



ERS | *monograph*

Respiratory Physiology: New Knowledge, Better Diagnosis

Edited by Raffaele Dellacà
and Andrea Aliverti

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Raffaele Dellacà and
Andrea Aliverti

Editor in Chief
Peter M.A. Calverley

This book is one in a series of *ERS Monographs*. Each individual issue provides a comprehensive overview of one specific clinical area of respiratory health, communicating information about the most advanced techniques and systems required for its investigation. It provides factual and useful scientific detail, drawing on specific case studies and looking into the diagnosis and management of individual patients. Previously published titles in this series are listed at the back of this *Monograph*.

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Preface

Peter M.A. Calverley 

I have always been fascinated by how things work and therefore, as a doctor, by physiology. At the Scottish university I attended, physiology was known as The Institutes of Medicine, reflecting its central role in explaining how structure is translated into function. Respiratory physiology has been extremely successful in doing this, with our modern understanding of pulmonary gas exchange driven by the need for safe high-altitude flying in the Second World War, and lung mechanics by the earliest measurements of pleural pressure obtained from patients with tuberculosis treated with an artificial pneumothorax. From these unpromising beginnings sprang our present understanding of how the lungs work, and thanks to ingenious technological applications, a wide range of diagnostic tests and procedures now allow us to characterise pulmonary dysfunction, and both monitor and predict disease progression.



Respiratory physiology was at the cutting edge of medical progress from the 1950s to the late 1980s but thereafter, there was a feeling that all the problems had been solved. This was an error, as the advent of modern computing and data management meant that ideas that had been thought experiments in the past could be converted into practical tools for patient care.

Given these new advances in diagnosis, it felt appropriate that we dedicate an issue of the *Monograph* to offering the reader an accessible insight into this exciting new world of improved diagnosis and understanding of pulmonary disease. To this end, we have been fortunate to recruit two distinguished bioengineers (and long-standing members of the European Respiratory Society) with an interest in respiratory physiology to be our guest editors. Raffaele Dellacà and Andrea Aliverti have, in turn, recruited a very distinguished group of physicians and scientists to survey this rapidly changing field. The chapters presented here cover the more basic physiological considerations, such as gas exchange and pulmonary mechanics, but also look at the theory and practice that underlies familiar and novel diagnostic procedures, as well as the application of physiology to understanding the impact of pollution and assisted ventilatory support. All these chapters are worth

reading, as they offer unexpected insight into what we think we know already and what we will need to know in the future.

This issue of the *Monograph* shows why an understanding of respiratory physiology is still at the heart of our discipline and why it is likely to remain so as the 21st century progresses. So read on and enjoy the ride!

Disclosures: P.M.A. Calverley reports receiving grants, personal fees and non-financial support from pharmaceutical companies that make medicines to treat respiratory disease. This includes reimbursement for educational activities and advisory work, and support to attend meetings.

Guest Editors

Raffaele Dellacà

Raffaele Dellacà is a full Professor at the Department of Electronics, Information and Bioengineering at the Politecnico di Milano University (Milan, Italy), and Chair of the PhD programme in bioengineering. He is responsible for the Technologies for Respiration (TechRes) Laboratory and is a co-founder of Restech s.r.l. (Milan), a spin-off company of the Politecnico di Milano University, which develops and produces innovative devices for lung function tests.



Raffaele Dellacà received an MSc in control science engineering in 1997 at the Politecnico di Milano University. He then went on to receive a PhD in biomedical engineering in 2001 at the same institute.

Raffaele's main research interests lie in bioengineering of the respiratory system and medical devices, with particular focus on respiratory physiology, respiratory mechanics, modelling of the respiratory system's mechanical properties, development of new technologies for the analysis of respiration, mechanical ventilation, and anaesthesia in adults and newborns.

In 2005, Raffaele became a fellow of the European Respiratory Society (ERS) and was Raine Visiting Professor at the University of Western Australia (Perth, Australia) in 2016. He has acted as principal investigator, co-principal investigator or collaborator on several research projects sponsored by public agencies, foundations and companies. He is the author of more than 150 papers published in international peer-reviewed journals and has 19 patents, most of them used by commercial companies producing medical devices.

Andrea Aliverti



Andrea Aliverti is a full Professor at the Department of Electronics, Information and Bioengineering at the Politecnico di Milano University (Milan, Italy), where he has been Director delegate for technology transfer since 2023. Andrea is also responsible for Lares (the Respiratory Analysis Laboratory) at the Biomedical Technology Laboratory (TBMLab) of the same university.

Andrea received an MSc in electronic engineering and a PhD in biomedical engineering from the Politecnico di Milano in 1992 and 1997, respectively.

His main research interests include bioengineering of the respiratory system, physiological measurements, biomedical instrumentation, functional lung imaging, respiratory mechanics, wearable sensors and technologies for digital health, and artificial intelligence applied to physiological data, signals and images.

An active member of the European Respiratory Society (ERS), Andrea was Secretary and Chair of the Respiratory Structure and Function group, and Secretary and Head of the Clinical Physiology, Sleep and Pulmonary Circulation assemblies. He has been an Honorary Fellow of the ERS since 2020.

Andrea is also an active member of the Institute of Electrical and Electronics Engineers (IEEE), and has been the Conference General Chair of the IEEE-STAR (Sport, Technology and Research) International Conference since 2023.

Andrea is the author of more than 300 papers, the editor of four books and has 15 patents. He received the ERS COPD Award in 2004, the Vertex Innovation Award in 2018 and the Andrew P. Sage Award of IEEE Transactions on Human-Machine Systems in 2021.

Introduction

Raffaele Dellacà  and Andrea Aliverti

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This *Monograph* traces the rapid evolution of respiratory physiology, highlighting how technological advances and interdisciplinary approaches have reshaped our understanding of lung function, gas exchange and ventilatory mechanics <https://bit.ly/ERSM107intro>

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The development of respiratory physiology has been characterised by a slow initial phase followed by much faster development, most of which took place over the last 70 years. The most significant advancements were facilitated by novel technologies that were able to provide new observations and measurements of relevant structures and variables. For example, the microscope allowed Marcello Malpighi to discover the alveoli and capillaries in 1661 (“De pulmonibus observationes anatomicae”). But it wasn’t until 250 years later that August and Marie Krogh were able to understand and prove the O₂ diffusion in the lung by performing accurate measurements using new devices that they created – the micro tonometer and the Krogh spirometer.

For many years, the development of respiratory physiology was ensured by scientists able to combine excellent technical skill with physiology, allowing them to create new devices and methods for addressing their research questions. This transdisciplinary approach drove significant advances that reshaped our understanding of lung function, gas exchange, respiratory mechanics, and the interactions between the respiratory and cardiovascular systems. These advancements have led to improved diagnostic tools, more precise disease phenotyping and enhanced clinical interventions. The contributions of physiologists such as John West, Hermann Rahn, Jere Mead, Peter Macklem and Joseph Milic-Emili have significantly advanced our understanding of pulmonary function. The recent technical advances in sensors, data processing, imaging techniques, computational modelling and molecular biology have boosted our ability to assess and interpret respiratory function to unprecedented levels. Interestingly, the increased complexity of the methods and techniques has widened the disciplines and competencies involved, with the research in respiratory physiology today involving biomedical engineers, physicists and biochemists, as well as physiologists.

Respiratory physiology is a key clinical domain that directly affects patient care. The ability to assess lung function accurately and efficiently has key implications in diagnosing, monitoring and planning the treatment of a wide range of respiratory conditions. Historically, pulmonary function tests such as spirometry and body plethysmography have been central to diagnosing respiratory disorders, but newer technologies have provided deeper insight into lung mechanics

and gas exchange. For example, oscillometry has emerged as a noninvasive technique that allows precise evaluation of airway obstruction, even in conditions affecting the small airways. Gas washout techniques, on the other hand, are a powerful tool for assessing ventilation distribution and detecting early signs of lung disease. These new methodologies have refined disease assessment, allowing the definition of more detailed phenotyping, and providing clinical tools that gather more comprehensive information on the patient's respiratory system and condition. Technologies that allow detailed measurement of chest wall kinematics, such as optoelectronic plethysmography, have enhanced our understanding of chest wall mechanics in several breathing disorders.

The impact of exercise on respiratory function has been another area of significant interest in recent decades. The integration of CPET into clinical practice has transformed the assessment of exercise intolerance, particularly in patients with chronic respiratory diseases such as COPD, ILD and pulmonary hypertension. Improved technologies and interpretation of the results of these tests not only increase successful diagnosis but also inform rehabilitation strategies and therapeutic interventions aimed at improving functional capacity and quality of life.

Imaging has long played a critical role in respiratory physiology, and recent technical advances have particularly impacted this field and expanded its capabilities. Traditional chest radiography and CT have been indispensable in evaluating lung structure, but emerging modalities such as synchrotron radiation CT and functional MRI are creating new opportunities for a better understanding of structure–function relationships, providing detailed insight into lung ventilation, perfusion and microstructural changes. The integration of imaging with physiological measurements is paving the way to a more comprehensive approach to respiratory diagnostics, in which structural abnormalities can be correlated with functional impairment to enhance clinical decision-making.

The continued evolution of respiratory physiology is driven by a combination of technological innovation, interdisciplinary collaboration and a growing emphasis on personalised medicine. As our understanding of lung function deepens, the potential for developing targeted therapies and individualised treatment plans becomes more attainable. This *Monograph* aims to provide an up-to-date and comprehensive overview of respiratory physiology, integrating classical concepts with the latest advancements. It highlights cutting-edge diagnostic techniques and their application in respiratory medicine, and bridges the gap between basic physiology and clinical practice, offering insight into how physiological principles inform disease diagnosis and management. It also explores emerging challenges and research directions, including the impact of environmental exposures, ageing and sex-related differences in lung function. Finally, it serves as an educational resource for clinicians, researchers and trainees in pulmonary medicine, physiology and related fields.

This *Monograph* is structured into four sections addressing: 1) new knowledge; 2) advancements in technology and methods for measuring lung function; 3) the state of the art of the rapidly evolving world of respiratory functional imaging techniques; and 4) current knowledge on the way in which factors such as sex and environmental exposure impact on respiratory physiology.

A proper understanding of all of these elements is crucial for improving diagnostic accuracy, enhancing patient outcomes, and informing treatment strategies in respiratory medicine. By integrating classical principles with contemporary advancements, and fundamental principles with emerging concepts, we hope that this *Monograph* will provide a unique resource for

clinicians, researchers, healthcare professionals and those engaged in respiratory medicine and research, with the ultimate goal of enhancing diagnostic precision, fostering scientific discovery, and, ultimately, improving outcomes for patients with respiratory disorders.

Disclosures: R. Dellacà reports the following, outside the submitted work: patents on applications of oscillometry owned and licensed from Politecnico di Milano University to Vyaire and Restech; stocks owned and being a member of the Board of Directors of Restech s.r.l., a Politecnico di Milano spin-off company; and research grants to Politecnico di Milano by Vyaire and Chiesi. A. Aliverti is inventor of several patents regarding optoelectronic plethysmography, forced oscillations, wearable systems for respiratory monitoring.

ChatGPT-4o was used as a basic writing assistant in the composition of this chapter, during January 2025.

List of abbreviations

CF	cystic fibrosis
CO	carbon monoxide
CO ₂	carbon dioxide
CPET	cardiopulmonary exercise testing
CT	computed tomography
D_{LCO}	diffusing capacity of the lung for carbon monoxide
FEV ₁	forced expiratory volume in 1 s
FRC	functional residual capacity
FVC	forced vital capacity
ILD	interstitial lung disease
MRI	magnetic resonance imaging
O ₂	oxygen
RV	residual volume
$\dot{V}_{\text{CO}_2}$	carbon dioxide production
$\dot{V}_{\text{O}_2}$	oxygen uptake



Respiratory physiology: a patient perspective

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Patients' knowledge of respiratory physiology improves over time and is pivotal for correct diagnosis and treatment. When patients understand how their symptoms relate physiologically, they can communicate with their providers. <https://bit.ly/ERSM107>

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This chapter aims to capture patients' understanding of respiratory physiology, how it has an impact on diagnosis, the balance between physiology and patient experience, and patients' hopes for the future. It combines data from the published literature, insights from a patient survey, and interviews with people affected by respiratory conditions from across Europe. Findings show that patients with knowledge of respiratory physiology receive more timely diagnosis and appropriate treatment and have better outcomes. Findings also show that patients' knowledge of respiratory physiology improves over time, empowering them to better advocate for their needs. Patients feel that clinicians rely too much on standardised respiratory physiology tests and that there needs to be more consideration of patient-reported outcomes and individual patient characteristics; they would like these to be considered as part of the diagnosis and treatment process. Patients would like better access to reliable respiratory physiology information. They tend to seek support elsewhere if, over time, they do not feel heard and if their experiences are not considered. Patients' priorities for future improvement include joint consideration of respiratory physiology and patient-reported symptoms and more individualised care. We must listen to and understand these patients' voices to provide the best possible care.

Background

This introductory chapter aims to set the scene for this *Monograph* by outlining views of people with respiratory disease. It aims to shed light on patients' knowledge of respiratory physiology, its influence on the diagnostic processes, and the balance between physiology and patient-reported outcome measures (PROMs) during monitoring and treating respiratory diseases. Specifically, we will highlight patients' perspectives on creating a more balanced approach and what happens when there is a divide between physiology and patient experience.

This chapter was developed by the European Lung Foundation (ELF) and draws on the experience of the ELF patient advisory groups and patient organisations from the ELF patient organisation network. The authors have included the perspectives of people living with respiratory diseases and representatives of patient support and advocacy organisations from Italy,

Greece, the Netherlands and the UK, as well as an umbrella federation representing respiratory disease patients in 26 European countries (European Federation of Allergy and Airways Diseases Patients' Associations). By involving patient representatives from various countries and settings, we aim to reflect differences in patients' experiences from different demographic backgrounds and health systems. Our participants were diagnosed with a mix of respiratory conditions, including asthma, aspergillosis, bronchiectasis and COPD. These patients are active members of the patient community; therefore, there may be some selection bias as they chose to contribute to this *Monograph* chapter.

This chapter will provide insight into the lived experiences of people with respiratory diseases, highlighting their challenges and priorities for the future in their own words.

Working methods and data sources

In discussion with the editors, ELF selected four questions:

- 1) What is patients' understanding of respiratory physiology?
- 2) How does their knowledge have an impact upon diagnosis and disease management?
- 3) What are patients' views of the balance between physiology and patient-reported outcomes?
- 4) What are their hopes for the future?

ELF administered an online cross-sectional survey in November 2024. ELF is a patient-driven organisation with a network of >200 patient organisations across Europe. It also has 18 patient advisory groups with members representing all respiratory conditions from 34 European countries. The survey was sent out to 243 patients of many demographic backgrounds and with many respiratory conditions, of whom 73 completed the study. The survey comprised eight questions, out of which three were closed:

- 1) Do you feel that your understanding of your lungs and lung condition affected your diagnosis? (yes/no) If "Yes", please tell us how.
- 2) Do you feel your understanding of your lungs and lung condition has impacted your treatment? (yes/no) If "Yes", please tell us how.
- 3) Do you think learning more about your lungs and condition has helped you manage it better? (yes/no) If so, in what ways?

We began by descriptively exploring closed-question responses. We then thematically analysed the open-ended questions, applying the questions agreed upon at the onset as a framework for the analysis. We subsequently carried out a thematic analysis with qualitative data.

We also invited 10 patients who completed the survey to participate in in-depth interviews, of whom five agreed. The aim was to ask more about the balance between physiology and patient-reported outcomes. The interviews were 30–40 min long.

In addition, ELF reviewed previously published qualitative literature to understand patient experiences and perspectives more broadly. The themes presented in this chapter are based on the combined perspectives from the authors' experiences, knowledge of their patient communities, and data from published sources. For the literature searches, two databases, Medline and CINAHL, were searched for relevant studies to supplement the personal experiences explored in the survey and in-depth interviews with selected items from the literature. Search terms included "respiratory physiology", "patient perspective", "understanding", "diagnosis", "treatment", "patient-reported outcomes", "knowledge", "education" and "quality of

care”. Citations were deemed relevant if they included the patients’ perspectives of the topic and subtopics. Only citations published in the English language were included. We also mainly included citations published between 2000 and 2023, to ensure relevance to current experience.

Study population

The final sample responses comprised 73 patients. Some patients reported having more than one condition. The total representation was the following: sarcoidosis 35.6% (n=26), “other” 28.8% (n=21), asthma 26.0% (n=19), bronchiectasis 24.7% (n=18), aspergillosis 20.5% (n=15), COPD 13.7% (n=10), chronic cough 9.6% (n=7), idiopathic pulmonary fibrosis 9.6% (n=7), pulmonary fibrosis 6.8% (n=5), sleep apnoea 6.8% (n=5), lung cancer n=4.1 (n=3) and α_1 -antitrypsin deficiency 1.4% (n=1).

Findings from closed questions

Nearly half of the patients (47%, n=34) expressed that their knowledge and understanding of their condition and respiratory physiology affected their diagnostic process. Most respondents (74%, n=54) reported that their knowledge and understanding had an impact on their treatment, and 88% (n=64) reported that their knowledge and understanding had an impact on how well they could manage their condition. These data are shown in table 1.

The empirical findings of the survey show that patient knowledge is essential throughout the diagnostic, treatment and self-management process. The findings indicate that patients’ knowledge of their condition improves, as a higher proportion reported that their understanding had an impact on their treatment and disease management after the diagnosis, and the longer they had lived with the condition. Therefore, it is more likely to affect their treatment and how well they manage the condition.

Themes from qualitative analysis

The empirical findings were reflected in the qualitative findings. For the qualitative analysis, responses were analysed from 73 respondents and five transcripts from in-depth interviews. The qualitative findings were structured around four overarching themes. The next sections of this chapter address our qualitative findings with respect to these themes:

- 1) Patients’ understanding of physiology
- 2) Importance of respiratory physiology in disease diagnosis, treatment and outcomes
- 3) Patients’ view of the balance of using respiratory physiology *versus* PROMs
- 4) Patients’ concerns and hopes for future improvements

TABLE 1 Patients’ views on the importance of understanding their lungs and lung condition

Survey question	Response	
	Yes	No
Do you feel that your understanding of your lungs and your lung condition affected your diagnosis?	47%, n=34	53%, n=39
Do you feel your understanding of your lungs and lung condition has impacted your treatment?	74%, n=54	26%, n=19
Do you think learning more about your lungs and condition has helped you manage it better?	88%, n=64	12%, n=9

Patients’ understanding of respiratory physiology and underlying mechanisms

“My knowledge was everything. I would not have been diagnosed had I not taken the time to learn about physiology through patient advocacy groups on the importance of learning about the symptoms and physiology.”

Qualitative findings from surveys and interviews show that patients’ understanding and knowledge are critical during diagnosis, especially when choosing and agreeing on the “right” treatment and self-management. The findings show that, over time, many patients become experts in respiratory physiology and their condition. Patients described researching published evidence, attending conferences, and participating in online forums where they could discuss their symptoms. Patients reported that new knowledge empowered them and helped to speed up the diagnosis process. Responses from study participants and published evidence have reported that patients actively seek out knowledge. Over time, they become better able to communicate their symptoms and achieve better outcomes. Examples of patient responses to our survey and interviews in relation to this first theme are shown in table 2.

These findings and previous evidence show the extent to which patients seek to understand the relationship between their lived experience and physiology. This helps them communicate their needs and preferences to healthcare providers and shows how they can more actively influence treatment plans.

In line with the published evidence [1], survey responses have shown that healthcare professionals need to provide more information about symptoms and side-effects of treatment [2]. Patients would like to receive more information directly from their provider rather than relying on patient advocacy groups and forums or searching on the Internet. Much information and knowledge is accessed *via* social media, discussions with other patients, and patient organisations (table 3). Some patients think that lack of information is a problem, particularly for those from disadvantaged groups, such as lower socioeconomic groups and older patients.

Barriers to accessing information

Patients report that they would prefer if healthcare providers gave more information about their respiratory condition. Instead, digital platforms, including websites and mobile applications, have become increasingly popular for educating patients about respiratory diseases. Published evidence, including a systematic review, found that patients primarily seek telemonitoring devices to learn

TABLE 2 Do you feel your understanding of respiratory physiology affected your diagnosis and treatment?

“I had to learn more about physiology to get diagnosed. It made it more clear to me why the current standardised tests are not always well equipped to diagnose my asthma correctly.” (Patient 1; interview; asthma)
“Once I understood my lung condition better, it was easier to get a change of medication or referrals to a specialist respiratory team than if I lacked that knowledge; it also gave me the correct terminology to unlock support.” (Patient 3; survey; asthma, bronchiectasis, COPD and sleep apnoea)
“I did an enormous amount of research on my asthma. Over time, I was involved in research and discovered things. For me, that made me understand the mechanisms of my asthma better. That made me communicate better with my doctor about my asthma in a consistent way.” (Patient 1; interview; asthma)
“My knowledge of the physiology and symptoms influenced my decisions and how I talked to the doctors.” (Patient 8; survey; sarcoidosis)
“Knowledge, adherence, and self-management depend on different things; one does not grant the other. Knowledge, of course, helps in managing scare and emotional behaviours.” (Patient 4; interview; ILD)
“Knowing more about my illness gave me a better understanding, and I can now interpret the symptoms better.” (Patient 22; survey; pulmonary hypertension)

TABLE 3 How do you learn more about and find information about your lungs and lung condition?

"Since my diagnosis, I have actively sought information from various websites and charities, attended specialist respiratory and cancer conferences, read books, and participated in webinars and talks." (Patient 6; survey; asthma, lung cancer and sleep apnoea)
"Searching the Internet for sources from the UK, Europe, the USA and Australia, visiting virtual conferences, and reading articles have helped." (Patient 16; survey; bronchiectasis)
"Both UK charities like Asthma+Lung UK and the European Lung Foundation." (Patient 24; survey; COPD)
"First, I used Doctor Google and support groups and quickly learned to only use trusted websites. I was lucky enough to become a charity trustee and became involved in pharmaceutical company patient advisory boards." (Patient 7; survey; idiopathic pulmonary fibrosis)
"I started attending conferences and webinars, mainly from the Hellenic Thoracic Society and the EU-PFF (European Pulmonary Fibrosis Federation)." (Patient 13; survey; idiopathic pulmonary fibrosis)
"I made it my business to learn more and gained most of my knowledge through my support group, expert speakers, and other group members." (Patient 15; survey; rheumatoid arthritis-associated ILD)
"Initially, I bombarded a consultant with questions in a 10-min meeting, then preferably used online respiratory support information like ELF or Asthma+Lung UK." (Patient 24; survey; COPD)
"Aspergillois Trust website, Facebook research, and papers online." (Patient 31; survey; aspergillois)

more about their condition. Whilst these platforms can enhance patient experience, knowledge, and behaviour change, improving quality of life [3], there are limitations. The review identified these as developing online relationships with healthcare providers, lower digital capabilities, and poor computer/smartphone/tablet access [4]. Several studies have reported that some patients experience education and knowledge access barriers. For instance, patients with lower digital literacy and who lack access to digital devices do not benefit equally from technology-enabled education [5]. Therefore, these approaches may not be practical for them.

Patients feel that these obstacles need to be overcome for equity in access. Similarly, patients who were interviewed discussed different environmental and social circumstances. They emphasised that providers should be mindful that health inequalities and demographic factors can all affect how people with symptoms access and interact with healthcare services (table 4). Published evidence and findings from interviews with patients also reported concerns about

TABLE 4 Access to information and patients' concerns about disadvantaged groups

"Health services and patient organisations must collaborate to create information at the right language level to make it more accessible to all patient groups. There is an advised language level that is understandable by the majority. If you want to make it accessible, use this language level, use many pictures, and explain things differently." (Patient 1; interview; asthma)
"If your clinician shows you the asthma airways and explains it, only a small group of patients will understand. This is due to low health literacy." (Patient 2; interview; bronchiectasis)
"The way I look at it, we are worried. We are educated, well-spoken patients, and if we talk about how difficult it is for us to get the care we need, we are apprehensive about people who do not know the symptoms. We get concerned because these patients are not taken seriously enough or helped well enough because essential information is missing, and they do not know how to give it or do not know the answer." (Patient 3; interview; bronchiectasis)
"There are enormous disadvantages and inequality in healthcare. Unfortunately, even if you are higher educated in lower income groups, the impact of your living situation already makes you more disadvantaged. Even if there is not much health difference, the differences start to show at age 35." (Patient 1; interview; asthma)
"Younger with higher levels of education have easier access to information and, thus, knowledge. Nonetheless, respiratory physiology is very technical, requiring willingness and a few basic notions, without which the topic could appear scary to many people." (Patient 4; interview; ILD)

patients who may be disadvantaged due to their socioeconomic background, financial status or age [6]. They noted that more needs to be done to provide information in a clear and easy-to-access way for all groups to reduce health inequalities.

Patients recommend providing more support and knowledge to empower patients to communicate about their symptoms and better manage their condition. Therefore, providing more information and signposting to reliable sources of information is essential. Mainly, communicating information in a way that is more accessible to all groups, including those from lower socioeconomic backgrounds, could reduce the adverse effects of health inequalities (table 4).

Importance of respiratory physiology in disease diagnosis, treatment and outcomes

“The more I learn, the more it helps me discuss options with my consultant. The knowledge has empowered me to cope better with a long-term chronic condition.”
(Patient on the importance of knowledge and understanding of the condition)

Both published evidence and patient interviews have shown that patient knowledge affects diagnosis, treatment adherence and outcomes. Studies show that patients with chronic respiratory diseases, such as COPD, asthma, ILD and bronchiectasis, who have limited understanding of their symptoms have delayed diagnosis [7]. For instance, an extensive study of the COPD diagnostic process in the UK found that 58% of patients with COPD presented with respiratory symptoms up to 5 years before diagnosis, which suggests a much-delayed diagnosis [8]. Conversely, studies have shown that patients with better knowledge of their respiratory diseases are more likely to seek timely medical help and adhere to treatment protocols [9].

Patients described that understanding physiology is essential for disease management. In cases where some described the process of getting the correct diagnosis as lengthy, they had to educate themselves better to understand the relationship between their symptoms and physiological tests, mainly to communicate discrepancies between the two to their healthcare provider effectively. Patients frequently reported that they were misdiagnosed and that they felt unheard. Patients reported that healthcare providers did not consider their experiences enough. Patients reported becoming aware that clinical judgement is based on clinical trial data, which does not always reflect the disease burden from the patient’s experience. Table 5 includes illustrative findings from the survey and semi-structured interviews describing the impact of patient knowledge on the treatment process. Survey findings also showed that improved knowledge and understanding help patients to manage their condition better (table 6).

Patients also described where they think the significant gaps in knowledge are and what type of information they would want more of. Some patients described not understanding the relationship between their experiences and physiology early post-diagnosis. They explained that more information is required to help them connect the two (table 7). Patients would like more information from their clinicians about the relationship between respiratory physiology, the underlying mechanisms, and symptoms. They would also like better signposting to reliable

TABLE 5 Has your knowledge impacted your treatment?
“Knowing the medications available makes it easier to make a more informed decision with my consultant. Knowing the side-effects of medication is very important to me. The knowledge has empowered me to cope better with a long-term chronic condition.” (Patient 71; survey; aspergillosis and asthma)
“I have a good knowledge of different medications and side-effects so that I can discuss this with my consultant on a more informed basis.” (Patient 3; survey; asthma, bronchiectasis, COPD and sleep apnoea)

TABLE 6 Do you think learning more about your lungs and condition has helped you manage it better? If so, in what ways?

"Yes, because I know how vital chest clearance is and how to eat a healthy diet and avoid viral infections (e.g. paying attention to hand hygiene). Also, I want to stay up to date with vaccinations." (Patient 68; survey; bronchiectasis)
"Yes. It helps me to consider my environment/the impact of my conditions in life." (Patient 69; survey; aspergillosis and bronchiectasis)
"The more information I have about the disease, the better I understand it and the more easily I can deal with the symptoms." (Patient 18; survey; aspergillosis and asthma)
"Yes, keeping a better distance from sick people. Avoid crowded areas and avoid managing hard soil/garden work. Try not to get stressed and eat, rest and sleep well." (Patient 67; survey; aspergillosis, bronchiectasis and asthma)
"Knowing how pharmaceutical drugs can be supplemented with specific exercises and what ingredients in dishes may help or hinder breathing, such as loosening mucus viscosity through hydration, cinnamon and turmeric, is helpful." (Patient 3; survey; COPD and sleep apnoea)
"The longer I live with the disease, the more I feel confident that I can interpret my symptoms." (Patient 4; survey; sarcoidosis)

sources of information, as this is key to better diagnosis and treatment experience. Patients reported that consultations should allow sufficient time and resources to provide this information (table 7).

Patients' views of the balance of using respiratory physiology *versus* PROMs

"I know a lot about the physiological monitoring of asthma, but I also know how big the divide is between the physiological monitoring and the patient experience." (Patient's experience of the diagnostic process)

Patients described a divide between the results from physiological monitoring and their personal experiences. Patients reported feeling that clinicians can rely too much on physiological measurements and that a more holistic approach is necessary for correct diagnosis and treatment. Findings from interviews show that the disagreements between patients and clinicians are due to, for instance, discrepancies between physiological monitoring data (such as spirometry results and O₂ levels) and PROMs such as breathlessness. Patients explained that their diagnosis and treatment experience would improve if they were better incorporated into the process.

TABLE 7 Type of information that patients would like to receive

"With physiology, you must explain what symptoms this physiology creates and how to recognise these symptoms. Combining good information from patient organisations, hospitals and healthcare providers with consistent details is crucial." (Patient 2; interview; bronchiectasis)
"It is better to provide more information to patients directly because it is the clinicians' responsibility to inform them about their condition. If patients look for irrelevant data online, they create more significant problems." (Patient 5; interview; lung cancer)
"If patients are not sleeping well or are exhausted after breakfast and a shower, they may not know it is related to their asthma. It is not easy to combine what is happening to you and how to explain it to your doctor as a patient. You need help to build up this knowledge, which cannot be found online." (Patient 3; interview; bronchiectasis)
"More is needed to help the patients understand the specifics of your disease. You can only expect your patients to know everything if you tell them what to look for." (Patient 14; survey; sarcoidosis)
"Clinicians must sufficiently inform their patients about the condition when it is diagnosed; they need to tell you what symptoms could be and how to recognise them." (Patient 5; interview; lung cancer)
"When I get a new drug, I would like to know what it is not supposed to do." (Patient 1; interview; asthma)

Evidence shows that PROMs enhance diagnosis and support disease control [10]. Some studies have also demonstrated that routine feedback of PROMs to healthcare providers and patients can lead to moderate improvements in quality of life and patient–physician communication [11]. PROMs in routine monitoring have shown the potential to improve symptom detection and patient-centred care.

Patients reported that agreeing on the “right” treatment after an accurate diagnosis is more complicated if clinicians rely too much on physiology. Some patients experienced that their symptoms were not improving and that, therefore, they felt that they were not receiving the proper treatment. Some patients reported that it was challenging to communicate to their health professionals that the treatment was not working, especially if they suggested that the physiology measurements were not describing the whole picture and that their diagnosis, type of medication or dosage may need adjusting. Patients reported that their clinicians often had a perfect knowledge of physiology and that they usually expected physiological tests to match this. Disagreements about the next steps could occur when there was a difference between observations, physiology, and patients’ experiences (table 8).

“They insisted that I do lung function tests even though they made me worse. Moreover, I told them that I was not doing them because they made me ill and made me worse. I felt overlooked and made to think that I was a difficult patient.” (Patient talking about when physical tests make them worse)

Findings from the open survey questions and interviews with patients mentioned additional challenges, including situations when patients did not want to do physiological tests because they felt that it made their condition worse and that they suffered from side-effects for an extended period afterwards. Some patients often reported that they could not see the benefit of testing, while their healthcare provider relied on these tests.

Patients’ experiences reflect the most recent published research, which found a poor correlation between physical tests and PROMs during severe acute exacerbations of COPD [12]. This was

TABLE 8 What is the balance between physiology and patient-reported outcomes?
“There needs to be more than just looking at the physiology and data because if you only do that, you will miss much of what is going on with your patient and may completely misdiagnose them.” (Patient 1; interview; asthma)
“I believe that disease-specific PROMs [patient-reported outcome measures] should be developed to have better results.” (Patient 4; interview; ILD)
“In severe or moderate asthma, physiological measures alone are insufficient to diagnose a patient well. You still need to listen to the patient and learn what is happening to them.” (Patient 1; interview; asthma)
“My asthma did not conform to guidelines presumptions, so they had to think outside the box.” (Patient 26; survey; asthma)
“Lower FEV ₁ in some patients is not experienced as bad, and these tests must sufficiently reflect this diversity. If you go in for lung function research and do breathing exercises in the machine based on weight, age, etc., they calculate the 100% line. Though the guidelines advise that you look at the patient best, not standardised best, this rarely happens. FEV ₁ increase or decrease does not necessarily reflect the disease burden experienced by the patient.” (Patient 1; interview; asthma)
“Relying too much on physiology measurements can negatively impact everything. It can affect the medication dosage.” (Patient 5; interview; lung cancer)
“My O ₂ levels are acceptable, but my CO ₂ levels are off; we need more patient-related outcomes to understand the disease’s burden. Clinicians do not always consider patient-reported outcomes.” (Patient 3; interview; bronchiectasis)
“I know about physiology, but these standardised outcome measures are only sometimes as closely related to a patient’s experience as the doctor might hope.” (Patient 6; survey; asthma, lung cancer and sleep apnoea)

the first study to correlate physical tests and PROMs during a severe acute exacerbation of COPD. The study included multiple tests and PROM surveys. Because of the poor correlation, the study concluded that future studies should focus on developing a core outcome set, including physical tests and PROMs.

Similarly, a review of PROMs for asthma found that they are fundamental to more accurate assessment. The study noted that although PROMs are used to understand asthma control in clinical management guidelines, evidence of how they are used and their effectiveness is limited. The study recommends more urgent research to understand how these can be used practically in clinician settings as part of routine care [13].

Published evidence and patients' views are that combining physical tests and PROMs is a more comprehensive way to make assessments, especially in cases where they are not well correlated. Therefore, future research studies should focus on developing a comprehensive outcome set that includes physical tests and PROMs. Findings also suggest more training and research into how these are used in clinician settings. These core outcome sets could be constructed using and building on existing questions from previously validated surveys. A holistic approach during assessments may also result in more patient-centred care, which patients want.

Patients' concerns and hopes for future improvements

"Things improve if you combine the patient experience with the clinician's expertise and see how you can create an image that fits both."

Our research identified priorities for research and care throughout discussions with the patient group and through examining the published literature.

Improving the balance between physiology and PROMs

While some improvements have been reported, more work is needed to combine respiratory physiology science with patients' lived experience to improve the diagnostic and treatment processes and patient outcomes. More research is also needed to implement this in clinical settings. Patients' concerns are that diagnostic and treatment decisions are sometimes characterised by too much reliance on physiology testing and a lack of attention to their experiences and preferences and them as individuals. Instead, patients would like better integration strategies to balance physiology and patient-reported experience. This is also supported by published evidence, which has found a knowledge gap.

Supporting patients' involvement in decision making

Patients also reported that more openness toward PROMs and joint discussions is needed because this would give a more accurate diagnosis and a better treatment plan. Patients would like open communication that allows for more balanced decision making. Patients expect to be involved in decision making. Similarly, published evidence found that combining patients' experience in the diagnosis and treatment process as part of shared decision making in asthma management improves patient outcomes. The study found that negotiating patients' treatment decisions improves adherence to asthma pharmacotherapy and clinical outcomes [14].

Visual representations, analogies and simplified language make information accessible to all patients

Our findings show that patients feel that better access to information is pivotal because it empowers them and enables shared decision making. To facilitate better access to information,

clinicians could adopt simple language visual aids. Published evidence shows that using formats such as pictograms and presenting statistical information more intuitively can facilitate better understanding. Studies also found that presenting information in a way that informs without causing undue anxiety by framing risks and benefits in a balanced manner helps patients grasp the consequences of different outcomes [15].

Patients described a positive experience when they could discuss their symptoms and concerns. For example, some patients felt disappointed if the clinician resisted when asked about alternative treatment options. As a result, patients are also concerned that some disadvantaged groups are at even greater risk of misdiagnosis due to existing health inequalities. In the future, patients will want more awareness and knowledge among clinicians about how to provide more personalised care. Therefore, healthcare professionals must prioritise educating patients early in the diagnostic process.

Conclusion

The themes capture the patient's perspective on the knowledge and understanding of respiratory conditions, the balance of respiratory physiology *versus* PROMs for monitoring and hopes for the future.

Many patients with respiratory conditions develop a good understanding of physiology as they live longer with their condition. This understanding is essential in their diagnosis and disease management. They describe various challenges throughout the diagnostic process. Some situations in which patients find challenges are that clinicians rely too much on standardised physiology tests and that the patient must “fit the mould”. Patients were aware of new tools, including PROMs and observations, and felt their providers could use them more frequently.

Patients seek support and knowledge online and through patient advisory groups if their health professionals need more information and support or if disagreements occur. Patients know the risk of misinformation but feel they need more options. Patients wish that more information was provided by their clinician from the onset. Patient organisations across Europe provide vital information, signposting and supporting people with respiratory conditions due to what patients describe as under-resourced health systems.

Patients want increased investment in research to develop better diagnostic processes with joint standardised measurements, PROMs and observations. They would also like information to be made more accessible, considering the patient demographic.

We hope this chapter has provided valuable insight into the experiences and priorities of those living with respiratory conditions and that it helps healthcare professionals consider how best to meet their patients' needs.

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Advances in gas exchange physiology and pathophysiology

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Recent research has led to new insights into and methods for assessing lung/tissue gas exchange. These advances apply to cardiopulmonary diseases generally, offering noninvasive or minimally invasive techniques to quantify/understand gas exchange. <https://bit.ly/ERSM107>

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Although respiratory gas exchange is a mature field, recent research offers new insight into and new techniques for patient evaluation. While some gases suffer pulmonary diffusion limitation, others do not, and this is explained by comparing their solubility in the alveolar wall to their solubility (dissociation curve slope) in blood. This chapter revisits the well-established concepts of shunt and deadspace with new computerised analyses and measurement approaches, showing that in COVID-19, abnormal gas exchange reflects not just shunts but also alveolar deadspace, suggesting vascular obstruction (sometimes persisting for months). It is likely that so-called “silent hypoxia” in COVID-19 reflects normal variation in hypoxic ventilatory responsiveness; in most patients, arterial CO₂ tension is either normal or reduced. There is little strong evidence to suggest that COVID-19 impairs neurological pathways of breathing control. Finally, this chapter reviews O₂ transport from air to mitochondria as an interactive system (lungs, heart, blood and tissues) in which all components function in ways that affect each other. This systems approach enables separation and quantification of central and peripheral contributions to impaired O₂ transport.

Introduction

Although the fundamentals of pulmonary gas exchange have long been established, recent research offers new insight into the understanding of lung pathophysiology and techniques for patient evaluation. This chapter focuses on the latest insights in diffusion limitation, ventilation/perfusion ($\dot{V}/\dot{Q}$) mismatch, shunt and alveolar deadspace, which, combined with new analysis approaches and an integrative view of gas exchange at the whole-body level, provide useful awareness of new problems.

Gas exchange is a result of passive diffusion caused by a partial pressure difference between alveoli and the pulmonary capillaries, resulting in, at equilibration, alveolar O₂ tension ($P_{A_{O_2}}$) equal to end-capillary O₂ tension/arterial O₂ tension ($P_{a_{O_2}}$). The alveolar–arterial partial pressure

difference for O₂ (AaDO₂) is the primary index of gas-exchange efficiency, with P_{AO_2} calculated as:

$$P_{AO_2} = [(P_B - P_{H_2O}) \cdot F_{IO_2}] - (P_{ACO_2}/R) + [P_{ACO_2} \cdot F_{IO_2} \cdot (1-R)/R] \quad (1)$$

where P_B is barometric pressure, P_{H_2O} is saturation vapor pressure of water (47 mmHg at 37°C), F_{IO_2} is inspiratory O₂ fraction, P_{ACO_2} is alveolar CO₂ tension (P_{CO_2}) and R is the respiratory exchange ratio. P_{ACO_2} is estimated from the arterial CO₂ tension (P_{aCO_2}), assuming a (reasonably in health) lack of alveolar–arterial CO₂ difference. P_{AO_2} is approximated as:

$$P_{AO_2} = [(P_B - P_{H_2O}) \cdot F_{IO_2}] - (P_{ACO_2}/R) \quad (2)$$

R is often assumed, introducing error. Measurement of the ratio of O₂ consumption to CO₂ production with expired gas analysis is preferred. P_{H_2O} is calculated from body temperature [1]. Contributors to the AaDO₂ are diffusion limitation, $\dot{V}/\dot{Q}$ mismatch and shunt.

Diffusion limitation

Gases cross the alveolar blood–gas barrier, in either direction, by diffusion [2]. While there are additional processes that are often included in assessing diffusion (in particular, the reversible chemical reactions of O₂ (and CO) with haemoglobin (Hb) and the formation of H⁺ and HCO₃[−] from CO₂ [2]), this section only addresses diffusive transport across the barrier.

Diffusive movement in any circumstance, including between alveolar gas and capillary blood, is fuelled by thermal kinetic energy (E). At the same temperature, all gases have the same kinetic energy, $E = \frac{1}{2} M \cdot v^2$, where M is molecular mass and v is the velocity of diffusive motion. Therefore, v is inversely proportional to $M^{0.5}$. Diffusive transport is also proportional to: 1) the gas-exchanging surface area (A); 2) the solubility (α) of the gas in Hb-free fluids within the blood–gas barrier (cells/matrix); and 3) inversely to the thickness of the blood–gas barrier (T). Therefore, if D_L represents the diffusive conductance of a gas:

$$D_L = (k \cdot A \cdot \alpha) / (T \cdot M^{0.5}) \quad (3)$$

where k is a constant reflecting the innate diffusivity of the blood–gas barrier. Once gas has crossed the alveolar capillary membrane it is removed by blood flow at a rate of $\beta \cdot \dot{Q} \cdot P_{C'}$, where β is the dissociation curve slope of the gas and $P_{C'}$ is the end-capillary gas partial pressure.

In the 1980s, PIPER and SCHEID [3, 4] demonstrated that for any gas with a linear dissociation curve, or solubility, the $P_{C'}$ was an exponential function of D_L , $\dot{Q}$ and β , and that it is the ratio of D_L to $\dot{Q}$ conductance (i.e. $D_L/(\beta \cdot \dot{Q})$) that determines the degree of diffusion equilibration:

$$P_A - P_{C'} = (P_A - P_{\bar{V}}) \cdot e^{-D_L/(\beta \cdot \dot{Q})} \quad (4)$$

where P_A and $P_{\bar{V}}$ are the alveolar and mixed-venous partial pressure difference for the gas. $P_A - P_{C'}$ is the alveolar to end-capillary partial pressure difference caused by diffusion limitation.

Substituting D_L from equation 3 into the exponent of equation 4 yields:

$$D_L/(\beta \cdot \dot{Q}) = (k \cdot A \cdot \alpha) / (T \cdot M^{0.5}) / (\beta \cdot \dot{Q}) \quad (5)$$

The key point is that in any given lung, different gases “see” the same k , A , T and $\dot{Q}$ and so will move by diffusion at different rates according to their own value of the compound ratio $(\alpha/\beta)/M^{0.5}$.

Given that differences in $M^{0.5}$ (especially among O_2 , CO and CO_2) are small, the relative speeds of diffusive gas exchange comparing different gases in a given lung are overwhelmingly determined by α/β , i.e. the ratio of the solubility of the gas in the (Hb-free) blood–gas barrier to its solubility in blood (or, for O_2 , CO and CO_2 , their average dissociation curve slopes between P_A and P_V). It is enlightening to compare α/β for common gases. Table 1 displays the key variables (including α/β) involved in diffusion for O_2 , CO and CO_2 , and two inert gases (one with low α and one with high α). Table 2 shows the percentage reduction in diffusive movement of a gas based on the value of $D_L/(\beta \cdot \dot{Q})$. Figure 1 compares diffusion limitation across the lung for these gases.

In health, during peak normoxic exercise, diffusing capacity roughly doubles [3] because of capillary recruitment [10] and distension [11], increasing pulmonary capillary blood volume, which in turn increases the surface area for diffusion [12]; however, cardiac output increases relatively more, often four-fold. The mixed venous partial pressure of O_2 (P_{VO_2}) is also considerably reduced, which increases β from about 0.08 to about 0.2 [3, 4]. The increase in $(\beta \cdot \dot{Q})$ is thus almost 10-fold, much larger than the approximately two-fold rise in the D_L of O_2 (D_{LO_2}). Hence, during exercise, $D_L/(\beta \cdot \dot{Q})$ falls considerably, often to ~ 2 , causing a modest but significant diffusion limitation (table 2; reviewed in [13]). This becomes even more evident at altitude when gas exchange occurs on the steep portion of the O_2 dissociation curve, raising β to ~ 0.4 and reducing $D_L/(\beta \cdot \dot{Q})$ to ~ 1 [6–9].

However, in lung disease, despite reduced D_{LCO} , diffusion limitation for O_2 does not usually occur (at rest) until diffusing capacity is $<50\%$ of normal (figure 1) [14]. It should also be recognised that $\dot{V}/\dot{Q}$ mismatching alone will reduce CO uptake and thus D_{LCO} without any actual diffusive impairment present. A reduction in D_{LCO} therefore does not necessarily imply diffusion limitation for O_2 . That said, patients with ILD and a reduced D_{LCO} (but not those with COPD) do exhibit O_2 diffusion limitation, even during very modest exercise [14], because of low D_{LCO} , limited capillary recruitment and distension with exercise, and the increases in $\dot{Q}$ and β . Thus, reduced D_{LCO} may, but does not always, result in diffusion limitation [12] depending on the ratio $D_{LO_2}/(\beta \cdot \dot{Q})$. Resting diffusion limitation in disease is seen only in ILD [14] and possibly in liver cirrhosis (due to enlarged intrapulmonary blood vessels [15, 16]).

In summary: 1) for all gases, the value of $D_L/(\beta \cdot \dot{Q})$ determines the degree of diffusion limitation through equation 4; 2) in any given lung, the ratio α/β (where α is gas solubility in the Hb-free blood–gas barrier, and β is solubility in blood) determines the relative vulnerability to diffusion limitation; and 3) for O_2 , CO_2 and CO , β is the average slope of their dissociation curve between arterial and mixed venous blood.

$\dot{V}/\dot{Q}$ matching

$\dot{V}/\dot{Q}$ matching such that well-ventilated lung regions are also well perfused, is a primary determinant of gas exchange efficiency and is disrupted in lung disease. Although the overall (mean) $\dot{V}/\dot{Q}$ is ~ 1 , the heterogeneity of that mean is paramount in determining gas exchange efficiency. $\dot{V}/\dot{Q}$ mismatch is an important contributor to the $AaDO_2$ because the mean P_{AO_2} is determined by the distribution of $\dot{V}$, but P_{aO_2} is determined by the distribution of $\dot{Q}$ from each unit (figure 2).

TABLE 1 Vulnerability of specific gases to alveolar–capillary diffusion limitation

Gas	Molecular weight	Water solubility (α), mL·100 mL ⁻¹ ·mmHg ⁻¹	Blood or mean β , mL·100 mL ⁻¹ ·mmHg ⁻¹	α/β	D_L capacity, mL·min ⁻¹ ·mmHg ⁻¹	$D_L/(\beta\cdot\dot{Q})$
CO	28	0.0024	~60	0.00004	35	0.01
O ₂	32	0.0031	0.083	0.037	42	8.4
CO ₂	44	0.067	0.8	0.084	785	16.4
Inert gas with low α (SF ₆)	146	0.0007	0.0007	1.0	4.5	107
Inert gas with high α (acetone)	58	40.0	40.0	1.0	407 000	170

The ratio of solubility (α)/dissociation curve slope (β), as a component of the ratio of diffusive conductance (D_L) to perfusive ($\beta\cdot\dot{Q}$), is the principal determinant of vulnerability when comparing gases within any given lung. This ratio (α/β) varies remarkably across the five gases presented in this table, from 0.00004 for CO to 0.037 for O₂, and to 1.0 for both sulfur hexafluoride (SF₆) and acetone. It is this variation that allows the multiple inert gas elimination technique to assess ventilation/perfusion mismatch without interference from diffusion limitation (that may affect O₂) [5]. Note that if D_L is expressed as usual in mL·min⁻¹·mmHg⁻¹ and $\dot{Q}$ in L·min⁻¹, β must be expressed in mL·L⁻¹·mmHg⁻¹ and not in the more common unit of mL·100 mL⁻¹·mmHg⁻¹.

TABLE 2 Percentage reduction in diffusive movement of a gas based on the value of $D_L/(\beta \cdot \dot{Q})$

$D_L/(\beta \cdot \dot{Q})$	Reduction in diffusive movement, %
>10	0
8	0
5	1
4	2
3	5
2	14
1	37
0.5	61
0.1	90
0.01	99

D_L is lung diffusing capacity expressed in $\text{mL} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$; β is the blood solubility (inert gases) or the average slope of the dissociation curve (for CO , O_2 , CO_2) expressed in $\text{mL} \cdot \text{L}^{-1} \cdot \text{mmHg}^{-1}$; $\dot{Q}$ is cardiac output, in $\text{L} \cdot \text{min}^{-1}$. The left column shows the values of $D_L/(\beta \cdot \dot{Q})$; the right column is the corresponding value of $100 \times e^{-D_L/(\beta \cdot \dot{Q})}$. In healthy young adult resting humans, $D_L/(\beta \cdot \dot{Q})$ is about 8. This implies essentially zero diffusion limitation. However, with exercise, or ascent to altitude, and especially with both, $D_L/(\beta \cdot \dot{Q})$ can fall to as low as 1, producing a significant amount of diffusion limitation. This reduces both arterial O_2 tension and O_2 delivery to the tissues, which together contribute to the well-known reduced exercise capacity at altitude [6–9].

Passive mechanisms underlying $\dot{V}/\dot{Q}$ matching

Passive mechanisms dominate in ensuring $\dot{V}/\dot{Q}$ matching in the healthy lung. Blood vessels and airways follow a closely matched fractal branching structure, allowing passive matching. The lung sags underneath its own weight, behaving like compressible foam [18, 19], with blood and lung tissue both contributing to compression [20]. The result is that the alveoli in the nondependent lung are stretched larger than those in the compressed dependent lung [21], and thus lie on different portions of the pressure/volume curve during tidal breathing. Despite greater inflation, the stiffer nondependent alveoli are more poorly ventilated than less-expanded dependent alveoli, leading to a gravitational $\dot{V}$ gradient [22]. There is also a gravitational gradient in $\dot{Q}$, which is reduced in the nondependent lung by: a loss of vascular $\dot{Q}$ pressure [2]; and an increase in vascular resistance from alveolar stretch, which compresses capillaries [2].

Furthermore, as the pulmonary capillaries run in the walls of the alveoli, there is less tissue density and therefore less $\dot{Q}$ per unit volume in the nondependent lung, all of which contribute to a gravitational gradient. Since the $\dot{Q}$ gradient is larger than the $\dot{V}$ gradient, the $\dot{V}/\dot{Q}$ ratio is higher in the nondependent than dependent lung (figure 3) [22].

Posture

While the use of prone posture in mechanically ventilated patients with acute respiratory distress syndrome is not new, the COVID-19 pandemic focused attention on this intervention [25]. The physical shape of the lung is not symmetric between prone/supine postures, interacting with gravitational lung deformation to affect $\dot{V}$ and $\dot{Q}$. This asymmetry is trapezoid-like, with the lung hanging from the shorter side in supine posture and the longer side in prone posture [23]. While stretch and compression occur in both postures [26], the volume of the lung at any gravitational plane differs [23], interacting with gravity, and resulting in more uniform pleural pressure gradients in prone posture; modelling studies show this without even considering the chest wall or heart, *i.e.* from tissue deformation [23], although the heart acts to further compress the lung in supine posture [27]. The pleural pressure gradient effects have the potential to affect gas exchange. In pulmonary oedema, lung stretch/compression may be further increased [28]; a higher lung weight increases consolidation in the dependent lung [29], particularly in supine posture [28].

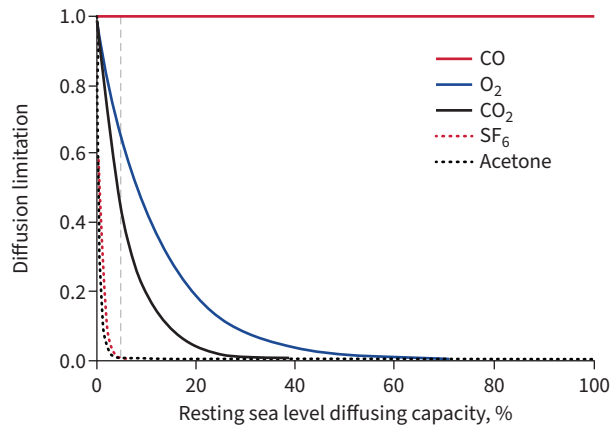


FIGURE 1 This figure shows the dominant effect of the solubility (α)/dissociation curve slope (β) (plus a small effect of molecular mass (M)) on the degree of diffusion limitation (see equation 5) as the diffusion conductance (D_L) is reduced from normal (100%) to zero for five specific gases. CO is essentially completely diffusion-limited even at normal diffusing capacity, which explains why it is the best gas to measure diffusing capacity. O_2 becomes diffusion-limited (at rest, at sea level) only when D_L falls below about 50%; CO_2 is somewhat more resistant to diffusion limitation than O_2 , while inert gases are essentially invulnerable to diffusion limitation. The vertical dashed line shows that inert gases experience no diffusion limitation even when D_L is reduced to just 5% of normal, a situation incompatible with life due to grossly limited O_2 uptake. SF_6 : sulfur hexafluoride.

In large animals, there is a reduction in regional $\dot{V}/\dot{Q}$ mismatch in prone posture because the gravitational gradient in $\dot{V}$ becomes more uniform [30, 31]. This change in $\dot{V}$ is also seen in some human studies [24] but not all [32–34]. This may be because in the latter, alveolar $\dot{V}$ was inferred from specific $\dot{V}$ or the distribution of inhaled labelled particles; both may be affected by the changes in the intrapleural gradient between postures.

Prone posture may also reduce high $\dot{V}/\dot{Q}$ regions, particularly in the nondependent lung [24], partly because of increases in $\dot{Q}$ [26]. This is consistent with reductions in deadspace [35] in prone COVID-19 patients. In patients with COVID-19, awake proning improves dorsal $\dot{V}$ and gas exchange [36]. Early proning in noninvasively ventilated patients with moderate-to-severe COVID-19 respiratory failure is positively associated with outcome [37].

Active mechanisms

O_2 and CO_2 may have small effects on $\dot{V}/\dot{Q}$ matching, with both alveolar hypoxia and hypocapnia inducing bronchoconstriction [38–42]. Hypercapnia affects gas mixing by inhibiting increases in collateral airways resistance [43], but hyperoxia has little effect [44–46]. The most important active mechanism that improves $\dot{V}/\dot{Q}$ matching [47] is hypoxic pulmonary vasoconstriction (HPV) [48]. HPV is thought to be active in all humans [49], is initiated when P_{AO_2} falls below ~ 95 mmHg, and is maximal at an O_2 tension (P_{O_2}) of ~ 40 mmHg [50–52]. HPV increases the local $\dot{V}/\dot{Q}$ by redistributing $\dot{Q}$ away from region(s) of low $\dot{V}/\dot{Q}$ to areas of higher P_{AO_2} . This lowers the $\dot{V}/\dot{Q}$ in the recipient areas until a dynamic equilibrium between stimulus–response is reached.

Although HPV is active in patients [53, 54], it fails to normalise P_{O_2} . In normal lungs, there is sufficient regional P_{O_2} heterogeneity that some HPV is expected [50]; however, the extent is unknown. Studies often rely on isolated cell/pulmonary arterial strip preparations [52, 55],

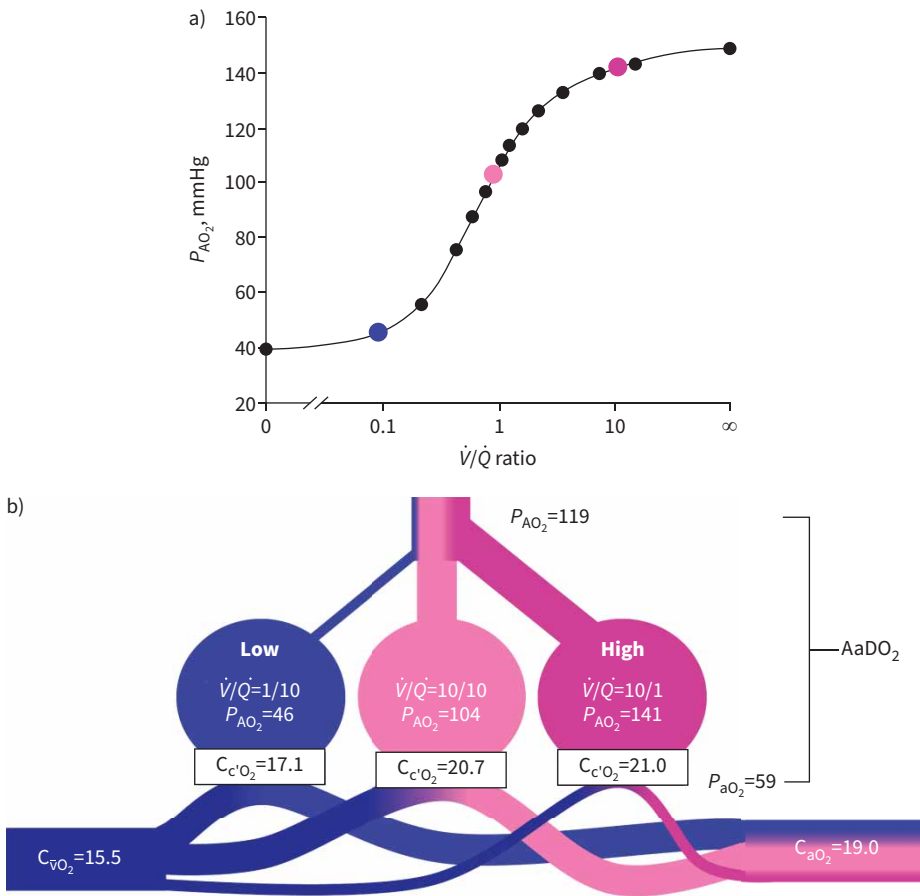


FIGURE 2 Conceptual relationship between a) ventilation/perfusion ratio ($\dot{V}/\dot{Q}$) and alveolar O_2 tension (P_{AO_2}), and b) the impact of $\dot{V}/\dot{Q}$ mismatch on the alveolar–arterial partial pressure difference for O_2 (AaDO₂). Regions of low $\dot{V}/\dot{Q}$ contribute relatively large amounts of poorly oxygenated blood to pulmonary end-capillary blood, while doing little to affect alveolar gas. This is not compensated for by regions of high $\dot{V}/\dot{Q}$ because these units are on the flat part of the dissociation curve and there are little further changes in O_2 content despite large increases in O_2 tension (P_{O_2}). This is shown numerically at the bottom in a simplified three-compartment lung, with compartments of low (1:10), matched (1:1) and high (10:1) $\dot{V}/\dot{Q}$. The mean P_{AO_2} is the average of each unit's alveolar P_{O_2} weighted by the ventilation of each unit, i.e. $[(1 \cdot 46) + (10 \cdot 104) + (10 \cdot 141)] / [1 + 10 + 10] = 2496 / 21 = 119$ mmHg. Arterial P_{O_2} (P_{aO_2}) is P_{O_2} derived from the weighted average of the end-capillary contents from each unit then converted to P_{O_2} , i.e. $[(10 \cdot 17.1) + (10 \cdot 20.7) + (1 \cdot 21.0)] / [10 + 10 + 1] = 19.0$ mL·dL⁻¹ = P_{O_2} of ~59 mmHg. The net result is an AaDO₂ of 60 mmHg. $C_{c'O_2}$: end-capillary O_2 content; $C_{\bar{v}O_2}$: mixed venous O_2 content; C_{aO_2} : arterial O_2 content. Reproduced and modified from [17] with permission.

microsphere administration in animals [56], lung imaging [57, 58] or changes in pulmonary arterial pressure/vascular resistance [59, 60] during hypoxic exposure; this presents safety issues in patients and may be undesirable for other reasons [61]. Consequently, the extent that HPV optimises $\dot{V}/\dot{Q}$ relationships in health and disease is not fully established.

A recent imaging study noninvasively quantified HPV in normal subjects [62]. Redistribution of $\dot{Q}$ was induced by breathing hyperoxia sufficient to raise the P_{AO_2} above the HPV threshold everywhere [50–52]. The mean squared difference from a voxel-by-voxel subtraction of MRI images obtained when breathing 21% and 30% O_2 , was used as an index of HPV. Those with

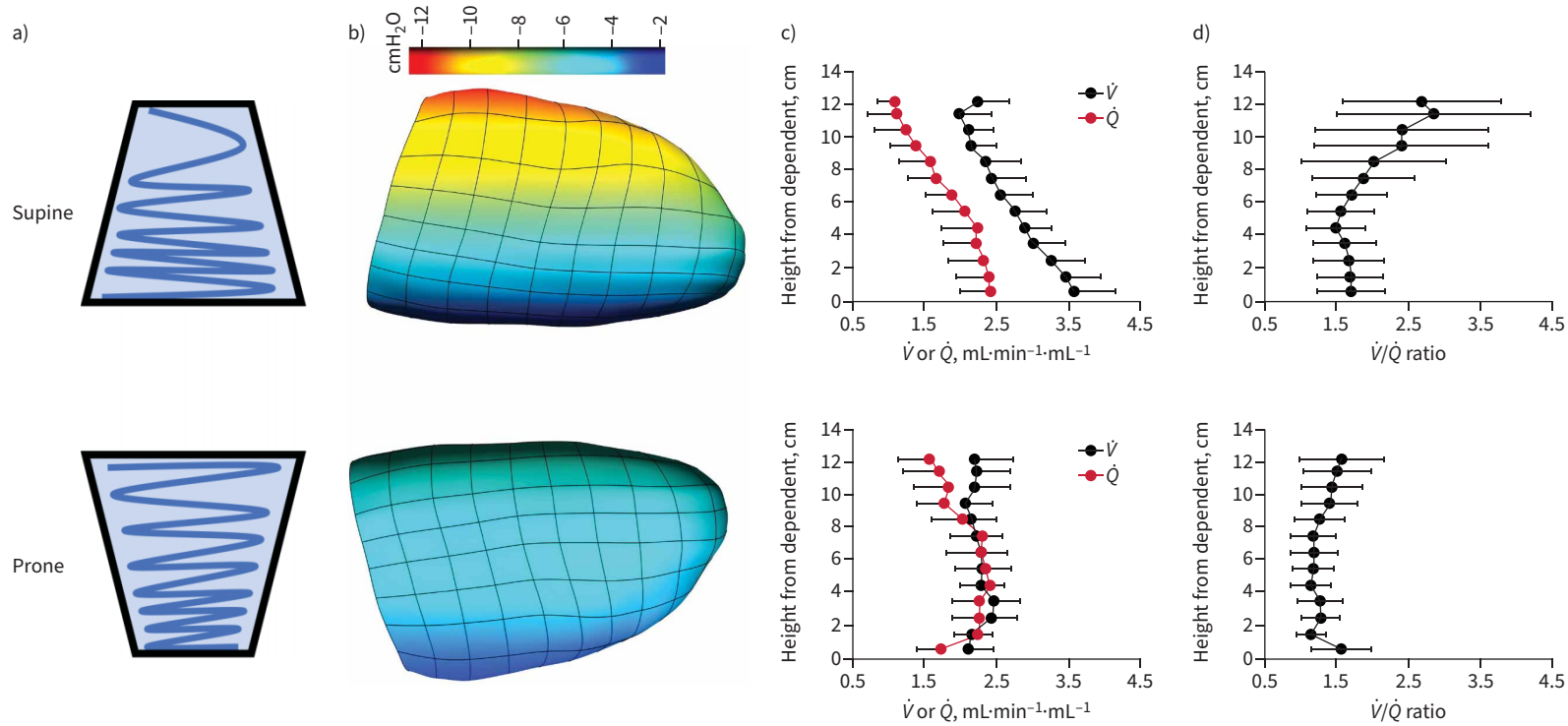


FIGURE 3 The effects of posture on ventilation/perfusion ($\dot{V}/\dot{Q}$) matching. a) The asymmetry in the physical shape of the lung modelled as a trapezoidal spring, hanging from the shorter side in supine posture and the longer side in prone. The shape interacts with gravity such that the gravitationally nondependent portion is more stretched in supine. b) This affects intrapleural pressure gradients as modelled from multidetector CT imaging data by TAWHAI *et al.* [23], which are more uniform in prone position. c) Data from quantitative MRI imaging [24] showing postural differences in alveolar $\dot{V}$ and $\dot{Q}$. Note that the x- and y-axes are reversed in the images so that vertical height increases up the figure. Error bars represent the average standard deviation of $\dot{V}$ or $\dot{Q}$ within the isogravitational plan (not between-subject variability) and thus are also a metric of spatial heterogeneity. The gravitational gradient in $\dot{V}$ is more uniform in prone posture, as is the gradient in $\dot{Q}$. d) In prone posture, there is less spatial heterogeneity in $\dot{V}/\dot{Q}$, as evidenced by both a more vertical distribution and smaller error bars. Panel b) is reproduced and modified from [23] with permission.

greater redistribution of blood flow in hyperoxia had less $\dot{V}/\dot{Q}$ mismatch in normoxia, suggesting greater HPV. By avoiding hypoxic exposure, this approach is suitable for evaluating HPV in patients (figure 4).

Shunt

A veno-arterial shunt occurs when mixed venous blood reaches the systemic circulation without being oxygenated in the pulmonary capillaries [63], increasing the AaDO₂. Shunts can be: 1) extrapulmonary, *e.g.* heart or elsewhere; 2) post-pulmonary, *e.g.* bronchial circulation/thebesian veins; or 3) intra-pulmonary, from regions of the lung that are perfused but not ventilated or *via* aberrant intra-pulmonary arterio-venous anastomoses (IPAVAs) that allow red blood cells to bypass the capillaries.

IPAVAs are found in many mammalian species including humans [64, 65], where they are typically up to 200 μm in diameter, *i.e.* ~ 20 times larger than a pulmonary capillary [66]. IPAVAs are assessed using agitated saline contrast echocardiography by injecting microbubbles into the systemic venous circulation and imaging the presence of bubbles in the arterial circulation. Transpulmonary passage of bubbles in the left atrium after three to five cardiac

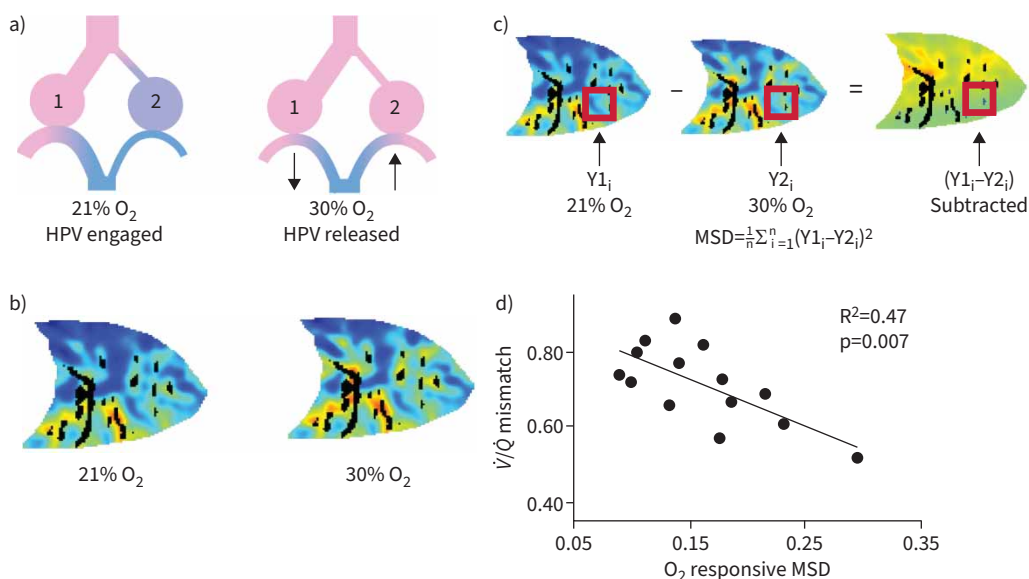


FIGURE 4 Assessment of hypoxic pulmonary vasoconstriction (HPV). a) Effect of hyperoxia on the distribution of pulmonary blood flow in a hypothetical two-compartment lung model. In normoxia (left), well-matched ventilation ($\dot{V}$) and perfusion ($\dot{Q}$) result in a high alveolar P_{O_2} (P_{AO_2}) in unit 1. Airway narrowing to unit 2 reduces the local $\dot{V}/\dot{Q}$ and P_{AO_2} , and HPV is activated, decreasing local $\dot{Q}$, mitigating the gas exchange consequences of $\dot{V}/\dot{Q}$ mismatch and diverting blood towards unit 1. After breathing 30% O₂ (right) the P_{AO_2} in both lung units is increased above the threshold for HPV. This has the effect of decreasing blood flow to unit 1 as flow is diverted to unit 2 with the release of HPV. b) $\dot{Q}$ images are acquired in normoxia and after 15 min of breathing 30% O₂. Images are quantified. Large vessels not reflecting $\dot{Q}$ are removed and images are normalised to account for global differences in blood flow and cardiac output. c) Images are subtracted voxel-by-voxel to calculate the mean square difference (MSD) between normoxic and hyperoxic images, reflecting redistribution. d) Individuals with a greater MSD had less variance in spatial $\dot{V}/\dot{Q}$, suggesting that hypoxic pulmonary vasoconstriction is activated more in some individuals than others, affecting $\dot{V}/\dot{Q}$ relationships. Y_{1i} : mean signal in voxel "i" in condition number 1; Y_{2i} : mean signal in voxel "i" in condition number 2. Panels a) and d) are reproduced and modified from [62] with permission.

cycles is taken as evidence of IPAVAs as it is assumed that otherwise bubbles larger than the pulmonary capillary ($\sim 10 \mu\text{m}$) are trapped upstream of the capillaries and that smaller bubbles degrade below detection limits [67].

Establishing what shunt type is responsible for an abnormal AaDO_2 may be difficult. The role IPAVAs play in increasing the AaDO_2 during exercise [68] has been hotly debated because the multiple inert gas elimination technique (MIGET) [69], the gold standard for quantifying pulmonary gas exchange, has not documented significant shunt in healthy humans [70]. However, scoring systems devised to putatively quantify shunt that might arise from IPAVAs in humans show that bubbles traversing the pulmonary circulation at rest [71] are increased by inotrope infusion [72, 73], exercise [74] and hypoxia [75].

One animal study compared anatomic shunt, quantified by transpulmonary passage of rigid $25\text{-}\mu\text{m}$ microspheres, to contrast echocardiography, and gas exchange shunt measured by MIGET [76]. Hypoxia and inotropes were also administered. Intravenously injected microspheres were recovered in the arterial circulation, suggesting IPAVAs, and this anatomic shunt when present (whether intra-pulmonary or intra-cardiac) was captured by MIGET. However, while agitated saline contrast echocardiography had high sensitivity to detect IPAVAs, the correlation with microspheres was relatively poor, and there was low specificity for an anatomic shunt of a magnitude important for gas exchange ($>1\%$). Echocardiography frequently detected trivial gas exchange shunts and the scoring system was unhelpful in distinguishing small from large shunts. Consequently, while agitated saline contrast echocardiography can be used to evaluate the presence or absence of IPAVAs, it does not assess their magnitude or their effect on gas exchange.

This is important for research on the pathophysiology of gas exchange in COVID-19 pneumonia. In acute respiratory distress syndrome, MIGET shows that hypoxaemia arises because of shunt (absent $\dot{V}$) and regions of low $\dot{V}/\dot{Q}$ [1]. This is also the case in COVID-19 pneumonia, although there is some suggestion that post-pulmonary shunt may also be increased [77]. A study using contrast enhanced transcranial Doppler with intravenous agitated saline contrast, showed that patients with severe COVID-19 had arterial microbubbles, and noted a $P_{\text{aO}_2}/F_{\text{IO}_2}$ ratio that was inversely correlated with the number of bubbles, suggesting shunt from IPAVAs. While this is consistent with pulmonary microvascular dilatations reported at autopsy [3], the limits of agitated contrast echocardiography and the inability of the technique to quantify the magnitude of flow in IPAVAs mean that no conclusions can be drawn about their contribution to hypoxaemia in COVID-19 pneumonia.

Noninvasive estimation of pulmonary gas exchange efficiency

Since the seminal description by Riley and Courmand of the three-compartment model of pulmonary gas exchange [63], the AaDO_2 has been used as a metric of gas exchange inefficiency. In a “perfect” lung in which $\dot{V}$ and $\dot{Q}$ are matched, and in which there is no diffusive limitation to O_2 crossing the alveolar-capillary membrane, AaDO_2 must be zero. In normal subjects, the presence of $\dot{V}/\dot{Q}$ mismatch at rest results in the presence of an AaDO_2 , despite absent diffusion limitation. However, measuring the AaDO_2 requires arterial blood sampling, and determination of the alveolar P_{AO_2} and gas exchange ratio R , all of which are difficult in a clinical setting. WEST and PRISK [78] recently described a novel estimate of pulmonary gas exchange efficiency termed the O_2 deficit (OD), essentially a noninvasive surrogate for AaDO_2 .

Measurement of the OD uses a rapidly responding miniaturised gas analyser, pulse oximetry and small microcomputers in a single package. Measured end-tidal P_{O_2} (the minimum value of

expired P_{O_2} during tidal breathing) is used as P_{AO_2} , and P_{aO_2} is estimated from arterial saturation (S_{pO_2}), yielding the OD as $P_{AO_2} - P_{aO_2}$. In doing so, the OD differs slightly from the standard AaDO₂, which is weighted towards units in the lung with low $\dot{V}/\dot{Q}$, whereas the OD adds information from high $\dot{V}/\dot{Q}$ units (figure 5). However, because of the flatness of the O₂–Hb dissociation curve at high values of P_{O_2} , units with high $\dot{V}/\dot{Q}$ add little to the O₂ content of arterial blood and the consequence of the difference is small.

A limitation of using S_{pO_2} to estimate P_{aO_2} is that the O₂–Hb dissociation curve is very flat at high P_{O_2} . Modelling of the resulting errors showed accurate estimation of P_{aO_2} , providing the S_{pO_2} was $< \sim 95\%$ [80]. If the S_{pO_2} is $> 95\%$, then clearly gas exchange efficiency is not greatly compromised, and measuring OD may be unnecessary.

To account for the effects of P_{CO_2} on the O₂–Hb dissociation curve (Bohr effect), the device simultaneously measures end-tidal P_{CO_2} , allowing correction. However, disturbances caused by non-zero base excess and altered body temperature are not accounted for, although these effects are usually relatively small [80].

Studies by WEST *et al.* [81] have demonstrated that physiologically plausible measurements can be obtained from both normal subjects and patients with a variety of lung diseases [78, 82]. A study of normal subjects undergoing hypobaric hypoxic exercise showed good agreement between the OD and the AaDO₂ [83]. This approach has proved useful in field research where direct measurement of the AaDO₂ is challenging. In studies of deep breath-hold diving, an increase in the OD was positively associated with the depth of the dive [84, 85]. The approach was also used in field studies at high altitude [86].

A prospective study of patients presenting with COVID-19 showed the approach to be more accurate than pulse oximetry alone in predicting the need for supplementary O₂, and for

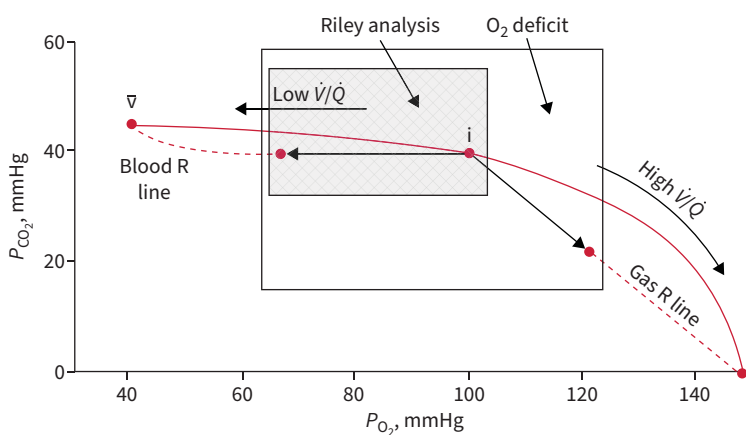


FIGURE 5 Classic O₂–CO₂ diagram with the ventilation/perfusion ($\dot{V}/\dot{Q}$) line joining the points for mixed venous and inspired gas. The traditional Riley analysis is based on the composition of arterial blood and ideal alveolar gas, and is strongly weighted towards lung units with low $\dot{V}/\dot{Q}$ that lie to the left of the ideal point (hatched area). By contrast, the new test also includes contributions from lung units with high $\dot{V}/\dot{Q}$ that are located to the right of the ideal point. P_{O_2} : O₂ tension; P_{CO_2} : CO₂ tension; $\bar{v}$: mixed venous point; i : ideal alveolar point; I : inspired point. Reproduced and modified from [79] with permission.

subsequent hospitalisation [87]. This may be because of simultaneous measurement of both arterial and alveolar values, since the presence of $\dot{V}/\dot{Q}$ heterogeneity serves to simultaneously decrease P_{aO_2} and increase P_{AO_2} .

The three-compartment lung revisited

Over 75 years ago, Riley and Cournand developed the classic three-compartment analysis to quantify gas exchange [63]. The three compartments were: 1) shunt (shunt flow/cardiac output ($\dot{Q}_S/\dot{Q}_T$), perfused but unventilated lung); 2) deadspace (deadspace volume/tidal volume (V_D/V_T), ventilated but unperfused lung); and 3) the “ideal” (normal) compartment (designated as i) containing all of the non-deadspace $\dot{V}$ and all of the non-shunt blood flow, and which therefore performed all of the gas exchange in the lung. They showed that from mass conservation equations applied to O_2 concentrations (C) in measured arterial (a) and mixed venous ($\bar{v}$) blood and calculated ideal (i) compartment end-capillary blood, shunt could be estimated as:

$$\dot{Q}_S/\dot{Q}_T = (C_{iO_2} - C_{aO_2}) / (C_{iO_2} - C_{\bar{v}O_2}) \quad (6)$$

From equivalent mass conservation equations applied to expired gas, CO_2 was used to calculate deadspace from measured mixed expired P_{CO_2} (P_{ECO_2}) and calculated ideal P_{CO_2} (P_{ICO_2}):

$$V_D/V_T = (P_{ICO_2} - P_{ECO_2}) / P_{ICO_2} \quad (7)$$

Or, using what is known as the Enghoff modification (replacing hard-to-measure P_{ICO_2} with measured P_{aCO_2}) [88]:

$$V_D/V_T = (P_{aCO_2} - P_{ECO_2}) / P_{aCO_2} \quad (8)$$

However, this well-established analysis has been recently updated because there are approximations that under some circumstances may be significant [89]. First, all gases are affected by shunt, low $\dot{V}/\dot{Q}$ regions, high $\dot{V}/\dot{Q}$ regions and deadspace [90, 91], so assuming that all O_2 abnormality is only due to shunt and low $\dot{V}/\dot{Q}$ regions and not to deadspace or high $\dot{V}/\dot{Q}$ regions is not correct. Ditto the assumption that all CO_2 abnormality is only due to deadspace and high $\dot{V}/\dot{Q}$ regions. Second, the anatomic deadspace that is normally present is often the largest contributor to V_D/V_T , meaning quantifying the V_D/V_T caused by $\dot{V}/\dot{Q}$ inequality (i.e. alveolar deadspace) requires separate measurement of the anatomic deadspace and subtraction of that from the total. Third, while the Enghoff modification makes the calculation V_D/V_T feasible, it tacitly assumes that there is no arterial–alveolar P_{CO_2} difference, which is not true, especially when high $\dot{V}/\dot{Q}$ regions exist.

Gas exchange software that encodes the dissociation curves for O_2 and CO_2 [91–94] allows these shortcomings to be overcome by redefining the three-compartment analysis to allow for the way in which shunt and deadspace both affect both O_2 and CO_2 exchange [89]. The data that are required are mean P_{AO_2} and P_{ACO_2} defined as P_{O_2} and P_{CO_2} at the midpoint (by volume) of the expirogram in a conventional single-breath recording [89], as routinely obtained during CPET, and simultaneously measured P_{aO_2} and P_{aCO_2} , resulting in measurements of both the AaDO₂ and aADCO₂. The software then finds the unique values of shunt and alveolar deadspace that produce both measured AaDO₂ and aADCO₂. By measuring mixed-alveolar P_{O_2} and P_{CO_2} directly, anatomic deadspace is eliminated as a contributor to AaDO₂ and aADCO₂, enabling direct assessment of alveolar deadspace. Figure 6 illustrates the concept underlying this approach. It shows how any combination of AaDO₂ and aADCO₂ is associated with a unique value of shunt and deadspace.

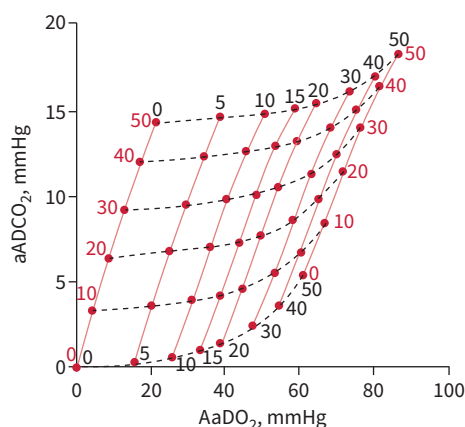


FIGURE 6 This figure plots alveolar-arterial O_2 tension differences ($AaDO_2$, abscissa) against arterial-alveolar CO_2 tension differences ($aADCO_2$, ordinate) over a wide range of possible values. A large number of ($AaDO_2$, $aADCO_2$) pairs is shown by the filled red circles, with each point corresponding to a particular shunt and deadspace pair. Isopleths of shunt and deadspace are shown by the curved dashed black lines for points of equal shunt, and red lines for points of equal deadspace. To use this framework, suppose an $AaDO_2$ of 40 mmHg is measured. It can be seen that this $AaDO_2$ can be caused by many combinations of shunt and deadspace: 20% shunt with zero deadspace; 15% shunt with 10% deadspace; 10% shunt with 30% deadspace; 5% shunt with 50% deadspace. But, if the $aADCO_2$ is simultaneously measured, the unique shunt that produces a 40 mmHg $AaDO_2$ and the measured $aADCO_2$ is identifiable, as is the unique alveolar deadspace. For example, an $AaDO_2$ of 40 mmHg and an $aADCO_2$ of 10 mmHg indicates a shunt of 10% and an alveolar deadspace of 30%. Reproduced and modified from [89] with permission.

Gas exchange in COVID-19

While COVID-19 remains prevalent, its severity has decreased as the virus has mutated, clinical management has improved and most have developed some degree of immunity from infection and/or have received immunisation. Gas exchange is not as much of a concern as it was in the early stages of the pandemic. At that time, COVID-19 commonly caused pneumonitis (often severe), led to alveolar filling with fluid/cell debris, and resulted in airway blockage by secretions [95]. As with other forms of acute lower airway infections, this sometimes led to severe arterial hypoxaemia. The consequences were clear: absent or greatly reduced regional $\dot{V}$ in impacted areas despite continued $\dot{Q}$ produced intrapulmonary gas exchange shunts. As has been known for more than 75 years, such shunts mean that desaturated pulmonary arterial blood passes through unventilated regions to the pulmonary veins, lowering P_{aO_2} . However, COVID-19 brought to light several new ideas on gas exchange, which we will discuss here.

Hypoxaemia without dyspnoea: “silent hypoxia”

Reports that some hypoxaemic COVID-19 patients were not dyspnoeic [96–98] raised the question of whether the virus had a direct inhibitory effect on the central nervous system (including chemoreceptors or the autonomic nervous system), which attenuated the usual ventilatory response to hypoxia [99]. However, as pointed out in some studies, substantial variability is seen in the normal ventilatory response to hypoxia [100, 101]. Typically, dyspnoea is not experienced until P_{aO_2} is ≤ 60 mmHg. Even then, many have little ventilatory response until P_{aO_2} is ~ 40 mmHg. In the absence of a ventilatory response, shunt will also raise P_{aCO_2} (because the exchange of all gases, not just O_2 , is impaired by shunt), and while this increase is much less than the fall in P_{O_2} , it would also be expected to stimulate $\dot{V}$, returning P_{aCO_2} to normal, or even to below normal given the added stimulus of hypoxaemia.

It has now been shown that P_{aCO_2} is indeed normal/reduced in acute COVID-19 (absent pre-existing pulmonary disease, such as COPD). Furthermore, “silent hypoxia” was not found to be an identifiable characteristic in patients with COVID-19 infection, many of whom had higher respiratory rates than non-COVID-19 patients [102].

In summary, while some patients have substantial hypoxaemia without dyspnoea, most evidence suggests ventilatory responsiveness is not impaired by the virus.

Gas-exchange disturbances in acute COVID-19

The parenchymal pathology of acute COVID-19 means that arterial hypoxaemia is expected (and indeed, observed), due to poorly and/or unventilated regions of the lung [103, 104]. Much less apparent is the extent of ventilated but poorly perfused/unperfused regions of high $\dot{V}/\dot{Q}$ (alveolar deadspace). By measuring the alveolar–arterial differences for both O_2 and CO_2 simultaneously in mild-to-moderate COVID-19 patients and applying the above analysis [89], elevated alveolar deadspace was observed in most of those studied [105, 106]. This fits with pathology reports of small vessel intravascular thrombosis [107], and is distinct from large vessel pulmonary thromboembolism, which was excluded in the cohorts studied. BUSANA *et al.* [77] studied more severely affected patients in the intensive care unit, using the multiple inert gas elimination technique, and found shunts of 7–54% and elevated deadspace values of 35–75% of V_T (including anatomical deadspace, typically ~30% of V_T).

Clearly, there is great variability in both parameters, but both shunt and deadspace are elevated in severe COVID-19, again suggesting not just alveolar filling causing shunt but also vascular obstruction causing deadspace.

Post-COVID-19 gas exchange

It is well-documented that recovery from COVID-19 can be complicated by persistent symptoms/signs. In a small group of patients who had sustained mild-to-moderate disease, gas-exchange disturbances persisted in most, lasting many months into recovery [106]. These were characterised more by persistent high $\dot{V}/\dot{Q}$ areas than by low $\dot{V}/\dot{Q}$ areas, suggesting that recovery from alveolar filling may be more complete than recovery from (small vessel) vascular obstruction. The implications of persistent small vessel abnormalities are presently unknown, but post-COVID-19 gas exchange merits continued assessment.

O₂ transport: an integrated, interactive gas-exchange system

O_2 moves from the air to the mitochondria through a series of well-known steps [108]: 1) ventilation; 2) alveolar-capillary diffusion; 3) cardiovascular transport of O_2 in blood from the lungs to the tissues and back; 4) diffusion from the tissue microvessels to the mitochondria; where 5) O_2 is used to generate adenosine triphosphate in a reaction that follows the law of mass action and Michaelis–Menten kinetics. Most O_2 transport research has focused on just one of these steps/tissues/organs at a time, with particular attention given to the heart. Consequently, it is easy to lose sight of the critical interactions of these five steps, which involve the lungs, heart, blood and the many different tissues and their mitochondria. However, applying only the principle of mass conservation of O_2 at every step along its journey from air to mitochondria, it has become clear that the amount of O_2 that gets to the mitochondria depends on all these steps working together as a system rather than as a collection of independent organs. Each step affects the other steps. Fortunately, generating a mass conservation equation for O_2 at every step allows the entire transport chain to be characterised by a series of five simultaneous equations, one for each step [109]. This enables quantitative assessment of those interactions. The equations contain,

as independent variables, F_{IO_2} , ventilation, D_L , cardiac output, Hb, and the O_2 -dissociation curve, tissue diffusing capacity (D_M if the tissue is muscle) and lastly the hyperbolic Michaelis–Menten relationship between O_2 consumption and mitochondrial P_{O_2} (described by its P_{50} and its maximal rate of mitochondrial O_2 consumption when O_2 is in excess ($\dot{V}_{max}$)). There are five dependent variables in these five equations: alveolar, arterial, venous and mitochondrial P_{O_2} , and $\dot{V}_{O_2}$ itself. Accordingly, the listed independent variables, by means of the five mass conservation equations, determine the unique values of the five dependent variables.

How their interactions happen is perhaps best appreciated visually [110]. It is helpful to plot the trajectories of just two of the five equations mentioned above (*i.e.* those underlying steps 3 and 4) as in figure 7a. This figure, applied to muscle under conditions of maximal exercise when mitochondrial P_{O_2} is likely to be in low single digit levels [108, 112], shows how maximal $\dot{V}_{O_2}$, occurring at the point of intersection of the two equations, is determined by the values of, and interactions between, the many variables. P_{aO_2} , Hb and blood flow together define the blue curve, while D_M and mitochondrial P_{O_2} define the red line. In turn, P_{aO_2} is determined by ventilation, blood flow and alveolar-capillary diffusion. Figure 7b exemplifies application of the analysis to patients with COPD, before and after training, with control subject data also shown.

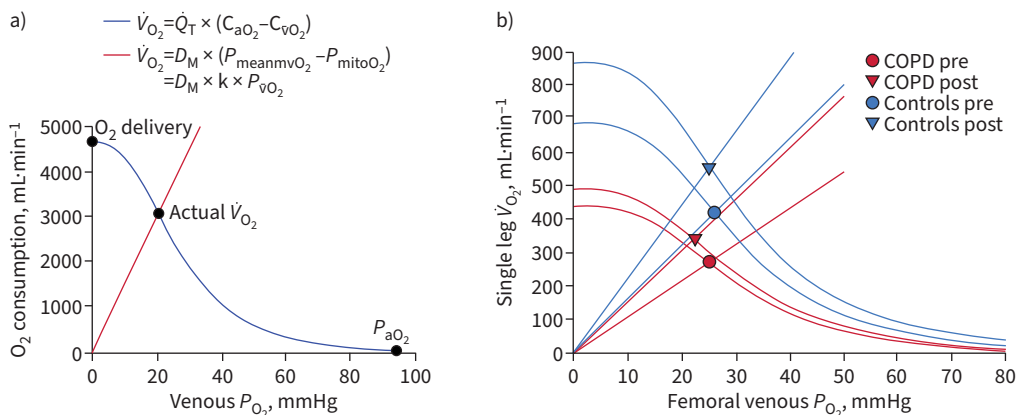


FIGURE 7 a) Convective delivery of O_2 to the maximally exercising muscles, with the curved blue line representing the Fick principle equation. This line shows that: 1) at any muscle venous O_2 tension (P_{O_2}) there can be only one value of $\dot{V}_{O_2}$ that satisfies the Fick equation for the given values of cardiac output, arterial P_{O_2} (P_{aO_2}) and arterial O_2 , but; 2) all points on the blue line satisfy the Fick principle. This panel also presents the diffusive delivery of O_2 from the blood vessels to the mitochondria via the red straight line of the positive slope, which reflects the law of diffusion ($\dot{V}_{O_2} = \text{diffusing capacity } (D_M) \times (\text{mean capillary } P_{O_2} - \text{mean mitochondrial } P_{O_2})$). Mitochondrial P_{O_2} is very low and may be neglected in this analysis [108]. In addition, mean capillary P_{O_2} has a relatively constant proportional relationship (k) with venous P_{O_2} (P_{vO_2}), which allows $\dot{V}_{O_2}$ to be expressed as a function of P_{vO_2} . The red line shows how diffusive transport of O_2 would increase linearly with P_{vO_2} , but that at any P_{vO_2} , there is only one value of $\dot{V}_{O_2}$ that diffusion can support. The key message is that the point of intersection of the red and blue lines is the only location on the entire plot where O_2 mass is conserved such that at the same P_{vO_2} , both diffusive and convective O_2 transport yield the same values for $\dot{V}_{O_2}$, and thus ensure O_2 mass conservation. If the conditions reflect maximal exercise, the intersection point indicates maximal $\dot{V}_{O_2}$ and the associated value of venous P_{O_2} . b) This part of the figure shows the principle applied to normal subjects and patients with COPD both before and after several weeks of exercise training [111]. The data show that while both groups display substantial and similar increases in the slope of the line representing diffusion, only the normal subjects show significant improvement in blood flow, as indicated by upward movement of the curved lines. $\dot{Q}_T$: blood flow; C_{aO_2} : O_2 content in arterial blood; C_{vO_2} : O_2 content in venous blood; $P_{\text{meanmv}O_2}$: mean P_{O_2} in mixed venous blood; $P_{\text{mito}O_2}$: mitochondrial P_{O_2} . Part a) reproduced and modified from [110] with permission. Part b) reproduced and modified from [111] with permission.

Key observations are as follows:

- Every one of the independent variables affects the performance of the O₂ transport system (*i.e.* $\dot{V}_{O_2}$, and the P_{O_2} values mentioned above). Thus, the old question “What is *the* limiting factor to maximal O₂ consumption?” is not the right question to ask, because all independent variables have an effect.
- A change in any one independent variable affects the performance of every O₂ pathway step, and usually in the opposite direction. For example, an increase in cardiac output will increase O₂ flow to the tissues, but may at the same time reduce red cell transit time in the lung and tissue microvessels, potentially causing diffusion limitation for O₂ in both locations, which may offset the benefit of the higher blood flow rate in transporting O₂.
- Figure 7 can be applied “in reverse” to individual patients (*i.e.* by measuring the dependent variables) to determine values, and responses to therapy, of each of the independent variables. This is shown in figure 7b, where patients with COPD and normal control subjects are compared before and after exercise training.

Conclusion

In summary, while the fundamentals of pulmonary gas exchange have long been established, revisiting the field and applying new approaches brings novel and useful means to quantitatively measure aspects of pathophysiology, and provide measurements that have potential bedside application. These improvements highlight the fact that disruptions to both ventilation and perfusion, and to diffusion, can be quantified and serve to highlight the importance of a comprehensive view of pulmonary gas exchange.

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Advances in airway and parenchymal physiology and pathobiology

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Complex changes in the structure and function of airways and lung parenchyma occur in different respiratory diseases. Their interactions have profound and sometimes unpredictable effects on the pulmonary function tests used in clinical practice. <https://bit.ly/ERSM107>

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This chapter reviews the multiscale structure–function relationships of the lung parenchyma, from fibres through surface tension to alveolar septal network, with an emphasis on abnormalities such as emphysema, pulmonary fibrosis, pneumonia and lung metastases, and with consideration of implications for their clinical assessment. Structural abnormalities of the airway wall observed in obstructive disease are described, as are the implications for function at a local tissue level and how these may manifest *in vivo* when considering the heterogeneity of structure–function. We also look at length adaptation, a major property of airway smooth muscle: changes in cell length initially disassemble the contractile apparatus, resulting in a reduction of contractility, which is followed by reassembly of the contractile apparatus and recovery of contractility. This confers on the muscle the ability to modulate airway calibre in response to deep inspirations. Finally, the impact of structure–function characteristics and airway–parenchymal interdependence on standard lung function tests is examined, and we highlight how interferences between various mechanisms may affect overall lung function differently, depending on the underlying disease and severity.

Introduction

The human lung is a complex organ, the function of which is determined by the interactions between structures with different morphological and mechanical properties. A major difference between the lung and other internal organs is that the lung is continuously submitted to external forces throughout life, and dysfunction may occur whenever pathological changes alter the structure or the mechanical behaviour of the airways, lung parenchyma, or both.

This chapter will first describe the structural and functional abnormalities of lung parenchyma in the healthy lung, and in several main parenchymal diseases, such as emphysema, pulmonary

fibrosis (PF) and lung cancer. Next, we will discuss the structural abnormalities of the airway wall in obstructive disease, and the implications for function at a local tissue level in the presence of heterogeneity. We then provide an overview of length adaptation of airway smooth muscle (ASM), with emphasis on how changes in cell length regulate the contractile apparatus in response to deep inspiration (DI). The last section of the chapter examines the impact of structure–function characteristics and airway–parenchymal interdependence on standard lung function tests, highlighting how different mechanisms interact to influence overall lung function, depending on the severity of underlying disease. The way in which disease impacts clinical lung function testing will be illustrated throughout the chapter.

Lung parenchymal structure, mechanics and functional abnormalities

Alveolar structure

The lung parenchyma, by definition, is the structure beyond the respiratory bronchioles, which includes the alveolar ducts, with occasional branching, and the alveoli, where gas exchange occurs (figure 1). This complex structure has been optimised for efficient gas exchange *via* passive diffusion, which requires a large surface area and a small distance between air in the alveoli and capillary blood. The demands of gas exchange impose severe constraints on alveolar structure, including alveolar size and septal wall thickness [1, 2]. For example, the large surface area ($\sim 70 \text{ m}^2$) requirement is satisfied by packing ~ 480 million alveoli into the volume of the lung, with diameters at total lung capacity (TLC) reaching $200 \mu\text{m}$ in the adult human lung; in

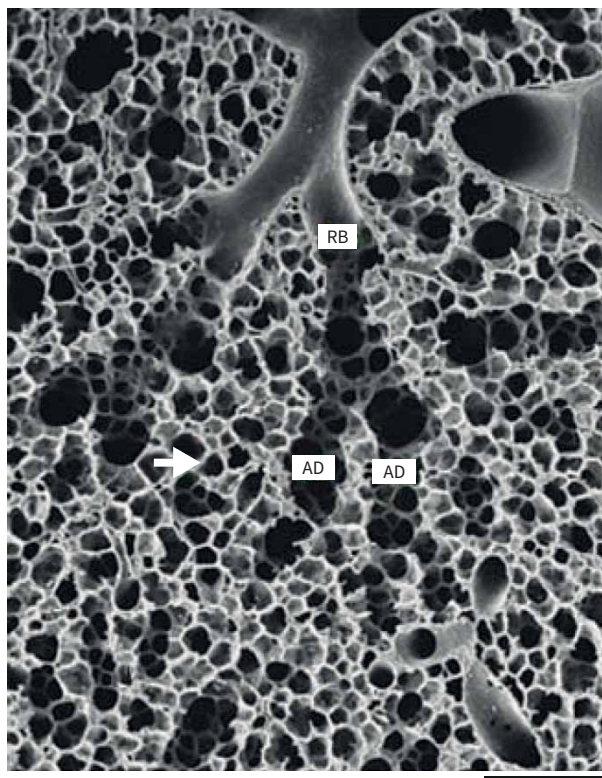


FIGURE 1 Image of the lung parenchyma showing a respiratory bronchiole (RB) and two alveolar ducts (AD). The arrow points to a single alveolus. Scale bar: $500 \mu\text{m}$. Reproduced and modified from [1] with permission.

contrast, the short diffusion distance ($\sim 1 \mu\text{m}$) means that the septal walls separating neighbouring alveoli only need a thickness of $< 8 \mu\text{m}$ [3]. This complex interconnected network of thin-walled tubes and spherical gas exchange units displays a fractal organisation [2]. The evolution of the lung tissue has not only fine-tuned parenchymal structure for gas exchange but has also resulted in proper composition of its extracellular matrix (ECM), including elastin and collagen, with mechanical properties that allow easy stretching and force transmission during breathing [4].

Taken together, the healthy lung parenchyma is a well-designed structure suitable to serve an organism's needs both at rest and at maximum effort [1].

Multiscale mechanics

Lung tissue mechanical function derives from ECM and alveolar structure, the mechanical properties of the constituents and their interactions [5]. The primary load-bearing structural protein of the alveolar ECM is thought to be the fibril-forming collagens, including types I and III. The very stiff, rod-like collagen molecules [6] are arranged into hierarchical fibrils and fibres that are stabilised by cross-links [7]. These fibres form: the axial fibre system running from the airways down to the alveolar ducts; the peripheral fibres emanating from the visceral pleura; and the network of fibres in the septal walls linking the axial and peripheral networks [7]. During development [8, 9] and in diseases such as COPD [10] and PF [11], the collagen amount and type can vary in lung tissue. As a result, collagen fibres have been associated with the structural stability and biomechanical properties of the parenchyma. Collagen fibres are also wavy at low and intermediate lung volumes [12]. As the lung inflates, fibres become straighter and, as lung volume approaches TLC, the waves gradually disappear. This recruitment process stiffens the septal walls [13], resulting in stiffer parenchyma, and leading to the saturation-like plateau of the pressure–volume (P – V) curve of the lung [14, 15].

During normal breathing, the elastic fibres provide the majority of recoil pressure of the lung parenchyma [16]. These fibres contain elastin, a soft and resilient biopolymer, surrounded by microfibrils. The stiffness of elastic fibres is at least 100 times lower than the stiffness of fully straightened collagen fibres [6]. The easy stretchability of elastic fibres derives from the entropic nature of the extension of the hydrophobic molecule, coupled with dense cross-linking, which together produce a perfect linear stress–strain curve, with near zero hysteresis up to a strain of $\geq 150\%$ [6]. These fibres are also mechanically associated with collagen fibres [17], perhaps through microfibrils or proteoglycans [18–20]. The fibres form a fully connected network in the parenchyma [12], meaning they play a major role in lung elasticity at low-to-medium lung volumes [16], and are primarily responsible for lung compliance during breathing. Due to their superb mechanical characteristics, it has been suggested that elastic fibres behave mechanically as an ideal linear spring that does not dissipate energy during cyclic stretching [6]. Hence, elastic fibres are ideally suited to supporting breathing throughout life.

Several additional factors contribute to parenchymal mechanics. The most important is the pre-existing stress, or prestress, in the septal walls due to transpulmonary pressure [21]. The pleura transmits the mechanical stresses of transpulmonary pressure to the subpleural alveolar septal walls, which in turn transmit these stresses to the neighbouring alveoli, and their next neighbours, and so on, forming a complex interdependent prestressed network. The actual stress transmission occurs through the fibre network [22]. The total stress in alveolar tissue therefore arises from the dynamic stresses caused by breathing, superimposed onto the static prestress. Consequently, all adherent cells, such as epithelial, endothelial, and fibroblast cells, feel and respond to these stresses *via* mechanotransduction, affecting many cellular functions [23, 24].

An important function of the prestressed alveolar network is that it structurally supports small airways, preventing them from collapse in the normal lung.

In addition to prestress and ECM, lung mechanics is also influenced by the surface tension of the air–liquid interface lining the alveolar surface, especially at low lung volumes [25–28]. Because pulmonary surfactant lowers surface tension as lung volume decreases, alveoli remain stable at end-expiration [28].

Other constituents of the parenchyma that contribute to a lesser extent to lung mechanics include the compressibility of proteoglycans [29] and the elastic properties of adherent cells [30–32].

The effects of disease on alveolar structure and function

How do diseases affect the ECM, the septal walls and their network organisation? Emphysema, one of the major subtypes of COPD, is known to attack and remodel both the elastic and collagen fibres [33–35], which, due to prestress (particularly in the apex of the lung), become prone to rupture [36]. Once fibres in the wall fail, ECM fragments are exposed that can drive further progression [37]. At some point, the wall itself ruptures, redistributing the mechanical load it carried among neighbouring septal walls, and increasing the likelihood that these walls will rupture [38]. This process leads to airspace enlargement, a characteristic feature of emphysema, locally softening the tissue. Furthermore, residual inflammation in the enzymatically weakened septal walls can undergo cyclic stretch-induced fatigue, leading to rupture and causing continued airspace enlargement and functional decline [39], potentially even in the absence of stimulus. In PF, activated myofibroblasts remodel the septal walls [40] through mechanotransduction-driven scar formation [41], resulting in uncontrolled collagen deposition [42] and cross-linking [43]. Subsequent stiffening of the alveolar tissue further activates myofibroblasts in a positive feedback loop [44]. Alveoli affected by pneumonia are usually oedematous, which hinders normal surfactant function [45]. However, little is known about how ECM remodelling influences alveolar mechanics in lung cancer.

In a recent study, BANERJI *et al.* [46] introduced novel technology: the crystal ribcage. This technology makes it possible to perform multiscale imaging and probing of the mechanics of single alveoli in the mouse lung as a function of heterogeneous remodelling of the ECM and septal walls in several diseases, including lung metastasis (nodular *versus* infiltrative tumour growth), emphysema and fibrosis. In the study, nodular and infiltrative tumours, also known as co-optive tumours, were created in the lung through intravenous injection of murine breast cancer cell lines. Pneumonia was induced *via* bacterial infection with *Streptococcus pneumoniae* serotype 3, and fibrosis was modelled using bleomycin treatment (figure 2). In the nodular phenotype, tumours were observed to “push” into the lung tissue and bulge out of the pleural surface, whereas infiltrative tumours replaced existing lung tissue and were superficially located on the pleural surface. While this differential growth pattern has been reported previously in brain tumours [47], there is poor understanding of its presence in the context of the lung and how it may affect the surrounding alveoli. Compared with the healthy lung, in pneumonia, alveoli were filled with neutrophils, whereas in fibrosis, injured regions demonstrated thickened septal walls with gross heterogeneity.

BANERJI *et al.* [46] achieved mechanical probing of individual alveoli by assessing the pressure–diameter (P – D) curves, which revealed a large inter-alveolar heterogeneity in the healthy lung, as well as distinct functional impairments specific to each disease (figure 2b). For example, nodular tumours demonstrated how growth-induced forces, one of the physical hallmarks of cancer [48, 49], affect neighbouring tissue. Alveoli that contained nodular tumours (100–

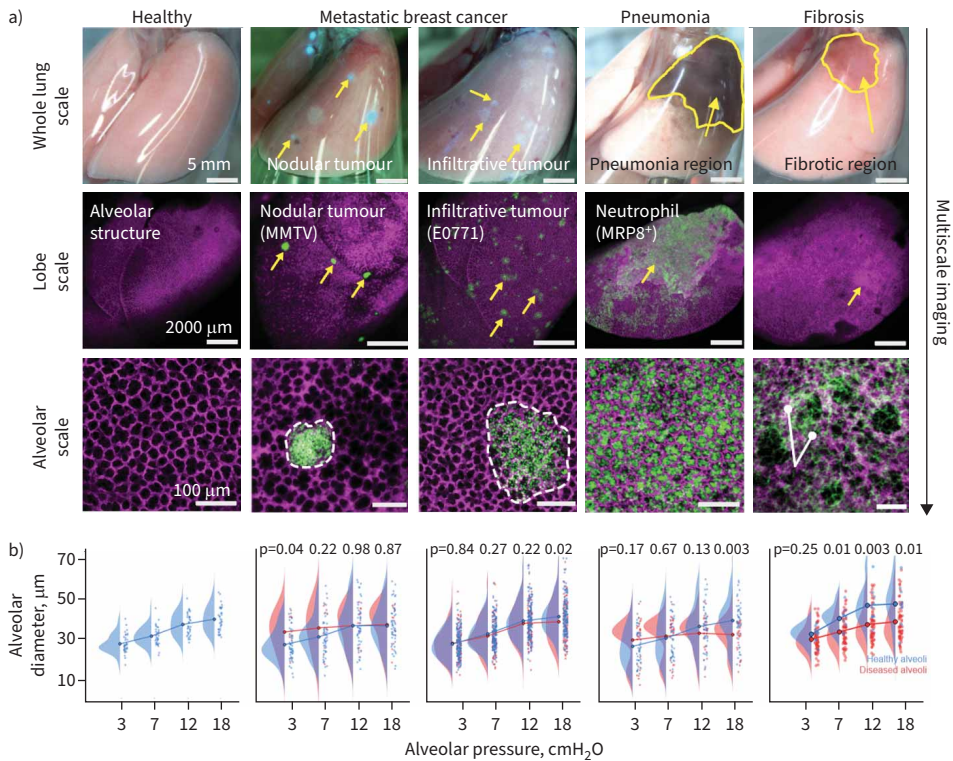


FIGURE 2 a) Multiscale imaging of the lung, from whole organ down to alveoli, in health and disease, and b) the corresponding alveolar pressure–diameter values measured *via* the crystal ribcage platform, with healthy alveoli indicated in blue and diseased alveoli indicated in red. MRP8: reporter for neutrophils. Reproduced and modified from [46] with permission.

200 μm in diameter) were unable to inflate, rendering them non-functional for gas exchange. These alveoli remained maximally dilated across the quasi-static alveolar pressure range, indicating that cancer cells occupied the entire airspace and forced alveoli to remain fully distended. These functional impairments extended to one or two layers of the adjacent alveoli in the peritumoral region. Larger nodules (1–2 mm in diameter) caused alveoli to become stretched and compacted up to 200 μm from the tumour boundary, while maintaining larger diameters at greater distances. In sharp contrast, infiltrative tumours did not impair the mechanical function of alveoli that were inside or adjacent to the tumour. These alveoli behaved similarly to the healthy ones, suggesting that this type of tumour preserved existing tissue architecture, and did not compromise mechanical function. Interestingly, when the elasticity of the lung and tumour areas were mapped with a multiscale mathematical model, both the tumour and nearby normal lung tissue showed substantial strain stiffening, a sign of collagen recruitment, with the tumour regions stiffening at a higher rate [50].

In the pneumonia model, infected alveoli exhibited functional deficits over the range of quasi-static alveolar pressures (3–18 cmH_2O). Alveolar diameters remained largely constant, resembling healthy alveoli at 7 cmH_2O . This loss of expansion and contraction capacity resulted in a very low compliance that may be attributed to neutrophil infiltration, pulmonary oedema, likely caused by surfactant inactivation [45] and compromised vascular integrity [51], allowing

fluid influx into the airspaces. Finally, in the fibrosis model, the injured alveoli demonstrated a heterogeneous structure with thickened septal walls. The P - D curves displayed reduced diameters at higher alveolar pressures compared with normal alveoli, consistent with a stiffening phenotype and lower compliance, as reported in human PF [52].

These findings underscore the structural and functional mechanical alterations in individual alveoli across different pulmonary diseases, highlighting the distinct mechanisms of ECM and septal wall remodelling and dysfunction.

Assessment of parenchymal function

The clinically measurable functional index of the parenchyma is lung compliance, the slope of the lung's P - V curve, which is an emergent property arising from the mechanical properties of the ECM's fibres, their structural arrangement, the surfactant system and their multiscale interactions. The P - V curve of a single alveolus is determined by elastin stiffness and collagen waviness in the septal wall and the surface tension along the wall [14]. In health, the P - V curve of the lung is essentially the sum of the individual alveolar P - V curves, corresponding to the gravitational position of each alveolus [53]. Lung compliance is therefore sensitive to the mechanisms that govern the P - V curve of individual alveoli.

In parenchymal diseases such as emphysema, PF, pneumonia and lung cancer, the characteristics of the single alveolar P - V curve can change drastically and in a highly heterogeneous manner (figure 2). Hence, the P - V curve and lung compliance also reflect the spatial distribution of disease and the interdependence of diseased and normal alveoli.

In general, it is difficult to extract specific information on alveolar remodelling from a single quasi-static lung compliance measurement. When compliance is measured as a function of frequency, the static to dynamic compliance ratio [54] or the variation of compliance or reactance with frequency [55] may convey information about changes in alveolar mechanics. However, it is likely that this is a result of time-constant inequalities involving the spatial distribution of alveolar compliance and airway resistance, without carrying specific insight into the remodelling of individual alveoli [56]. It is therefore useful to supplement pulmonary function tests with CT or MRI, which can reveal regional variations in tissue pathology.

Airway remodelling: structural and functional abnormalities

Origins

A loose definition of airway remodelling is any structural feature of the airway wall which is abnormal in terms of its cross-sectional thickness (area or equivalent volume in three-dimensions) and the composition of its constituent elements. That is, structures may have grown (or shrunk), which in turn alters their mechanical properties. The mechanism(s) of airway remodelling have not been conclusively established, although the majority of scientists favour inflammation as the driver of pathology [57]. A second possibility is structural changes mediated through activation of mechanotransduction pathways, such as that occurring during bronchoconstriction [58]. Finally, evidence that airway remodelling is observed early in life and even prior to diagnosis of obstructive disease [59, 60] raises the possibility that abnormalities of the airway wall are a remnant of some developmental disorder [61]. Even COPD, which has long been associated with cigarette and environmental exposure, is now recognised as having a developmental foundation in some patients [62]. Nevertheless, airway remodelling may also be related to persistent and residual inflammation, even after a stimulus such as cigarette smoke is removed [63].

Structural modifications

There is variation in the broad structure of airways that make up the bronchial tree, where small airways lack cartilage, exhibit more predominant ciliation of the epithelium, and show a greater proportion of club cells and, conversely, fewer mucus-producing goblet cells [64, 65]. The point of distinction between a “large” and “small” airway is often defined as a lumen diameter of <2 mm, a somewhat arbitrary definition that tends to lead to broad hypotheses of small or large airway disease, which may be an oversimplification [66].

At the gross level, airway wall thickness has been shown to be increased in patients with asthma when imaged using CT [67, 68]. While CT can assess total wall thickness, histological assessment is required to resolve specific changes to wall compartments; in patients with asthma, there is an increase in the thickness of the inner and outer airway wall layers and therefore total wall thickness [69]. There is evidence that the airway epithelium is also thicker [70], with hyperplasia of goblet cells [71]. Beneath the epithelium, the basement membrane is thickened in patients with asthma, which is considered one of the most distinguishing features of the disease [72].

Several studies have reported changes to ECM expression within and external to the airway wall in patients with asthma [73, 74]. Changes to ECM within the ASM layer are particularly notable, including an increased expression in elastic fibres and fibronectin within the ASM of large airways from patients with fatal asthma, and an increase in elastic fibres within the ASM of small airways from patients with fatal asthma compared with non-fatal asthma [75].

Another striking change to the airway wall in asthma is an increase in the thickness of the ASM layer. When averaged across cases of asthma, remodelling of the ASM has been observed in both small and large airways [69]. Our understanding of ASM remodelling has since been advanced to consider phenotypes based on anatomical location; remodelling in the small airways or large airways only; remodelling in both the small and large airways; or absence of ASM remodelling altogether [76]. Hence, the presence and extent of ASM remodelling varies both between and within subjects with asthma (figure 3) [77], an observation that should be considered when designing therapies that strive to address these structural abnormalities.

COPD has similarities to asthma in airway remodelling, but also some important differences [78], particularly obliteration of the small airways [79–81]. Again, there is generalised



FIGURE 3 Variation in airway smooth muscle (ASM) remodelling between and within subjects with asthma. Each cluster is a different subject. Each dot is an airway with remodelled (orange) or normal (blue) ASM. FA: fatal asthma; NFA: non-fatal asthma; NA: non-asthmatic. Reproduced and modified from [77] with permission.

thickening of the airway wall [82], which in some patients includes the ASM layer [83], though perhaps not to the same degree as has been documented in asthma. Variation in ASM remodelling between patients with COPD has also been demonstrated after bronchial biopsy; interestingly, patients with high amounts of ASM responded positively to inhaled corticosteroids compared with patients who had relatively less remodelling [84]. An important distinction in COPD is that the volume fraction of ECM expands disproportionately and is inversely related to lung function [85]; this is in contrast to patients with asthma, where there is a proportional increase in ECM and smooth muscle that accounts for most of the area of the ASM layer [85]. Compositional changes to ECM in COPD are observed throughout the airway wall [86].

Functional changes at the cellular and tissue level

At the cellular and tissue level, structural impairment precedes functional impairment. The degree to which impairment at the local cell or tissue level translates to integrated organ function is dependent upon the distribution and magnitude of the pathology, and airway-to-lung interdependence, as will be discussed. Loss of the epithelial barrier's integrity will increase the sensitivity of the airway wall to a bronchoconstrictor agonist delivered *via* the lumen [87]. Furthermore, the capacity of the epithelium to repair is reduced in both patients with asthma [88] and COPD [89], leaving the airway susceptible to environmental exposures that drive inflammatory processes.

As the principal effector cell producing bronchoconstriction, a change in ASM function is a likely contributor to airflow limitation. Morphology and function of the ASM cells appears intimately associated with the external structural and mechanical environment established by the surrounding ECM. Mechanosensitivity to a specific ECM substrate alters ASM contractile capacity, with evidence of enhanced contraction in the presence of fibronectin [90]. As the stiffness of the external environment to which the ASM is exposed increases, so too does cell size (hypertrophy) and expression of cytoplasmic markers of contractility [91].

At the tissue level (whole airway segments), increased thickness of the ASM layer enhances force production and narrowing in airways from patients with asthma [92]. It has been argued that of all the various changes to the airway wall that characterise remodelling, an increase in ASM thickness is the most influential in driving an increase in bronchoconstriction [93]. Patients with fixed airflow limitation (COPD) exhibit a somewhat different response, with airways narrowing more, even without an increase in gross ASM thickness [94]. An increase in airway narrowing that is independent of ASM bulk is potentially related to the aforementioned changes to ECM within the ASM layer [94] and an associated increase ASM contractility [95].

Functional changes at the end organ level

It has long been understood that functional heterogeneity (namely, bronchoconstriction and heterogeneity of airway calibre) has important consequences for overall lung function; for example, increased heterogeneity of airway calibre, as a result of any of the previously discussed mechanisms that alter function at the cell or tissue level, leads to elevated resistance and elastance of the whole system [96, 97]. Paired with the notion that functional heterogeneity could be self-organised, that it arises from a structurally homogeneous beginning [98, 99], it is tempting to conclude that structural heterogeneity (*e.g.* of ASM thickness) is a secondary consideration. While it may be the case that this dynamic (or self-organised) heterogeneity is present *in vivo*, it is also certainly true that there is significant structural heterogeneity [77], and that overall patterns of functional heterogeneity are likely to emerge from a combination of the underlying structural heterogeneity working in concert with the dynamic self-emergence process [100]. This is supported by the observation that the locations of ventilation defects in asthma are largely, though not entirely, persistent over time [101].

Everything being equal, increased structural heterogeneity should lead to decreased lung function and increased temporal persistence of ventilation patterns [102]. However, this is an oversimplification, and potentially a gross one, because there are many ways in which airway structure can be heterogeneous. The simplest notion is to imagine that airway structure is independent and uncorrelated, in which case simple measures of dispersion (*e.g.* coefficient of variation) might suffice. In reality, that does not appear to be the case; there are significant correlations in remodelled airway structure both “along” and “across” flow pathways [77], and these correlations may have significant implications for resulting functional heterogeneity and lung function. The mechanism through which these structural correlations are generated is unclear, which is perhaps unsurprising given that the mechanism of remodelling itself is disputed. However, some possibilities have been suggested [103], and while the correlation in structure itself is only partially revealed, the fact that airway remodelling is correlated may offer a clue as to the underlying remodelling process itself. Moreover, the precise ways in which structural heterogeneity, in its many guises, manifests as functional heterogeneity, remains only partially solved [104].

The above largely focusses on the heterogeneity of ASM thickness, and airway calibre, as prototypical examples of structural and functional heterogeneity, respectively. There are, of course, other important aspects that manifest heterogeneously; for example, the location and extent of mucus plugs [105, 106]. The statistics and correlations of the location of these plugs are not perfectly understood but will seemingly have important contributions to overall functional heterogeneity.

Length adaptation: a smooth muscle property that may underlie some of the unique functions and dysfunctions of the lung

Smooth muscle has a working length range that is much greater than that of skeletal or cardiac muscle. This is essential for the proper function of hollow organs, like the stomach, bladder, uterus and digestive tract that regularly undergo large volume changes. The same property, when not regulated appropriately, could be a source for pathophysiology that underlies diseases like asthma and hypertension. To understand smooth muscle-related organ dysfunction, one needs to know the mechanism responsible for the muscle’s ability to retain contractility over a large length range.

When a striated muscle is stretched over 60% from its optimal length, it will not be able to generate active force because the actin and myosin filaments no longer overlap each other [107]. In contrast, smooth muscle lining the urinary bladder is able to generate force over a seven-fold length range [108]. In ASM, a constant active isometric force can be maintained over a three-fold length range [109]. To achieve this, the muscle relies on a mechanism called “length adaptation” [110].

A simple definition of length adaptation is that it is a process initiated by a change in cell length, which involves rearrangement of contractile and cytoskeletal filaments to ensure maximal overlap between actin and myosin filaments, in order to maintain optimal force generation. The process does not occur instantly after a length change; rather, it takes some minutes. There is evidence that immediately following a length change, the ability of ASM to generate force is reduced, which accompanies partial dissolution of myosin filaments [111]. When the length of the muscle stops changing, the muscle contractility recovers over time (20–30 min, with periodic stimulation), along with repolymerisation of myosin filaments [111]. This myosin filament evanescence is part of a much more complex process, in which dismantling

and rebuilding of the contractile apparatus occurs in adaptation to a change in cell geometry [61]. In ASM cells, there is evidence that myosin filaments and myosin monomers or dimers are in equilibrium in a dynamic polymerisation and depolymerisation process, facilitated by the existence of a pool of myosin monomers and dimers within the cytoplasm of smooth muscle cells [112, 113].

Functional evidence indicates that in ASM adapted to different lengths, shortening velocity, power output and adenosine triphosphate consumption of the muscle are linearly proportional to the cell length, whereas isometric force is length-independent [114]. To explain the observations, a conceptual model of length adaptation has been proposed, as illustrated in figure 4. The increased shortening velocity in ASM adapted to a longer length can be explained by the increased number of contractile actomyosin units in series. The increased power output and energy consumption can be explained by the increase in the overall number of contractile units in the muscle adapted to a longer length. The length-independent isometric force at different adapted lengths can be explained if length adaptation involves only addition and subtraction of contractile

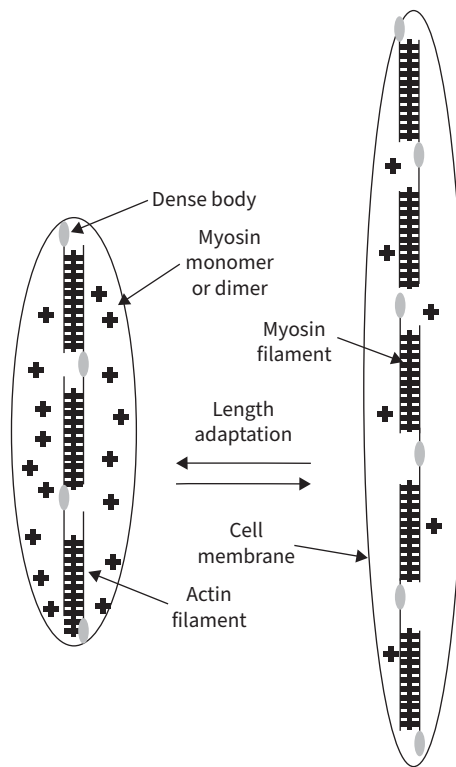


FIGURE 4 Reversible length adaptation in smooth muscle. When a muscle cell is lengthened or shortened from its original adapted length, disassembly of the contractile apparatus occurs, resulting in a loss of contractility. This is followed by reassembly of the contractile apparatus at the new cell length, so that the overlap between the myosin and actin filaments is optimal. This reassembly phase is accompanied by the recovery of the muscle's ability to generate force. The dynamic equilibrium between myosin filaments and myosin monomers and dimers facilitates the adaptation process. Note that a key feature of the adaptation process is that the contractile units can only be added or subtracted from the contractile unit array in series. This is required to accommodate the length-dependent behaviour of the muscle in terms of force, velocity and power, as described in the main text.

units in series, without changing the number of contractile units in parallel. Besides functional changes, structural changes in ASM adapted to different lengths have also been found. Using the number of myosin filaments in a cell cross-section (myosin filament density) to indicate the extent of myosin filament polymerisation, it has been shown that filament polymerisation rises as the adapted muscle length increases [114], supporting the assumption that more contractile units are present in smooth muscle cells adapted to longer lengths. The model shown in figure 4 presents a minimalist approach to explaining smooth muscle structure and function. As more structural and functional properties of smooth muscle are uncovered, more complexity will need to be added to the model to accommodate new evidence.

A shift in the length–force relationship of ASM is another consequence of length adaptation [111]. As illustrated in figure 5, adaptation of ASM to lengths shorter or longer than the reference length results in shifts to the left or right of the length–force curve respectively, while maintaining maximal force. In striated muscle, the length–force relationship cannot be shifted, because the underlying sarcomeric structure in striated muscle is fixed [107]. The fact that the same relationship in smooth muscle can be shifted indicates that the structure of the contractile apparatus in smooth muscle is not fixed, and can be altered by length adaptation. When fully adapted, ASM can generate maximal force. However, immediately after a length change (either shortening or lengthening), ASM can only generate submaximal force defined by the specific length–force curve associated with a specific length at which the muscle has been adapted (figure 5).

The length adaptation model described in figure 4 is compatible with the argument that smooth muscle cells possess material properties similar to that of plastic [109] or soft glass [115]. The model predicts that dissolution of contractile filaments in smooth muscle cells occurs after a change in cell length, which can be lengthening, shortening or length oscillation. During this phase of length adaptation, smooth muscle cells temporarily lose their ability to generate maximal force. It is likely that this is an explanation for the bronchodilatory and bronchoprotective effect of DI. The DI effect has been observed in human subjects [116, 117] and in *ex vivo* lungs [118, 119]. Furthermore, the distension of airway calibre during DI has been shown to be

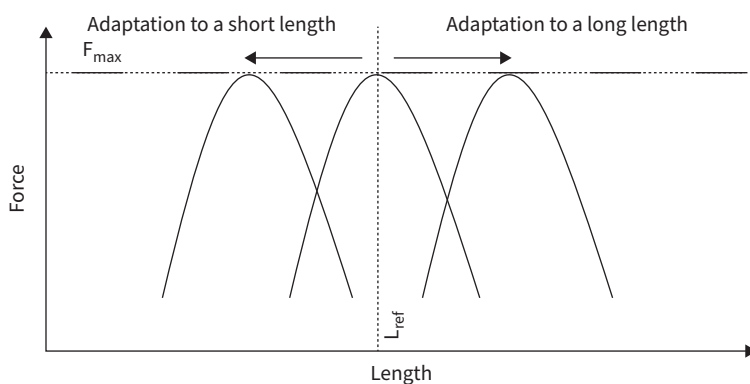


FIGURE 5 Shifting the length–force relationship due to length adaptation. After the muscle has been fully adapted to a reference length (L_{ref}), shortening or lengthening from L_{ref} will result in a temporary loss of contractility, along the curve specific to the muscle adapted to L_{ref} . However, if the muscle is held at the shortened or lengthened state for a prolonged period of time (e.g. 30 min), the curve will shift with little change in shape, so that the muscle can again generate the maximal force (F_{max}). The shift can be explained by the model shown in figure 4.

sufficient to stretch the smooth muscle cells encircling the airways and cause a temporary loss of contractility of these cells [119, 120]. These observations provide a plausible explanation for the bronchodilatory and bronchoprotective effects of DI.

The lack of beneficial effects of DI in asthmatic patients [117] could be a consequence of maladaptation of ASM to an abnormally short length, causing it to enter a “frozen state” [121], meaning DIs are unable to “fluidise” [122]. In human asthmatic lungs, it has been noted that ASM cells are hyperreactive [123]. This could be a consequence of “force adaptation”; that is, an increase of ASM contractility due to chronic activation of the muscle cells in the inflammatory environment of the asthmatic lung [124]. Chronic activation of ASM could put the muscle in a frozen state and become refractory to DI perturbation.

Airway hyperresponsiveness develops when lung volume is chronically reduced (for example, in cases in which chest strapping is used [125], when the subject is in the supine position [126] or when the subject is obese [127]). In such situations, ASM may adjust to abnormally short lengths. As illustrated in figure 5, maximal force can be achieved again after adaptation to a short length, resulting in exaggerated airway narrowing. When lung volume is increased, such as under positive end-expiratory pressure or continuous positive airway pressure, experimentally induced bronchoconstriction in healthy human subjects can be attenuated and airway reactivity in stable asthmatics can be reduced [128]. These can be taken as examples of adapting ASM at long lengths. As illustrated in figure 5, when ASM is adapted to a long length, shortening of the muscle results in less force generation (without adaptation), thus conferring bronchoprotection on the lung. Knowing the adaptive behaviour of smooth muscle, adaptation of ASM to short lengths should be prevented to preserve healthy lung function.

The impact of airway-to-parenchymal interdependence on lung function tests

In vivo, the airflow through the airways is critically dependent on the balance between factors that favour and factors that contrast with the airway tone [129, 130], the former being the force-generation capacity of ASM and the latter being the magnitude of internal and external elastic loads (figure 6). However, due to the complexity of lung structure-to-function changes in disease, their net effect on airway calibre may be highly variable and even contrary to expectations.

In this section of the chapter, we will examine how the mechanisms and structural characteristics that modulate airway narrowing may impact on standard lung function tests in health and disease.

The effects of lung inflation on airway calibre

Following the seminal work by NADEL and TIERNEY [116], which reported that DI temporarily reduces the resistance of constricted airways *in vivo*, a long series of studies confirmed that this phenomenon is consistent in healthy subjects but absent or minimal in asthmatic subjects. The lack of relationship between this and indices of airway inflammation [131] suggests that the impaired effect of DI in asthma is the result of reduced distensibility of the airway walls, possibly because of an increase in ASM and abnormal length adaptation, airway wall remodelling, or reduced stretching by the surrounding lung parenchyma [132, 133]. Whatever the mechanism, the different effects of DI between healthy and asthmatic subjects have an important impact on the clinical assessment of airway responsiveness, with spirometric measurement that requires a DI becoming more sensitive in separating asthmatic from non-asthmatic subjects than measurements performed during tidal volume, such as airway resistance [117, 134]. In 1995, SKLOOT *et al.* [135] found similar methacholine dose–response

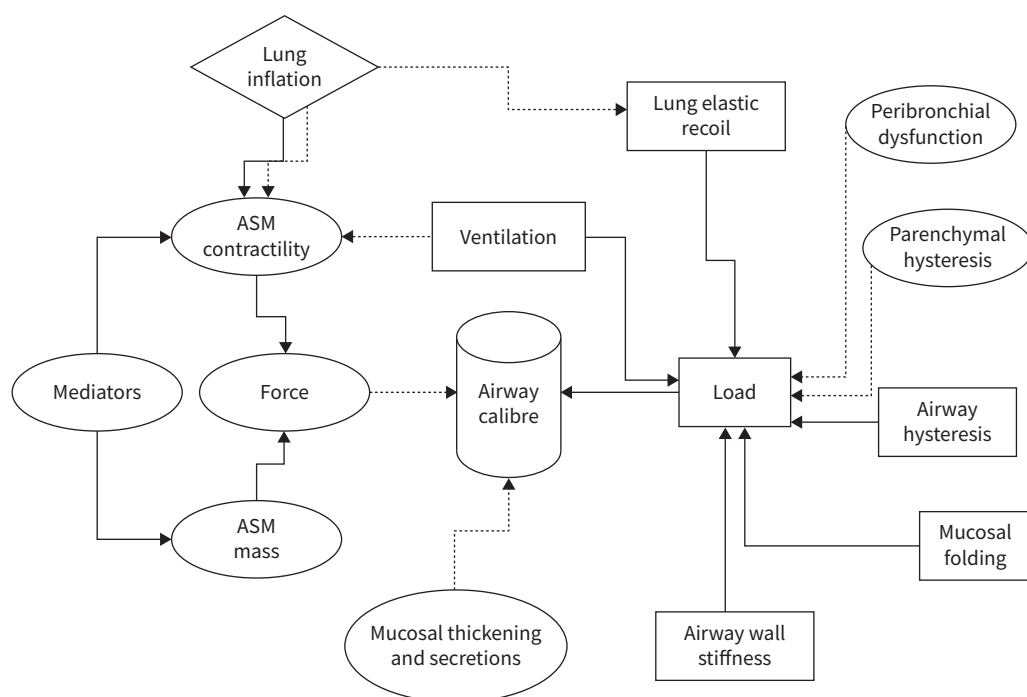


FIGURE 6 Schematic representation of factors favouring (in ovals) and opposing (in rectangles) airway narrowing. Continuous and dotted lines denote increasing and decreasing effects, respectively. Airway smooth muscle (ASM) force together with mucosal thickening and bronchial secretions are the mechanisms that directly decrease bronchial lumen. In contrast, the loads external and internal to the airway wall are the mechanisms that tend to limit ASM shortening, thus opposing airway narrowing. Note that lung inflation has a negative effect on load, as it transiently decreases elastic recoil, and an unpredictable effect on ASM, as it is capable of decreasing ASM contractility in health, but increasing it in asthma. Reproduced and modified from [129] with permission.

curves in asthmatic and normal subjects when DIs were strictly prohibited. This supports the idea that hyperresponsiveness in asthma may reflect an inability to dilate constricted airways rather than an increased response to stimuli [135]. This was only partially confirmed when volume-independent measurements were used, suggesting that both impaired bronchodilation and increased contractile response may contribute to airway hyperresponsiveness [131]. A bronchoprotective effect of DIs taken before a single dose of methacholine was observed in healthy but not asthmatic subjects [136]. However, the bronchoconstrictor response inferred by measurements that do not require full lung inflation was enhanced rather than attenuated by prior DIs both in healthy and asthmatic subjects [137].

Several studies suggest that the effects of DI on the assessment of lung function are widely variable. In asthmatic subjects with spontaneous bronchoconstriction [138] and in COPD [139], forced expiratory flows were found to be lower during a manoeuvre beginning at maximal rather than partial lung inflation, which was explained by a discrepancy between the hysteric properties of airways and lung parenchyma [132, 138, 140]. The practical consequence of this short-lasting bronchoconstrictor effect is that the effect of bronchodilators may be underestimated by classical spirometric measures [141]. In COPD subjects with extensive emphysema, FEV₁ may not increase after bronchodilator inhalation, despite an increase in FVC. The potential mechanisms for this isolated volume response are: space competition between

airways; distended alveoli; and a decrease in airway calibre with lung inflation due to longitudinal stretching in the presence of reduced radial tethering force [141]. Finally, in a small subset of asthmatic subjects, a decrease in FEV₁ was reported to occur with subsequent spirometric manoeuvres, which was explained by an increase in ASM tone caused by DI triggering a myogenic mechanism [142–144].

Another important mechanism linking DI to lung function assessment in clinical practice is intrathoracic gas compression. During forced expiration, alveolar pressure increases, and gas is compressed within the lung, thus causing lung volume and lung elastic recoil to decrease. The latter will result in a reduction of driving pressure and distending pressure at choke point, thus explaining why FEV₁ measured at the mouth is less than it would be if measured by body plethysmography. The magnitude of this effect is determined by the amount of gas within the lung and airway resistance. Practical consequences are that FEV₁ tends to overestimate the severity of airflow obstruction in emphysema [145] and airway responses to pharmacological interventions in subjects with larger lungs [146].

The effects of breathing pattern and operational lung volume

Studies in animals and humans have provided clear evidence that increasing the operational lung volume or tidal volume reduces airway resistance and can attenuate or reverse the response to bronchoconstrictor stimuli.

In vitro, dynamic swings can blunt the response of ASM to contractile stimuli through mechanisms that reduce its force-generation capacity [147, 148], depending on the magnitude and frequency of pressure oscillations. In healthy humans, bouts of five tidal volumes terminating at ~80%, ~90% and 100% of TLC have been shown to cause gradual bronchodilation that lasts for ≥11 min [149]. Increasing ventilation by breathing a CO₂-enriched mixture [150] or through hypobaric conditions [151] significantly blunts the bronchoconstrictor response to methacholine.

Breathing voluntarily at increased lung volume has also been shown to attenuate induced bronchoconstriction in healthy subjects [152, 153]. However, the major determinant appeared to be the operational lung volume, whether attained by increasing FRC or tidal volume [153]. Conversely, breathing voluntarily at low lung volume enhanced the bronchoconstrictor response [152]. Analogously, airway responses to bronchoconstrictor stimuli are enhanced in healthy subjects using chest strapping [125], in the supine position [126] and in obesity [127], with all conditions associated with breathing at low lung volume.

Collectively, the available literature supports the notion that changes in lung volume are strong a mechanism of airway calibre modulation. However, while increasing operational lung volume has a beneficial effect that opposes airway narrowing, the effects of DI may vary according to disease conditions.

The effects of ventilation heterogeneity: topographical and temporal patterns

It has long been understood that ventilation is not uniform in obstructive lung diseases [154], but its impact on global lung function tests was not investigated until the beginning of this millennium, when imaging studies documented the occurrence of large and important ventilatory defects during bronchospasm [98, 155, 156].

Integrative modelling approaches suggested that even minimal anatomical variations between peripheral airways of the same generation could cause responses to a constrictor agent that

differed in magnitude and time, resulting in airflow diverting from more to less constricted airways, thus paradoxically increasing the size of the latter (parallel inhomogeneity) [99]. This mechanism may explain the insensitivity of forced expiratory flows to lung inhomogeneity during induced bronchoconstriction [157]. Similar results were also achieved by considering the hypothesis that constriction of more central airways will cause hypoventilation of the subtending regions (serial heterogeneities), thus magnifying the effects of parallel heterogeneities occurring within the peripheral airways [155]. Two major studies then attracted the attention of the medical community on this issue. The first demonstrated that replications of such events were associated with the occurrence of severely hypoventilated regions once a given threshold of variability was exceeded [98]. The second found that ventilation heterogeneity was a major determinant of airway hyperresponsiveness in asthma, independent of airway inflammation [158]. However, in a recent study [159] airway hyperresponsiveness and abnormalities of single- or multiple-breath nitrogen washout [160] were frequently present in subjects with suspicion of asthma and normal spirometry, but were not necessarily associated in individual subjects. This lack of concordance suggests that these tests reflect different pathological aspects of the disease.

Ventilation heterogeneity has also been examined under the time domain on the ground that the respiratory system exhibits mechanical fluctuations over different time scales. In a seminal study by QUE *et al.* [161], the variability of respiratory impedance measured using the forced oscillation technique for 15 min was much higher in asthmatic individuals than in healthy subjects. When the latter inhaled a constrictor agent in supine position, the impedance variability became similar to that of asthmatics, confirming the idea that ASM contraction and unloading are basic mechanisms modulating bronchial tone. Importantly, the impedance time series exhibited power law features with similar exponent of the relationship between groups, suggesting that the phenomenon is replicable at different time scales. The study by QUE *et al.* [161] paved the way for the investigation of temporal variability, which represents a major characteristic of bronchial asthma. Studies using peak expiratory flow were able to predict asthma attacks 1 month in advance [162], as well as asthma worsening after withdrawing steroid therapy [163]. Monitoring the daily variability of oscillatory resistance not only allowed the prediction of airway narrowing 1 week in advance [164] but also made it possible to separate asthmatic from healthy subjects more accurately and over a shorter period of time than by monitoring peak expiratory flow [165].

Based on the above studies, it is hoped that the topographical and temporal variabilities of airway narrowing will take a crucial place in the armamentarium of fundamental markers for asthma diagnosis and monitoring in clinical practice.

Conclusion

In health, lung function is optimised by the mechanical interaction between airways and lung parenchyma. In disease, heterogeneous structural changes occur in both the lung parenchyma and the airways, impairing the ability of the whole organ to maintain a normal homeostatic state in response to external stimuli. In this context, an important role is played by the ASM length-adaptation property, with an abnormally short length placing the muscle in a “frozen” state, making it unresponsive to the broncho-relaxant effect of deep and tidal inspirations. Owing to the complex interactions between various structural units of the whole organ, the mechanical effects of breathing can either enhance or attenuate the functional impairment depending on the type and severity of underlying disease. This means the interpretation of lung function tests in clinical practice is not always univocal, nor is it compliant with the expectations and/or the recommendations of international guidelines.

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Advances in exercise physiology and pathophysiology

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Advances in technology and innovation have enabled noninvasive, continuous and real-world assessment of human biology, advancing our understanding of exercise physiology across the spectrum of health and disease, to optimise performance and health <https://bit.ly/ERSM107>

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Over the past two decades, technology and innovation have advanced the field of sport and exercise physiology. Noninvasive imaging techniques such as near-infrared spectroscopy, functional near-infrared spectroscopy, MRI, impedance cardiography and optoelectronic plethysmography have deepened our understanding of exercise physiology, benefiting athletic populations to optimise performance and recovery, as well as clinical populations to understand mechanisms of limitation and develop novel therapeutic targets. The rapid acceleration in wearable technology development has enhanced the ability to monitor a range of human physiological parameters in a continuous, real-time and nonintrusive manner across health and disease. Wearable technology aids athletes in monitoring training load and recovery to enhance performance, supports health management in the general population, and offers personalised medicine opportunities for those with long-term conditions including chronic respiratory disease. Despite this, extensive clinical trials are needed to demonstrate the validity, reliability and clinical relevance of digital biomarkers in chronic respiratory disease for regulatory and clinical endorsement.

Introduction

The field of exercise physiology has grown significantly over recent decades, leading to developments in knowledge, approaches and opportunities for applications in health and disease. At the forefront of this growth, is the evolution of technology and innovation [1]. Investigations of high-level human performance have provided valuable insights into physiological adaptations to extreme metabolic loading. Identification of the mechanisms and pathways that limit exercise capacity in highly trained individuals have aided the application of innovative methodology to better understand the pathophysiological limitations to exercise in pathological conditions. This mechanistic understanding has led to the identification of novel therapeutic targets and treatment approaches for people with long-term conditions [1]. For instance, in chronic respiratory disease, exercise physiology research has utilised technology to

explore responses to pharmacological and nonpharmacological interventions such as bronchodilators, O₂ and heliox supplementation, and noninvasive ventilation, as well as develop and evaluate rehabilitative strategies including interval training, nonlinear periodised exercise training, neuromuscular electrical stimulation and blood-flow restriction training, to optimise physiological adaptation in a wider range of individuals [2, 3].

In this chapter, we focus on the advances in noninvasive technology to evaluate physiological responses during exercise testing. These include imaging techniques such as near-infrared spectroscopy (NIRS), functional NIRS (fNIRS), MRI, impedance cardiography and optoelectronic plethysmography (OEP), which have informed exercise physiologists about the structure and function of skeletal muscle, the brain, and the cardiovascular and respiratory systems before, during and after exercise. Furthermore, the innovation in wearable biosensor technology allows researchers to collect a wealth of physiological data during real-world conditions. Importantly, several of the emerging technologies have been pioneered in the field of sports medicine and wellbeing, but they may offer significant value when applied to patients with respiratory disease as well. Accordingly, this chapter also reports on advances in wearable biosensors applicable to athletic and disease populations. It also reports on the quantity and array of data that can be obtained from laboratory and real-world conditions, which has created opportunities for the use of advanced analytical techniques in the field, including computational modelling, to create and interpret powerful data reflecting a wide range of physiological variables and responses [1]. Finally, the chapter outlines some key considerations to facilitate regulatory and clinical endorsement of real-world digital biomarkers in the field of chronic respiratory disease.

Imaging, bioimpedance and motion capture techniques

At the physiological level, imaging techniques, including NIRS, fNIRS, functional MRI (fMRI), cardiac bioimpedance and OEP have informed exercise physiologists about cerebral, cardiovascular, respiratory and muscle metabolic functions during exercise.

Near-infrared spectroscopy

NIRS has been used to assess tissue oxygenation, local O₂ consumption (a measure of oxidative metabolism) and blood flow in various human tissues including the brain and skeletal muscle [4]. In the muscle, NIRS provides a tool for assessing two major determinants of the capacity to exercise: local muscle O₂ delivery and O₂ utilisation. NIRS includes near-infrared light that can easily pass through tissues and directly measure the concentration (and changes in concentration) of chromophores such as oxyhaemoglobin and deoxyhaemoglobin. Since the early years of development, NIRS has undergone significant advancements and has several advantages over older approaches, including noninvasiveness, minimal susceptibility to movement artefacts and high spatiotemporal resolution [5].

The noninvasive nature of NIRS makes it an appealing technique for use in the laboratory, and wearable NIRS biosensors can be used during activities of daily living [6]. As athletic training methods advance, the use of wearable NIRS biosensors for precise training monitoring has become increasingly prevalent [7]. Athletes can adjust the intensity of their workouts based on real-time data about their muscle oxygenation levels, ensuring that they train at optimal levels without overexertion. Muscle oxygenation data can also indicate how effectively an athlete's muscles are utilising O₂ during prolonged activities, which is essential for endurance sports such as cycling, running and swimming [8].

In patients with COPD, studies have utilised NIRS to assess the effects of O₂ and heliox supplementation [9, 10], noninvasive ventilation [11] and pharmacological agents [12] on locomotor muscle O₂ availability, leading to a better understanding of the underlying haemodynamic pathophysiology.

Evidence shows that wireless, portable NIRS is a reliable method to detect COPD-related loss of gastrocnemius muscle oxidative capacity [13]. These findings support the reliability of noninvasive muscle oxidative capacity assessment by NIRS in COPD, which may help track the efficacy of interventions such as pulmonary rehabilitation that are designed to mitigate locomotor muscle deconditioning. In patients with respiratory disease, wearable NIRS sensors can be used during field walking tests to evaluate the degree of locomotor muscle deoxygenation as a contributing factor to walking intolerance.

Using NIRS surface optodes in combination with the light-absorbing tracer indocyanine green dye (ICG) injected as an intravenous bolus has enabled the assessment of respiratory muscle blood flow at rest and during exercise in athletes and patients with COPD [14]. Intravenously administered ICG binds to albumin and undergoes rapid metabolism, making it suitable for repeated measurements. NIRS-ICG allows the calculation of local muscle blood flow based on the rate of ICG accumulation in the muscle over time (figure 1). The NIRS-ICG method has been employed in various tissues, including respiratory and locomotor muscles, and the brain, in healthy people and those with respiratory disease [14]. To understand the circulatory regulation of respiratory muscles during exercise in healthy individuals and in patients with COPD, investigators originally assessed the perfusion of the intercostal muscles that provide the main pressures to displace the ribcage and increase end-inspiratory lung volume [15, 16]. Subsequent studies succeeded in assessing blood flow during exercise from the scalene, sternocleidomastoid and abdominal muscles alongside locomotor muscle blood flow [9, 17]. A series of studies allowing simultaneous assessment of respiratory and locomotor muscle blood flow showed that

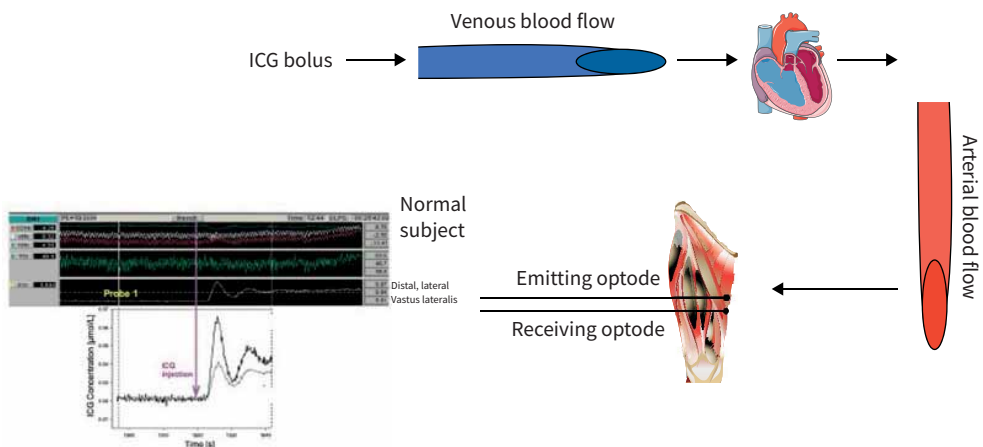


FIGURE 1 Schematic representation of the NIRS-ICG (near-infrared spectroscopy - indocyanine green dye) technique. From top left to bottom: an intravenous bolus of ICG is administered, it passes through the heart and lungs through the venous circulation, then *via* the arterial circulation it enters the systemic circulation and microcirculation. The NIRS optodes, which are positioned over the tissue, detect the ICG. Extinction coefficients in a matrix operation are employed and the ICG curve is isolated. The circles represent the NIRS optodes positioned over the tissue. Reproduced and modified from [4] with permission.

during strenuous exercise quadriceps muscle blood flow was not reduced in favour of the respiratory muscles in either athletes or patients, thereby challenging the theory of blood-flow redistribution from the locomotor to the respiratory muscles [14].

Functional near-infrared spectroscopy

Real-time measurement of dynamic brain activity during exercise has advanced our understanding of the role of exercise in brain health and function. fNIRS is a functional neuroimaging technique that exploits cerebral haemodynamics and has been widely used in the field of sports psychology to reveal the neural mechanisms underlying the effects of exercise-associated improvements in higher cognitive functions (*e.g.* working memory and inhibitory control) [18]. In sport and exercise science, fNIRS has primarily been used to measure the effects of exercise intensity on haemodynamic responses in the cerebral cortex. fNIRS has, however, a limited spatial resolution and only allows for the evaluation of brain activation patterns in cortical areas [19]. Accordingly, fNIRS has commonly been applied to monitor general changes in cerebral oxygenation (indicated by oxygenated haemoglobin) during incremental exercise at different intensities (*e.g.* low, moderate, hard and very hard), typically observed as a quadratic trend. During incremental exercise from low to moderate intensities, cerebral oxygenation increases, remains stable between moderate and hard intensities, then decreases at very hard intensities (near exhaustion) [20]. Wearable fNIRS systems that operate wirelessly (figure 2) have the potential to image the brain in real-world conditions, thereby allowing the development of a user-friendly brain-computer interface system [21]. Studies in patients with COPD using portable and wireless fNIRS provide evidence of reduced cognitive-motor capacity and poorer motor performance during walking compared with healthy age-matched individuals [22, 23].

Functional MRI

fMRI has been used to study the influence of acute physical exercise on subcortical structures, such as the hippocampus, which have a crucial role in cognitive processes (*e.g.* memory) [24]. fMRI enables the assessment of brain activation changes in cortical and subcortical areas and offers a higher spatial resolution compared with fNIRS, which results in superior source localisation [19]. fMRI applications have promoted the investigation of the effects of acute physical exercise on cognitive performance and their underlying neural correlates (*e.g.* changes in functional brain activation patterns). Accordingly, there is growing evidence that acute bouts of aerobic or resistance exercise leads to profound changes in functional brain activation and



FIGURE 2 Example of portable functional near-infrared spectroscopy device placement.

hence cognitive performance. However, behavioural improvements in response to acute physical exercise is dependent on different factors, such as the characteristics of participants (e.g. age), the cognitive domains tested (e.g. executive functions), the exercise characteristics (e.g. exercise intensity), and the time delays between the cessation of exercise and post-exercise cognitive testing. Functional brain imaging markers of breathlessness expectation have been investigated as predictors of therapeutic response to pulmonary rehabilitation in patients with COPD [25]. Breathlessness expectation refers to the brain's anticipation of breathlessness in response to cues, which can significantly influence the perception of breathlessness and its clinical outcomes. The study showed that breathlessness expectation-related brain activity before rehabilitation is a strong predictor of clinical improvement in breathlessness over pulmonary rehabilitation. It is therefore anticipated that monitoring brain markers of breathlessness expectation will be able to predict which patients have clinically important improvements in breathlessness over pulmonary rehabilitation. Such models could provide new insights into the mechanisms by which breathlessness may be targeted, paving the way for targeted behavioural and pharmacological interventions [25]. However, it should be noted that exercise testing in the horizontal position, as required by fMRI, poses limitations for research in respiratory disease because of the altered physiological demands compared with upright or seated positions. Nevertheless, exercise testing in the horizontal position provides valuable insights into brain responses and mechanisms that are not accessible through other modalities.

Impedance cardiography (cardio-impedance)

Cardio-impedance is a noninvasive, electrical, sensor-based wearable technique that has been gaining popularity over the last few decades for monitoring cardiac output during CPET, as well as real-world physical activities. Cardio-impedance works by detecting changes in electrical impedance across the thorax region, with changes in impedance used to calculate haemodynamic parameters such as stroke volume, heart rate and cardiac output (figure 3) [26]. Impedance changes occur as a result of fluctuations in blood volume during the cardiac cycle, which are influenced by the contraction and relaxation of the heart, thereby providing real-time insights into cardiovascular function [27]. Studies suggest that cardio-impedance provides an acceptable cardiac output measurement from rest to peak cycling exercise in patients with COPD [28]. Combining cardiac output with $\dot{V}_{O_2}$ measurements allows the calculation of whole-body arterio-venous O_2 difference, indicating the amount of O_2 primarily extracted by the working muscles during exercise. Hence, cardio-impedance has been employed in studies on respiratory patients to appreciate the cardiovascular limitations to exercise [29], the effects of pharmacological agents [30] and those of noninvasive ventilation on hemodynamic regulation during exercise [31]. In addition, there have been studies assessing the effects of exercise-based pulmonary rehabilitation on peak cardiac output during incremental exercise [32]. The portability of wearable cardio-impedance also facilitates the assessment of central hemodynamic responses during field walking tests in COPD and the choice of the optimal walking modality during exercise-based pulmonary rehabilitation sessions [33].

Optoelectronic plethysmography

OEP is a motion capture method to measure chest wall movements and estimate lung volumes on a breath-by-breath basis by modelling the thoraco-abdominal surface. OEP reconstructs the chest wall surface and then volume by placing several markers on the skin. Several infrared cameras capture the scene and track a number of infrared-reflective markers (figure 4). OEP has been extensively employed in studies looking at chest wall kinematics and volume changes in chest wall compartments in healthy subjects in relation to age, gender, weight, posture and different physiological conditions [34]. In COPD patients, OEP has been used to test the degree of dynamic hyperinflation during exercise [35] and the effects of bronchodilators [36], O_2 and

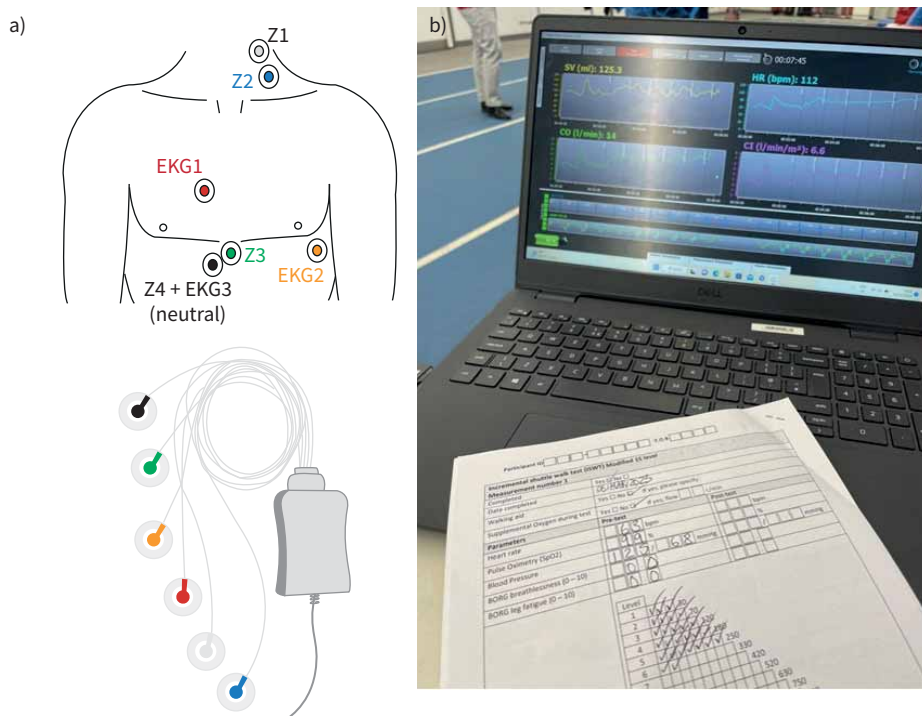


FIGURE 3 Impedance cardiography. a) Placement of six electrodes of the physio-flow: two on the left carotid (Z1 and Z2), two in the breast area (EKG1 and EKG2), and two in the chest area (Z3 and Z4 + EKG3 (neutral)). b) Portable impedance cardiography device and a screenshot of variables recorded by the portable device, including heart rate, stroke volume, cardiac output and cardiac index.

heliox supplementation [9, 37], as well as noninvasive ventilation [38], on dynamic chest wall hyperinflation. Furthermore, the application of OEP has allowed the assessments of total and compartmental chest wall volume changes following a programme of pulmonary rehabilitation in patients with COPD [39]. OEP may be used in the future to assist in the understanding of breathing patterns in respiratory conditions that are difficult to diagnose objectively, such as dysfunctional breathing in long COVID, and therefore it may have the potential to contribute to an improvement in therapeutic strategies.

Wearables and bio-signals in sport

Wearable technologies, such as accelerometers, Global Positioning System (GPS) trackers, heart rate monitors, electromyography sensors, skin temperature sensors and sweat analysers, are widely employed to monitor sports performance, optimise training and predict potential injuries [40]. Additionally, similar technologies are employed in sport and exercise to promote wellbeing and fitness as part of everyday life [41]. These devices provide real-time data during real-world training or competition on various physiological parameters, facilitating athletes and coaches to tailor training programmes to enhance performance and minimise the risk of injury.

The integration of multiple sensors, including heart rate monitors, temperature sensors and motion detectors, has been demonstrated to be effective in elite sports settings, for example, during the Tokyo 2020 Olympic games [42]. These sensors collect comprehensive data on physiological responses, which are used to monitor athletes' health and performance during

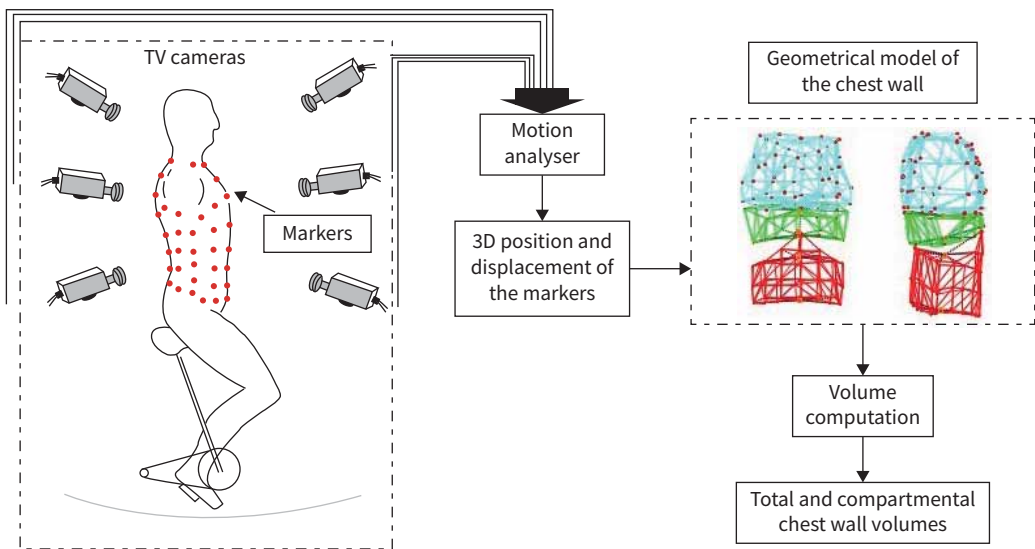


FIGURE 4 Optoelectronic plethysmography: the principle of measurement. Cameras record the movement of 89 retro-reflective markers placed over the front of the chest wall and the back from the clavicles to the pubis. Each marker is tracked by six video cameras (Smart system BTS, Milan, Italy), three in front of the subject and three behind.

events. For instance, heart rate, core body temperature and spatial-temporal variables were tracked to provide insights into athletes' endurance and biomechanical efficiency [42].

Wearable sensors are fundamentally Internet of things devices, comprising three main components: the sensor (hardware), the processing unit (software) and a network for connectivity [43, 44]. Recent advancements in hardware have improved the accuracy and reliability of these devices. Finally, recent developments in data analytics and artificial intelligence allows the extraction of more accurate and informative parameters [45].

Types of wearables

Sensors such as electromyography, electrocardiogram (ECG), electroencephalogram [46], galvanic skin response, oximetry [47], temperature, humidity, environmental sensors [46], biomechanical sensors, accelerometers, gyroscopes and magnetometers are widely used in sports [42, 44]. These sensors can be placed on various body parts including apparatus worn on the head, trunk, wrists, lower body, shoes and pockets. They can be configured in several forms such as devices attached with a strap or tape, or as patches themselves, providing versatile and comprehensive monitoring of several parameters [42, 44].

Analytics

Wearable sensors in elite sports utilise advanced data analytics and processing techniques to extract valuable insights from raw data. Low-level processing involves initial steps such as noise reduction, signal filtering, normalisation, and feature extraction to prepare the raw sensor data for more complex analyses. This stage ensures the accuracy and relevance of the data for further processing [40]. Simple approaches in signal processing can include basic analytics pipelines. For instance, calculating parameters such as peaks in acceleration signals can identify initial contacts in gait [48]. Other examples include the calculation of simple statistics such as mean, median, standard deviation and coefficient of variation of sensor readings (bio-signals) to

provide initial insights into performance metrics, and time-series analysis to identify trends and patterns over specific periods. Machine learning enabled processing builds on this foundation by applying algorithms to detect intricate patterns and dependencies, classify activities and make predictions. Techniques such as supervised learning, unsupervised learning and feature selection are used to develop models that can provide valuable metrics leading to actionable insights [46]. Furthermore, deep learning enabled processing goes even further, using neural networks to model complex relationships in the data. A few examples include the use of convolutional neural networks, recurrent neural networks and autoencoders, which are employed to process spatial and temporal data, enabling advanced capabilities that further aid the creation of valuable insights from the data [46]. It should be noted, however, that movement artefacts are a notable concern, especially in real-world settings. To address this, hardware solutions like motion-resistant sensor designs [49] and software approaches such as advanced signal processing [50] and artefact correction algorithms [51] can be employed to mitigate these effects and enhance data accuracy.

Personalised training programmes

Personalised sport and exercise training programmes can be crafted by artificial intelligence (AI) systems that analyse individual data, ensuring that exercise regimens are tailored to specific needs and performance levels [52, 53]. Real-time feedback during workouts enables immediate adjustments to enhance exercise efficacy and safety [52, 53]. Hence, adjustments in training programmes are made dynamically, analysing real-time data from wearables. This enables the creation of daily tailored workouts, recovery protocols and nutritional plans, ensuring optimal training loads and preventing overtraining.

AI-driven predictive analytics play a crucial role in injury prevention by evaluating physiology and biomechanics to foresee and mitigate potential injuries, thus safeguarding athletes from overstrain. According to systematic evidence, artificial neural networks and decision tree classifiers are commonly employed methods [54, 55]. These models utilise variables such as training load, ground reaction force patterns, heart rate variability (HRV), O₂ saturation, sleep quality and body temperature to predict injury risk. These variables are critical because they provide comprehensive insights into an athlete's physical condition and are related to physiological stress. For example, HRV is related to stress on the cardiac system, hence an athlete with elevated HRV might be advised to avoid high-intensity tasks that day to promote recovery and prevent overtraining. However, the scientific evidence to support existing models is limited since their application is offered as a product [56]. It should be highlighted that the choice of variables must be relevant to the outcomes they predict and collected in real-time so that the models can be generalised, and their validity supported. For example, using advanced language models such as OpenAI's Generative Pre-Trained Transformer 4 for prescribing exercise programmes cannot provide personalised programmes based on the athletes' real-time physiological measurements, despite its capability to make informed decisions based on its knowledge base [57]. AI-driven predictive analytics may provide a solution to smartly process patient-specific respiratory data captured in daily life by unobtrusive sensor technologies for optimised, personalised treatment including pulmonary rehabilitation, as well as the prediction of exacerbations and the assessment of health outcomes to improve quality of life in these patients.

General population and wellbeing

Wearable sensors are incorporated in products offered by companies like Apple and Fitbit, which are increasingly used to monitor a variety of bio-signals, contributing to overall health and wellbeing by providing insights into several physiological parameters. The Apple Watch records photoplethysmography (PPG) and ECG signals to monitor heart rate and detect irregular heart rhythms, such as atrial fibrillation (AFIB). The Apple Heart and Movement Study

leverages extensive PPG and ECG data to train models that offer real-time health monitoring and predictive analytics for better health management [58]. Additionally, the Apple Watch measures blood O₂ levels, which is crucial for assessing respiratory and cardiovascular health, and provides detailed sleep analysis by tracking sleep stages and offering insights into sleep quality. Similarly, Fitbit devices utilise PPG technology for continuous heart rhythm monitoring and AFIB detection, validated in a large virtual study demonstrating high accuracy [59]. Considering that more than half of the global population use a smart device [60], monitoring bio-signals for wellbeing is feasible without further extensive infrastructure investment.

Management of chronic respiratory diseases

Chronic respiratory diseases (CRDs), such as COPD and asthma, are characterised by pathophysiological impairments that lead to poor prognosis and reduced quality of life [61]. Monitoring bio-signals associated with these impairments is crucial for early detection, disease management and prognosis. The advent of wearable technology and data science offers new opportunities to monitor these bio-signals continuously and noninvasively in any environment, providing valuable insights into disease progression and patient health. As illustrated earlier, recent advancements have introduced novel bio-signals relevant to CRDs obtained from wearables. A few examples include noninvasive measurement of tissue blood flow and O₂ saturation, which provide insights into peripheral O₂ delivery and utilisation [62]; breathing frequency, which provides insights into respiratory rate and ventilatory efficiency [63, 64]; and HRV, which offers information on autonomic nervous system function and overall cardiovascular health. These wearables facilitate continuous data collection, capturing large volumes of physiological data over extended periods.

Continuous monitoring of physiological data provides a comprehensive picture of a patient's health status, capturing variations and trends that may be missed by periodic "snapshots" such as laboratory assessments. Consequently, recent advances in wearables for sports and general wellbeing could help us develop predictive models for adverse events, such as exacerbations, and provide real-time feedback to guide the titration/adjustment of personalised pharmacological and nonpharmacological interventions. A recent meta-analysis on wearable technology interventions shows that wearable technology can significantly improve physical activity and exercise capacity in patients with COPD, particularly when combined with interventions such as health coaching or pulmonary rehabilitation. However, studies on the detection of COPD exacerbations are heterogenous, with mixed outcomes and high attrition rates, suggesting further work is needed in this field to integrate wearable technology into the core management of COPD [65].

Insights from regulatory-approved and nonapproved biomarkers and software tools

Despite the growing evidence into digital endpoints [66] and the progress towards the adoption of digital endpoints as biomarkers for supporting decision-making in drug development and clinical practice, there are no approved digital endpoints reflecting bio-signals in CRDs. Considering that there are no digital biomarkers obtained from bio-signals in CRDs, scientists focus on other digital tools approved in CRDs. In COPD, the hybrid tool combining objectively measured physical activity with the patient's subjective experience of physical activity (Daily-PROactive and Clinical visit-PROactive Physical Activity instruments) has received a favourable qualification opinion from the European Medicines Agency (EMA) [67]. Besides CRDs, to the best of our knowledge, the qualification of stride velocity 95th centile (SV95C) in 2019 was the first advancement in this field [68]. This biomarker quantifies the ambulation ability of patients with Duchenne muscular dystrophy in interventional studies [69] and has obtained approval from the EMA [70].

In the general population and regarding the diagnostic aspect, the software for detecting AFIB in both the Apple Watch and Fitbit devices has received approval from the US Food and Drug Administration (FDA). Apple received FDA approval for its ECG app and AFIB detection features, which leverage the watch's optical sensors to monitor heart rhythm continuously and alert users to potential irregularities [71]. Similarly, Fitbit's software, which also uses PPG sensors to detect AFIB, received FDA clearance [72]. The approval for Fitbit's algorithm allows it to passively monitor heart rhythms and notify users of potential AFIB events. Continuous glucose monitoring systems as wearable devices have received favourable opinions from FDA in monitoring glucose and informing the user of hypoglycaemia or hyperglycaemia [73–75]. Implantation devices, providing real-time monitoring of measures such as the CardioMEMS HF System (Abbott), monitor pulmonary artery pressure in patients with heart failure. A small sensor is implanted that measures and records the pulmonary artery pressure [76].

Moreover, there is a lot to be learned from digital tools and endpoints that failed to receive favourable regulatory approval. An example is the instrumented Part III of the Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) through Verily's watch, assessing motor function in patients with Parkinson's disease [77]. The FDA declined the application, stating that the device and its associated motor examination "are limited in their capacity to evaluate meaningful aspects of concepts of interest that are relevant to the patients' ability to function in day-to-day life" [78]. This decision reflects the FDA's preference for the MDS-UPDRS Part II, which assesses motor aspects of daily living experiences. "A change in rigidity or finger tapping in the MDS-UPDRS Part III cannot be directly interpreted as being meaningful to patients. However, a change in speech, eating, and dressing (as assessed in the MDS-UPDRS Part II) represents a meaningful change in how patients function in daily life," the FDA explained. Trium's Actibelt wearable device measures real-world walking speed to assess mobility in patients with multiple sclerosis, but the EMA has not yet accepted this measure because the clinical significance of changes in walking speed for patients' daily lives has not been adequately demonstrated [79]. Interestingly, the Mobilise-D consortium aiming to connect digital mobility assessment for regulatory and clinical endorsement has received two support letters from the EMA [80]. However, establishing that the novel digital endpoints (in this case digital mobility outcomes) can be granted regulatory approval, the responsiveness of such outcomes to successful interventions (pharmacological or nonpharmacological) needs to be established before regulatory approval is granted [80].

From the lab to the real world and towards regulatory approval

Translating digital endpoints from laboratory research to real-world applications involves rigorous testing and validation to meet regulatory standards, for example, adherence to the ALCOA+ (attributable, legible, contemporaneous, original, accurate, complete, consistent, enduring, available) data principles [81] and good software practice for software as medical device (SaMD) set by the FDA [82] and EMA [83]. This process includes extensive clinical trials and real-world evidence generation to demonstrate the biomarkers' measurement properties, accuracy, reliability, clinical relevance and importance to patients. Successful cases of digital biomarkers that obtained regulatory approval can inform researchers of how to better plan and utilise development and validation processes of novel digital endpoints. The validation of novel digital biomarkers includes an array of validation processes, which are required starting from the development of relevant digital endpoints to the specific population requirements. Considering the physiologic impairments in COPD that are measurable and targeted by interventions (such as bronchodilator therapy and pulmonary rehabilitation), bio-signals reflecting the function of the cardiorespiratory and peripheral muscle systems are thus viable and relevant potential digital endpoints. The technical validity of such metrics needs to be extensively validated providing that the hardware

and software pair measures what it is supposed to measure in agreement with gold standard methods. Furthermore, the clinimetric properties of such outcomes needs to be proven by 1) assessing their construct validity against known constructs based on the respective diseases; 2) assessing their predictive capacity against clinically relevant events such as hospitalisations or exacerbations; 3) establishing a minimum clinically important difference anchored against intervention-induced change or disease-progression-induced change; and 4) establishing the sensitivity of such digital endpoints following intervention is required to be established through controlled evidence as well. Finally, the importance of such outcomes to patients should be established and can be included in the process of developing such outcomes (*i.e.* reverse engineering of biomarkers [84]), going further than just establishing the acceptance of the device.

Besides specific biomarkers captured by wearable devices, diagnostic software for bio-signals, such as NIRS, may prioritise usability and practicality to ensure adoption in clinical practice and real-world settings. Key considerations include ease of use, affordability and seamless integration into existing equipment and workflows, such as those used for CPET. Recent advancements in CPET software have demonstrated the potential for real-time and automated data analysis, improved accessibility and streamlined use in both research and clinical environments [85]. In addition, emerging AI models [86] are trained with the aim of enhancing versatility by adapting to diverse laboratory settings and wearable devices. Future advancements should address variability in data types from different equipment, preprocessing requirements and scalability, while ensuring that AI model features are widely applicable, easy to acquire, and accessible to empower broader applicability across diverse protocols and environments.

Conclusion

In conclusion, advances in technology have been imperative to the growth of exercise physiology across the continuum of human biology, from optimising elite performance to treating individuals with long-term pathological conditions. At the physiological level, imaging techniques, including NIRS, fNIRS, MRI, cardiac bioimpedance and OEP, have deepened understanding of the cerebral, cardiovascular, respiratory and muscle metabolic functions during exercise, enabling the development and evaluation of interventions to promote physiological adaptation. Furthermore, due to the growing innovation in wearable technology, athletes and coaches can now continuously and noninvasively track critical training and performance metrics, providing valuable data to adjust training loads, recovery periods and prevent injuries. Beyond elite sport, the ability to monitor almost any biological variable remotely in real-time, combined with advanced analytical techniques, has the potential to transform monitoring, detection and prediction within health and disease, allowing for more personalised and preventative medicine. In chronic respiratory disease, further clinical trials are required to validate digital biomarkers and establish their use within routine clinical care.

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Spirometry and body plethysmography

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Spirometry and plethysmography are the bastions of the lung function laboratory, but are they still relevant in the modern world? Can long-standing controversies ever be resolved? And can AI ever really replace human interpretation of the tests? <https://bit.ly/ERSM107>

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Spirometry and body plethysmography have a long history and have stood the test of time. However, limitations in the measurement techniques and their clinical relevance mean lung function results must be interpreted in the broader clinical context. Ongoing controversies need to be resolved, particularly regarding the role of race/ethnicity in predicting “normal” lung function. A long track record in the lung function laboratory should not mean that spirometry and body plethysmography are beyond scrutiny: instead, we must continue to evaluate the place of these traditional measurements in the modern world. In this chapter, we revisit the history of spirometry and body plethysmography, the current recommendations for measurement and interpretation, and the likely future of these techniques.

Introduction

Attempts have been made to measure lung volumes for centuries. Early scholars recognised that measuring the volume of air moving in and out of the lung was key to understanding its function. This notion has stood the test of time, as evidenced by our continued reference to measured lung volumes as “lung function”. However, the measurements we now take for granted have a challenging history: the physical principles of measuring lung volume were well known, but it took ingenuity and technological advances to translate these principles into practical methods. As a testament to these pioneering efforts, spirometry and body plethysmography are still considered the gold standards for measuring lung function. Experts from around the world have come together to ensure standardised approaches to measurement and interpretation. However, despite their long history, these techniques attract ongoing controversies around what should be considered “normal” lung volumes, whether they have true clinical relevance for individual patients, and what role they may have in a world full of increasingly complex physiological measurements.

Development of spirometry and body plethysmography: how did we get here?

Spirometry

The birth of modern-day spirometry is mostly attributed to British surgeon John Hutchinson in 1846 [1]. Although he was not the first to measure lung volume or understand its association with lung disease, he both coined the term “spirometer” and used his device to make several familiar observations: that lung volume is related to age, sex and height; that lung volume measurements are highly reproducible within individuals; that accurate measurement required maximal effort; and even that up to three repeat measurements are required to achieve an optimal result [1]. It took many decades, however, for spirometry to be properly applied to the investigation of lung diseases. The classification of obstructive *versus* restrictive ventilatory impairment was introduced by Cournand and Richards [2] in 1941. Tiffeneau and Pinelli [3] were the first to describe what is now known as the FEV₁, and, soon after, Gaensler [4] showed that FEV₁ correlated remarkably well with maximum breathing capacity (what we now refer to as maximum voluntary ventilation). Tiffeneau *et al.* [5] later proposed the ratio of FEV₁ to vital capacity as an index of ventilatory obstruction. The familiar maximal flow–volume curve arose from the work of Fry and Hyatt [6] in the 1950s, and was made possible by sensitive equipment that could measure flow in real time [7].

The endurance of spirometry over the decades can be attributed to both its simplicity and its relevance to human health and disease. Several large, longitudinal cohort studies have shown FEV₁ and FVC to be predictors of mortality and a number of non-pulmonary diseases such as cardiovascular disease and cancer [8–10]. Fletcher and Peto [11] showed how the rate of decline in FEV₁ over time is heterogeneous and is influenced by exposure to cigarette smoke. Moreover, serial measurements of spirometry from childhood to older age have increased our understanding of lifetime lung function trajectories, and how the lung function obtained in early adulthood can determine the risk of lung disease decades later [12].

Body plethysmography

Determining total lung volume has long been possible by inert gas dilution techniques, but these only measure the “accessible” lung volume and are therefore of limited value in obstructive lung diseases. As far back as 1882, the German scientist Eduard Pflüger understood that total lung volume could conceivably be measured by Boyle’s Law [13], which states that gas volume is inversely related to pressure at a given temperature. Attempts to exploit this phenomenon using a sealed chamber had been hampered by factors such as changing temperature, humidity, metabolic exchange of gases, and measurement artefact. In 1956, Arthur DuBois managed to overcome many of these challenges by using sensitive equipment to simultaneously measure alveolar pressure (equal to mouth pressure in a closed system) and box pressure during a short compression–decompression manoeuvre [14]. Relating alveolar pressure to air flow also allowed calculation of airway resistance. Hence, modern body plethysmography was born (described beautifully in DuBois’ own words for those who are interested [15]).

The main role of body plethysmography is to quantify FRC, from which total lung capacity (TLC) and its subcomponents can be derived. It complements spirometry by differentiating true restrictive ventilatory impairments (*i.e.* reduced TLC) from other causes of a reduced FVC such as severe obstruction with airway closure. It also helps further characterise obstructive ventilatory impairment by demonstrating hyperinflation and gas trapping. Plethysmographic lung volumes may have clinical relevance: for example, increased RV relative to TLC is one of the earliest manifestations of COPD [16] and is an independent predictor of mortality [17].

Interpretation of lung function test results: state of the art

Lung function interpretation must consider the technical quality as well as the clinical context of the measurements. The most recent update to the European Respiratory Society (ERS)/American Thoracic Society (ATS) technical standard on lung function interpretation emphasises three key aspects, including classification of measurements in accordance with what is expected in a healthy population, identification and classification of a physiological or functional impairment, and integrating the results with clinical data to inform and guide therapy [18].

Normal values

Healthy population based reference equations have been derived to define an expected range of values in different individuals for the various lung function tests. Hundreds of published reference equations exist in different populations, and the Global Lung Function Initiative (GLI) collaboration aimed to standardise the reference equations for spirometry, D_{LCO} and lung volumes [18–21]. These reference equations are internally consistent, but there is no single equation applicable to all populations. Debate around the issues with reference equations is discussed later in this chapter. The recent ERS/ATS standards recommend the use of the GLI reference equations to define the “limits of normal” using the 5th and 95th percentiles (corresponding to a -1.645 and $+1.645$ z-score, respectively) [18], rather than fixed percentage or ratio thresholds.

Interpreting lung function: physiological and clinical context

Spirometry measures volume or flow as a function of time and quantifies the maximal volume of air that an individual can inspire and expire with maximal effort [22, 23]. The main parameters measured include the volume delivered during a forced expiration after a full inspiration (FVC), the expired volume in the first second of an FVC manoeuvre (FEV_1), the ratio of FEV_1 /FVC, and peak expiratory flow (PEF). Abnormalities of the thoracic cage (respiratory muscles or skeletal structures), the lung parenchyma, or upper or lower airways can lead to varying effects on the FEV_1 , FVC and PEF and different patterns of ventilatory abnormality.

Airflow obstruction is manifested by a disproportionate reduction in the FEV_1 relative to the FVC with a subsequent reduction in the FEV_1 /FVC ratio, *i.e.* an obstructive ventilatory impairment [23]. It implies a pathological reduction in the rate of forced airflow from the lungs [18] and is distinct from expiratory airflow limitation (which is a normal physiological phenomenon). FEV_1 /FVC below the lower limit of normal (LLN; *i.e.* 5th percentile) defines an obstructive ventilatory impairment. The GLI reference ranges for FEV_1 /FVC are relatively independent of population differences and the percentile-based method provides a more statistically robust assessment than using a fixed cut-off point (see later in this chapter) [18].

The standard definition of airflow obstruction is based on the maximal forced expiratory manoeuvre, but other measurements from spirometry can indicate obstruction. Inspiratory capacity (IC) measured from spirometry (the difference between end-expiratory lung volume (EELV) and the maximal inspired volume) is an indirect measure of obstruction since it is reduced in the presence of resting hyperinflation, although it may be less sensitive to severe hyperinflation if EELV and TLC increase in parallel. Resting IC represents the mechanical constraints of tidal breathing during exercise, and thus correlates with exertional dyspnoea [24]. Reduced IC is an established predictor of respiratory and all-cause mortality in COPD [25, 26]. Meanwhile, the forced expiratory manoeuvre may lead to airway closure and underestimation of the vital capacity which, in turn, may increase the FEV_1 /FVC ratio making the test less sensitive to obstruction. An alternative is to determine FEV_1 relative to the slow vital capacity (SVC). SVC exceeds FVC by an average of 250 mL in normal lungs and 375 mL in people with

obstruction [27]. It demonstrates bronchodilator responsiveness [28] and is influenced by age and body mass index (BMI) [27, 29]. The ratio of FEV₁ to SVC can reclassify patients with normal spirometry as having airflow obstruction. Reclassified patients have clinical and radiological features similar to those with obstruction defined by reduced FEV₁/FVC ratio [27, 30].

Obstruction in the intra- or extrathoracic central airways may manifest as a reduced PEF but normal FEV₁ and FVC. A key tool for diagnosing these types of obstruction is the maximal flow–volume loop, where specific patterns can further help differentiate between types of obstruction (figure 1) [31]. For example, an inspiratory plateau with relatively normal expiratory curve suggests variable extrathoracic central airway obstruction, while an expiratory plateau with normal inspiratory curve points to variable intrathoracic central airway obstruction. Finally, plateaus in both inspiratory and expiratory curves suggest a fixed central airway obstruction.

A reduced FEV₁/FVC despite normal FEV₁ may be observed in healthy individuals and may represent a normal physiological variant due to unequal growth of the airways and lung parenchyma (“dysanapsis”) [32]. However, new evidence suggests this may indicate a predisposition to future obstructive lung disease [33]. A reduced FEV₁ and/or FVC with a normal FEV₁/FVC has been described as a “nonspecific” pattern and is thought to represent airway

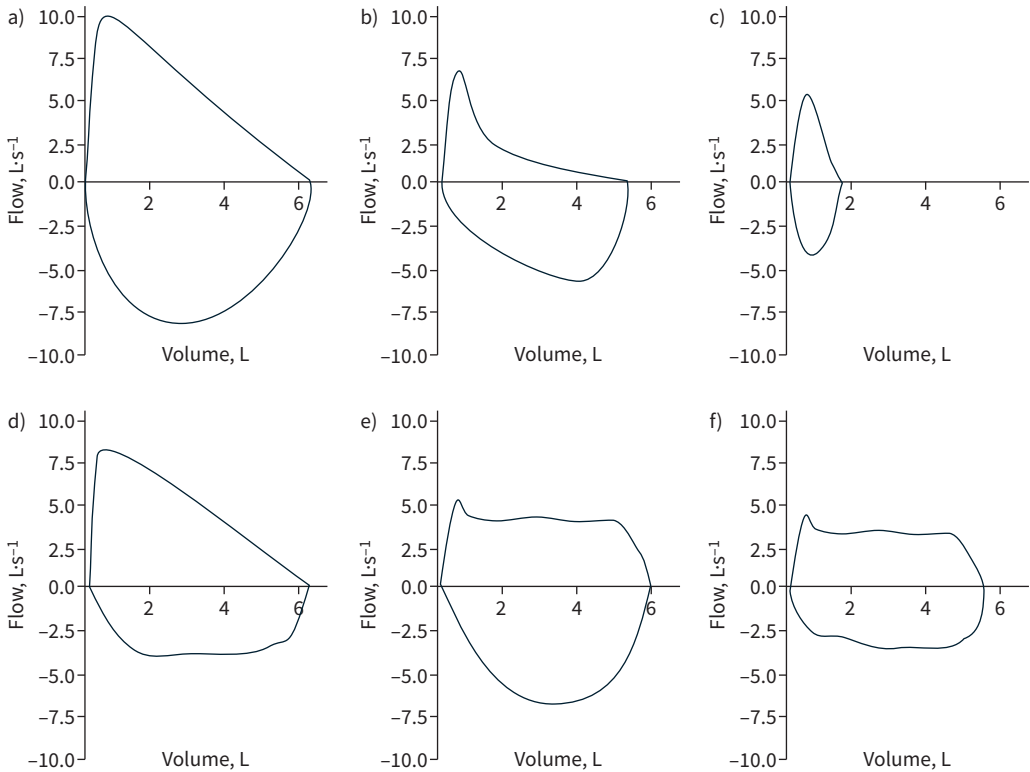


FIGURE 1 Patterns of flow–volume loops to aid diagnosis using spirometry. a) Normal. b) Obstructive ventilatory impairment showing reduced peak expiratory flow and concave expiratory curve. c) Restrictive ventilatory impairment showing reduced vital capacity and convex expiratory curve. d) Variable extrathoracic central airway obstruction showing flattened inspiratory curve but normal expiratory curve. e) Variable intrathoracic central airway obstruction showing flattened expiratory curve but normal inspiratory curve. f) Fixed intra- or extrathoracic central airway obstruction showing flattening of both inspiratory and expiratory curves.

occlusion or collapse [34], but this description is deemed to be an over-simplification (and is now often referred to as preserved ratio impaired spirometry (PRISm)). The nonspecific spirometric pattern may be due to reduced patient effort or a restrictive ventilatory impairment, or may reflect early signs of small airways dysfunction [35]. However, measurement of TLC is required to confirm a restrictive ventilatory impairment.

Spirometry may indicate a restrictive ventilatory impairment if the FVC is reduced, the FEV_1/FVC ratio is normal or elevated, and the flow–volume curve shows a convex shape (figure 1). However, a reduced FVC does not always correlate with true restrictive ventilatory impairment, and lung volume measurements are needed to confirm whether TLC is also below the LLN. Typically, most simple restrictive disease processes demonstrate a proportional reduction in FEV_1 , FVC and TLC. Patterns of more “complex restriction” may show a reduction in FVC that is out of proportion to the reduction in TLC and a disproportionately elevated RV. This complex restrictive ventilatory impairment can be seen in diseases where lung emptying is impaired, such as neuromuscular disease or chest wall restriction, or in obstructive lung diseases characterised by airway closure.

Mixed obstructive and restrictive ventilatory impairment exists when lung function demonstrates both obstructive and restrictive patterns and is defined by a reduction in both the FEV_1/FVC and TLC below the LLN. Mixed impairments are seen in conditions like combined pulmonary fibrosis and emphysema, or in individuals with COPD who develop restrictive abnormalities such as chest wall disorders, respiratory muscle weakness or pleural effusions.

Mis- or over-interpretation of results

Pulmonary function testing is done for a variety of reasons and to answer different clinical or scientific questions, and no single test can answer every such question with complete perfection. Consequently, the interpretation of pulmonary function tests requires incorporation of the relevant clinical or scientific background and expertise, in conjunction with an awareness and reflection regarding the underlying biological variability of the test and its margins of uncertainty.

The relevance of “abnormal” results

Values within the 5th and 95th percentile limits (*i.e.* -1.645 and $+1.645$ z-scores) are considered to be in the range expected from a healthy population measured in a standardised way. This range depicts a compromise between falsely ascribing an “abnormal” label to a healthy person and failing to identify clinically significantly abnormal lung function (*i.e.* balancing sensitivity and specificity). In other words, a value below the 5th percentile or LLN does not necessarily represent a pathophysiological process, nor can it be used in isolation to diagnose an underlying disease. Rather, it merely indicates that the measurement falls outside the range expected for a healthy person of the same age, height and sex. Equally, a single lung function test that falls within the normal range does not exclude an underlying pathology.

There is a strong recommendation against using fixed cut-offs such as 80% of predicted for FEV_1 or 0.70 for the ratio of FEV_1/FVC , as these approaches both dismiss the variance attributable to age [36] and lead to systematic misinterpretation of results, especially for female, paediatric and elderly populations [37]. The use of z-scores for identifying airflow obstruction is advocated by the latest ERS/ATS standard [18], although replacing FEV_1 % predicted with z-scores to categorise the severity of obstruction (mild: -1.65 to -2.5 ; moderate: -2.51 to -4.0 ; severe: < -4.1) has attracted some controversy [38, 39], particularly when the FEV_1/FVC ratio is $<LLN$ but FEV_1 is > -1.65 z-scores: in this situation, it can be difficult to distinguish between

normal physiology (large FVC, or large lung volume relative to airway volume, *i.e.* dysanapsis) and mild but clinically relevant obstruction. This classification may continue to evolve with future iterations of the standards, but in the meantime it highlights the critical importance of the clinical context in interpreting lung function. Importantly, any staging of severity should be grounded in clinically relevant outcomes (ideally, outcomes other than just mortality).

Obesity can complicate the interpretation of lung function since expiratory reserve volume and FRC can both be reduced in obese patients [40], although TLC is generally not reduced until BMI exceeds $40 \text{ kg}\cdot\text{m}^{-2}$ [40]. The measured lung volumes may be outside the expected normal range, but typical patterns of obstructive and restrictive ventilatory impairments may differ in this context, and both types of impairment may occur in morbid obesity [41]. It is up to the clinician to decide whether reduced lung volumes in an obese patient are relevant in the greater context of the patient's presentation.

Change over time

The change in lung function between tests may be just as clinically relevant as a one-off result. For example, consider a change over time from the 90th percentile to the 10th percentile: the latter result is still within the limits of normal, yet clearly constitutes a clinically significant change [18]. Assessment of pulmonary function over time requires specific knowledge of the respective short- and long-term biological variabilities as well as limits of reproducibility of a respective lung function parameter. While it would be ideal to be able to evaluate temporal changes in lung function measures based on a person's baseline level of pulmonary function prior to having developed a disease [18], this is commonly not possible in clinical practice. Regardless of baseline pulmonary function, however, accelerated decline in lung function is associated with poor clinical outcomes [42, 43] and serial measurements in an individual over time (*e.g.* at every doctor visit, similar to blood pressure measurements) may help overcome some of the limitations of reference equations.

Quality control

As described in the ERS/ATS standards, spirometry and body plethysmography both require careful calibration of the equipment and standardisation in how they are conducted. Both tests require a great deal of cooperation from the subject, making them unsuitable for some patient populations, including young children. Robust quality control is necessary to ensure validity and reproducibility. Note that current guidelines do not recommend the validation of reference equations in individual lung function laboratories with small sample sizes of healthy people [18], nor the use of biological controls as part of quality control procedures in pulmonary function laboratories, which currently lacks scientific evidence [44].

Spirometry

The 2019 update of the ERS/ATS standardisation of spirometry document [22] states that test quality should be the first step in interpreting spirometry. When acceptability criteria are met, repeatability should be demonstrated in at least two measurement efforts. Note that FEV₁ and FVC should be rated separately. Based on these characteristics, quality grading from A to F should be applied (where A represents the highest level of quality and F means no acceptable spirometry efforts have been achieved). The 2019 spirometry standards online supplement provides some standardised operator statements that can help report on test quality and the level of confidence in the results. A range of patient- or measurement-derived errors can contribute to poor quality [45].

Plethysmography

As for spirometry, the ability of an individual to follow instructions is an essential requirement for lung volume acquisition by body plethysmography and many patients find aspects of the test difficult. For FRC to be measured accurately, a stable EELV during relaxed tidal breathing must be established. This aspect can be challenging for some patients with severe obstructive lung disease. Plethysmographic lung volumes are less repeatable than those of spirometry. Of note, since TLC and RV are calculated from FRC and linked spirometry, measurement errors from each of these parameters may compound, resulting in a larger variability than that for FEV₁ and FVC alone. Other potential sources of error for body plethysmography are loss of lip-seal during closed-shutter panting, incorrect size or frequency of efforts against the shutter (recommended range 0.5–1 Hz), cheek distortion, the deep inspiratory vital capacity manoeuvre not closely linked to the closed-shutter panting, or incorrect assignments of closed-shutter loops or resting EELV [44].

Current ERS/ATS standards: how and why have they changed?

The 2022 ERS/ATS interpretation standard [18] was significantly changed from the 2005 document [46]. Importantly, its purpose had evolved from a clinical document aiding diagnosis to a technical standard focused on measurement quality. There were several important changes, which we will discuss in the following sections.

Reference equations

The 2022 document changed its recommendation to use the GLI reference equations, and noted that more emphasis needs to be placed on determining ancestral origin and the impact this may have.

Bronchodilator response

There is a significant departure from the 2005 document by changing the recommendation for determining “bronchodilator response” to >10% of predicted value in FEV₁ or FVC, compared to the former $\geq 12\%$ and 200 mL increase in FEV₁ and/or FVC. The former recommendation had very little supporting evidence, and was limited by the fact that absolute and relative changes in FEV₁ and FVC are inversely proportional to baseline lung function, and biased by height, age and sex, in both health and disease. Ultimately, the most clinically relevant method is the most appropriate method; given the fact that bronchodilator response does not differentiate between types of airways diseases, exactly what is most relevant is still unclear.

Minimal clinically important difference

Data are limited on the minimal clinically important difference (MCID) for spirometry and body plethysmography. The MCID is likely to be different for each of the measured variables (FEV₁, FVC, TLC, etc.) and for different diseases. The 2022 document had two recommendations: in adults, the FEV₁ quotient (FEV₁Q), which relates FEV₁ to a minimum level of lung function that is compatible with life [47]; and in paediatric patients, a new concept, the change score, which adjusts for longitudinal changes in FEV₁ z-score and conditions on the initial FEV₁ value. Again, much evidence needs to be generated to support the proposed approaches.

Severity of lung function impairment

Severity of lung function impairment was simplified from five categories to three and based on a z-score approach as opposed to percentage of predicted. Again, it is stated that there is limited evidence to support the use of severity scoring as there is no evidence for relating the scale to clinical outcome. It is likely that severity scales will be disease specific, adding to the complexity.

Reference equations and geographical ancestry

Historically, reference equations were based on obtaining a sample of a population that could be described as “normal”, for which then a reference equation (or equations) was developed. This meant there were literally hundreds of reference equations from which to choose. It was not until the pioneering work of the GLI, formed from a joint task force of the ERS and ATS, that reference equations were developed from multiple datasets combined [21]. At this time, it was clear that in order to develop a reference equation, the minimum number of subjects required was much larger than originally thought. Therefore, previous recommendations of testing a small sample and developing your laboratory-specific equations were debunked. For spirometry, GLI reference equations were developed for various populations from around the world, including European ancestry, African-American and Asian, to name a few. However, what determines an individual’s lung volume is highly complex [48]; height, age, sex and geographic ancestry still only explain a portion of the variance in FEV₁. Other known determinants such as epigenetics, environment, structural racism and nutrition are not captured by these equations.

In 2023, the ATS published a research statement calling for the discontinuation of race/ethnicity-based interpretation of pulmonary function tests [49]. This was in response to the influence of structural racism on pulmonary function, and the fact that there is no real evidence that the lungs of people of different ancestries behave differently from each other. Indeed, the association between race/ethnicity and lung function is most likely the combined effects of social and environmental determinants of lung function; these are not easily measured let alone fully understood, and race/ethnicity is merely a convenient surrogate for these factors.

It was therefore recommended that the GLI “global” equations be used as opposed to equations specific to geographic ancestry. GLI global is effectively a reference equation generated by all the various GLI datasets combined, irrespective of geographic ancestry. Much discussion in the literature followed, especially around valid concerns that having such an approach reduces the fidelity of the equations especially regarding classification of disease. For example, using GLI global may reduce the likelihood of airflow obstruction for various populations, which might deny medications to people who may benefit from them. The idea of applying medical treatments based on race/ethnicity is clearly unacceptable.

It is clear that using race/ethnicity does reduce variation in the normative equations, which means that, in its absence, the question arises as to what else should be used to ensure the utility of reference equations. Understanding, measuring and incorporating all the determinants of “normal” lung function may ultimately be too difficult a task. We are therefore left with how to address this issue so that it is equitable for all. One possibility is to reduce our dependence on reference equations for guiding clinical care. For example, rather than asking how abnormal the spirometry is, perhaps simply ruling in or out obstruction based on the FEV₁/FVC should be the focus. This would be particularly advantageous since current evidence suggests that reference values for FEV₁/FVC are very similar across geographical ancestral groups. Additionally, FEV₁Q has also been determined to be independent of geographical ancestry [50] and could be an approach to track values over time. Finally, tracking an individual’s lung function against their own measures is likely to be a more appropriate approach, rather than comparing measured values to reference equations.

Ultimately, what matters is how a clinician interprets the results belonging to the patient sitting in front of them. Since the issue of ancestral determinants of lung function is unlikely to be resolved any time soon, we suggest clinicians take an approach that examines the results from different perspectives (table 1).

TABLE 1 A suggested approach to applying race/ethnicity-specific and race/ethnicity-agnostic reference equations**Most patients**

Use GLI global reference equation

Interpret the FEV₁/FVC ratio according to the current ERS/ATS interpretation strategy [18]Define a grey zone around the lower limit of normal, likely to be 1 z-score centring around -1.64 z-scores for FEV₁ and FVCIf the FEV₁ or FVC are outside of the grey zone then interpret the FEV₁/FVC ratio according to the current ERS/ATS interpretation strategy [18]If the FEV₁ or FVC are inside the grey zone, then cautionary statements are madeUse FEV₁Q for determining clinically important deterioration**Patients in whom ancestry is clear**

Race/ethnicity-specific GLI equations may be used with relative confidence

Interpret the lung function test in line with the current ERS/ATS interpretation strategy [18]

GLI: Global Lung Function Initiative; ERS: European Respiratory Society; ATS: American Thoracic Society; FEV₁Q: FEV₁ quotient.**The future of spirometry and body plethysmography****Artificial intelligence in pulmonary function testing**

In 2019, TOPALOVIC *et al.* [51] demonstrated what many of us had long suspected: artificial intelligence (AI; specifically, machine learning) could outperform respiratory physicians in the accurate interpretation of pulmonary function tests. The fact that a computer would be better than a human in a highly algorithmic task with very clear parameters should come as no surprise. In fact, most modern pulmonary function systems include some degree of automated interpretation from artificial but unintelligent computer software. However, the nervousness about this finding suggests a common misconception: AI is coming for our jobs.

Instead, it is more likely that AI will complement, if not enhance, the work of respiratory physicians. For example, DAS *et al.* [52] showed that respiratory physicians being assisted by AI improved their diagnostic accuracy by 8–10%. Interestingly, the diagnostic accuracy of some of the physicians in this study appeared to be hampered by the AI interpretations. This highlights the fact that clinical experience may not be completely superseded by AI. A large-scale trial is currently underway investigating the role of AI in spirometry interpretation and decision support for primary care physicians in the UK.

AI may be used to facilitate large-scale analytics such as mining health records for lung function data [53] or lung function quality control programmes [54, 55]. Cost efficiencies might be found using AI: for example, using spirometry to predict lung volumes and thus obviating the need for the more expensive plethysmography [56]. AI may be deployed to assist diagnosis using lung function performed in remote settings [57]. An exciting possibility is the use of AI avatars to assist patients in performing lung function tests, much in the way that this technology has been implemented to help guide medical care in chronic diseases [58]. Indeed, avatar-based virtual reality is capable of changing a subject's breathing pattern [59], and therefore might be used to help train or optimise patient performance during lung function testing.

Where do we go from here?

Despite being almost 180 years since Hutchinson described his spirometer, it was somewhat surprising to see the lack of evidence for many aspects of the measurement and interpretation of spirometry and lung volumes when it came to writing the updated ERS/ATS technical standards. Unfortunately, it has put us in a false sense of security of what these measurements actually

mean and the clinical utility for the patient that is sitting in front of their healthcare provider. The interpretation of bronchodilator response and the issues around reference equations described in this chapter are two examples of where significant research and rethinking of our approach is needed. The use of absolute change in FEV₁ in pharmaceutical trials, which is significantly biased by age, height, sex and geographical ancestry, is another example that requires our attention. Finally, compared with our colleagues in other disciplines, we in respiratory medicine have real difficulties in making the measurements and their interpretation accessible to the wider community. Compare lung function tests to the ECG, which has been routine in primary care for much of its 120-year history. Although these shortcomings could be seen as a threat, we see this as a very real positive opportunity for research.

Moving forward, we propose that spirometry and lung volumes need a reset or refresh, or even a rebrand. Modern measurement techniques such as ultrasonic flow sensors have a level of accuracy and precision that is far beyond that of even 20 years ago. Technical standards have difficulty in keeping up with these changes; however, there is a real need to question what level of precision we need in our measurements for clinical purposes. For example, performing multiple manoeuvres in spirometry improves accuracy and reduces noise, but does this really change the final measurement (and therefore interpretation) significantly? The same can be said for indirectly measured lung volumes: the gold standard body plethysmograph is big and costly, but if detecting a restrictive ventilatory impairment is the goal, could a normal FVC and a normal single-breath dilution be all that is required for clinical purposes? Even further, in a person without airflow obstruction on spirometry, a single-breath measure of alveolar volume is potentially equivalent to that of a body plethysmograph for measuring restriction [60]. Addressing some of these issues would reduce the time and cost of doing these tests and make the tests more accessible to primary care. Finally, instead of presenting a long list of numbers that are not intuitive to clinicians outside respiratory medicine, we need to anchor our tests to clinical outcomes. With AI at our fingertips, lung function tests could be presented as a likelihood of a condition, or the physiological answer to symptoms/traits, as opposed to numbers and acronyms.

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Oscillometry: theoretical bases

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Oscillometry is a simple noninvasive way to assess respiratory mechanics during spontaneous breathing. The technique's theoretical basis provides clues about the best way to optimise the measurements and interpret the results correctly. <https://bit.ly/ERSM107>

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This chapter focuses on the theoretical basis of oscillometry, specifically the methodology for data acquisition, data processing, and the modelling used to interpret the results. First, we explain the difficulty of noninvasively measuring respiratory mechanics during spontaneous breathing and describe the principles of oscillometry, which solves this problem by applying small amplitude, high-frequency oscillations through a mouthpiece. Secondly, technical issues related to implementing these principles in oscillometry devices are discussed: specifically, the generation of oscillations, the device requirements for minimally disturbing the patient's breathing, and the data processing to compute respiratory impedance. Next, the theoretical aspects and limitations of the mathematical models used to interpret oscillometry data are discussed. Among them are the ability of models to selectively capture pathophysiological mechanisms, the sensitivity of model parameters, and the potential effect of the extrathoracic upper airway wall compliance in routine oscillometry. Finally, a simple interpretation of oscillometry data based on both theoretical and experimental evidence is proposed for clinical application.

Introduction

Oscillometry, also known as the forced oscillation technique, is a methodological approach for measuring and interpreting the mechanical properties of the respiratory system over a wide range of frequencies (f). Its practical applications extend from pathophysiology research to clinical assessment of patient status.

This technique was first developed in the 1950s [1] and has grown in popularity over the subsequent decades, reflecting its noninvasive effort-independent nature and relative simplicity from the patient's perspective. With the availability of recently issued updates on technical recommendations [2] and clinical applications [3], this extensive history has led to a wealth of theoretical and practical literature, particularly in review format [4, 5]. This chapter focuses on the theoretical basis of oscillometry, specifically the methodology for data acquisition and the modelling for interpreting the results.

Measurement of respiratory mechanics during spontaneous breathing

Noninvasively measuring respiratory mechanics while the patient is spontaneously breathing is not straightforward. Figure 1a shows a simplified diagram of the respiratory system illustrating that breathing, *i.e.* circulation of airflow ($\dot{V}$) through the airway and the associated change in lung volume (ΔV), is caused by the change in force (F) exerted by the contraction/relaxation of respiratory muscles. The mechanical energy produced by these muscles is required to overcome the forces opposed to breathing, mainly the resistance of the airways owing to air viscosity and the elastic forces needed to inflate the lungs and chest wall. As shown in figure 1b, these basic mechanical properties can be schematically represented by a dashpot with resistive coefficient η and a spring with elastic coefficient k , respectively. Hence, the force (F) applied by the respiratory muscles to result in flow $\dot{V}$ and volume change ΔV is:

$$F = \eta \cdot \dot{V} + k \cdot \Delta V \quad (1)$$

The mechanical diagram in figure 1b can be alternatively represented by an electrical-like circuit as in figure 1c. In this analogy, the mechanical variables pressure, flow and volume are equivalent to voltage, electrical current and charge. The variable F is therefore replaced by a pressure (P); a resistor with resistance R_{rs} (respiratory system resistance) replaces the dashpot,

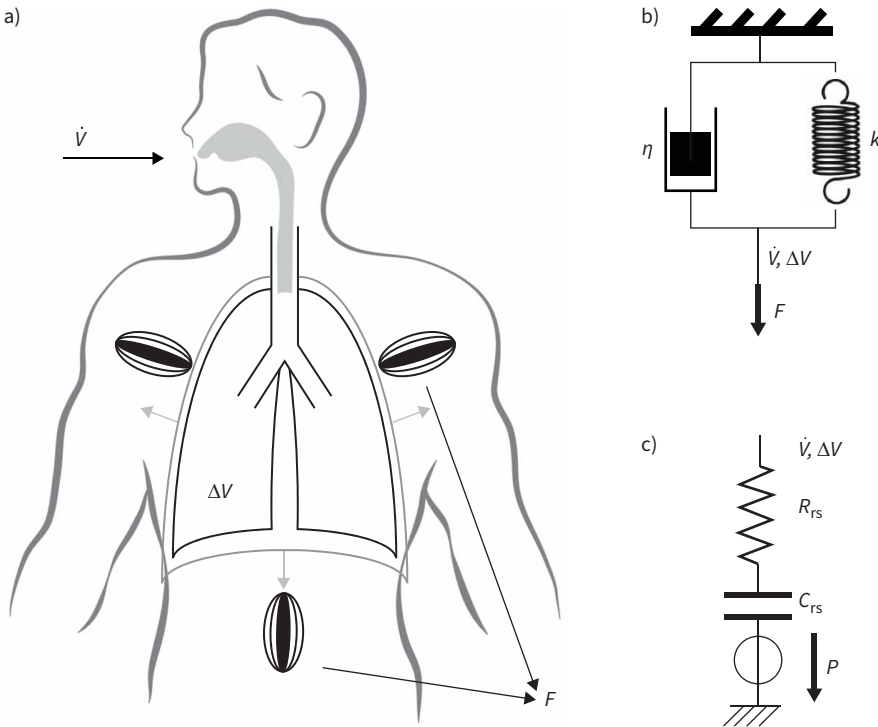


FIGURE 1 Measurement of respiratory mechanics in spontaneously breathing patients. a) Diagram of the respiratory system, where $\dot{V}$ is breathing flow, ΔV is lung volume change, and F is the force exerted by respiratory muscles. b) Mechanical model of the diagram in a: a dashpot with constant η and a spring with constant k represent the resistive and elastic properties of the respiratory system. c) Electrical representation of the mechanical model in b: P is the pressure applied across the system, and R_{rs} and C_{rs} are the respiratory system resistance and compliance, respectively.

and the spring is replaced by a compliance, C_{rs} (respiratory system compliance). Of note, when moving from the mechanical to the electrical model, the type of connection of the individual elements changes from parallel to series. Then, the model equation is similar:

$$P = R_{rs} \cdot \dot{V} + \Delta V / C_{rs} \quad (2)$$

Double mechanical/electrical representation, as in figure 1b and c, is possible because the corresponding driving equations are identical. The fact that electrical-like models are employed when studying respiratory mechanics might be surprising. Still, it can be explained historically because the conceptual tools of frequency analysis in oscillometry come from the engineering field of electrical circuits.

The resistive and elastic parameters of the respiratory system cannot be noninvasively determined from signals recorded during spontaneous breathing (equations 1 and 2). Indeed, whereas $\dot{V}$ (and thus derived $\Delta V = \int \dot{V}$) can be measured by a pneumotachograph at the airway opening (mouthpiece, mask or endotracheal tube), the force F or pressure P exerted by the respiratory muscles cannot be measured. To skip this difficulty, an alternative procedure would be to derive the resistance (R_L) and compliance (C_L) of the lungs only (instead of the total respiratory system) by estimating oesophageal pressure (P_{oes}) by an oesophageal balloon or a catheter-tip pressure sensor ($P_{oes} = R_L \cdot \dot{V} + \Delta V / C_L$). This way, linear fitting of P_{oes} , $\dot{V}$ and ΔV signals would determine R_L and C_L . However, despite being of paramount importance for development of respiratory physiology, this is an invasive procedure that is hardly used in clinical routine.

Overcoming the previously mentioned limitations to assess respiratory mechanics noninvasively during normal spontaneous breathing is possible by using oscillometry.

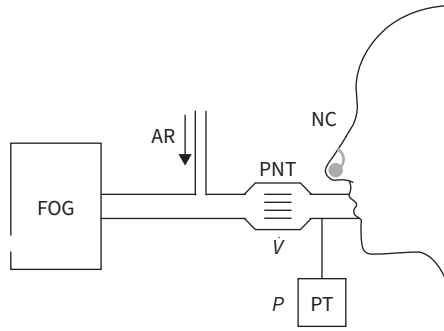
Principle of oscillometry

Oscillometry is based on the fact that a linear, stationary system's response to a sinusoidal input is an output that is also sinusoidal with the same frequency. Consequently, the system can be analysed independently for each frequency component (f_{os}) of its input and output signals. When oscillometry is applied to characterise its mechanical properties, the respiratory system is explored in the frequency domain using oscillatory pressure ($P_{os}(f_{os})$) and flow ($\dot{V}_{os}(f_{os})$) signals, each characterised by their amplitude and phase angle. Using common frequency analysis tools, the mechanics of the respiratory system is described by its mechanical impedance, $Z_{rs}(f_{os})$:

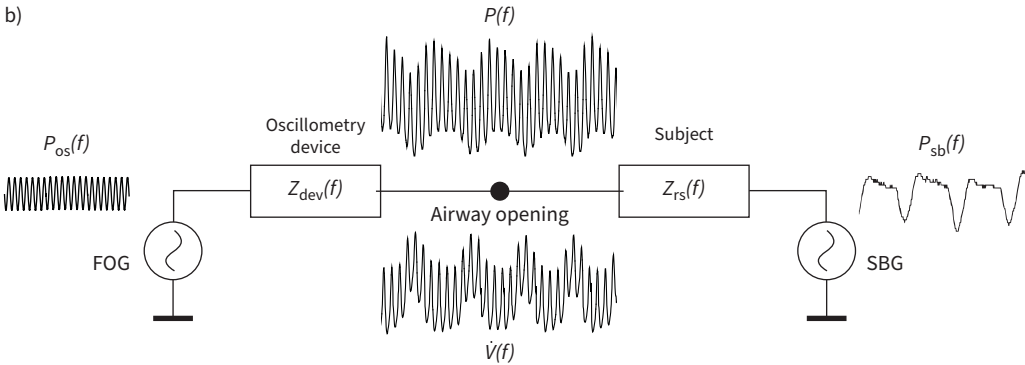
$$Z_{rs}(f_{os}) = P_{os}(f_{os}) / \dot{V}_{os}(f_{os}) \quad (3)$$

In practice, oscillometry is based on applying a small amplitude forced oscillation pressure at the airway opening using a device (figure 2a) that minimally interferes with the patient's spontaneous breathing, *i.e.* has a low mechanical impedance (device impedance; $Z_{dev}(f)$). Figure 2b shows the corresponding diagram, illustrating that two pressure generators (the oscillometry device and the patient's respiratory muscles) operate simultaneously, inducing total flow ($\dot{V}(f)$) and pressure ($P(f)$) at the airway opening, measured at the mouthpiece that connects the oscillometry device and the patient's respiratory system. If the patient's muscles were passive, the pressure $P_{os}(f)$ induced by the oscillometry device generator would be $P_{os}(f) = Z_{rs}(f) \cdot \dot{V}_{os}(f)$, where $\dot{V}_{os}(f)$ is the flow induced by the oscillometry device. If the oscillometry device were not working, the spontaneous breathing flow ($\dot{V}_{sb}(f)$) generated by the respiratory muscles would induce a pressure ($P_{sb}(f) = -Z_{dev}(f) \cdot \dot{V}_{sb}(f)$). According to the principle of superposition in linear and stationary systems, when both pressure generators are acting simultaneously, pressure is $P(f) = P_{sb}(f) + P_{os}(f)$, and

a)



b)



c)

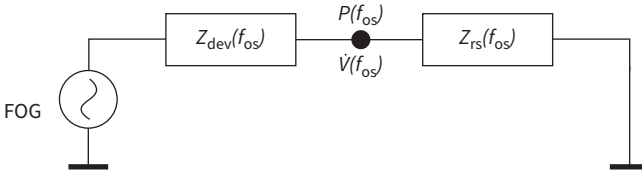


FIGURE 2 Principle of oscillometry. a) The oscillatory pressure from a forced oscillation generator (FOG) is introduced into the patient's respiratory system through a mouthpiece while the patient breathes spontaneously wearing a nasal clip (NC). The FOG presents a low impedance to the patient's breathing, and an air renewal flow (AR) is circulated to avoid rebreathing. A pneumotachograph (PNT) and a pressure transducer (PT) measure flow ($\dot{V}$) and pressure (P) at the entrance of the respiratory system. b) Diagram of the oscillometry setting. The FOG and the spontaneous breathing generator (SBG) work simultaneously on the assembly consisting of the impedances of the oscillometry device ($Z_{dev}(f)$) and of the respiratory system ($Z_{rs}(f)$), connected in series. Pressure ($P(f)$) and flow ($\dot{V}(f)$) are measured at the entrance of the respiratory system (represented by a black dot). $P_{os}(f)$: pressure induced by the oscillometry device; $P_{sb}(f)$: pressure induced by spontaneous breathing. c) Diagram of the oscillometry setting where only the oscillometry signal components (f_{os}) are considered. In this case, the diagram in b) is simplified since for f_{os} the SBG is not active. Thus, $Z_{rs}(f_{os}) = P(f_{os})/\dot{V}(f_{os})$ regardless of whether the patient is breathing spontaneously.

circulating flow is $\dot{V}(f) = \dot{V}_{os}(f) + \dot{V}_{sb}(f)$. Hence, the impedance computed from the measured signals ($Z_m(f)$), where $K(f) = \dot{V}_{sb}(f)/\dot{V}_{os}(f)$, is:

$$Z_m(f) = (P_{os}(f) + P_{sb}(f))/(\dot{V}_{os}(f) + \dot{V}_{sb}(f)) = \quad (4.1)$$

$$= (Z_{rs}(f) \cdot \dot{V}_{os}(f) - Z_{dev}(f) \cdot \dot{V}_{sb}(f))/(\dot{V}_{os}(f) + \dot{V}_{sb}(f)) = \quad (4.2)$$

$$= (Z_{rs}(f) - Z_{dev}(f) \cdot K(f))/(1 + K(f)) \quad (4.3)$$

Since it is not possible to distinguish the contribution from each of the mechanical generators from the signals measured at the airway opening, equation 4.3 indicates that, in general, the measured impedance $Z_m(f)$ would be an unknown combination of $Z_{rs}(f)$ and $Z_{dev}(f)$. Hence, $Z_m(f)$ would approach $Z_{rs}(f)$ as the ratio $K(f)$ is minimised. The fundamental basis of oscillometry is to use an oscillation signal with frequency components f_{os} within a band that exceeds the frequencies generated by the respiratory muscles during spontaneous breathing. Then, as $\dot{V}_{sb}(f_{os})=0$ and thus $K(f_{os})=0$, equation 4.3 results in $Z_m(f_{os})=Z_{rs}(f_{os})$. Therefore, by applying pressure oscillations at frequencies higher than those generated by the respiratory muscles, oscillometry allows the exact measurement of respiratory system mechanics at f_{os} even though the patient spontaneously breathes. To fulfil this requirement, the forcing frequencies should be higher than 4–5 Hz for spontaneously breathing adults [2]. When the respiratory rate increases, such as in children or adults during tachypnoea, higher frequencies should be used, typically ≥ 8 Hz, while for newborns the lowest frequencies should be ≥ 10 Hz. It is important to underline that the relevant characteristic of the breathing pattern to consider when selecting the oscillatory frequency is the frequency content of the spontaneous flow and pressure signals, rather than the respiratory rate *per se*. For example, a severely obstructed patient may have a long respiratory time but a highly unbalanced duty cycle, with a very short inspiratory time, associated with high-frequency contents of the pressure and flow signals, and a prolonged expiratory time, required to accommodate the reduced maximum expiratory flow. In such conditions, the fast inspiratory waveform may conflict with the use of a 5 Hz oscillatory forcing signal, and higher forcing frequencies should be considered. Frequency content overlap may be revealed by unexpected shapes of the $Z_m(f)$ spectra at the lowest frequencies. In the remainder of this chapter, f_{os} will be noted simply as f , assuming that frequency will always refer to the frequencies of the oscillatory forcing signal.

$Z_{rs}(f)$ describes the system through the amplitude ratio and phase lag between pressure and flow and as such is a complex magnitude: $Z_{rs}(f)=R_{rs}(f)+j\cdot X_{rs}(f)$, where $j=(-1)^{1/2}$. Its real part, respiratory resistance ($R_{rs}(f)$), represents the component of oscillatory pressure in phase with flow, and its imaginary part, respiratory reactance ($X_{rs}(f)$), represents the component of pressure out of phase with flow.

Figure 3 schematically depicts the typical pattern of respiratory impedance in a healthy adult for a frequency band commonly explored in oscillometry. $R_{rs}(f)$ does not depend on frequency, and $X_{rs}(f)$ has negative values at the lowest frequencies, crosses zero at the resonance frequency (f_R), of ~6–10 Hz, and increases linearly at the higher frequencies. $Z_{rs}(f)$ can be interpreted by a resistance (R)–inertance (I)–elastance (E) model, where R represents the airflow viscous resistance, I mainly accounts for the inertial properties of the gas in the airways and E (the reciprocal of compliance: $E=1/C$) corresponds to the elastance of the respiratory tissues and the compressible gas within the lung ($X_{rs}(f)=2\cdot\pi\cdot f\cdot I-E/(2\cdot\pi\cdot f)$). In addition to the $X_{rs}(f)$ values, reactance is usually characterised by f_R and by the area under the reactance curve (AX) from the lowest frequency to f_R (figure 3a). Figure 3b illustrates the pattern of $R_{rs}(f)$ and $X_{rs}(f)$ typically found in patients with several respiratory diseases, *e.g.* COPD, asthma and acute respiratory distress syndrome. $R_{rs}(f)$ exhibits a negative frequency dependence (ΔR_{rs}) and there is a shift of $X_{rs}(f)$ to more negative values than in healthy subjects, with increased f_R and AX (figure 3b).

Application of oscillometry

This section briefly comments on some theoretical issues regarding the oscillometry measurements. There are different technical solutions to implement practically in the measurement setting (figure 2a) and in processing the recorded signals P and $\dot{V}$ used to

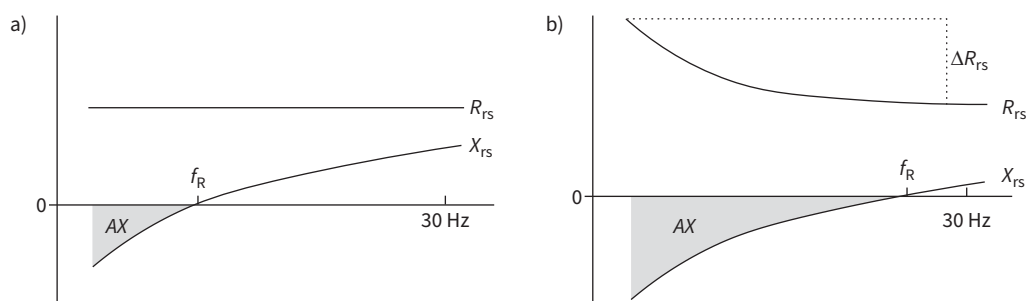


FIGURE 3 Frequency dependence of respiratory system resistance (R_{rs}) and reactance (X_{rs}) in a) a typical healthy subject and b) a patient. In patients, R_{rs} is characterised by a negative frequency dependence (ΔR_{rs}), and X_{rs} exhibits higher resonance frequency (f_R) and area under the reactance curve (AX), compared with healthy subjects.

compute $Z_{rs}(f)$. Except for some general recommendations [6], recently updated [2], there is no well-defined standardisation, and consequently, the practical solutions adopted by different investigators and companies commercialising oscillometry devices are different. As methodological details are not fully disclosed, from the technical viewpoint most currently employed devices appear to be black boxes. Using different implementations (*e.g.* regarding oscillation generation, sensors and data processing) may lead to results that do not agree with each other. Indeed, there were clear differences in performance when several devices were tested on the bench under well-controlled conditions, for instance, when measuring the impedance of passive and active analogues of the respiratory system [7, 8]. Hence, paying attention to these potential sources of equipment variability and quality control is particularly important in oscillometry. The technical standards published by the European Respiratory Society Task Force [2, 9] provide the basis for harmonising the results of devices compliant with such standards.

Oscillation generation and signal measurement

The core of an oscillometry device is the electromechanical component that generates the oscillation pressure to measure the patient's impedance. Ideally, the device should be able to generate the same $P_{os}(f)$ regardless of the patient's impedance, either directly or using electronic control. For the most common setting (figure 2a), a woofer loudspeaker is a simple and quite effective option, normally not requiring active control, since these electromechanical devices are, by construction, force (and therefore pressure-like) generators. The moving part of the loudspeaker should have a sufficiently high effective surface so that the oscillatory displacement required to generate $P_{os}(f)$ keeps it within its normal working position even when the lowest patients' load is tested (*i.e.* when low patient load requires high flows to produce the desired oscillatory pressure), a requirement particularly relevant at the lowest frequencies. It is important to note that selecting the loudspeaker characteristics is not independent of the circuit design used to generate air renewal in the oscillometry device and minimise the device dead space (figure 2a). Indeed, in addition to presenting low impedance at the breathing frequencies to reduce the mechanical load to patient breathing, this circuit should present a high impedance at oscillatory frequencies. Otherwise, compensating for an excessive oscillatory flow shunted to room air would stress the loudspeaker. Hence, there are constraints to reducing the size and power of the loudspeaker, which limits the extent to which the size of a loudspeaker-based oscillometry device can be reduced. However, a recent alternative has proposed using a servo-controlled blower for oscillation generation [10], which is suitable for hand-held oscillometry devices. It should be mentioned that in applications where oscillometry is applied

when the patient is treated with ventilatory support (*e.g.* continuous positive airway pressure (CPAP) or mechanical ventilation), a loudspeaker-based forced oscillation generator is subjected to more demanding conditions. Indeed, to withstand the therapeutic positive pressure applied at the airway opening, and thus to keep its moving element within normal working position, requires including purely mechanical [11, 12] or servo-controlled [13] components in the setting. Alternatively, a servo-controlled valve in a blower-based device can simultaneously generate the treatment pressure and $P_{os}(f)$ [13, 14]. A more elegant and simpler solution is that the mechanical ventilator itself is designed to optionally add a $P_{os}(f)$ on the ventilation pressure it applies to the patient by appropriately controlling either the blower rotation in blower-based ventilators or the inspiratory/expiratory valves in proportional valves-based ventilators [14–17].

Different types of $P_{os}(f)$ signals can be used in oscillometry. Monofrequency (*i.e.* sinusoidal) was the type originally employed [1] and is the simplest (figure 4a and d). Sinusoidal forcing is optimal from the signal-to-noise ratio viewpoint but provides R_{rs} and X_{rs} at one single frequency; thus, exploring the respiratory system for a wide frequency band would require successive measurements at different frequencies. However, monofrequency oscillometry (*e.g.* at 5–10 Hz) could be most suitable for some applications, for instance when R_{rs} and X_{rs} are employed to monitor the time course of respiratory mechanics along the breathing cycle to detect either airway obstruction in sleep apnoea studies [18, 19] or expiratory flow limitation (EFL) in COPD patients [20–22]. Moreover, there is increasing evidence that Z_{rs} at 5 Hz is sufficient for assessing airway obstruction in several clinical applications [23, 24]. For more general applications, using a composed oscillation signal containing several frequencies of interest is preferable. Different types of multifrequency signals can be used in oscillometry. One of them, random noise, includes a continuous and uniform distribution of energy over the frequency band of interest. This signal was employed in a seminal paper in oscillometry methodology [25] and subsequent works, but it is hardly employed currently. Periodic multifrequency signals are particularly well suited and hence mostly used. They are the superposition of the different sinusoidal components of interest (figure 4b and e). Notably, the amplitude of each frequency component can be independently set for optimising the signal-to-noise ratio: first, to compensate for the transfer function of the pressure generation setting, *i.e.* to adapt the voltage signal driving the loudspeaker for achieving the desired amplitude composition of $P_{os}(f)$ at the airway opening; secondly, to enhance the lowest frequency oscillatory components to minimise the potential effect of the highest breathing components (*i.e.* to ensure that $K(f_{os})$ in equation 4.3 is negligible). The phase angle distribution among the different sinusoidal components can be optimised to reduce the peak-to-peak of the signal for a given amount of oscillation energy, thus reducing the risk of potential nonlinearities in the respiratory system [26]. A particular case of periodic multifrequency signal employed in oscillometry is a succession of impulses (figure 4c and f). This signal has a lower (fundamental) frequency, which is the inverse of the period of impulse repetition, and components at the harmonics of the fundamental frequency (with amplitude distribution depending on the impulse duration). This type of signal has a suboptimal ratio between peak-to-peak and energy. Hence, to have the same signal-to-noise ratio in a signal as in figure 4b, the impulse amplitude should be much higher, risking potential nonlinear effects. It is interesting to note that by using a signal composed of a combination of sinusoids with frequencies that are not sums and not differences from each other, it is possible to avoid frequency crosstalk (and thus artefacts) induced by potential effects of respiratory system nonlinearities [27].

A pressure sensor and a flowmeter are used to measure pressure (P) and flow ($\dot{V}$) at the airway opening (figure 2a). Contrary to spirometry, where flowmeters based on different technologies are also used (*e.g.* based on turbine, ultrasound or thermal/heat transfer), the measurement of $\dot{V}$ in oscillometry is commonly carried out by a differential pressure transducer sensing the

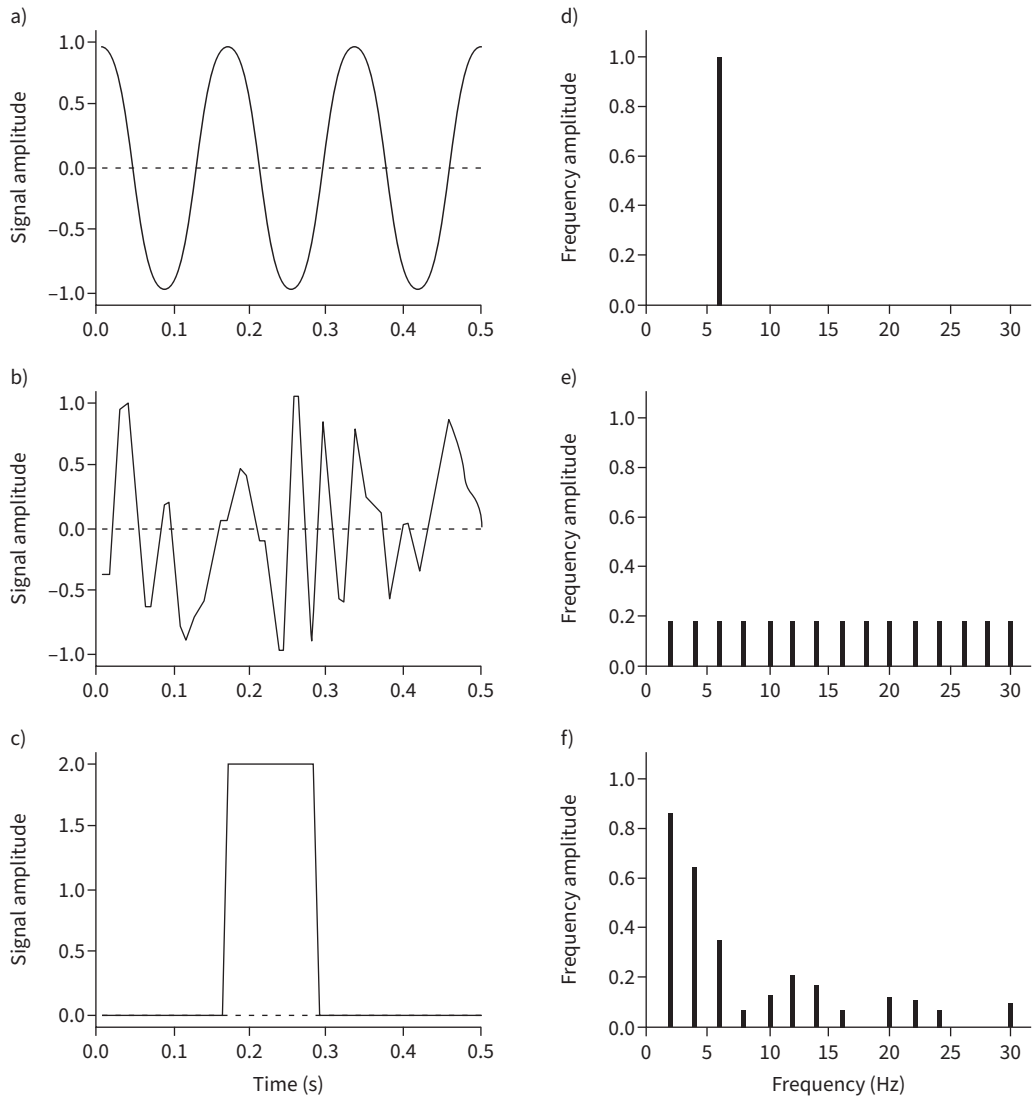


FIGURE 4 Periodic excitation signals used in oscillometry (the 0.5 s data sections shown are cyclically repeated). a) Sinusoidal (6 Hz). b) Multifrequency with equal amplitudes at 2, 4, 6 Hz, etc. (up to 30 Hz; randomly selected phase angles). c) Periodic positive impulses (e.g. 120 ms duration). d–f) Amplitudes of the frequency components of a–c), respectively. Note that the impulse in c) has double the amplitude of signals in a) and b).

pressure drop caused by flow through a pneumotachograph. As with any differential transducer, its common-mode rejection ratio (including all its connecting tubes) should be sufficiently high (>60 dB) for all the oscillatory frequencies [28, 29]. Moreover, both pressure transducers must be insensitive to vibrations potentially transmitted by mechanical coupling from the forced oscillation device. Otherwise, artefactual signals would be recorded, thus corrupting the impedance measured. The mechanical impedance of a mesh-screen pneumotachograph (Lilly type) is a pure resistance (R_{pnt}). Capillary-based pneumotachographs (Fleisch type) are a resistance (R_{pnt})–inertance (I_{pnt}) system. As real transducers have no ideal frequency response

(flat amplitude and zero phase lag), corrections should be applied as required. The pressure signal ($P_m(f)$) provided by the transducer when measuring $P(f)$ is $P_m(f) = H_p(f) \cdot P(f)$, where $H_p(f)$ is this transducer's frequency response. The signal measured $\dot{V}_m(f)$ when measuring flow $\dot{V}(f)$ is $\dot{V}_m(f) = Z_{pnt}(f) \cdot H_v(f) \cdot \dot{V}(f)$, where $H_v(f)$ is the frequency response of the pressure transducer at the pneumotachograph. Hence, the impedance ($Z_m(f)$) computed from the measured signals $P_m(f)$ and $\dot{V}_m(f)$ is:

$$Z_m(f) = P_m(f) / \dot{V}_m(f) = H_p(f) \cdot P(f) / (Z_{pnt}(f) \cdot H_v(f) \cdot \dot{V}(f)) = Z_{rs}(f) \cdot H_p(f) / (Z_{pnt}(f) \cdot H_v(f)) \quad (5)$$

Therefore, correction for the dynamic response of the sensing elements is carried out as:

$$Z_{rs}(f) = Z_m(f) \cdot (Z_{pnt}(f) \cdot H_v(f) / H_p(f)) \quad (6)$$

Only if using an ideal screen pneumotachograph and the frequency responses of both pressure sensors (including their connecting tubes to the measurement site) were exactly the same, would the correction factor relating $Z_{rs}(f)$ and $Z_m(f)$ in equation 6 be 1. However, this correction factor cannot be neglected, depending on the specific equipment employed, especially as frequency increases.

Signal processing

In most oscillometry applications it is assumed that $Z_{rs}(f)$ remains constant along the breathing cycle, a hypothesis quite reasonable in healthy subjects and confirmed by oscillometry data. Indeed, although compliance and resistance vary with lung volume, deviation from linearity is small in healthy humans spontaneously breathing around FRC. Moreover, superimposing a small oscillation (<1 hPa) reasonably keeps a healthy respiratory system under almost linear conditions. However, this can be no longer valid, particularly in obstructed patients, when lung volume is increased above FRC during mechanical ventilation or CPAP therapy [30], or during increased ventilation, tachypnoea or exercise. Despite depending on volume or flow, $R_{rs}(f)$ and $X_{rs}(f)$ may vary along the spontaneous breathing cycle, for instance in patients with COPD or sleep apnoea, given the complexity of their pathology (see the later section of this chapter "Within-breath variations of Z_{rs} ").

Data processing in the time and frequency domains is a discipline applied to many fields, with a wide variety of algorithms, each best suited for different characteristics of the system to be studied. In particular, the choice of algorithm depends on whether the system is considered stationary or time-dependent. In the first case, signal filtering and frequency analysis are selected to process all the signals in a data recording. If the system is expected to be time-dependent, the algorithms analyse the signals over a time window with a duration defined according to the system's rate of change, as illustrated by the simplified example in figure 5. Figure 5a shows a section of the flow ($\dot{V}$) signal recorded at the pneumotachograph (figure 2a) that would correspond to an oscillometry measurement with a 5-Hz sinusoidal oscillation in a simulated patient whose impedance was simply a resistance of $5 \text{ hPa} \cdot \text{s} \cdot \text{L}^{-1}$. The pressure signal (P_{os} ; not shown in the figure) is a 5-Hz pressure with amplitude $\pm 1 \text{ hPa}$. Figure 5b shows the corresponding oscillatory flow $\dot{V}_{os}$ obtained after adequately filtering $\dot{V}$ to eliminate its spontaneous breathing component. Figure 5c shows the time course of R_{rs} computed by analysing P_{os} and $\dot{V}_{os}$ using a moving time window. The graphs in figure 5d–f show the same data, but when the resistance of the simulated subject is flow-dependent according to $R_{rs} = 5 \text{ hPa} \cdot \text{s} \cdot \text{L}^{-1} + 30 \text{ hPa} \cdot \text{s}^2 \cdot \text{L}^{-2} \cdot |\dot{V}|$. Contrary to the case where R_{rs} is constant, $\dot{V}$ and $\dot{V}_{os}$ show that the amplitude of the 5-Hz oscillometry component varies inversely with $|\dot{V}|$. Accordingly,

the computed impedance (figure 5f) shows that R_{rs} changes along the breathing cycle, with differences between inspiration and expiration. This graph also shows the value of R_{rs} ($6.75 \text{ hPa}\cdot\text{s}\cdot\text{L}^{-1}$) that would be obtained if the signal processing was carried out under the hypothesis that respiratory impedance was constant along the breathing cycle. Data processing providing the time course of Z_{rs} seems advisable, since it may help detect changes along the breathing cycle that are relevant to clinical applications (see the later section of this chapter “Within-breath variations of Z_{rs} ”).

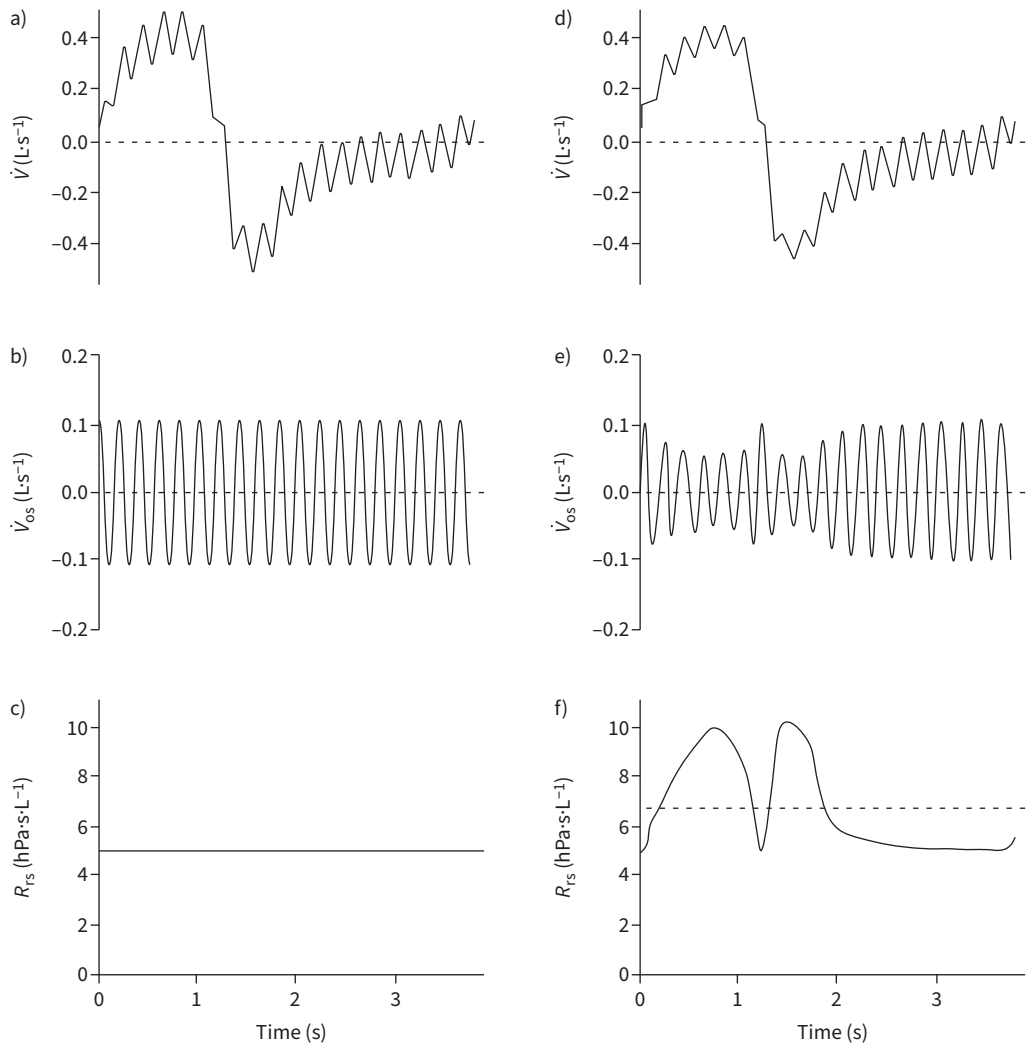


FIGURE 5 Within-breath variation of respiratory mechanics. a) Flow ($\dot{V}$; positive for inspiration) measured along a breathing cycle, showing the superposition of the 5-Hz oscillatory and spontaneous breathing components, corresponding to the oscillometric measurement of a constant resistance. b) Oscillatory flow ($\dot{V}_{os}$) derived by digital filtering of $\dot{V}$. c) Instantaneous respiratory system resistance (R_{rs}) computed from $\dot{V}_{os}$ and the corresponding oscillatory pressure (P_{os} ; not shown). d–f) Same as in a–c), respectively, when measuring a flow-dependent resistance. The dashed line in f) is the mean value of R_{rs} along the whole breathing cycle.

Interpretation of oscillometry data

Different applications of oscillometry, both in pathophysiology research and clinical studies, have shown that, in general, patients' $R_{rs}(f)$ and $X_{rs}(f)$ differ from the typical pattern in healthy subjects (figure 3a), depending on disease type and severity (figure 3b). Given that $Z_{rs}(f)$ is the transfer function characterising the respiratory system mechanics, it is theoretically possible to determine the parameters of a mechanistic model with pathophysiological interest that fits the measured $Z_{rs}(f)$ data. For instance, such a model may feature peripheral airway resistance, airway wall elastance, tissue viscoelasticity, or lung non-homogeneities. However, care should be taken when using a model for oscillometry data interpretation.

Model and parameter identification or inverse modelling

An essential difficulty in trying to derive pathophysiological interpretations from model fitting to $Z_{rs}(f)$ data is that completely different models from a mechanistic viewpoint can equally fit a given experimental data set. This is particularly relevant when interpreting the typical altered pattern of $R_{rs}(f)$ and $X_{rs}(f)$ shown in figure 3b.

One of these models, also known as the Mead model or serial inhomogeneity model (figure 6a), assumes that airway resistance is split into a central resistance (R_c) and an increased peripheral resistance (R_p), and that increased R_p enhances the shunting role of the intrathoracic airway wall elastance (E_{sh}). This interpretation based on increased R_p is conceptually appealing for analysing $Z_{rs}(f)$ data in most patients with obstructive diseases such as asthma or COPD. A second model (figure 6b), typically known as the Otis model or parallel inhomogeneity model, assumes that

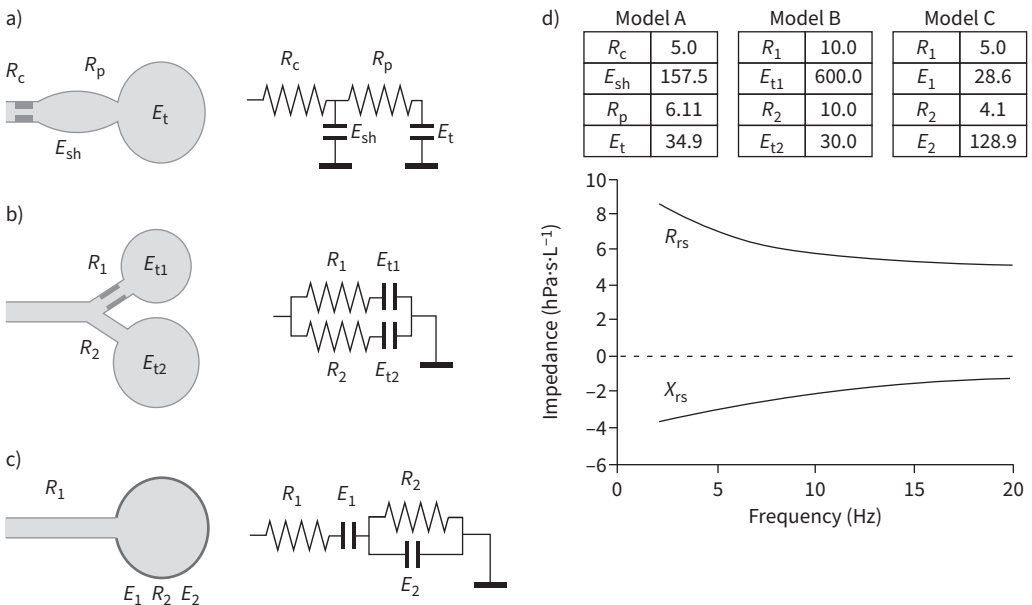


FIGURE 6 Respiratory models for interpreting oscillometry data. a) Serial inhomogeneous model (model A) including central (R_c) and peripheral (R_p) resistances and airway wall (E_{sh}) and tissue (E_t) elastances. b) Parallel inhomogeneous model (model B) including the airway resistances (R_1 , R_2) and tissue elastances (E_{t1} , E_{t2}) of two parallel compartments. c) Homogeneous model (model C) including airway resistance (R_1) and a viscoelastic tissue compartment characterised by parameters R_2 , E_1 and E_2 (Kelvin body). d) Example of parameters in models A, B and C that have the same plotted respiratory system resistance (R_{rs}) and reactance (X_{rs}). Resistances are in hPa·s·L⁻¹ and elastances in hPa·L⁻¹, respectively.

the patient's respiratory system consists of two parallel components characterised by different degrees of resistance and tissue elastance (R_1 and R_2 , and E_{t1} and E_{t2} , respectively). The most obvious and simple example would be that one lung remains healthy (R_1 , E_{t1}), and the other one is diseased (*e.g.* unilateral pneumonia) with increased resistance (R_2) and elastance (E_{t2}). Figure 6c shows a third model that is homogeneous but with respiratory tissues presenting viscoelastic properties, contrary to the two previous models featuring tissues as a pure elastance (E_t , E_{t1} or E_{t2}). In addition to a simple airway resistance (R_1), the model has a viscoelastic tissue element, known as a generalised Kelvin body of first-order or linear standard solid, where the elastances (E_1 and E_2) represent a stress proportional to strain and the resistance (R_2) corresponds to a stress proportional to the time derivative of strain.

It would be very useful for interpreting oscillometry data if each of the three mechanistic models in figure 6 had its particular $R_{rs}(f)$ and $X_{rs}(f)$ frequency dependence pattern. In that way, assessing whether one of the models better fits the specific oscillometry patient data would provide clues on the pathophysiological mechanism determining the respiratory mechanics of that patient. However, the theoretical analysis of the three models in figure 6 shows that, although each has its particular structure and parameters, they are described by the same canonical equation [31]. Indeed, for the three models, the differential equation describing respiratory mechanics in terms of driving pressure $P(t)$, flow $\dot{V}(t)$ and volume $V(t)$, and their time derivatives $\dot{P}(t)$ and $\ddot{V}(t)$, is of the general form:

$$P(t) + a \cdot \dot{P}(t) = b \cdot V(t) + c \cdot \dot{V}(t) + d \cdot \ddot{V}(t) \quad (7)$$

Parameters a , b , c and d are determined by the resistances and elastances of each of the models: the serial (R_c , R_p , E_{sh} , E_t) and parallel (R_1 , R_2 , E_{t1} , E_{t2}) inhomogeneous models and the viscoelastic (R_1 , R_2 , E_1 , E_2) model [31]. When translating equation 7 from the time domain into the frequency domain, it results that the three models have the same $Z_{rs}(f)$ function defined by parameters a , b , c and d :

$$R_{rs}(f) = ((c - a \cdot b) + a \cdot d \cdot (2\pi f)^2) / (1 + a^2 \cdot (2\pi f)^2) \quad (8.1)$$

$$X_{rs}(f) = ((d - a \cdot c) \cdot 2\pi f - b / 2\pi f) / (1 + a^2 \cdot (2\pi f)^2) \quad (8.2)$$

For example, figure 6d shows parameter sets for the three mechanistic models exhibiting identical $R_{rs}(f)$ and $X_{rs}(f)$. The fact that the three models are equivalent means that if equations 8.1 and 8.2 can fit the $Z_{rs}(f)$ data of a patient, it is impossible to distinguish whether the observed $R_{rs}(f)$ and $X_{rs}(f)$ are caused by an increase in peripheral resistance or parallel inhomogeneity or alteration of tissue viscoelasticity. It is noteworthy that more complex oscillatory measurements could help distinguish the mechanism involved. For instance, the patient could be asked to breathe gas mixtures with different viscosities (air, He–O₂, SF₆–O₂). However, such potential measurements are beyond typical routine oscillometry.

One of the conceptual advantages of oscillometry is that exploring the respiratory system for a range of frequencies far beyond the typical breathing rates and using parametric models to fit the data may allow us to better understand the different mechanisms determining respiratory mechanics in a patient. However, a requirement for such an aim is that $R_{rs}(f)$ and $X_{rs}(f)$ data provide sufficient information for reliably determining the parameters of interest. Specifically, the frequency band explored should be suitable for detecting the mechanism, *i.e.* optimal parameter sensitivity. This requirement is easily illustrated by the simplest case of the R – I – E model used to characterise $Z_{rs}(f)$ in healthy subjects. The question is to what extent the frequency band of the measurement may determine the reliability of the derived R , I and E parameters.

A straightforward way of assessing the sensitivity (S) of a given model parameter is to compute the partial derivative of the model function with respect to the parameter. Hence, for the R – I – E model:

$$S_R = |\partial R_{rs}(f)/\partial R| = |\partial R/\partial R| = 1 \quad (9.1)$$

$$S_I = |\partial X_{rs}(f)/\partial I| = 2\pi \cdot f \quad (9.2)$$

$$S_E = |\partial X_{rs}(f)/\partial E| = 1/(2\pi \cdot f) \quad (9.3)$$

This shows that the sensitivity of R does not depend on f , the sensitivity of I linearly increases with f , and that of E inversely decreases with f . In other words, equations 9.1–9.3 indicate that there is no preferred frequency band for measuring R and that to more accurately determine I and E , the optimal frequencies are the highest and the lowest, respectively. Of note, when fitting data on models with more than one parameter, calculating confidence intervals for each parameter independently may underestimate the confidence resulting from joint estimates of all parameters simultaneously. In this case, appropriate mathematical approaches should be used [32].

Another useful approach for assessing parameter sensitivity is Monte Carlo analysis, as illustrated in figure 7. A population of 50 healthy patients was simulated by adding zero-mean random noise ($SD=0.30 \text{ hPa}\cdot\text{s}\cdot\text{L}^{-1}$) to a typical example of $R_{rs}(f)$ and $X_{rs}(f)$ ($R=3 \text{ hPa}\cdot\text{s}\cdot\text{L}^{-1}$, $I=0.01 \text{ hPa}\cdot\text{s}^2\cdot\text{L}^{-1}$, $E=30 \text{ hPa}\cdot\text{L}^{-1}$) between 2 and 20 Hz, as shown in the example of figure 7. Then, R , I and E for each simulated patient were estimated from $R_{rs}(f)$ and $X_{rs}(f)$ using two different frequency bands' data: 2–10 Hz and 12–20 Hz. Figure 7 shows that R was estimated with the same reliability regardless of computing it from the low- or high-frequency band, as indicated by the coefficient of variation within the 50 simulated identical patients. By contrast,

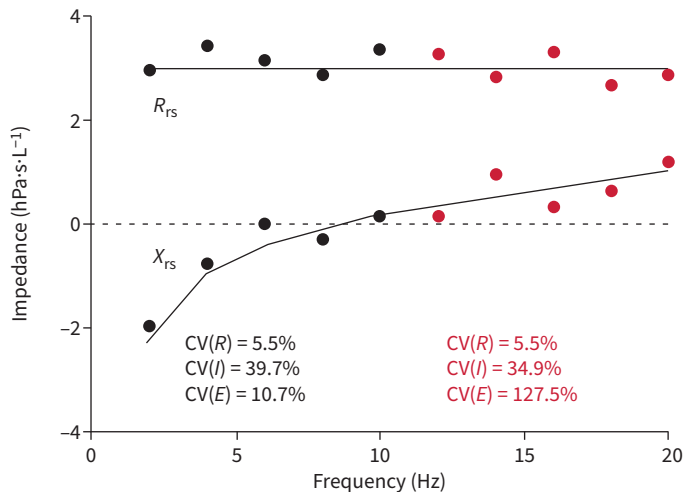


FIGURE 7 Parameter sensitivity for the simple resistance (R)–inertance (I)–elastance (E) model representing the impedance (respiratory system resistance (R_{rs}) and reactance (X_{rs}); solid lines) of a healthy subject ($R=3 \text{ hPa}\cdot\text{s}\cdot\text{L}^{-1}$, $I=0.01 \text{ hPa}\cdot\text{s}^2\cdot\text{L}^{-1}$, $E=30 \text{ hPa}\cdot\text{L}^{-1}$). Dots are an example of the data of one of the 50 simulated patients with added random noise in a Monte Carlo analysis. The model fitting was performed independently for the lowest (2–10 Hz; black) and highest (12–20 Hz; red) frequency bands. The coefficients of variation (CV) correspond to the parameter fitting from the 50 simulated patients.

and in agreement with the previous S analysis (equations 9.1–9.3), the estimation of E was by far much more reliable when derived from the low-frequency data (10.7% coefficient of variation) than from the highest frequencies (127.5% coefficient of variation). Although the differences were minor among frequency bands, the contrary was found regarding I estimation.

The kind of simple analyses illustrated in figure 7 for the case of the R – I – E model should be carried out when using more complex models. Parameter sensitivity analysis should ideally be carried out before designing the measurement to determine the optimal frequency band to be explored for more reliably assessing the target parameters (*i.e.* the investigated mechanism). However, in many practical cases, $R_{rs}(f)$ and $X_{rs}(f)$ are already available for a given frequency band (*e.g.* 4–30 Hz in routine oscillometry). In this case, *a posteriori* parameter sensitivity analysis should be carried out to determine the expected reliability of the parameters derived from the available data [32].

Effect of the extrathoracic upper airway shunt

The effect of the nonrigid walls of the extrathoracic upper airways (including the cheeks) has to be considered when interpreting the R_{rs} and X_{rs} measured by the routine setting based on applying the forced oscillation pressure through a mouthpiece (figure 2a). Indeed, part of the oscillatory flow measured at the airway opening is shunted by the extrathoracic upper airway walls and does not enter the patient's lung. As shown in figure 8a, the impedance of the extrathoracic upper

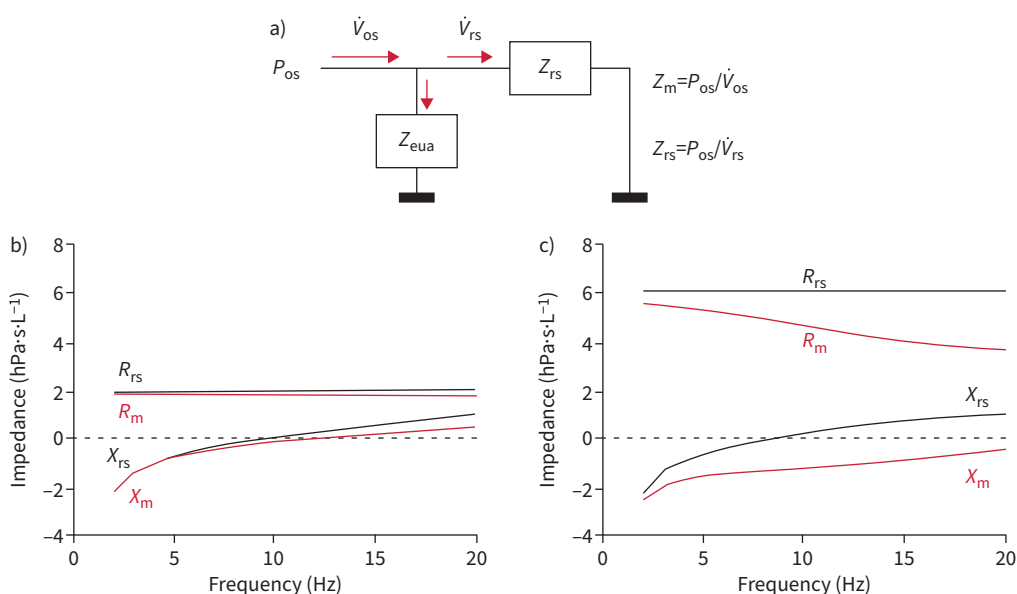


FIGURE 8 Effect of the extrathoracic upper airways in respiratory impedance. a) Diagram showing that the impedance of the extrathoracic upper airways (Z_{eua}) is placed in parallel with the respiratory system (lung and chest wall) impedance (Z_{rs}). Part of the oscillatory flow ($\dot{V}_{os}$) measured by the pneumotachograph (figure 2a) is shunted by the compliance of the extrathoracic upper airways. $\dot{V}_{rs}$ is the flow entering the lungs and P_{os} is the oscillatory pressure. Thus, measured impedance (Z_m) is different from respiratory impedance (Z_{rs}). b) Respiratory system resistance (R_{rs}) and reactance (X_{rs}) in an example of a healthy subject ($R=2 \text{ hPa} \cdot \text{s} \cdot \text{L}^{-1}$, $I=0.01 \text{ hPa} \cdot \text{s}^2 \cdot \text{L}^{-1}$, $E=30 \text{ hPa} \cdot \text{L}^{-1}$) (black lines). Data in red show the measured impedance (R_m and X_m) when considering the effect of typical extrathoracic upper airways ($R=7 \text{ hPa} \cdot \text{s} \cdot \text{L}^{-1}$, $I=0.025 \text{ hPa} \cdot \text{s}^2 \cdot \text{L}^{-1}$, $E=870 \text{ hPa} \cdot \text{L}^{-1}$). c) Same as in b) for the case of a patient with increased resistance ($R=6 \text{ hPa} \cdot \text{s} \cdot \text{L}^{-1}$, $I=0.01 \text{ hPa} \cdot \text{s}^2 \cdot \text{L}^{-1}$, $E=30 \text{ hPa} \cdot \text{L}^{-1}$).

airways ($Z_{\text{eua}}(f)$) is placed in parallel with the impedance of the lung and chest wall (i.e. $Z_{\text{rs}}(f)$). Accordingly, the impedance measured ($Z_{\text{m}}(f)$) from the recorded signals is:

$$Z_{\text{m}}(f) = Z_{\text{rs}}(f) \cdot Z_{\text{eua}}(f) / (Z_{\text{rs}}(f) + Z_{\text{eua}}(f)) = Z_{\text{rs}}(f) / (1 + Z_{\text{rs}}(f) / Z_{\text{eua}}(f)) \quad (10)$$

In general, this measurement artefact ($Z_{\text{m}}(f) \neq Z_{\text{rs}}(f)$) is more critical as $Z_{\text{rs}}(f)$ increases. It is mild in healthy subjects, as shown in figure 8b by assuming a typical value of $Z_{\text{eua}}(f)$ modelled as an $R-I-E$ compartment with $R=7 \text{ hPa}\cdot\text{s}\cdot\text{L}^{-1}$, $I=0.025 \text{ hPa}\cdot\text{s}^2\cdot\text{L}^{-1}$, $E=870 \text{ hPa}\cdot\text{L}^{-1}$ [33]. By contrast, in patients (commonly with increased $Z_{\text{rs}}(f)$) the effect of the extrathoracic upper airways can be considerable. Indeed, figure 8c illustrates the case of a patient simply exhibiting a general increased obstruction, thus showing no frequency dependence of actual $R_{\text{rs}}(f)$. Remarkably, measured $R_{\text{m}}(f)$ would exhibit a considerably negative frequency dependence, and there would be a clear reduction in $X_{\text{m}}(f)$, which could be erroneously attributed to increased peripheral airways (e.g. using the model in figure 6a). Simulations in figure 8b and c assume that the oscillatory measurement is carried out with unsupported patient cheeks, which is not the conventional procedure. However, detailed studies show that even when the cheeks are supported, the shunt effect of the extrathoracic airways is still remarkable in patients [34, 35]. Hence, using models that do not account for the shunt of the extrathoracic upper airways to interpret oscillometry data measured with the conventional setting may result in misinterpretations.

It is possible to eliminate the effects of $Z_{\text{eua}}(f)$ on $R_{\text{rs}}(f)$ and $X_{\text{rs}}(f)$ measured with the conventional setting in those applications in which the aim is to assess within-patient changes in respiratory mechanics (e.g. within the breathing cycle, after bronchoprovocation/dilatation tests or monitoring along disease evolution). Indeed, assuming that $Z_{\text{eua}}(f)$ remains constant for a given patient, measuring the change ($\Delta Y_{\text{m}}(f)$) in measured admittance ($Y_{\text{m}}(f)=1/Z_{\text{m}}(f)$) provides data freed from the extrathoracic upper airway artefact [36, 37]. According to the circuit in figure 8a, $1/Z_{\text{m}}(f)=1/Z_{\text{rs}}(f)+1/Z_{\text{eua}}(f)$. For two measurements (1 and 2) in the same patient:

$$\begin{aligned} \Delta Y_{\text{m}}(f) &= Y_{\text{m}2}(f) - Y_{\text{m}1}(f) = 1/Z_{\text{rs}2}(f) + 1/Z_{\text{eua}2}(f) - (1/Z_{\text{rs}1}(f) + 1/Z_{\text{eua}1}(f)) \\ &= 1/Z_{\text{rs}2}(f) - 1/Z_{\text{rs}1}(f) = Y_{\text{rs}2}(f) - Y_{\text{rs}1}(f) = \Delta Y_{\text{rs}}(f) \end{aligned} \quad (11)$$

Thus, the absolute change $\Delta Y_{\text{m}}(f)$ is equal to $\Delta Y_{\text{rs}}(f)$ under the reasonable hypothesis that the extrathoracic upper airways impedance remains unchanged ($Z_{\text{eua}2}(f)=Z_{\text{eua}1}(f)$). However, it should be noted that the values $Y_{\text{m}2}(f)$ and $Y_{\text{m}1}(f)$ remain with the measurement artefact.

Interestingly, the artefact ($Z_{\text{m}}(f) \neq Z_{\text{rs}}(f)$) induced by the extrathoracic upper airways can be virtually avoided by using a different measurement setting already proposed 40 years ago [38]. It is known as the “head generator” and consists of applying the forced oscillation using a transparent box surrounding the head of the patient, adjusted to the patient’s neck, with no need to support the patient’s cheeks during the measurement. In this way, virtually the same pressure is applied at the internal and external sides of the extrathoracic upper airway walls (the only pressure difference would be the small pressure drop across a low impedance pneumotachograph ($Z_{\text{pnt}}(f)$)). Then the impedance measured with the head generator ($Z_{\text{m,HG}}(f)$) [38] is:

$$Z_{\text{m,HG}}(f) = Z_{\text{rs}}(f) \cdot (1 + Z_{\text{pnt}}(f)/Z_{\text{eua}}(f)) \quad (12)$$

Thus, $Z_{\text{m,HG}}(f)$ is virtually equal to $Z_{\text{rs}}(f)$ since $Z_{\text{pnt}}(f)$ (typically $0.1\text{--}0.2 \text{ hPa}\cdot\text{s}\cdot\text{L}^{-1}$) is two orders of magnitude smaller than $Z_{\text{eua}}(f)$. Although detailed studies in healthy subjects and

patients validated the suitability of the head generator device for providing $Z_{rs}(f)$ data freed from the extrathoracic upper airway artefact, this oscillometry setting has not been subsequently applied, probably because it is not as simple as the conventional approach.

The fact that the conventional oscillometry setting results in $R_{rs}(f)$ and $X_{rs}(f)$ values that include the extrathoracic upper airways, and not just the impedance of the lung and chest wall, may reduce the sensitivity of the technique to detect impedance changes within a patient and makes model interpretation of the data more complex. However, this does not at all reduce the high value of the measured data both for pathophysiological research and clinical practice.

An alternative approach for interpreting Z_{rs} by mathematical models: the morphometric models or in silico respiratory mechanics models and forward modelling

Mathematical models for interpreting oscillometry data by fitting experimental data on model equations have provided a useful tool for exploring the clinical significance of Z_{rs} , but have also showed limitations. The main limiting factor is related to the complexity of the respiratory system's behaviour in disease settings. Most respiratory conditions have an impact on the lung with heterogeneous/patchy patterns, unbalancing the system, and making simple models with few parameters unsuitable to describe a system with thousands of different time constants. In these situations, the mathematical models able to describe oscillometry data would require too many parameters to allow their identification from data fitting. In this case, the model parameters are said to be “not observable”, *i.e.* it is impossible to univocally determine the condition of the entire system starting from the system's outputs [39]. To overcome these limitations, an alternative approach known as “forward modelling” was applied to oscillometry data, starting from the early 1990s [40]. With this approach, a large and complex morphologically coherent mathematical model is developed by including equations for describing all factors that contribute to the overall mechanical behaviour of the lung. These models comprise equations for describing every single airway by considering both fluid dynamic properties and airway wall mechanics as a function of its geometry as well as the characteristics of acini/alveoli [41]. Each element equation is built by combining the knowledge of lung morphology provided by airway tree physical casts made in animal lungs (see the seminal studies of WEIBEL [42] and HORSFIELD *et al.* [43]) and, more recently, high-resolution CT imaging, with data from experimental studies on the mechanical properties of airway and parenchymal tissues, leading to morphologically and mechanically accurate *in silico* models of the lung [41, 44, 45]. These models are then used to simulate lung response to oscillometry tests for predicting oscillometry results in healthy conditions and during pathological alterations.

Morphometric lung models provided the theoretical foundations for a better understanding of the effects of heterogeneous constriction on lung impedance in asthma [41], for highlighting the clinical significance and sensitivity of X_{rs} to small airways disease [45] and, in general, to support the clinical interpretation of deviations of Z_{rs} from healthy reference values.

Within-breath variations of Z_{rs}

The approaches we have described implicitly assume that the respiratory system is a time-invariant system, *i.e.* that the mechanical properties of the respiratory system do not change during breathing. In reality, during breathing the mechanical properties are time-variant, as they depend on several factors, such as lung volume and flow, and the status of the airway smooth muscles and respiratory muscles, and these sources of time-dependent changes are strongly affected by type and severity of respiratory conditions.

Oscillometry has the unique feature of performing measurements with very high temporal resolution compared to spontaneous breathing, as the measurement rate depends on the frequency of the oscillatory signal rather than the respiratory rate. As the oscillatory frequency is commonly higher than the rate of change in respiratory mechanics during the breathing cycle, it is possible to characterise the system through the time course of the within-breath respiratory impedance. Several mathematical methods can be used but, whichever approach is used, the result is a time course of R_{rs} and X_{rs} that describes within-breath variations of mechanical conditions [20, 46–48].

Although this approach was suggested many years ago, the importance of within-breath or intra-breath oscillometry has only been recognised in recent decades. In particular, it provides several advantages: 1) it can be used to accurately detect and quantify the presence of tidal EFL when large negative swings of X_{rs} appear during the expiratory phase only, as a result of the development of choke points through the airways (figure 9) [20–22, 49]; 2) when tidal EFL or dynamic airway collapse is present, it allows evaluation of the impedance values during the inspiratory phase only, therefore excluding from the evaluation the expiratory artefacts due to choke points and allowing more sensitive and specific testing of lung conditions [50]; 3) it allows the characterisation of flow and volume dependence of Z_{rs} , providing additional information on lung mechanics and phenotypes of airway obstruction [51–53]; and 4) it enables identification and exclusion from the data analysis transient artefacts caused by cough, swallowing, involuntary glottis closure, *etc.*, before averaging results.

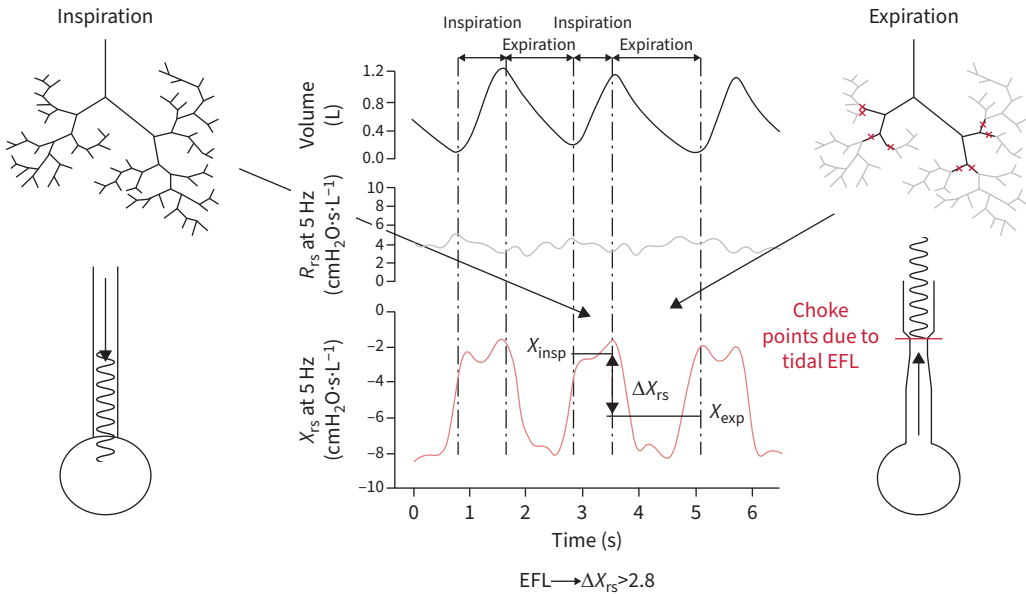


FIGURE 9 Experimental tracing from a representative flow-limited patient and definition of the index used to characterise the within-breath changes of respiratory system reactance (X_{rs}) during a single breath. Respiratory volume and total respiratory input resistance (R_{rs}) and X_{rs} at 5 Hz are reported as a function of time for a few spontaneous breaths. X_{insp} : mean value of X_{rs} during inspiration; X_{exp} : mean value of X_{rs} during expiration. Since reactance was expected to decrease during expiratory flow limitation (EFL), as choke points prevent oscillation from reaching the peripheral lung, the difference between X_{insp} and X_{exp} , named ΔX_{rs} , is used to identify the presence of tidal EFL when its values are greater than a pre-determined threshold of 2.8 $\text{cmH}_2\text{O}\cdot\text{s}\cdot\text{L}^{-1}$. Reproduced and modified from [20] with permission, and with information from [21, 49].

Within-breath oscillometry has also been proposed as a noninvasive tool for tailoring the treatment pressure when combined with CPAP or noninvasive ventilation, specifically for the real-time automatic detection of inspiratory dynamic obstruction in obstructive sleep apnoea syndrome [18, 54] and the automatic adjustment of positive end-expiratory pressure (PEEP) to abolish tidal EFL and minimise intrinsic PEEP [14, 55, 56]. For all these reasons, using within-breath oscillometry should become standard in clinical applications.

Interpretation of Z_{rs} for clinical applications

The use of inverse and forward mathematical models, combined with experimental data from preclinical and clinical studies, allows us to draft a general framework for a first, rough interpretation of the results from an oscillometry test from the patterns of deviation from normal expected reference data. A frequency-independent increase of R_{rs} with normal X_{rs} can be associated with homogeneous or upper or central airway obstruction. A frequency-dependent R_{rs} , with R_{rs} at the lower frequencies higher than at the higher frequencies, suggests heterogeneous airway narrowing. It is important to emphasise that the frequency dependence of R_{rs} (commonly quantified with indices like R_{5-20} (R_{rs} at 5 Hz minus R_{rs} at 20 Hz), equivalent to ΔR_{rs} in figure 3b) is increased when there is heterogeneity of time constants within the lung from any cause. This occurs when peripheral airway obstruction is present but also in the presence of shunt (*e.g.* ineffective or poor cheek support), making this index sensitive but not specific for small airways disease. A negative shift of X_{rs} suggests a loss of communicating lung volume, as occurs in peripheral airway constriction [41, 45], airway closure [57–59], or lung volume de-recruitment [12, 60]. While for most obstructed patients both R_{rs} and X_{rs} are commonly affected, a negative shift of only X_{rs} may be suggestive of changes in only the lung periphery, such as in small airways disease. In case of airway obstruction, a within-breath approach should be used to identify specific phenotypes of patients. For instance, within-breath analysis can recognise patients experiencing tidal EFL [61] or dynamic airway compression, or patients with vocal cord dysfunction [62, 63], and focus on the inspiratory impedance only, to properly characterise lung condition avoiding artefacts. For an extensive analysis of clinical applications of oscillometry, please refer to chapter 7 of this *Monograph* [64].

Conclusion

The theoretical bases of oscillometry provide clues that help us optimise the measurement and interpretation of respiratory system impedance. The equipment employed, including the forced oscillation generator, transducers and data processing, should be adapted to the specific application of oscillometry, which expands from measurements in animal models and pathophysiology studies in humans to clinical applications (analysis of lung function, domiciliary patient follow-up, noninvasive and invasive ventilation, and sleep). The different possible technical implementations of oscillometry devices, currently not fully standardised and generally undisclosed, result in potential variance in results, thus requiring equipment characterisation and quality control. Given the complexity of the diseased respiratory system and some practical measurement constraints, particularly in routine applications (*e.g.* extrathoracic upper airways), the interpretation of oscillometry data in terms of mechanistic models should be made carefully to avoid misinterpretations. Thus, reporting the within-breath cycle time course of raw $R_{rs}(f)$ and $X_{rs}(f)$ data seems to be most useful, since it can allow further reanalyses.

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Oscillometry: clinical significance and applications

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Oscillometry is a practical lung function test, applicable in the clinic or ICU and for patient home monitoring, in paediatric and adult care. The strong clinical associations and technical development mean it should now be in routine use. <https://bit.ly/ERSM107>

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Respiratory oscillometry (or the forced oscillation technique) is a highly practical lung function test that can be applied in a wide range of clinical scenarios in children and adults, including the clinic, intensive care unit, patient home monitoring and emergency departments. Oscillometry measurements complement spirometry in detecting abnormal lung function, measuring effects of treatment such as inhaled corticosteroids or bronchodilators, and changes due to disease activity. Use of oscillometry is informed by a large body of evidence showing strong clinical associations, high reproducibility and high signal-to-noise ratio. Oscillometry's practical implementation is supported by the European Respiratory Society technical standards document and availability of commercial hardware. Oscillometry research continues to accelerate, further supporting its clinical use and developing parameters that will add to its clinical utility. This chapter summarises the current state of the art of the clinical application of oscillometry, which demonstrates why it is ready for routine clinical use.

Introduction

The utility and usefulness of respiratory oscillometry has increased greatly, in both clinical practice and research in airways diseases. Indeed, oscillometry is increasingly familiar to clinicians and researchers as a lung function measurement that has strong correlations with clinically important outcomes and is responsive to drug treatments. As a measurement that reflects the mechanics of eupnoeic breathing, it complements our gold standard parameter of spirometry in alerting the clinician to abnormal airway function.

Oscillometry is performed by superimposing an oscillatory pressure signal into a spirometry circuit, while measuring the pressure and flow signals with high temporal resolution. It is

usually done during quiet, resting breathing and so it is a bonus and advantage that it is easy for the patient to complete. This allows an assessment of lung function in those unable to perform spirometry and ensures that patients are happy for repeat measurements, which contrasts to how patients feel about performing repetitive maximal spirometry.

We argue strongly that clinical practice and research in obstructive airways diseases must have more than spirometry, for advances in management to occur. The current paradigm, which almost exclusively relies on symptoms, which define asthma control and exacerbations for instance, will impede the development of new drug classes, because symptoms are too nonspecific and heterogeneous in their underlying pathophysiology. For example, symptoms are highly influenced by psychological state, by perception and by abnormal breathing patterns, and not only by bronchoconstriction and mucus hypersecretion. The “treatable traits” or “comprehensive phenotyping” approach makes this very clear: symptoms mean and represent different things in different people, and asthma means different things to different doctors. Like any test or biomarker, abnormal airway function does not have to lead directly to a specific treatment, otherwise we would defer clinical decision making to machines. For example, CT coronary artery calcium scores are a continuous measure of abnormality that informs preventative strategies. When, and not if, oscillometry becomes a routine test in clinical practice and research, it will be accepted as a measurement to better assess potential benefit of novel treatments.

In this narrative review, we highlight the relevant knowledge of oscillometric impedance (*i.e.* impediment to flow for a given driving pressure), which will help clinicians and clinical researchers interpret results in the context of their patients. Although the mathematical basis of respiratory impedance is complex, the conceptual basis is much less so (as described in the next section of this chapter). For example, impedance is entirely consistent with the principles of quantifying the work of breathing and, hence, relevant to severe exacerbations of airflow obstruction and to invasive and noninvasive ventilation. Respiratory system resistance (R_{rs}), reactance (X_{rs}), elastance (E_{rs}) and inertance (I_{rs}) are based on the simple equation of motion and clinically provide a way of conceptualising the components of impedance. These components also provide insight into elevations in impedance, such as a loss of lung volume *versus* the narrowing of conducting airways, which are very different pathological processes. We briefly provide a summary of why impedance parameters are very clinically relevant, given the consistency and strength of correlations with clinical outcomes and responsiveness to treatment. Finally, availability of commercial devices, with well-thought-out software, adequate technical support and published technical standards [1], makes oscillometry measurement practical, and means that oscillometry is now ready for routine clinical use. We therefore describe how oscillometry enhances patient care in adult and paediatric airways disease clinics, in the respiratory function laboratory and in high-dependency and intensive care units.

What is oscillatory impedance?

Simple explanations of the concept of oscillatory impedance

Oscillometry measures the impediment to moving air in and out of the lungs. The total impediment is termed impedance (abbreviated as Z). During resting breathing, which is typically at a frequency of about 12 breaths per min (0.2 Hz) in adults, the respiratory muscles overcome this impediment (impedance or Z) by generating sufficient intrathoracic pressure (work of breathing). Oscillometry is like cyclical breathing, but at a higher frequency, typically measured at ~ 5 cycles $\cdot$ s $^{-1}$ (5 Hz) and higher. Rather than respiratory muscles generating the required pressure swings, an external pressure oscillation is applied at the airway opening to generate oscillatory airflow, from which the respiratory system's mechanics are derived. The reader is referred to chapter 6 of this *Monograph* for an in-depth description of the theoretical basis of impedance measurements [2].

The two components of oscillatory impedance are resistance (R) and reactance (X) of the respiratory system. Most clinicians are very familiar with R , it being a quantification of the impediment to flow of air in and out of the airway tree. X is a parameter that is unfamiliar to most; however, it turns out to be very clinically important given its clinical associations and behaviour. The theoretical bases and derivation were covered in detail in chapter 6 of this *Monograph* [2], where the authors describe resistance as being in phase with flow changes, while reactance is in phase with volume changes. A brief, simplified explanation of X follows.

Explaining the critically important parameter of reactance

The way in which R and X make up total impedance (Z) of the respiratory system can be represented diagrammatically in figure 1. The length of the red line is impedance, which is determined by the resistance (x-axis) and reactance (y-axis), and their relationships are simple (if we remember our school geometry for right-angled triangles). Worsening X is, by convention, directed downwards on the y-axis. Reactance can be thought of as the impediment of expanding the respiratory system during inhalation and is proportional to the volume of lung that is in communication with the airway opening, which has appropriately been referred to as “communicating volume”. Severe airway obstruction due to disease reduces “communicating volume”, making it harder to breathe with the same tidal volumes (greater impediment and worsening X).

How about depicting X in a more intuitive way, by plotting it upwards rather than downwards, which flips the diagram vertically (figure 2)? Using figure 2, we could make the analogy of a cyclist riding up a hill. The cyclist’s legs have to generate pressure to overcome the friction (resistance) of the tyres on the road, but also against gravity going up the hill (reactance). Breathing thus requires respiratory muscles to work to overcome friction (R) and to expand the “communicating volume” (X).

In summary, Z is the total impediment to air movement in and out of the lungs. R is the resistive component due to friction, while X is for clinical purposes the “communicating

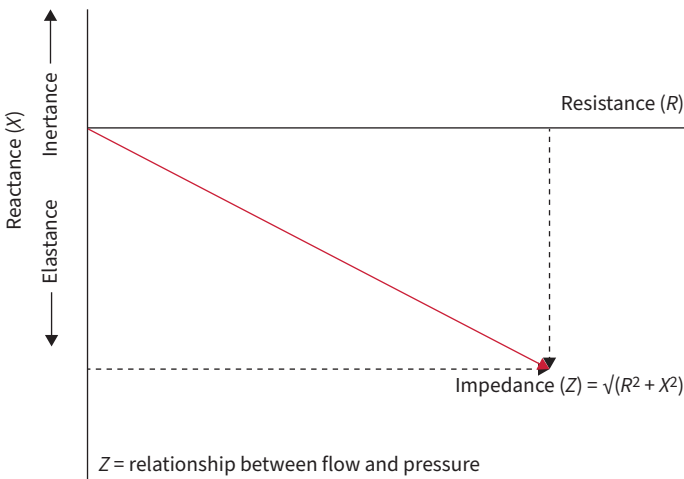


FIGURE 1 Diagrammatic representation of impedance (Z). The resistive component (R) is on the horizontal axis, with increasing R towards the right. Reactance (X) is on the vertical axis, in which increasing inertance is upwards and increasing elastance (or stiffness) is downwards. The magnitude of Z , R and X can thus be easily calculated and conceptualised.

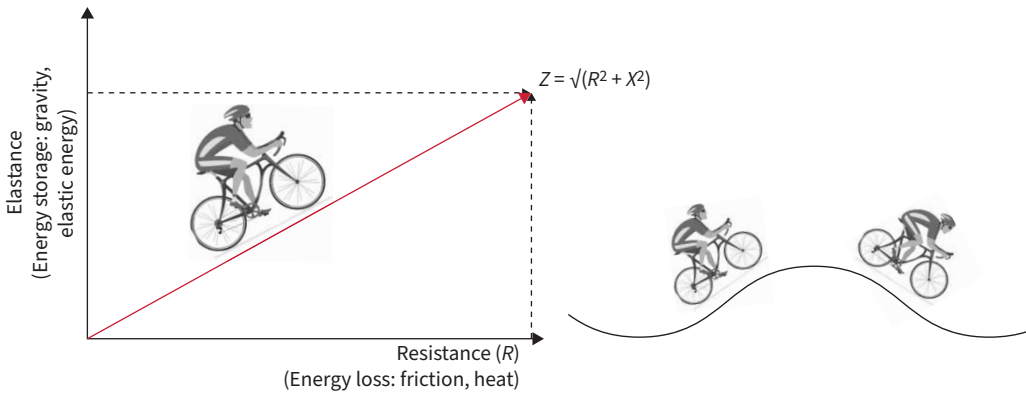


FIGURE 2 Diagrammatic representation of oscillatory impedance (Z). Increasing elastance (the dominant component of reactance (X)) is represented in the upwards direction on the vertical axis, rather than its usual downwards direction. The length of the red line is Z as in figure 1. Any increase in elastance or resistance (R), or both, increases the length of the red line. Each component contributes separately to the work of breathing and total Z . Elastance is increased when severe airway narrowing and closure make the subtended lung “inaccessible” to the oscillations (or to normal breathing). The smaller “accessible” volume thus requires greater pressure to achieve the same inspired volume.

volume” of the lung. In airways diseases, X is typically abnormally negative, indicating a reduction in “communicating volume”. The next concept to consider is what pathophysiological processes affect R and X .

What physiology do resistance and reactance represent in airways diseases?

In the common airways diseases COPD and asthma, which are where oscillometry has been best studied, R is determined by airway calibre. A higher R means narrower airways. Static lung compliance is classically increased in airways diseases (both asthma and COPD) [3, 4], which causes airway narrowing, dynamic collapse of airways and reduced pressures within the lung to drive air movement. This results in patchy, heterogeneous and uneven ventilation of the lung, and is well known to clinicians as expiratory gas trapping on CT and heterogeneous ventilation in ventilation/perfusion nuclear imaging. From a respiratory mechanics point of view, this explains how “communicating volume” is reduced in airways diseases.

This pathophysiology also explains “frequency-dependent” behaviour of R and X . This phenomenon was studied in obstructive airways diseases by WOOLCOCK *et al.* [5], who called it “frequency dependence of compliance” [6]. This same phenomenon is captured by oscillometry, whereby R and X vary according to oscillation frequency (see chapter 6 of this *Monograph* [2]).

It is possibly the fact that X is sensitive to changes in “communicating volume” that makes it an informative measurement of airway function. Hence, the lowest practical frequency at which it is measured, 5 Hz (X_{rs5}), has been shown to be an important measure of airway function by way of the large volume of data of its clinical associations.

Frequently used oscillatory impedance parameters

Respiratory system resistance (R_{rs}) and reactance (X_{rs}) can be measured simultaneously, at a number of frequencies, from ~5 Hz and higher in adults and from ~10 Hz and higher in

children (see chapter 6 of this *Monograph* [2]). The fundamental measurements are a mean value over the entirety of the acquisition. The behaviour of R_{rs} with frequency (frequency dependence) is captured as a subtraction of high-frequency from low-frequency R_{rs} , e.g. $R_{rs5}-R_{rs19}$. The behaviour of X_{rs} with frequency is captured as the “area under the reactance curve” (AX; see chapter 6 of this *Monograph* [2]). Measuring R_{rs} or X_{rs} in inspiration and expiration is termed “intra-breath” analysis (see chapter 6 [2]). In obstructive airways diseases, expiratory flow limitation (EFL) may occur due to expiratory airway collapse and a decrease in X_{rs5} of $\geq 2.8 \text{ cmH}_2\text{O}\cdot\text{s}\cdot\text{L}^{-1}$ in expiration is an accurate indicator of EFL [7].

What does oscillometry tell us in airways diseases?

Understanding why X_{rs5} is a clinically and physiologically relevant measure of airway function will give clinicians confidence in using this end-point in routine practice. The theoretical importance of X measurements in airways disease is supported by the large body of evidence showing its relationships with important clinical outcomes. It will be discussed in subsequent sections.

Oscillometry and the general population

Investigations of pulmonary dysfunction on a large scale or in general populations are now possible and attractive, given the ease and speed of performing the test. Several large studies are now being reported. BERGER *et al.* [8] reported a study of 439 healthy, asymptomatic subjects between the ages of 21 and 85 years that lived or worked in Manhattan, NY, USA, from which predictive equations for impulse oscillation system (IOS) oscillometry have been reported. The analysis showed a progressive dependence of resistance and reactance on body mass index when compared to weight. Age was found not to be significant. No comparisons to spirometry were reported. QVARNSTRÖM *et al.* [9] reported the use of IOS oscillometry and spirometry as part of the Swedish CARDioPulmonary bioImage Study (SCAPIS), a national population-based study of 10 360 randomly selected participants. They report post-bronchodilator abnormal oscillometry in 16% compared to abnormal spirometry in 19%, with an overlap of only 32% between these two measures of lung function. A majority of those that reported respiratory symptoms did not have abnormal lung function, whereas abnormal lung function by either measure was also shown to occur in the absence of symptoms. Nevertheless, abnormal oscillometry is frequently linked to an increased burden of symptoms and increased sick leave.

A recent paper by VENERONI *et al.* [10] provides considerable insight into the utility of oscillometry in detecting disease in the general population and points to the promise of oscillometry as a means of better assessing lung health across the lifespan. This analysis was performed on data collected by the LEAD (Lung, hEart, sociAl, boDy) study, and assessed respiratory symptoms, disease, spirometry and oscillometry in 7560 persons aged ≥ 18 years. The overall prevalence of abnormal spirometry was 13%, whereas the overall prevalence of abnormal oscillometry was 19.9%. Of those with respiratory symptoms or pulmonary disease, 38% had either abnormal spirometry or oscillometry, with oscillometry abnormalities somewhat more frequent (27% versus 22%). The presence of abnormal oscillometry was greater with more symptoms (even if only one symptom), and greater still in the presence of diagnosed disease. Interestingly, in the LEAD study, the diagnosis of COPD was associated with increased occurrence of expiratory oscillometry abnormalities, whereas asthma was associated with abnormal inspiratory measures. The authors concluded that assessing oscillometry in a general population would be an important addition to the better understanding of the genesis of lung disease and the factors that have an impact on lung health.

Taken together, these population-based studies demonstrate the utility of applying oscillometry to the investigation of disease pathogenesis, and the complementarity with spirometry. The results show that oscillometry is sensitive to even minor pathological processes within the lung, and clearly measures something quite different from spirometry. While spirometry measures the maximum functional and structural capacity of the lung, oscillometry measures the impedance to airflow during resting respiration. Changes in the size and static dimensions of the airways have an impact on spirometric findings, whereas oscillometry measures effects of physiology on resting respiration, such as neural tone, both central and peripheral airway dimensions, and inhomogeneity of airway narrowing (also referred to as heterogeneity). Accordingly, it appears that measuring both spirometry and oscillometry yields a much more complete assessment of lung function.

Clinical correlates

Oscillatory impedance is a clinically relevant measure of lung function because it has many clinical correlates in airways disease, and yet does not overlap much with spirometry, which strongly suggests complementarity. At a population level, respiratory symptoms or a diagnosis of airways disease have higher R_{rs} and lower X_{rs} [11, 12]. Abnormalities are also discordant with spirometry [10, 13], which indicates its complementarity.

In the clinic, spirometry will always be important diagnostically, *i.e.* to diagnose obstruction and to assess the severity of impairment. Oscillatory impedance should not replace spirometry, but together they provide a better picture of the physiological impairment. What we know now is that impairment of spirometry does not provide the full picture, *e.g.* hyperinflation is important in COPD but not captured by spirometry, whereas it influences impedance measurements [14, 15]. Another example is in patients attending a severe asthma clinic, where abnormal oscillometry (but not spirometry) was associated with worse asthma control [16, 17], with treatment requirements [18] and severe asthma exacerbations [19].

Oscillometric bronchodilator responsiveness

Spirometry bronchodilator responses are a fundamental characteristic of all airways diseases. Bronchodilator responsiveness (BDR) is characteristic in asthma, but it is neither sensitive nor specific. Clinicians debate whether positive BDR automatically indicates a diagnosis of asthma, or whether it is simply a physiological abnormality of either asthma, COPD or bronchiectasis. Others argue that this is irrelevant and that a label-free, treatable traits approach should be used [20]. Nevertheless, spirometric BDR in asthma and COPD has relevant clinical associations. In COPD, positive spirometry BDR is associated with greater FEV₁ response to oral corticosteroids [21–24], to inhaled corticosteroids (ICS)/long-acting β_2 -agonists (LABA) [25] and to ICS alone [26], reduced risk of severe exacerbation [27] and less hospitalisation and all-cause mortality [28], although there is conflicting evidence in terms of mortality [29]. In asthma, positive BDR is associated with both increased [30] and decreased [29] mortality risk, greater improvements in FEV₁ response to ICS [31, 32], worse asthma control [33] and greater exacerbation risk [34].

Normal limits for oscillometric response to bronchodilator have been relatively well studied in healthy cohorts [11, 35, 36]. When expressed as percentage change, the lower limit of decrease in R_{rs} is highly consistent between these three studies, at 30% of baseline. When expressed as absolute values, again this is consistent between the two studies in which it was reported [11, 36], at $1.4 \text{ cmH}_2\text{O} \cdot \text{s} \cdot \text{L}^{-1}$. One study reported the change also as z-scores [11], which was -1.4 z-scores. The normal limit for X_{rs} bronchodilator response was $0.55 \text{ cmH}_2\text{O} \cdot \text{s} \cdot \text{L}^{-1}$, again being very consistent between studies [11, 36], or 45% [35, 36] or 1.4 z-scores [11]. These are lower than the currently recommended cut-offs of -40% for R_{rs} and 50% for X_{rs} [1]. Although the

differences in percentages are very small, there were twice the number of subjects who had either wheeze or asthma diagnosis when using the above cut-offs, compared with the recommended cut-offs [35]. The more recent studies might influence future updates of the European Respiratory Society technical standards for respiratory oscillometry, and future evidence may inform an optimal way of expressing bronchodilator response (absolute, percentage or z-scores).

As a measure of BDR in asthma, oscillometry has a greater signal-to-noise ratio than spirometry, *i.e.* the response to bronchodilator is greater in comparison to its within-subject variability [37]. In addition to its associations with wheeze and asthma diagnosis [11, 35], positive oscillometric BDR is also associated with poor asthma control [38, 39]. These studies support the addition of BDR to the measurement of oscillometry in the clinic.

In smokers with normal spirometry and without asthma, oscillometric BDR was common, particularly in X_{rs} , occurring in around 20%, and was associated with respiratory symptoms [11]. In the presence of obstructed spirometry, however, responsiveness was increased to nearly 45%. Similarly, in a large community sample in Guangzhou, China [40], ~50% of moderate-to-severe COPD subjects exhibited positive BDR, again predominantly involving X_{rs} . In smokers without COPD, 20% showed responsiveness [40], a similar percentage to the smokers with normal spirometry and no asthma in the aforementioned study [11]. This very much reinforces the notion that COPD is bronchodilator responsive, which is much more readily apparent in oscillometry. Of potentially greater importance is that oscillometric BDR was associated with CT measures of emphysema and with greater decline in spirometry over time [40].

Responsiveness to treatment

Using oscillometry to assess treatment of patients with airways diseases is helpful to clinicians in helping assess overall response to treatment. As well as being very sensitive to short-acting bronchodilators, oscillometry also changes in response to ICS treatment, with or without long-acting bronchodilators. Whether oscillometry responses parallel improvements in other clinical outcomes in airways diseases, *e.g.* reduced exacerbations or lung function decline with treatment, has not been studied, but future such studies need to be completed. Improvements in R_{rs} and X_{rs} with treatment, or lack thereof, should contribute to the decision to continue, discontinue or increase doses. The lack of objective measures to guide treatment decisions, which are based mostly on subjective symptom responses, makes management uncertain and variable between practitioners, and makes outcomes imprecise in clinical trials or the clinic. Using oscillometry in combination with symptoms, spirometry and inflammatory markers will allow greater precision and consistency in management and clinical trials.

In keeping with oscillometric parameters being sensitive measures of airway function, they are responsive to inhaled combination ICS/LABA or long-acting bronchodilators in COPD [41–45] and in asthma [46–48]. All studies consistently show statistically significant changes in low-frequency R_{rs} (~10–25%), frequency dependence of resistance (~20–70%), X_{rs} (~25–60%) and AX (~50–60%). These were also universally accompanied by clinically significant improvement in patients' symptoms.

Repeatability

Minimal physiologically important difference can be estimated from variability of measurements in healthy subjects, both within a single testing session where there are multiple replicate measurements, or between measurements on multiple days. The estimates of such variability are summarised in table 1. The data suggest that R_{rs} and X_{rs} are highly reproducible tests within an individual, the variability values being around 0.7 and 0.4 $\text{cmH}_2\text{O}\cdot\text{s}\cdot\text{L}^{-1}$, respectively. This is

TABLE 1 Variability expressed as coefficients of repeatability and bronchodilator responses of oscillometric measurements in healthy subjects

	Repeatability (95% limits of agreement)				BDR, ULN or LLN	
	Within-session		Between-session		JETMALANI [§] [11]	
	RUTTING [#] [49]	THERKORN [¶] [50]	OOSTVEEN ⁺ [36]	THERKORN [¶] [50]	RUTTING [#] [49]	
Subjects n	31	129	38	129	31	577
R_{rs5} cmH₂O·s·L⁻¹	±0.60	±0.36	±0.94	±0.52	±0.64	-1.4
R_{rs5} z-score					±0.8	-1.4
FDR cmH₂O·s·L⁻¹	±0.32	±0.30		±0.34	±0.30	
X_{rs5} cmH₂O·s·L⁻¹	±0.41	±0.26	±0.55	±0.26	±0.37	0.6
X_{rs5} z-score					±1.0	1.4
AX		±2.90	±4.79	±3.34		

BDR: bronchodilator responsiveness; ULN: upper limit of normal; LLN: lower limit of normal; R_{rs5} : respiratory system resistance at 5 Hz; FDR: frequency dependence of resistance ($R_{rs5}-R_{rs19}$ or $R_{rs5}-R_{rs20}$); X_{rs5} : respiratory system reactance at 5 Hz; AX: area under the reactance curve. [#]: repeatability was calculated *post hoc*, since these were not in the original paper, from three visits on three separate days, as 2× root mean squared error (RMSE); [¶]: calculated as 2×RMSE from two separate days; ⁺: measurements were made 15 min apart; [§]: BDR as a comparison against repeatability.

about half the magnitude of the 95% limits for BDR of healthy individuals (table 1). This is in keeping with data suggesting high signal-to-noise ratio for oscillometry [37], hence greater sensitivity compared with spirometry.

Variability and minimal clinically important difference in disease

In addition to repeatability and BDR, respiratory impedance would be expected to vary over time in COPD, and particularly in asthma. The amount of variation over weeks is summarised in table 2.

The question of what is a clinically significant change in R_{rs} or X_{rs} parameters is difficult to answer, particularly given the lack of published data. ABDO *et al.* [53] published the only analysis that addresses this question of minimal clinically important difference (MCID) in asthma, but only for R_{rs5-20} and AX. They used a threshold of $0.4 \times SD$ of the population to determine clinical significance, because it is a commonly used threshold, which equated to improvements ≥ 0.6 cmH₂O·s·L⁻¹ and ≥ 6.5 cmH₂O·L⁻¹ as MCIDs for R_{rs5-20} and AX,

TABLE 2 Variability (95% limits of agreement) between two or more visits

	Subjects n	R_{rs5} cmH ₂ O·s·L ⁻¹	FDR cmH ₂ O·s·L ⁻¹	X_{rs5} cmH ₂ O·s·L ⁻¹	AX cmH ₂ O·L ⁻¹
Asthma					
GONEM [18]	18	±0.8	±0.4	±0.8	±5.0
Xu [51]	119	±1.1	±0.8	±0.66	±5.4
CHAN [52]	42	±0.7	±0.4		±3.9
RUTTING [49]	53	±0.74	±0.44	±0.60	
COPD					
Xu [51]	73	±1.72	±1.42	±2.92	±24.66
RUTTING [49]	36	±0.72	±0.48	±1.08	

R_{rs5} : respiratory system resistance at 5 Hz; FDR: frequency dependence of resistance ($R_{rs5}-R_{rs19}$ or $R_{rs5}-R_{rs20}$); X_{rs5} : respiratory system reactance at 5 Hz; AX: area under the reactance curve.

respectively. Interestingly, in stable asthma, these values are quite similar to the between-visit repeatability. This raises the possibility that the MCIDs are in the vicinity of the values for between-visit repeatability.

Clearly, more repeatability and MCID studies are needed, which ideally would involve a range of parameters, expressed as z-scores, absolute values and percentages, including within-breath parameters. They would need to be related to a clinically important patient outcome, *e.g.* asthma symptoms, breathlessness score, activity levels or exercise capacity, and have sufficiently large numbers and compare different devices, to allow generalisability. Additionally, MCID studies need to be conducted in other diseases of the respiratory system: COPD, CF, ILD, *etc.*

Home monitoring in asthma and COPD

There is a great need to monitor the activity of airways diseases at home. Oscillometry presents an opportunity to do that better. The present use of symptoms and exacerbations at occasional clinic visits is imprecise, of variable quality and entirely subjective. Lung function testing is helpful in the clinic, which we argue is greatly improved by the combined use of oscillometry with spirometry. Obstructive airways diseases are characterised by day-to-day variability of airway calibre, which on occasions may be severe leading to exacerbations, and in some, worsens progressively over time. And yet, this is not measured objectively. Blood sugar, blood pressure, lipids and cardiac rhythms can be monitored frequently or continuously, providing important data that allow precise measurement of disease activity and hence appropriate treatment. Oscillometry can provide such disease monitoring on a day-to-day basis, which would greatly improve assessments in the clinic.

Home monitoring using oscillometry is highly feasible in asthma and COPD [54, 55], as well as in children [56], where patients are able to make technically satisfactory recordings on a daily basis. This has become even more feasible and practical to implement with the advent of small, portable, hand-held oscillometry devices that are cost-effective. Use of a single-frequency oscillation allows hardware simplification, and R_{rs} and X_{rs} at a single low frequency, between 5 and 8 Hz, provide sufficient information for monitoring purposes. The CHROMED study was a randomised controlled trial of oscillometry home monitoring that triggered acute treatment for an exacerbation, in COPD patients. Although it did not substantially reduce exacerbations, there were fewer repeat hospitalisations and so, in this severe and difficult to manage group of patients, home monitoring may help a subgroup [57].

The paradigm of home monitoring is to detect a change on consecutive days that is outside the normal variability. In this regard, it is very similar to peak flow monitoring in asthma and so a period of monitoring during clinical stability is needed, since variability is an individual trait, and action plans based around home monitoring also therefore need to be individualised. Automated analyses and alerts are possible with digital data, and also desirable in terms of practicality. However, research in this area is still embryonic. A sub-analysis of data from the CHROMED study suggested that inspiratory X_{rs} should be the primary parameter, and that change over a shorter time interval (3 days rather than 7 days) would be more sensitive in early detection of deterioration [58].

Asthma should be an ideal condition to monitor with home oscillometry, because it is characterised by day-to-day fluctuations in airway function. Indeed day-to-day variability in inspiratory R_{rs} was highly discriminating in separating healthy individuals from those with asthma [59, 60], whereas a single measurement of impedance was not. Furthermore,

oscillometry is a sensitive way to measure change, *e.g.* to bronchodilator inhalation, in asthma. In a study of 10 patients with clinically stable and mild asthma, individualised R_{rs} patterns of day-to-day variability were used to predict episodic large increases in R_{rs} that may or may not have been associated with increased asthma symptoms, *i.e.* asthma exacerbation. The accuracy of 0.91 suggests that such an approach may be clinically useful. However, further research is needed to explore home monitoring as a diagnostic aid (in the same way that peak flow rate recordings are used), to optimise these analysis methods and to define patient groups who would benefit.

Oscillometry in paediatric patients

The tidal breathing nature of oscillometry offers particular advantages to the diagnosis and management of lung disease in children, particularly during the preschool years, where success rates of more complex lung function tests remain low. For example, 93% of very preterm born children obtained acceptable and repeatable oscillometry measures at 4 years of age, while the same group had success rates of 30% in FEV_1 , 23% in FVC, 4% in plethysmography and 0% in D_{LCO} [61]. Additionally, lung pathology is often mild in early lung disease with a focus on the small airways: the so called “silent zone” that often goes undetected by spirometry. Thus, oscillometry may offer important early information about lung disease in children and accordingly has been applied across several paediatric applications. However, the ability of oscillometry to discern between health and disease in young children is dependent on the disease population being studied and the outcome measure being utilised (*e.g.* R_{rs} , X_{rs} , AX and resonance frequency) [62], as will be discussed in the next sections.

Prematurity-associated lung disease

Oscillometry is the most effective and practical method to monitor lung function in preschool-aged children born prematurely. Apart from being highly feasible, children born prematurely have shown increased R_{rs} , more negative X_{rs} and increased AX and resonance frequency compared with preschoolers born at term [61]; these differences extend through childhood [63] and into adolescence [64]. Deficits are greater in those with a neonatal diagnosis of the chronic lung disease of infancy, bronchopulmonary dysplasia (BPD) [61, 65, 66]. While all oscillometry outcomes are abnormal in those born very prematurely, highly abnormal X_{rs} is clinically relevant since it is particularly sensitive at distinguishing term-born from preterm-born preschoolers [62] and correlates with respiratory symptoms [62, 63, 67]. However, the pathophysiology underpinning increased lung stiffness in this population remains unclear. It may reflect failed alveolarisation, structural damage to the parenchyma, or inflammatory changes to the lungs, although impedance parameters do not correlate with structural lung disease on chest CT in this population [64]. Nevertheless, with serial measures of oscillometry from early to mid-childhood, X_{rs} and AX deteriorated in those with BPD in parallel with declines in spirometry measures. Faster decline was noted in preterm-born children exposed to environmental tobacco smoke and those with respiratory symptoms, but even in the absence of structural lung damage on chest CT scans [68].

The normal limits for bronchodilator responses in healthy children are well described. BDR is evident in approximately one-third of children born very preterm. There are slightly more children with positive BDR by spirometry than by oscillometry but, similar to what has been found in adults, there was high discordance between BDR measured by oscillometry and by spirometry [69]. The clinical significance of BDR, irrespective of measurement technique, is uncertain in terms of prognosis or treatment response, but BDR by oscillometry alone was associated with wheeze.

Preschool wheeze and childhood asthma

As is the case in adults, oscillometric parameters differ in disease cohorts compared with healthy ones, but they are not diagnostic due to overlap and conflicting data. Some studies showed no differences in impedance parameters between healthy controls and preschool wheezers using oscillometry [70–72]. Other studies, including a study by OOSTVEEN *et al.* [73], showed that R_{rs} was increased in preschoolers with early transient wheeze compared with never-wheezers, and was even higher in persistent wheezers, although there was significant overlap. These findings are likely to be due to differing wheeze criteria and the severity of patients included in each study (*e.g.* wheeze on a single occasion *versus* persistent wheezers), as well as the uncertainty around which preschool wheezers are destined to develop asthma. There is some evidence, however, that impedance may have prognostic utility. In a longitudinal study of 255 Finnish children with asthma-like symptoms at 4–7 years, abnormal R_{rs5} z-score at baseline predicted the persistence of abnormal lung function and asthma diagnosis in adolescence [74]. However, intra-breath parameters, whereby impedance is separated for inspiration *versus* expiration, allowing separate analyses, may be more sensitive than the mean values for the entire acquisition (see chapter 6 of this *Monograph* [2]). Such analyses discriminated between preschoolers presenting acutely to an emergency department with acute wheeze and healthy controls, and between recurrent wheezers and healthy controls [75]. Further studies of clinical utility of this analysis are needed.

However, the real strength of oscillometry in the wheezy and asthmatic patient population lies in the ability to assess children for BDR and bronchial hyperresponsiveness, which are both important tools in the diagnosis of asthma beyond the preschool years [76]. Cut-off criteria for a positive BDR in clinical practice are well-established for children (*i.e.* 40% decrease in R_{rs} at 5 Hz, 50% increase in X_{rs} at 5 Hz, and 80% decrease in AX relative to baseline) [1]. BDR based on oscillometry parameters is superior to that based on FEV₁ at differentiating asthmatic from healthy children [73, 77, 78], while in general, BDR does not differ between wheezy (*i.e.* with no formal diagnosis of asthma) and non-wheezy preschoolers [73, 79]. Provocation studies in children are feasible in patients as young as 3 years old using oscillometry [80, 81]. However, conclusive data indicating which outcome measures and threshold values best reflect a positive bronchial challenge test are currently lacking in this age group. Bronchial challenge tests in research settings in children using a variety of challenge agents show reduced testing times and cumulative dose of challenge agent (compared to spirometry) [80–86]. However, further work around harmonising testing protocols and defining robust, clinically meaningful challenge end-points is required to bring this application into busy paediatric respiratory clinics.

Oscillometry is sensitive to drug treatment in children with asthma. For example, significant improvements in R_{rs} , X_{rs} and/or AX have been noted in clinical trials of montelukast [87], ICS [88] and combination ICS/LABA [88], with oscillometry outcomes being more sensitive than spirometry. Furthermore, R_{rs} is superior to both FEV₁ and methacholine testing (by FEV₁) in predicting asthma exacerbations in 4–7-year-old children with intermittent asthma [86]. Thus, oscillometry probably has a clinically useful role in identifying and monitoring exacerbations and treatment responses in children with asthma.

CF and other paediatric lung conditions

The majority of studies in young children with CF reveal normal oscillometry indices [89–91], and associations with other clinical parameters are variable. Therefore, the clinical utility of oscillometry in CF remains uncertain, particularly when other clinical tests (such as multiple-breath washout) have shown superior ability to detect early CF lung disease and successfully monitor improvement with intervention [92]. Paediatric data on the clinical utility of oscillometry

in other paediatric patient populations, such as (non-CF) bronchiectasis, primary cilia dyskinesia, neuromuscular disease, perinatally acquired childhood HIV lung disease, post-tuberculosis lung disease and sickle cell disease, are severely lacking.

Summary

In summary, oscillometry has the potential to play a vital role in assessing early lung function, diagnosing common paediatric lung conditions, monitoring progression of early lung disease and evaluating treatment responses during an important window of opportunity for lifelong lung health trajectory.

Oscillometry during invasive and noninvasive mechanical ventilation

Respiratory oscillometry provides significant advantages for assessing lung mechanics in patients on respiratory support. It can be performed without interrupting ventilation, even in non-sedated patients, allowing continuous assessment. This is achieved either by incorporating the oscillation signal into the ventilation waveform itself [93, 94] or by superimposing an oscillation signal onto the ventilation waveform [95, 96]. In most cases, oscillometry can be integrated into the mechanical ventilator without requiring additional setup to perform the measure. Since oscillometry involves minimal lung volume changes, it can measure lung mechanics by reducing nonlinearities (turbulent flow, which causes errors in impedance calculations). Because oscillation frequencies are much higher than breathing frequency, impedance can be tracked over the ventilation cycle. Applying multiple frequencies may allow the interpretation of lung mechanics in terms of pathophysiological models. Oscillometry provides real-time insights into the mechanical properties of the lungs and can be applied to monitor patient conditions, to evaluate the impact of treatments and interventions and eventually guide them, and to optimise ventilation settings.

Patient monitoring

Monitoring during respiratory support is essential for tracking the disease progression, recovery and prognosis, and for designing prompt and effective interventions. Oscillometry measurements can be easily made in hospitalised adults undergoing either invasive or noninvasive respiratory support [97–99]. However, clinical studies are still needed to determine the clinical utility of such monitoring in management or prognostication. Nonetheless, in preterm newborns, there are substantial data supporting the use of impedance measurements in the first days of life. Measurements of X_{rs} during ventilation are related to total days of ventilatory support and development of BPD [96, 100–103]. Furthermore, X_{rs} measured in the first 2 h of life predicts surfactant need [100].

Evaluating responses to treatments and interventions

In infants receiving respiratory support, oscillometry has been applied to evaluate the impact of several treatments, such as surfactant administration [104], bronchodilator therapy [105], corticosteroids for BPD [106] and postural change [95]. In adults receiving ventilatory support, oscillometry can be useful for monitoring bronchodilator treatment [107], assessing effectiveness of positive airway pressure to mitigate bronchoconstriction [108], and immediate post-operative monitoring [109–111]. There is a strong argument to use oscillometric impedance to assess ventilatory support, particularly in infants where there few or no useful alternatives. Impedance therefore provides measurements that aid clinical decision-making with respect to specific interventions, *i.e.* ceasing interventions that are ineffective or continuing beneficial treatments.

Optimising ventilatory parameters

Oscillometry is useful for adjusting ventilation settings with the key focus being optimising airway pressures. In fact, repeated oscillometry measurements during recruitment manoeuvres or at various airway pressures help clinicians to tailor pressure ventilation settings. This approach has been applied to various respiratory diseases, using a number of oscillometry indices and interpreting them in the context of the specific pathophysiological condition.

Optimising lung volume recruitment in infants and adults

When dealing with acute pathologies characterised by lung volume de-recruitment (such as acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) or neonatal respiratory distress syndrome), success of the ventilatory strategy is critically dependent on identifying the distending pressure that optimises lung recruitment while minimising lung distention. It is important to maintain lung recruitment through repeated recruitment manoeuvres. This is especially difficult given the wide range of lung conditions encountered in the intensive care unit.

Results of animal studies support the use of oscillometry for monitoring lung volume recruitment and optimising lung mechanics [112]. In experimental models of ALI/ARDS, X_{rs} at 5 Hz was a reliable index of the percentage amount of de-recruitment as assessed by CT and can monitor recruitment/de-recruitment regardless of the model and distribution pattern of lung collapse [113]. Moreover, monitoring X_{rs} during a decremental positive end-expiratory pressure (PEEP) trial allowed the identification of the minimum PEEP level required to prevent lung de-recruitment after a recruitment manoeuvre, with high sensitivity and specificity as determined by CT [114]. The PEEP optimisation strategy based on X_{rs} minimised tidal recruitment and distention [115], resulting in better oxygenation and reduced histopathological evidence of lung injury [116] in comparison with the standard protocol.

Applying oscillometry to optimise mechanical ventilation is especially valuable in neonatal intensive care. Newborns have more fragile lungs, with limited indicators of lung function and a higher risk of long-term complications. In the first days of life, their rapidly changing respiratory conditions demand continuous ventilation adjustments. Oscillometry provides information on lung mechanics in preterm infants that is critical in assessing effectiveness of recruitment manoeuvres, determining the optimal pressures and monitoring how these factors evolve over time [93, 117, 118]. This may be particularly useful during neonatal extracorporeal membrane oxygenation, when pulmonary gas exchange cannot be used to determine effectiveness of ventilation [119].

Another important application may be personalising resuscitation manoeuvres at birth, when urgently recruiting the lung while not provoking barotrauma is crucial. Oscillometry has been used to monitor lung mechanics during different recruitment manoeuvres in preterm lambs [120, 121]. It also provides information on lung aeration [120], and may guide the definition of personalised strategies, given the heterogeneity of conditions and mechanical response to the same strategy that is observed in preterm infants [122].

Use of oscillometry to optimise PEEP in COPD

Within-breath oscillometry identifies tidal EFL during ventilatory support [123, 124] that causes intrinsic PEEP and increases the work of breathing. Applying external PEEP to match intrinsic PEEP can reduce this burden, normalise the breathing pattern, improve gas exchange and decrease patient-ventilator asynchrony. However, excessive PEEP can increase the work of breathing and lead to haemodynamic compromise.

Algorithms to automatically titrate PEEP based on the continuous oscillometric monitoring of tidal EFL have only recently been implemented in commercial mechanical ventilators. In COPD, the optimal PEEP varies substantially between patients and changes with posture and exertion, which underscores the importance of continuous titration [125]. This treatment minimised transdiaphragmatic pressure swings and neural respiratory drive in patients with COPD and chronic hypercapnic respiratory failure [126]. This treatment also reduced the sleep time with transcutaneous CO₂ >45 mmHg and halved the number of ineffective breaths [127, 128].

Finally, detecting tidal EFL may also help set the airway pressure for obesity hypoventilation syndrome [129], since detecting inspiratory flow limitation [130] can help in autotitrating continuous positive airway pressure for obstructive sleep apnoea [131, 132].

Summary

In summary, respiratory oscillometry during respiratory support offers a valuable tool for optimising lung mechanics, minimising ventilator-induced injuries, and improving patient outcomes. Its full integration into routine clinical practice may require further advancements in commercially available devices. Further work is needed to increase measurement accuracy, eliminating effects of ventilatory circuits, different airway interfaces, leaks, nasal breathing and glottic activity, perhaps with the aid of artificial intelligence [133]. Further clinical studies would be welcome to define patient-related outcomes and patient selection.

Conclusion

Respiratory oscillometry is a practical way to measure lung function that, when combined with spirometry, lung volumes and diffusing capacity, provides a more complete picture of the physiology that is highly clinically relevant. The use of commercially produced devices in research is now being established in clinical practice, particularly in paediatric lung diseases and adult airways diseases. The large collective of clinical studies shows that oscillometric impedance measures are highly clinically relevant. The challenge is for more clinicians to use this test as part of routine practice, which requires them to learn more of the underlying principles of oscillometric impedance and lung physiology. The benefits, however, are great. Not only does it allow better clinical assessment, by complementing spirometry, treatable traits and inflammatory biomarkers, but also it is a measurement of function that can be used to track change and assess benefit of novel treatments. Oscillometry is at this moment ready for routine clinical use.

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Gas washout techniques

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Single- and multiple-breath inert gas washout techniques assess lung volume and peripheral airway function, with clinical utility demonstrated across a range of important obstructive respiratory conditions, including CF, asthma and COPD <https://bit.ly/ERSM107>

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The peripheral, or small, airways occupy >95% of the lung volume and are thought to be a key site of pathophysiology in various obstructive respiratory diseases. A lung function test such as spirometry cannot properly monitor these peripheral lung regions, and overlooks a crucial aspect, namely lung structural heterogeneity that may be modified by disease. Inert gas washout techniques, first described >75 years ago, were introduced to quantify ventilated lung volume and ventilation heterogeneity by monitoring a “tracer” gas of interest. In this chapter we outline the basic principles of inert gas washout using single- and multiple-breath tests, starting with the most basic question: why perform a washout test at all? We review the various analysis steps, the resulting indices and their interpretation. We then discuss the evidence of clinical utility in CF, asthma, COPD and bronchiolitis obliterans. Finally, we outline some important limitations of washout techniques.

Introduction

An important site of gas mixing within the lungs is the peripheral, or small, airways. These are airways with an internal diameter of <2 mm in adults, corresponding to approximately airway generations eight and beyond [1]. These airways occupy >95% of the lung volume, are a key site of pathophysiology in important obstructive respiratory diseases, and yet are largely overlooked by conventional lung function tests such as spirometry. Spirometry is based on the measurement of flow, which is primarily determined by airway resistance. However, only 10–20% of total airway flow-resistive pressure loss occurs in these peripheral airways [2]. Experiments in dog lungs have shown that complete obstruction of 50% of peripheral airways increased total airway resistance by only 10%, and had no effect on measured vital capacity [3]. Additionally, distal heterogeneous changes in lung function may be masked by increased flow through non-flow-limited distal airways [4]. Obstructive lung disease develops in a patchy manner and as such, generates uneven gas mixing (or ventilation distribution) as an early sign of accompanying lung function impairment. In this context, inert gas washout techniques were first referenced >75 years ago, as a

description of indices reflecting lung volume, ventilation heterogeneity and measures of airway deadspace volume, based on the information gained when monitoring a “tracer” gas of interest. Potential clinical utility has only recently been more widely appreciated.

In this chapter, we will outline the basic principles of inert gas washout, considering “why?” at each step along the way, to help the reader understand the reason certain choices are made during the execution or analysis phase of washout testing, and how these affect the interpretation of results. We will then discuss the evidence for clinical utility across important respiratory conditions.

So, let us start with the simplest question:

Why perform a washout test?

In a washout test, the subject inhales a gas mixture with a composition that is different from the one in the lungs, in order to wash out (part of) a lung-resident gas. By having a subject inhale 100% O₂, a standard nitrogen (N₂)-based single-breath washout (SBW) is produced, with a pre-test lung resident N₂ concentration equivalent to room air (≈78%).

If the washout test, with inspired and expired volumes (V_{IN} and V_{EX}), is performed to get an idea of lung volume (V_{LUNG}), then the lung V_{LUNG} from which 100% of O₂ is inhaled will be calculated by mass balance. Pre-test, the amount (or quantity) of N₂ is [pre-test N₂ conc× V_{LUNG}], which equals the sum of the amount of N₂ washed out [exhaled N₂ conc× V_{EX}] and the lung resident amount of N₂ at the end of an exhalation back to V_{LUNG} [end-exhaled N₂ conc× V_{LUNG}]. Hence:

$$\text{Pre-test N}_2 \text{ conc} \times V_{LUNG} = [\text{exhaled N}_2 \text{ conc} \times V_{EX}] + [\text{end-exhaled N}_2 \text{ conc} \times V_{LUNG}] \quad (1)$$

and thus, rearranging:

$$V_{LUNG} = [\text{exhaled N}_2 \text{ conc} \times V_{EX}] / [\text{pre-test N}_2 \text{ conc} - \text{end-exhaled N}_2 \text{ conc}] \quad (2)$$

In case of vital capacity SBW, V_{LUNG} represents residual volume (RV). In case of a tidal volume SBW, V_{LUNG} is functional residual capacity (FRC).

V_{LUNG} can also be obtained by washin of an exogenous test gas mixture such as helium (He) or methane, as is routinely done in a diffusing capacity test to obtain so-called alveolar lung volume. The choice of washin gas may depend on: gas availability; whether one wants to study the effect of gas diffusivity; or a potential comparison with an imaging modality in which a gas of similar diffusivity is used (MRI or CT performed with He or xenon). If the goal of the procedure is to obtain V_{LUNG} , a mass balance approach to calculation similar to that seen in equation 1 can be used, namely:

$$[\text{inhaled He conc} \times V_{IN}] = [\text{exhaled He conc} \times V_{EX}] + [\text{end-exhaled He} \times V_{LUNG}] \quad (3)$$

In case of a washin, the approach outlined in equation 2 for the washout phase can also be applied; the washin test is treated as if it were a washout by simply flipping the curve (figure 1). In fact, for most intent and purposes, a washin curve is usually transformed to a washout curve and calibrated (or normalised) to 100%, so that all lung concentrations are considered *versus* the maximum difference between lung resident and inhaled gases. In the case of tracer gases that are not present in the inhaled mixture but are resident in the lung, such as N₂ and argon, this normalisation is simply performed by dividing by their pre-test lung resident concentrations of

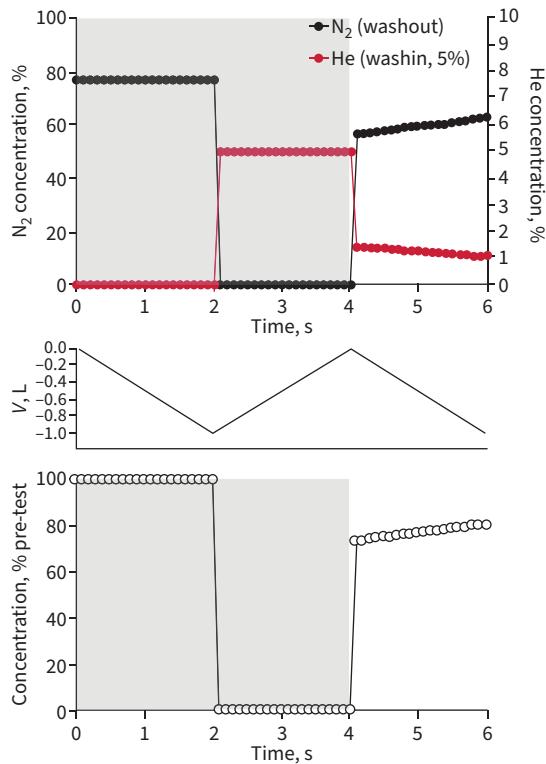


FIGURE 1 Illustration of how a single-breath washin test can be transformed to a single-breath washout test. Closed circles are concentrations of nitrogen (N_2) washout and helium (He) washin, in a hypothetical lung where both gases are subject to exactly the same degree of ventilation heterogeneity. When appropriately transformed (open circles), these two gases show an identical washout curve (see text for details). The grey zone covers the last pre-test exhalation and the single-breath test inhalation. V : volume.

$\approx 78\%$ and 0.9% , respectively. For example, in the washin of 5% inhaled He, the equivalent N_2 washout would be obtained by subtracting the exhaled He concentration from 5% and dividing it by 5% . Figure 1 shows how such a transformation would collapse N_2 washout and He washin curves onto the same washout curve in the hypothetical case that both gases were subject to exactly the same gas transport mechanism. In reality, the gas transport mechanism does differ between the gases, so the indices measured between gases should differ, but this transformation allows derived indices reflecting ventilation heterogeneity to be directly compared.

Note that in some cases, the lungs are first washed with an exogenous gas, for instance sulfur hexafluoride (SF_6), assuming complete homogenisation of SF_6 at the end of the washin, to then initiate the actual multiple-breath washout (MBW) test by washing out the SF_6 by air breathing [5]. In other instances, the washin phase of SF_6 is used for analysis [6].

Unless otherwise stated, N_2 washout will be considered from this point onwards.

Why perform MBW?

If exhaled N_2 at the end of just one exhalation were representative of N_2 concentration everywhere in perfectly diluted lungs, one exhalation would suffice to obtain an accurate

estimate of V_{LUNG} using equation 2. However, even in a normal healthy lung, one exhalation will underestimate the true V_{LUNG} , due to the presence of ventilation heterogeneity. This small degree of ventilation heterogeneity is present in all healthy lungs due to the effects of gravity [7] and the branching asymmetrical structure of the lung down to the region of the acini [8–10]. The degree of underestimation can be improved by performing a MBW, where the quantity of exhaled N_2 can be cumulatively collected over consecutive exhalations. The FRC estimate rapidly increases within the initial breaths, followed by a slower asymptotic rise in latter breaths (figure 2). In the case of N_2 washout, a horizontal FRC asymptote is never truly reached because when alveolar concentration towards the end of the N_2 washout is low enough, this will favour a diffusion gradient with respect to N_2 dissolved in the lung tissue [11, 12]. In this respect, N_2 is not a truly inert gas.

During MBW, the rate at which exhaled N_2 concentration decreases can be used to measure ventilation heterogeneity. The most commonly used index, the lung clearance index (LCI), is determined as the cumulative volume exhaled up to a given degree of N_2 dilution in the MBW, divided by FRC calculated at that same point. The historical approach, of extending MBW until

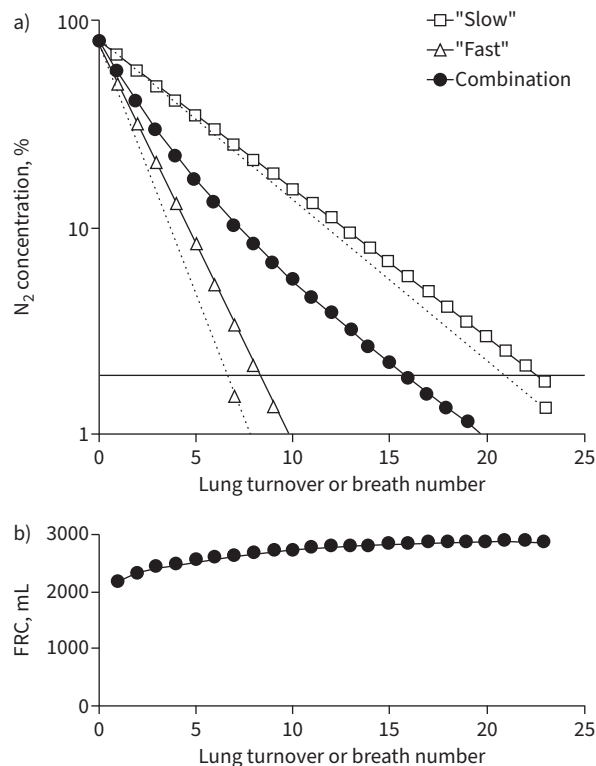


FIGURE 2 Effect of ventilation heterogeneity on washout curves and FRC. a) Nitrogen (N_2) concentration curves in a semi-log plot for a two-compartment model of 3 L with an inspired lung volume (V_{IN}) of 1 L. The “slow” compartment (low specific ventilation $V_{IN}/V_{LUNG}=0.18$; open squares) and the “fast” compartment (high specific ventilation $V_{IN}/V_{LUNG}=0.57$; open triangles) each produce a mono-exponential N_2 decline. Solid circles are the combination of slow and fast washout curves as measured at the model exit. Dotted lines are the mono-exponential curves according to equation 5, with V_{IN}/V_{LUNG} values corresponding to the slow and fast compartments. Horizontal line: 1/40th of pre-test N_2 concentration (=78%). b) FRC estimate versus breath number in the case of combined slow and fast compartments.

exhaled concentration reached 1/40th of pre-test concentration (horizontal line in figure 2), was based on signal-to-noise limitations of N₂ analysers and has since been retained in consensus discussions. Exhaled concentration can be expressed as: 1) mean expired concentration; 2) mean alveolar (typically calculated as mean concentration across the phase III slope); or 3) end-tidal [13]. There are arguments in favour of each option, but most published studies have used end-tidal. Whilst calculated FRC varies little with the choice of concentration, the LCI values obtained will be affected, increasing across the above three options. While mean expired and mean alveolar concentration based LCI are a measure of the rate of progressive breath-by-breath lung dilution, the end-tidal based LCI is confounded by the degree of within-breath flow asynchrony, irrespective of lung dilution [14].

The LCI is a measure of global (or overall) ventilation heterogeneity. The minimum value of the LCI computed for a perfectly mixed balloon (with no deadspace) in the physiological range of V_{IN}/V_{LUNG} , would sit between 4 and 4.5. A major determinant of the LCI is the amount of deadspace (*i.e.* V_D) generated by anatomy, equipment or physiology. Physiological deadspace is influenced by the molecular mass of the tracer gas, which determines its diffusive properties and the position of its diffusion front (more proximal for gases with lower molecular mass). For instance, even in healthy lungs, the LCI will be greater for SF₆ than for He, just because the physiological deadspace is greater for the former [15]. The non-linear dependence of the LCI on the ratio of deadspace over tidal volume has led authors to suggest the use of 1/LCI, termed ventilation distribution efficiency, which has the advantage of a linear relationship with V_D [16].

When plotting N₂ concentration in a semi-log plot, a linear decrease (*i.e.* an mono-exponential decrease) corresponds with the lung behaving as one homogeneous compartment, where at each subsequent V_{IN} inhalation of O₂, diluted N₂conc becomes:

$$N_2conc(breath) = N_2conc(breath-1) \times V_{LUNG} / (V_{IN} + V_{LUNG}) \quad (4)$$

Depending on the ratio V_{IN}/V_{LUNG} , which is also termed “specific ventilation” (ventilation per unit of lung volume), this is approximated by, but not identical to:

$$N_2conc(breath) = (pre-test N_2conc) \times \exp^{(V_{IN}/V_{LUNG}) \times breath} \quad (5)$$

Equation 5 is also how imaging modalities determine voxel-based three-dimensional maps of specific ventilation that can be linked to MBW [17, 18]. When the lung does not behave as a uniform compartment, each constituent uniform lung sub-compartment (or voxel in imaging) will generate a mono-exponential curve. But at the mouth, the resultant washout curve will be a sum of these concentration curves, weighted by the relative contribution of V_{IN} to the sub-compartment to overall V_{IN} (figure 2). Hence, the first portion of washout will mostly reflect the well-ventilated lung compartments, and when these are almost fully washed out, the washout mainly reflects the so-called “slow” (or not well ventilated) lung compartments.

While the LCI is derived from just one point of the washout concentration curve, the trajectory to that point may also be of interest. Two additional indices derived from a concentration washout curve attempt to capture this, based on earlier concepts, as follows:

- 1) The curvilinearity of the washout curve [19]. This index only depends on concentration differences resulting from differences in specific ventilation between various sub-compartment, irrespective of flow asynchronies between them.

- 2) Alveolar mixing efficiency (AME) of the first breath [20]. This index is a measure of more distal (or acinar) ventilation heterogeneity, which generates a lower than “expected” mean expired concentration at the very first breath. AME is quantified by:

$$\text{AME} = \text{N}_2 \text{conc} \times V_{\text{EX}} / (V_{\text{EX}} - V_{\text{DF}}) \times (\text{FRC} + V_{\text{IN}} - V_{\text{DF}}) / (\text{FRC} + V_{\text{Deq}}) \quad (6)$$

where V_{EX} is exhaled V, V_{DF} is Fowler V_{D} and V_{Deq} is post-capillary equipment V_{D} .

Why analyse phase III slopes in SBW?

The first reason for analysing the profile of an expiratory concentration curve within one breath is that one expects that ventilation heterogeneity induced by lung disease will not only result in different specific ventilations ($V_{\text{IN}}/V_{\text{LUNG}}$), generating concentration differences, but will also result in flow asynchronies. Collectively these contribute to convective-dependent inhomogeneity (CDI). Specific ventilation heterogeneity results in different concentrations in the lung that can also be visualised by lung imaging modalities incorporating hyperpolarised tracer gases; flow asynchrony cannot. At exhalation, the flow asynchrony is such that the best ventilated units (highest specific ventilation, lowest expiratory N_2 concentration) “empty first” and the worst ventilated units “empty last”, this results in a positive phase III slope. Specifically, a lung unit “emptying first” is shorthand for a unit whose outflow contributes relatively more to total expiratory flow at the beginning of exhalation *versus* the end of exhalation; for instance, from 54% at the beginning of exhalation, to 63% at the end of exhalation.

The reason for such flow asynchrony, and the fact that phase III slopes sampled at the mouth are generally positive, is not yet fully understood, but two mechanisms have been proposed that do produce positive phase III slopes. One is a gravitation-dependent mechanism, in which the interplay between the pleural pressure gradient and compliance of lower and upper lung zones makes the lower zones empty first [15]. The other mechanism is irrespective of gravity and is instead related to the fact that the volumes of compliant lung units subtended by the bronchial tree are not perfectly symmetrically distributed at each bronchial bifurcation. This creates differences in specific ventilation and flow asynchrony between parallel pathways to the acini [10]. In a normal lung, such “bronchial asymmetry of compliant lung units” results from the shape of the thoracic cage. In lung disease, bronchial asymmetry may be altered because airway narrowing, obstruction or remodelling may effectively close compliant units, thereby affecting the phase III slope [10].

Another contributor to the magnitude of the phase III slope has also been identified, which does not require any convective flow asynchrony [21]. The interaction of diffusion and convection in the distal (or peripheral) part of the lungs with structural asymmetry between lung units subtended by any two daughter branches, also results in a positive and characteristically curvilinear phase III slope [22]. This mechanism is termed diffusion-convective-dependent inhomogeneity (DCDI).

Why analyse phase III slopes in MBW?

CDI and DCDI have been shown to generate a very different pattern of phase III slope increase from one exhalation to the next, provided the phase III slope is normalised for exhaled concentration. Since a phase III slope reflects concentration differences inside the lungs, the decrease of the average concentration in the lungs by breath-by-breath dilution during MBW needs to be accounted for. This normalisation of the phase III slope (termed Sn_{III}), as with earlier indices described, can be achieved by dividing by the end tidal, mean alveolar or mean expired

N_2 concentration. CDI generates a Sn_{III} that steadily increases throughout MBW, whereas DCDI produces a Sn_{III} value at the first MBW exhalation, which reaches a horizontal asymptote within the first few breaths (figure 3). Hence, the steady Sn_{III} increase beyond the first breaths is used as a measure of CDI, and termed “ S_{cond} ” because CDI is operational in the conducting airways.

S_{cond} is defined as the Sn_{III} increase (calculated by linear regression) between lung turnovers 1.5 and 6.0, where the turnover for each breath is calculated as the ratio of cumulative expired volume of each breath over the FRC value for that MBW test. The first slope (minus a small correction for the CDI contribution to that breath) is proposed as a measure of DCDI (and termed “ S_{acin} ” due to its proposed location sitting close to the lung acinus). An alternate approach based on assessing the DCDI asymptote has also been proposed but is not widely used at present [23]. The dividing line between regions of the lung where conductive and acinar origins of ventilation inhomogeneity dominate (or their functional equivalents CDI and DCDI) is the diffusion–convection front, where the contributions of diffusive and convective transport balance each other. In the normal adult lung, the dividing line is typically at the acinar entrance. It might shift in diseased lungs, but based on first principles, it will be close to where a sudden increase occurs in the combined cross-section of all parallel airways.

To ensure a well-visualised phase III slope, adult protocols for MBW that focus on Sn_{III} analysis have targeted a fixed tidal volume (1.0–1.3 L) and respiratory rate (10–12 breaths·min^{−1}) [19]. For paediatric applications of Sn_{III} analysis, two amendments must be considered, as tidal volume is smaller and adopting a fixed tidal volume breathing protocol is not feasible [24]. Hence, lower tidal volumes are chosen in proportion to the child’s lung volume, and imposing a fixed tidal volume is abandoned. For adequate comparison of Sn_{III} values in adult and children of various ages, further normalisation is proposed [24]. The underlying assumption is that, provided normalisation of phase III slope for gas concentration already accounts for the effect of gas dilution in Sn_{III} (expressed in L^{−1}), multiplication of each Sn_{III} value by actual tidal volume (V_T) accounts for lung size, obtaining $Sn_{III} \times V_T$ (expressed as a dimensionless number) [13].

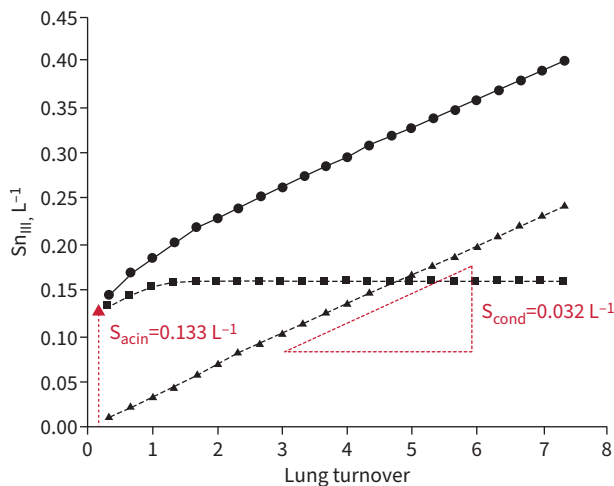


FIGURE 3 Normalised phase III slope (Sn_{III}) analysis. Sn_{III} versus lung turnover in case of combined conductive and acinar ventilation heterogeneity (circles) and corresponding indices S_{acin} and S_{cond} . Also represented are cases of only conductive (triangles) or only acinar (squares) ventilation heterogeneity.

Advancements in technique standardisation

The 21st century has witnessed a significant increase of interest in gas washout techniques because they offer both improved sensitivity in the detection of early obstructive lung disease in the setting of normal conventional lung function, and improved feasibility in younger ages (with typical success rates of >80% across wide age ranges) [25]. Prior to 2010, the vast majority of research was conducted on custom-built in-house systems, requiring operators with in-depth knowledge of washout testing and its pitfalls (*e.g.* erroneous baseline measurement, non-uniform mixed lung prior to washout testing). Since then, we have observed several important steps towards its integration into routine clinical care, culminating in an international collaborative effort to derive the first consensus recommendations for inert gas washout testing, published in 2013 and endorsed by both the European Respiratory Society (ERS) and American Thoracic Society (ATS) [13]. These recommendations outlined specifications for device design, validation and test procedure, requiring (for example) that equipment achieved a FRC measurement accuracy of 5% across a representative range of lung volumes, V_T and respiratory rates encountered in the age range intended for subsequent testing [13].

Collaborative work between industry and researchers has resulted in the widespread availability of commercial equipment for both N_2 and SF_6 based MBW, with individual devices at varying stages of validation [26–28]. In the process, updates to software have led to significant changes in MBW outcomes, as previously highlighted in the literature [29]. Encouragingly, close agreement of FRC has been achieved for both *in vitro* [26, 27] and *in vivo* models (*i.e.* plethysmography) [30]. For practical reasons, commercial devices have moved away from direct N_2 measurement to a molar mass measurement combined with concurrently measured O_2 and CO_2 concentrations [26] or just CO_2 concentration [31]. In the case of SF_6 , this indirect calculation has also been validated based on molar mass measurement [27] or by utilising a photoacoustic analyser [32].

Formal technical standards specifically for the more challenging preschool age range were published in 2018 [33]. More recently, commercial device validation has extended down to infancy [34]. Updated formal technical standards encompassing updated detailed recommendations for all age ranges, and reflecting the latest technical advances incorporated in the commercial devices, are planned for the near future.

Other adaptations to enable strong feasibility documented down to the infant age range (>80% of test occasions) include the use of facemask interfaces (with or without the use of therapeutic putty to achieve an adequate seal), distraction with a suitable show/movie to promote representative tidal breathing, and the potential to perform the test supine, either during quiet sleep or under mild sedation [35]. Due to concerns regarding alteration of infant breathing patterns during 100% O_2 administration [36, 37], research groups have largely focused on SF_6 for infant MBW. However, it has been recently shown that a reduction in tidal volume with 100% O_2 exposure is not accompanied by a change in end-expiratory lung volume [38], suggesting that N_2 MBW can also be used in this age range.

The availability of robust commercial equipment also meant reference data for healthy subjects could be obtained to define the normal range of common MBW indices across the entire age range [39–42].

The recent completion of a Global Lung Function Initiative (GLI) task force specific to MBW has for the first time produced GLI-style equations for LCI and FRC from the preschool age upwards [43]. One way to utilise the recently generated GLI reference equations for MBW is to

consider the equations (including their dependence on sex, age and, for FRC, height) as a guide and to use these to assess the capabilities of MBW data gathered locally before deciding about local implementation [44]. Potential reasons for a lack of alignment include aspects such as equipment deadspace or tracer gas used.

Evidence of clinical utility for early disease detection

CF

The peripheral airways are involved in CF pathophysiology from an early stage [45], and CF is the condition in which the clinical utility of MBW is best understood. Excitement about the LCI as a useful outcome measure in CF resulted from initial efforts to collate data across existing research studies [19], which outlined strong feasibility across a wide age range that abnormality of lung function using MBW was detectable in all age groups, even infants (*i.e.* from a young age), and that based on this cross-sectional data, the degree of abnormality increased over time (*i.e.* there was progression in underlying disease from an early age). This latter aspect was confirmed in subsequent longitudinal studies [41, 46, 47]. Alongside concurrent advances occurring in device standardisation, this led to the independent endorsement of the European Cystic Fibrosis Society (ECFS) Clinical Trials Network of the LCI as a primary outcome measure for CF clinical trials [48]. The subsequent establishment of a framework for training operators and for over-reading data collected during these large multicentre international studies [49] not only facilitated a successful outcome in the initial study (which examined the use of lumacaftor/ivacaftor in 6–11 year olds) [50] but has now been used in >30 clinical trials to date. These studies have outlined the health benefits from the preschool age upwards for a series of CF transmembrane conductance regulator (CFTR) modulators [48, 50–55]. The LCI has also outlined the beneficial effects of hypertonic saline in randomised controlled trials within infancy [56] and at preschool age [57]. The other significant attraction of the improved sensitivity of the LCI is the markedly reduced sample sizes required, compared with spirometry, for intervention studies [58].

The widespread availability, and efficacy, of CFTR modulators (>90% of the CF population are now eligible for a CFTR modulator based on their CFTR variants [59]) has led to a greater proportion of CF subjects with mild lung disease (as defined by normal spirometry). This shift to milder lung disease suggests that the role of MBW in disease monitoring may become more relevant over time. Recent data has shown that the previous strong correlation of the LCI with structural lung disease on CT in the pre-modulator era [60, 61], remains present when focusing solely on those with mild disease [62]. MBW also holds prognostic utility within the preschool age for prediction of later abnormal spirometry [46, 63, 64] and structural lung disease [64].

$S_{n_{III}}$ analysis in CF subjects shows a pattern of marked conducting airway abnormality (reflecting the “patchy” distribution of disease) evident from preschool age, and increased involvement of more distal airways over time [35]. Modelling has shown that a major determinant of raised LCI values is ventilation heterogeneity generated between convection-dependent units [65]. More severe disease required an adaption of $S_{n_{III}}$ -derived indices because, as predicted by modelling, $S_{n_{III}}$ levels off in case of extremely slow lung compartments which can be avoided if S_{cond} is determined in an earlier portion of the MBW. In mild disease, the prevalence of abnormality appears to be greater for S_{cond} than the LCI [62]; however, a number of practical challenges preclude clinical integration, and $S_{n_{III}}$ analysis remains largely confined to research studies [66].

Finally, reproducibility and sensitivity of MBW indices has been assessed in adult bronchiectasis [67], and the LCI was shown to correlate to the number of lung segments affected by bronchiectasis [68].

Asthma

Peripheral airway involvement in asthma is well documented in *post mortem* studies [69, 70]. Increased overall ventilation heterogeneity is a consistent finding in asthma, compared with controls, across both paediatric [71, 72] and adult [73] subjects. This arises predominantly within conducting airways, as evidenced by the MBW-derived elevated S_{cond} and the SBW-derived relative He *versus* SF₆ phase III slope changes that are seen post airway challenge [74].

S_{cond} was also shown to correlate with airway hyperresponsiveness, independently of airway inflammation [75], where it is important to point out that S_{cond} can represent conductive airways covering a wide range of bronchial generations. A recent structure–function study in which S_{cond} was simulated using CT-based geometry from adults with predominantly mild-to-moderate asthma, identified the proximal bronchioles as the site of the airway tree responsible for this S_{cond} elevation [76]. In independent modelling work, proximal airway involvement generated an avalanche-type flow redistribution compatible with the patterns of ventilation defects observed by nitrogen-13 PET imaging in asthma [77]. Elsewhere, it was shown that both S_{cond} and the LCI correlated with the degree of ventilation defects from hyperpolarised He MRI and the total airway count from CT imaging in patients with asthma of varying severity [78]. Interestingly, evidence of S_{cond} elevation is also seen as early as preschool age in those with more frequent wheeze episodes [79]; the fact that it is not reversed with bronchodilator use suggests that structural changes with airway remodelling may already be present at this stage. However, the prognostic role of phase III slope derived indices in the early prediction of persistent asthma remains unclear. In older subjects, the SBW phase III slope differentiated adult asthmatics with frequent exacerbations from those with stable asthma [80].

In a normal spirometry setting, pathological levels of gas trapping have been described in both children and adolescent asthmatics, suggesting peripheral airway involvement [81]. Increased gas trapping in the supine position (compared to sitting) may be a contributing mechanism to nocturnal worsening of asthma symptoms [71]. In severe asthma, S_{acin} is elevated [82] and correlates with peripheral eosinophilic inflammation [83].

Improvements in S_{acin} , without a change in S_{cond} , have been demonstrated in an adult asthma cohort with a change in preventer medication to an extra-fine particle inhaled corticosteroid [84]. Patients with abnormal S_{acin} had a lower FEV₁ than those with a normal S_{acin} (62% *versus* 90% predicted FEV₁). This suggests that therapeutic targeting using extra-fine particle medication may be beneficial in asthmatics with a more peripheral airway involvement. The recent ATLANTIS study (AssessmentT of smallL Airways involmeNT In aSthma) was designed to identify the optimal combination of physiological and imaging variables to best measure the presence and extent of peripheral airway involvement in adult asthma. It found that MBNW S_{NIII} indices correlated with asthma control and exacerbations on univariate but not multivariate analysis [85].

COPD

COPD has evolved beyond the traditional concept of a self-inflicted disease caused by tobacco smoking to include a number of other risk factors and distinct pathophysiological mechanisms, as summarised in a recent *Lancet* commission [86]. Within the *Lancet* paper, “nitrogen washout” was mentioned as a lung function test of interest in COPD identification. Its true utility may lie in detecting the pre-COPD phase proposed within this commission.

In smoking-related lung damage, N₂ SBW-derived phase III slope abnormality was originally suggested as a sensitive diagnostic tool [87], and the phase III slope difference between SF₆ and He appears even more sensitive to these effects [88]. When comparing MBW findings in adult smokers

with differing pack-year histories (10–40 pack-years), abnormalities in S_{cond} and S_{acin} were detectable as early as 10–20 pack-years [89]. As pack-year history increased, a disproportionate rise in S_{acin} was observed relative to S_{cond} . Smoking cessation maintained over a 12-month period, in a separate cohort, reduced S_{cond} values by 30% in the first week and by 42% at 1 year. No improvements in S_{acin} were detected [90]. Taken together, this suggests significant peripheral airway impairment, involving both small conductive and acinar airways with smoking-related lung. It also suggests that in the early stages, sustained improvements in the dysfunction of conducting airways are achievable through smoking cessation. However, even at this relatively early stage, the detectable abnormality in the acinar region appears to be irreversible. Whether the same pattern of impairment is observed within other COPD phenotypes is currently unclear, but is likely that they are influenced by the underlying pathophysiological mechanisms.

Relatively few studies have been conducted in COPD to date. In COPD patients with a mean FEV_1 of $60 \pm 18\%$ pred and long smoking histories, pronounced abnormalities in both S_{cond} and S_{acin} were observed [91]. S_{acin} correlated with diffusion capacity, which is also a reflection of distal acinar lung function, whilst S_{cond} correlated with more proximal airway function markers, such as FEV_1 , maximal expiratory flow at 25% and specific airway resistance. The FEV_1/FVC ratio correlated with both Sn_{III} indices. In a separate study, 6-week treatment with tiotropium led to improvements in FEV_1 and inspiratory capacity, without evidence of an improvement in phase III slope indices [92]; this may provide information about the site of action of tiotropium in this disease group.

Other conditions

Bronchiolitis obliterans (BO) is an obstructive lung disease that affects the peripheral airways and can occur after lung or haematopoietic stem cell transplantation (HSCT). Non-histological diagnosis is based on the (spirometry-focused) physiological and imaging criteria for BO syndrome (BOS). When comparing the SBW He phase III slope to exhaled biomarkers (CO and nitric oxide) in 65 post-lung transplant patients [93], the He phase III slope had the best sensitivity for early BOS (stage 0-p and stage 1), with elevation in the He phase III slope occurring >20 months before the corresponding decrease in FEV_1 [94]. Nonspecific bronchial reactivity is a known risk factor for subsequent BOS, and in these subjects, a disproportionately greater increase in the SBW He phase III slope post methacholine has been reported, *versus* the SF_6 phase III slope, not observed in controls with nonspecific bronchial reactivity [95]. MBW studies have reported elevated S_{acin} and S_{cond} in adult BOS subjects [96]. The use of MBW data in detecting BO/BOS in adult and paediatric post-bone marrow transplant subjects was recently summarised in a scoping review [97]. The review outlined the following themes: detectable peripheral airway abnormality in MBW occurred in a substantial proportion of subjects prior to HSCT; MBW indices post-HSCT were more frequently abnormal than spirometry when reporting group data, and among those with extrapulmonary chronic graft-*versus*-host disease and pulmonary involvement; and changes in MBW indices over time may be more indicative of pulmonary complications than absolute values at any given time-point.

Limitations of the technique

There are two important limitations of MBW tests. First, the total test visit duration becomes prohibitive in more severe disease. With increasing disease severity, the duration of washout (or washin) increases, as does the time taken for washin (or washout) between tests. Current consensus recommendations outline that three trials are required for Sn_{III} indices calculation. Whilst it has been suggested that two trials could provide an estimate of the overall LCI [98], equivalent data is lacking for Sn_{III} analysis. Another way to shorten the MBW test is by

adjusting the end-test threshold to an earlier point (e.g. 1/20th versus 1/40th of the starting tracer gas concentration). Theoretically, adoption of an earlier point may reduce sensitivity to detect the contribution of lung units with more severe impairments of ventilation distribution, since these only become apparent at the tail end of the MBW test. However, if the objective is just to quantify the acinar contribution to ventilation heterogeneity, one may merely use the first MBW expiration (i.e. SBW) to obtain a S_{acin} or its surrogate alveolar mixing efficiency [99]. In this case, the only reason to consider additional breaths, instead of just one, is to obtain a better estimate of FRC.

A second limitation of MBW testing again pertains to more severe disease, where the prevalence of non-ventilated lung regions increases, as evidenced by the increasing proportion of ventilation defects on novel imaging methods [100]. These non-ventilated lung regions do not participate during tidal breathing MBW, and therefore any reported MBW indices may underestimate the true extent of underlying disease. Interventions that recruit these more diseased lung regions (e.g. physiotherapy, intravenous antibiotics) may result in a paradoxical increase in the ventilation heterogeneity present [101]. Additions to the conventional MBW procedure have been proposed to quantify these non-communicating (or potentially very poorly communicating) regions of the lung [102]. Because these limitations of MBW testing are prohibitive to patients with severe lung disease, the main clinical utility of the standard MBW test probably lies in mild obstructive lung disease.

Conclusion

Strong feasibility across the age range, advancements in technique standardisation and the availability of robust commercial equipment requiring only medical air and O₂ gas access have led to a resurgence of interest in inert gas washout as a technique to detect and monitor peripheral lung disease. Evidence to outline clinical utility is at varying stages across a number of important obstructive lung diseases. Understanding the principles behind SBW and MBW versions of the technique is important when deciding how best to use the tool both in the research setting and clinically. Updated technical standards and clinical practice guidelines will further aid this integration into routine clinical care.

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Recent advances in respiratory muscle physiology and assessment

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Recent advances in different diagnostic and imaging techniques have provided new insight into respiratory muscle physiology and pathophysiology, the act of breathing and the limiting factors produced by different diseases <https://bit.ly/ERSM107>

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Recent advances in respiratory muscle physiology and assessment have enhanced our understanding of breathing mechanics, muscle adaptation and disease-related changes. New imaging techniques, including MRI and ultrasound, provide real-time insight into muscle structure and function. Optoelectronic plethysmography and electromyography have improved the analysis of respiratory muscle activation and coordination. Research highlights the impact of inactivity, ageing and chronic diseases like COPD on muscle performance, emphasising the importance of mitochondrial function and fibre composition. Studies on respiratory muscle fatigue and the metaboreflex reveal how breathing effort influences exercise tolerance and circulation. Emerging technologies, such as electrical impedance tomography and contrast-enhanced ultrasound, offer promising diagnostic and monitoring capabilities. These advancements are shaping personalised treatment strategies, improving disease management and optimising mechanical ventilation and rehabilitation approaches for individuals with respiratory conditions.

Respiratory muscle structure and function

Functionally, the respiratory muscles can be categorised into three groups: the diaphragm, the rib cage muscles and the abdominal muscles. These groups influence the chest wall and its compartments, namely the lung-apposed rib cage, the diaphragm-apposed rib cage and the abdomen (figure 1).

When the diaphragm contracts, it expands the abdomen and the lower part of the rib cage (abdominal rib cage). The rib cage muscles, including the intercostals, parasternals, scalenes and neck muscles, primarily affect the upper portion of the rib cage (the pulmonary rib cage) and play a role in both inspiration and expiration. In contrast, the abdominal muscles act on the abdomen and abdominal rib cage and function exclusively in expiration.

If one muscle group contracts in isolation or predominates over the others, undesirable effects, such as a “paradoxical” inward or outward motion during inspiration and expiration, may occur

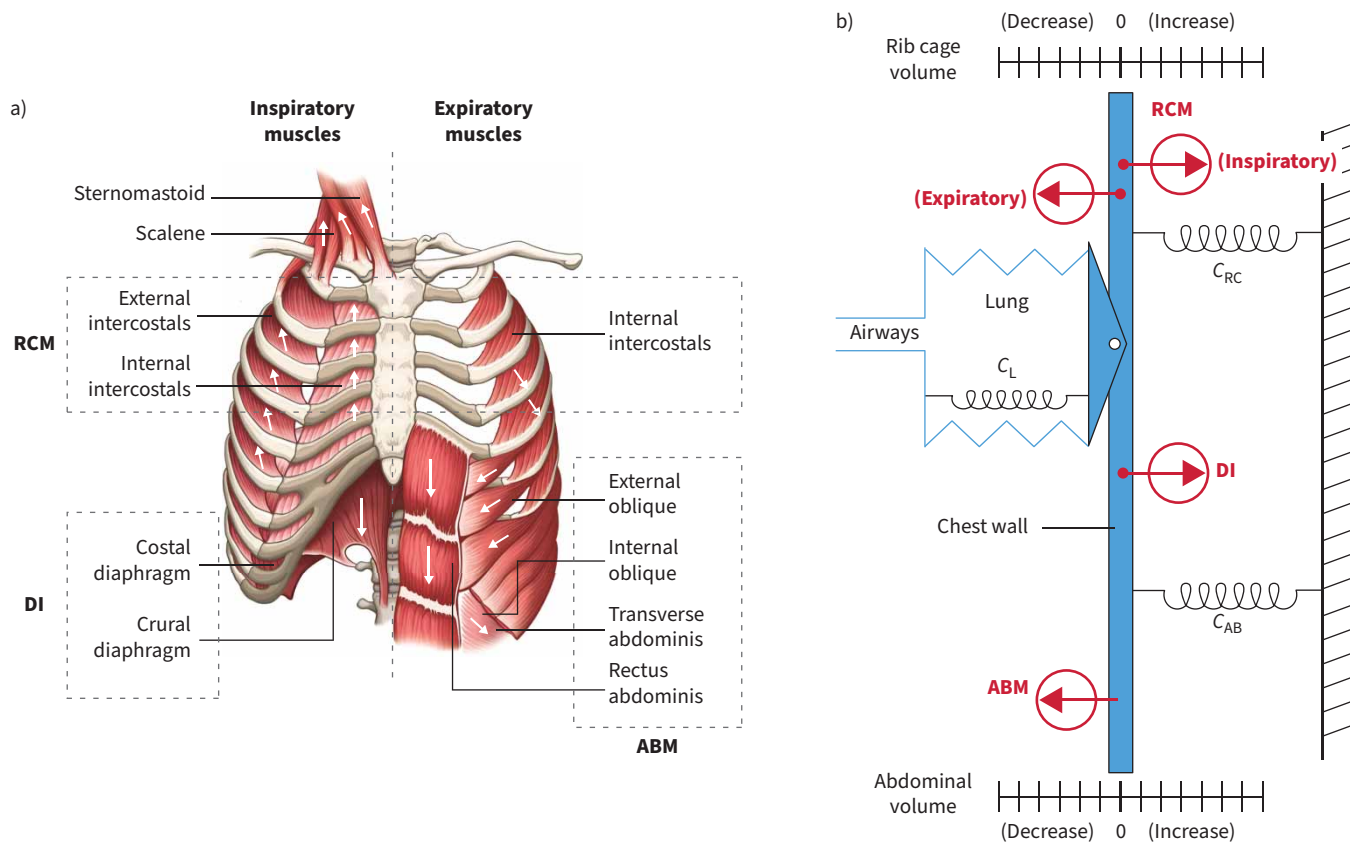


FIGURE 1 a) Schematic diagram of the respiratory muscles, divided into inspiratory and expiratory muscles, and grouped into rib cage muscles (RCM), diaphragm muscles (DI) and abdominal muscles (ABM). b) Simplified model of respiratory mechanics. Arrows and circles indicate the force (pressure) generators, *i.e.* the respiratory muscle groups, acting on the chest wall, on both its rib cage and abdominal part, and determining in rib cage and abdominal volume variations associated with lung volume variations. C_L : lung compliance; C_{RC} : rib cage compliance; C_{AB} : abdominal compliance. Reproduced and modified from [1] with permission.

in at least one compartment. To prevent such effects, a highly coordinated activation of two or all three muscle groups is essential. The term “dysfunctional breathing pattern” encompasses various patterns that deviate from normal breathing, including thoracic-dominant breathing (observed in asthma, COPD, heart failure and panic disorders), forced abdominal expiration (seen in COPD) and thoraco-abdominal asynchrony (associated with obstruction, neuromuscular diseases and respiratory failure) [2].

The diaphragm muscle

The diaphragm muscle is the primary respiratory muscle in mammals, playing an essential role in breathing and supporting various airway-clearance behaviours. Neural control of the diaphragm involves motor units, each of which comprises a phrenic motor neuron and the diaphragm muscle fibres it innervates. These motor units are classified based on their mechanical and fatigue properties, as well as their fibre-type composition. The diaphragm’s motor units are categorised into: slow, fatigue-resistant units (type S); fast, fatigue-resistant units (type FR); and more fatigable fast units (type FInt and FF). Type S units comprise type I fibres expressing MyHCSlow, while type FR units contain type IIa fibres expressing MyHC2A. Type FInt and FF units, which are less active, consist of type IIx/IIb fibres expressing MyHC2X and MyHC2B isoforms in varying proportions. Type S and FR motor units are essential for ventilation, operating with a high duty cycle, while type FInt and FF units are primarily recruited during airway-clearance and straining behaviours. The energy demands of diaphragm muscle fibres are met by mitochondria, whose volume density (mitochondria volume density) varies across fibre types based on activation frequency. Slow-twitch fibres (type I) and fast, fatigue-resistant fibres (type IIa) have a higher mitochondria volume density, reflecting their frequent activation and endurance properties. In contrast, type IIx/IIb fibres exhibit the highest adenosine triphosphate consumption rates during activation but are recruited infrequently. Mitochondrial biogenesis and structural changes, such as fragmentation, adapt to altered energy demands, playing a critical role in diaphragm muscle plasticity.

Ageing and chronic conditions like COPD significantly affect the diaphragm. Ageing disproportionately impacts type IIx and IIb fibres, leading to a reduction in cross-sectional area and force generation. However, type I and IIa fibres remain relatively spared, maintaining their role in ventilatory behaviours. In COPD, fibre composition shifts from fast-twitch to slow-twitch fibres, an adaptive mechanism to sustain breathing under chronic respiratory load. While compensatory, this remodelling may contribute to respiratory insufficiency.

Inactivity (during mechanical ventilation or where there is spinal cord injury, for example) leads to marked changes in diaphragm muscle fibre properties [3]. Studies show that 2 weeks of diaphragm inactivity reduce mitochondrial volume density and respiratory capacity in type I and IIa fibres [4]. These fibres, being highly active and vital for breathing, are disproportionately affected, potentially underlying difficulties in weaning patients off ventilators. BROWN *et al.* [5] highlighted that mitochondrial fragmentation and reduced succinate dehydrogenase activity in type I and IIa fibres occur due to inactivity, demonstrating the plasticity of diaphragm muscle fibres.

The diaphragm exhibits remarkable adaptability in response to varying mechanical and energy demands. Exercise training, including aerobic and resistance regimen, has been shown to improve diaphragm muscle strength and endurance, even in individuals with chronic diseases. During exercise, the diaphragm shifts its role from primarily generating pressure to facilitating airflow by increasing shortening velocity (see below). These adaptations optimise its performance under increased ventilatory demand.

Advances in histological and imaging techniques, such as confocal imaging and *in situ* hybridisation, have enabled detailed exploration of diaphragm structure and function. These methods have revealed fibre-type distribution changes in conditions like neuromuscular diseases, underscoring the role of type I and IIa fibres in sustaining ventilation. They also provide insight into the molecular and structural mechanisms underlying diaphragm muscle weakness in diseases and where there is chronic inactivity [6–8].

The rib cage muscles

The intercostal muscles assist with rib cage expansion during respiration, with the external intercostals involved in inspiration and the internal intercostals involved in expiration. Recent research has highlighted variations in muscle fibre composition between the external and internal intercostals. These fibres, which are a mixture of type I and type II, allow for both endurance and rapid contraction, depending on the respiratory demands. Studies like those by WEBER *et al.* [9] and JONES *et al.* [10] have demonstrated that intercostal muscles play an increasingly vital role in compensatory mechanisms during respiratory distress, such as in obstructive airway conditions. CT and ultrasonography studies over the past decade have shown that the intercostal muscles adapt to various respiratory conditions, such as COPD, often undergoing hypertrophy or atrophy depending on the chronicity and severity of the disease [11–13]. Additional studies have explored the synergistic roles of intercostal muscles with accessory muscles, such as the scalenes and sternocleidomastoids, in patients with restrictive lung diseases, further highlighting the importance of these muscles in maintaining adequate ventilation [14].

The abdominal muscles

The abdominal expiratory muscles consist of the transversus abdominis, internal oblique, external oblique and rectus abdominis. These muscles form the abdominal wall and, together with some rib cage muscles (*e.g.* the internal intercostals), constitute a vital part of the respiratory muscle pump. Under normal physiological conditions, the abdominal expiratory muscles remain largely inactive during tidal breathing. However, they become active during forceful expiration and coughing, or under conditions of high respiratory load. Their primary function is to increase intra-abdominal pressure, which pushes the diaphragm upward, thereby aiding lung deflation. During heightened respiratory demand – such as exercise, respiratory distress or mechanical ventilation – the abdominal muscles are recruited in a hierarchical manner [15]. Activation begins with the transversus abdominis, followed by the internal oblique, external oblique and finally the rectus abdominis. This recruitment reduces end-expiratory lung volume, enhances diaphragm efficiency by improving its length–tension relationship, and stores elastic energy within the respiratory system, facilitating the next inspiratory effort.

In critically ill patients, expiratory muscle activation is often seen as a result of increased respiratory load. For instance, during ventilator weaning, abdominal muscles are engaged to counterbalance inspiratory muscle fatigue and prevent dynamic hyperinflation. These muscles also significantly contribute to airway clearance through cough generation, producing the high intra-thoracic pressures necessary for effective sputum expulsion. Weakness of the abdominal expiratory muscles is common in critically ill patients and is associated with worse outcome, including prolonged mechanical ventilation, impaired cough strength, and increased risk of pneumonia and atelectasis. Conditions like sepsis and mechanical ventilation contribute to muscle atrophy and weakness [16, 17]. Dysfunction can exacerbate respiratory failure due to inadequate expiratory flow, airway collapse and poor airway clearance.

Respiratory muscle action and control at rest and during exercise

During quiet breathing in resting conditions, the diaphragm is the primary muscle generating inspiratory force, with limited contributions from the rib cage and abdominal muscles. Abdominal muscles are mostly inactive, allowing passive abdominal motion during expiration. The diaphragm generates pressure to expand the rib cage and lower abdominal contents, maintaining tidal volume with minimal effort. Inspiratory rib cage muscles also contribute but at a lower intensity compared with the diaphragm.

As exercise intensity increases, there is a significant shift in the pattern of respiratory muscle recruitment. The diaphragm transitions from a force generator at rest to primarily a flow generator during exercise [18]. There is a marked increase in diaphragmatic power, driven mainly by an increase in its velocity of shortening rather than pressure development. Diaphragm shortening is coordinated to optimise its force–length relationship and maintain efficient ventilation. Inspiratory and expiratory rib cage muscles are recruited early during exercise and progressively increase their activity with exercise intensity. The inspiratory rib cage muscles displace the rib cage to expand lung volume during inhalation. Expiratory rib cage muscles help maintain chest wall stability and assist in active expiration during higher workloads. Abdominal muscles are immediately recruited at the onset of exercise and progressively generate higher pressures as workload increases. These muscles contribute to active expiration by reducing end-expiratory lung volume, thereby optimising the diaphragm's length for the subsequent inspiration. During inspiration, the gradual relaxation of the abdominal muscles minimises transdiaphragmatic pressure (P_{di}), allowing the diaphragm to focus on generating airflow rather than pressure. The coordination of the diaphragm, rib cage and abdominal muscles minimises rib cage distortion and ensures efficient ventilation. The abdominal muscles counteract the impact of increased intra-abdominal pressure, while rib cage muscles stabilise the chest wall. This synergy allows for increased tidal volume and ventilatory capacity during exercise.

These findings were recently confirmed and extended by KIPP *et al.* [19], who measured work of breathing (WOB) during cycling and running using optoelectronic plethysmography (OEP) partitioned into contributions from the rib cage and abdomen based on their respective pressure–volume relationships. The study demonstrated that the diaphragm, rib cage and abdominal muscles contribute distinctively to WOB across different exercise intensities. At rest, WOB is minimal, with the diaphragm performing most of the inspiratory work. The rib cage contributes 83% to chest wall work, while the abdominal component contributes 17%. As exercise intensity increases, the total WOB rises significantly, with contributions from both the rib cage and abdomen increasing proportionally. Chest wall work increased nearly four-fold from rest to maximal cycling, with the rib cage contributing ~74% and abdomen contributing ~26% at peak intensity. Abdominal work increased by 84%, reflecting the greater reliance on active expiration at higher ventilatory demands. This was driven by the abdominal muscles assisting in reducing end-expiratory lung volume and optimising diaphragmatic length for inspiration.

When comparing cycling to running exercise, at matched ventilatory outputs, the work of the abdomen was significantly higher during cycling, suggesting greater abdominal muscle recruitment for ventilation in cycling. The rib cage's contribution remained similar across modalities, but running required a higher breathing frequency and a shorter inspiratory time, probably due to the mechanical constraints imposed by dynamic posture when running.

Respiratory muscles and expiratory flow limitation

In order to simulate the expiratory flow limitation (EFL) on respiratory muscle function and chest wall dynamics during exercise, several studies applied a Starling resistor to healthy

subjects, producing externally applied EFL (EFLe) [20–22]. EFLe reduced the shortening velocity of expiratory muscles, increasing expiratory muscle pressures, particularly in the abdominal muscles. This resulted in elevated abdominal pressures, creating a load that persisted into the inspiratory phase. Despite the reduction in shortening velocity, abdominal muscle power increased significantly due to higher force generation. This was attributed to central drive augmentation, probably driven by hypercapnia. The diaphragm contracted before inspiration began, working against the positive expiratory pressures imposed by EFLe. This pre-inspiratory diaphragmatic contraction increased P_{di} and contributed to inspiratory effort. Inspiratory rib cage muscles also exhibited increased activity, compensating for the additional mechanical load. EFLe altered the dynamics of chest wall compartments. The abdominal component's contribution decreased, while the rib cage component was tasked with maintaining ventilation. The rib cage–abdomen coupling was disrupted, affecting chest wall mechanics and requiring compensatory efforts from inspiratory muscles to stabilise breathing. Elevated end-tidal CO_2 levels were observed, indicating hypercapnia. This condition drove an increase in central respiratory drive, exacerbating the demand on respiratory muscles. Hypercapnia contributed to a “vicious cycle,” where increased expiratory muscle pressures further intensified central drive, reinforcing the respiratory effort required during EFLe. EFLe limited exercise performance due to severe dyspnoea and increased respiratory effort. The additional load on both inspiratory and expiratory muscles significantly impacted ventilatory efficiency. Dynamic hyperinflation was not a prominent feature but occurred in some individuals, adding to the inspiratory muscle burden.

A more recent study used double body plethysmography (DBP) (discussed further below) to investigate the impact of EFLe not only on respiratory muscle function and chest wall dynamics, but also on blood shifts during submaximal exercise in healthy individuals [23]. EFLe induced significant changes in respiratory mechanics, characterised by prolonged inspiratory and expiratory times compared with unrestricted breathing. Inspiratory pressures were lower, while expiratory pressures were higher under EFLe, leading to greater intrathoracic and intra-abdominal pressure swings. The diaphragm and abdominal muscles exhibited increased activity to maintain ventilation against the added resistance. These compensatory mechanisms optimised airflow but required higher respiratory muscle effort. Blood shifts between the trunk and extremities were significantly affected by EFLe. Intra-breath blood displacement increased substantially compared with unrestricted breathing. Inspiratory intrathoracic pressure reductions facilitated venous return to the trunk, while expiratory abdominal pressure expelled blood toward the extremities. However, the inspiratory draw into the trunk exceeded the expiratory outflow, causing progressive pooling of blood in the trunk. The amplified respiratory pump effect observed under EFLe resulted from a dominant contribution of lower inspiratory pressures over higher expiratory pressures. This mechanism enhanced the redistribution of blood into the thoracic cavity. The progressive thoracic blood pooling could impact cardiac preload and output, which may have implications for exercise performance and tolerance in clinical populations, such as those with COPD.

Respiratory muscles and cough

SMITH *et al.* [24] demonstrated that the operating volume (*i.e.* the lung volume at the start of a cough) significantly affects cough mechanics. Larger operating volumes led to increases in trunk volume displacement, peak flow and expelled air. The study identified three key phases in a cough: inspiratory, compressive and expulsive. The expiratory flow rate and volume expelled were maximised at higher operating volumes, with minimal changes in intrathoracic and intra-abdominal pressures. During a cough peal, subsequent coughs exhibited reduced trunk volume changes and peak flows, reflecting the decreasing operating volume. A coordinated activity of

respiratory muscles was present. The diaphragm initiated inspiration, while abdominal muscles generated forceful expiratory pressures during the compressive phase. The rib cage and abdominal components worked synchronously to maintain airway clearance. Cough peals involved rapid, repetitive muscle contractions, emphasising the efficiency of expiratory muscles in sustaining airflow and volume expulsion under dynamic conditions.

LoMAURO and ALIVERTI [25] expanded on these findings by exploring the haemodynamic effects of coughing. They measured blood shifts using DBP (discussed further later). During the compressive phase of a cough, abdominal pressures displaced ~200 mL of blood from the thorax to the extremities. In the expulsive phase, blood shifts reached 700 mL at total lung capacity. These shifts reflect the combined thoracic and abdominal pump mechanisms, which enhance venous return and support systemic circulation during vigorous coughing.

Both studies emphasise that coughing is not merely a protective reflex but also has significant haemodynamic consequences. The ability to generate effective expiratory flows and blood shifts depends on the coordinated function of respiratory muscles and sufficient operating volume. These findings have implications for understanding respiratory mechanics in diseases affecting cough efficiency, such as COPD and neuromuscular disorders, as well as for optimising therapeutic interventions like respiratory muscle training.

Respiratory muscle fatigue

Respiratory muscle fatigue is defined as a reversible decrease in the ability of the muscles to generate force, occurring when the energy demand exceeds the available supply. Despite their high endurance capacity, respiratory muscles, such as the diaphragm, intercostals and abdominal muscles, are prone to fatigue under conditions of intense physical activity or high ventilatory demand. Respiratory muscle fatigue is influenced by several factors. Increased ventilatory demand during high-intensity exercise elevates the work of breathing, leading to cumulative fatigue [26, 27]. Intense exercise can create competition for blood flow between locomotor and respiratory muscles. This blood flow redistribution may result in insufficient O₂ delivery to the respiratory muscles, exacerbating fatigue. Fatiguing inspiratory and expiratory muscle work can trigger a metaboreflex, causing sympathetically mediated vasoconstriction in the limbs to prioritise blood flow to the respiratory muscles.

Fatigue of the respiratory muscles impairs their ability to maintain effective ventilation, leading to increased dyspnoea, reduced exercise capacity and premature termination of physical activities. Expiratory muscle fatigue, in particular, disrupts airway clearance and affects postural stability. Inspiratory muscle fatigue has been shown to reduce limb blood flow, hasten locomotor muscle fatigue and impair exercise tolerance.

Recent studies have provided valuable insights into the mechanisms underlying diaphragm fatigue and its implications for exercise performance and respiratory health. In a pivotal study published in 2010, VERGÈS *et al.* [28] examined the effects of acute hypoxia on respiratory muscle fatigue in healthy individuals. The research aimed to determine whether reduced blood oxygenation directly contributes to diaphragm and abdominal muscle fatigue, independent of increased ventilation. The study found that hypoxia significantly exacerbated fatigue in both the diaphragm and abdominal muscles compared with normoxia and hyperoxia. In particular, the reduction in P_{di} immediately after hyperpnoea was more pronounced in hypoxia than in normoxia or hyperoxia. These findings suggest that acute hypoxia directly increases respiratory muscle fatigue, which has implications for exercise performance at high altitudes and in hypoxaemic patients.

The same authors also explored the effects of respiratory muscle training on exercise-induced respiratory muscle fatigue [29, 30]. The results indicated that respiratory muscle training increased the fatigue resistance of both inspiratory and expiratory muscles during exercise. Notably, subjects who exhibited significant diaphragm or abdominal muscle fatigue before training showed reduced fatigue after the training period. This enhancement in respiratory muscle endurance suggests potential benefits for athletic performance and individuals with respiratory muscle weakness.

Respiratory muscle fatigue is a significant factor limiting performance during intense exercise. Fatigue activates the respiratory muscle metaboreflex, redirecting blood flow from the limb muscles to the respiratory muscles. This redistribution can exacerbate peripheral fatigue and impair performance. Understanding the interplay between respiratory and limb muscles is critical to the development of interventions to enhance exercise tolerance [27, 31, 32]. The respiratory muscle metaboreflex is a physiological mechanism wherein fatigue of the respiratory muscles, particularly during strenuous activities, triggers reflexive cardiovascular responses. This reflex aims to preserve respiratory muscle function by redistributing blood flow from the peripheral locomotor muscles to the respiratory muscles, thereby ensuring adequate O₂ delivery. In a study by SHEEL *et al.* [27], the induction of diaphragmatic fatigue through inspiratory resistive breathing led to a significant reduction in blood flow to resting limbs. This finding highlighted the body's prioritisation of respiratory muscle perfusion over limb muscles during respiratory stress. The study demonstrated that when the diaphragm becomes fatigued, the body compensates by diverting blood flow from inactive limbs to support the overworked respiratory muscles, ensuring their continued function.

Further research by DEMPSEY *et al.* [26] explored the broader implications of the respiratory muscle metaboreflex on exercise performance. They found that increased work of breathing during intense exercise can lead to a significant reduction in limb blood flow, contributing to earlier onset of muscle fatigue and limiting exercise capacity. This work emphasised the competitive relationship between respiratory and locomotor muscles for blood flow during high-intensity activities.

Studies have also shown that inspiratory muscle training can attenuate the effects of the respiratory muscle metaboreflex. WITT *et al.* [33] demonstrated that inspiratory muscle training reduced the cardiovascular responses typically associated with high levels of inspiratory work, such as increased heart rate and mean arterial pressure. This suggests that strengthening the respiratory muscles through targeted training can mitigate the reflexive reduction in limb blood flow during strenuous exercise, potentially enhancing overall exercise performance.

Recent developments in respiratory muscle assessment

Over the past few decades, significant methodological advancements have improved our understanding of respiratory muscle function, aiding in diagnosis, phenotyping and treatment assessment for respiratory symptoms and neuromuscular diseases. Key progress has been made in breathing mechanics, respiratory muscle neurophysiology and imaging techniques, such as ultrasound and OEP.

Table 1 presents a schematic summary of different methods and techniques, classified into invasive and noninvasive, and volitional and non-volitional.

Physiology, technology and diagnosis of respiratory conditions are further discussed in chapter 17 of this *Monograph* [35].

TABLE 1 A summary of different methods and techniques of respiratory muscle assessment

Invasivity, volitional/ non-volitional	Functional parameter	Measurement method(s)
Noninvasive, non-volitional	Thoraco-abdominal kinematics determined by respiratory muscle action	Magnetometer (diameter), respiratory inductive plethysmography (cross-sectional areas), optoelectronic plethysmography (total and compartmental chest wall volumes)
	Diaphragm shape and displacement, muscle composition and muscle thickness	MRI
	Displacement of dome (excursion), length of apposition zone, thickness and thickening of the diaphragm	Ultrasound imaging
Noninvasive, volitional	Electrical activity of the respiratory muscles at rest and during exercise	Transcutaneous surface electromyography with surface electrodes
	Maximal static inspiratory and expiratory pressures	Pressure transducers with mask (or mouthpiece)
	Sniff nasal inspiratory pressure	Pressure transducers with nostril plug
	Peak cough flow	Oronasal mask/mouthpiece connected to a pneumotachograph or peak flow meter
	Respiratory muscle-related mechanics of breathing	Pulmonary function testing apparatus (e.g. measurement of upright and supine vital capacity)
	Tension time index	Pressure transducer plus pneumotachograph with mask (or mouthpiece)
Invasive, non-volitional	Paradoxical breathing index ($\Delta P_{ga}/\Delta P_{di}$)	Oesophageal and gastric balloon catheters with pressure transducers
	Inspiratory muscle effort during exercise	Oesophageal and gastric balloon catheters with pressure transducers
	Respiratory muscle endurance	Oesophageal and gastric balloon catheters with pressure transducers
	Strength of the diaphragm (peak P_{di})	Oesophageal and gastric balloon catheters with pressure transducers plus transcranial magnetic stimulation
Invasive, volitional	3D shape of the diaphragm	Volumetric CT imaging
	Strength of the inspiratory muscles (peak P_{oes})	Oesophageal balloon catheter with pressure transducers during sniff manoeuvre
	Strength of the diaphragm (peak P_{di})	Oesophageal and gastric balloon catheters with pressure transducers during sniff manoeuvre
	Strength of the expiratory muscles (peak P_{ga})	Gastric balloon catheter with pressure transducers during cough

Δ : change; P_{ga} : expiratory pressure; P_{di} : transdiaphragmatic pressure; 3D: three-dimensional; P_{oes} : oesophageal pressure. Reproduced and modified from [34] with permission.

Techniques for the assessment of respiratory muscle strength and activation

Measuring respiratory muscle strength is critical for diagnosing and monitoring patients with respiratory muscle dysfunction. The following methods have been improved and refined in recent years [36].

Maximal inspiratory and expiratory pressures

Maximal inspiratory pressure and maximal expiratory pressure are standard measures of respiratory muscle strength. Advances in pressure measurement devices and normalisation techniques have made these tests more reliable and applicable to a wide range of populations. Studies by LANZA *et al.* [37] have shown that normative values for maximal inspiratory pressure and maximal expiratory pressure now include considerations for age, gender and body mass index, improving diagnostic accuracy in clinical settings.

P_{di}

Direct measurement of the P_{di} provides a precise assessment of diaphragm function. New catheter-based pressure measurement techniques have enhanced the accuracy of P_{di} measurements. This technique is particularly useful in intensive care units, where monitoring respiratory muscle function is critical for managing ventilator weaning and extubation [38].

Sustained maximal inspiratory effort

Sustained maximal inspiratory effort testing is commonly used to assess respiratory muscle endurance. Advances in this test have focused on developing protocols that account for variables such as age and comorbidities, ensuring more accurate and standardised measurements. Recent studies have shown that sustained maximal inspiratory effort testing correlates with clinical outcome in patients with chronic respiratory diseases, such as COPD and ILD [39].

Electromyography

Electromyography has long been used to study the electrical activity of the respiratory muscles. Recent advances in electromyography signal processing have allowed for more precise measurement of respiratory muscle activation patterns, even in patients with severe respiratory dysfunction. Surface electromyography is now routinely used in research and clinical settings to monitor the recruitment and fatigue of respiratory muscles in real-time [40].

Transcranial magnetic stimulation

Transcranial magnetic stimulation is a noninvasive method used to study neural control of the respiratory muscles. Advances in transcranial magnetic stimulation technology have enabled more precise mapping of motor cortex control over the respiratory muscles, providing insight into how the central nervous system adapts to changes in muscle strength or function. Transcranial magnetic stimulation has been instrumental in understanding respiratory dysfunction in diseases like stroke, amyotrophic lateral sclerosis and spinal cord injury [41].

Techniques for the assessment of respiratory muscle metabolism

Near-infrared spectroscopy is widely used to assess the oxidative capacity of the respiratory muscles. This technique allows for continuous monitoring of muscle oxygenation and mitochondrial function. Recent studies have demonstrated its utility in tracking respiratory muscle endurance and fatigue in real-time, particularly in patients with COPD and other chronic respiratory diseases.

Muscle biopsy continues to be a valuable tool for understanding the metabolic properties of respiratory muscles. Recent research has focused on the role of mitochondrial function in respiratory muscle performance, revealing that mitochondrial dysfunction plays a key role in muscle weakness in diseases like COPD [42] and muscular dystrophies [43].

Advances in exercise physiology and pathophysiology are further discussed in chapter 4 of this *Monograph* [44].

Imaging techniques for respiratory muscles

Recent advances in imaging techniques have greatly enhanced our understanding of respiratory muscle structure and function. These innovations allow for noninvasive, real-time assessment of muscle performance and adaptation (figure 2).

MRI

MRI offers high spatial resolution and can assess both the structure and function of respiratory muscles. Recent studies have employed MRI to measure diaphragm volume and thickness in both healthy individuals and those with respiratory conditions.

Using MRI, researchers have shown that diaphragm dysfunction in diseases like COPD is associated with changes in diaphragm architecture, including reduced thickness and volume, which contribute to respiratory muscle weakness and increased work of breathing [46, 47]. Dynamic MRI techniques have also enabled real-time assessment of diaphragm motion during respiration, revealing new insights into the mechanical efficiency of the respiratory muscles [48]. T1 and T2 mapping and Dixon MRI have allowed quantification of fat infiltration and muscle composition in neuromuscular disorders, providing critical data on disease progression and therapeutic effects [49, 50]. Dynamic imaging with MRI allows for detailed analysis of diaphragm mobility and chest wall motion, highlighting the effect of posture in healthy subjects [51] and describing compensatory mechanisms in conditions like critically ill patients [52, 53], like Pompe disease [54, 55] and Duchenne muscular dystrophy [50].

Recent innovations in functional MRI have provided insight into regional differences in respiratory muscle activity during breathing and exercise. Functional MRI can now map blood flow and O₂ utilisation, revealing important metabolic information and detecting subtle changes in muscle performance that occur with disease progression or therapeutic interventions. Functional MRI and positron emission tomography have revealed reduced perfusion and impaired muscle function in diseases such as COPD. Exercise studies highlight increased perfusion with aerobic activity, linking metabolic demand to blood flow regulation [56]. Microvascular adaptations supporting perfusion and the potential of vasodilators to enhance blood flow and reduce fatigue in chronic diseases are noteworthy [57].

Ultrasound

Ultrasound has emerged as a reliable, noninvasive tool for assessing the thickness and movement of respiratory muscles, especially the diaphragm [58–60]. Recent studies confirmed the utility of ultrasound for monitoring diaphragm function in critically ill patients [61], and showed correlation with weaning success from mechanical ventilation [62–65]. The technique has also been used to assess diaphragm atrophy and dysfunction in conditions like COPD [66] and sepsis [67].

Advanced ultrasound techniques, such as speckle tracking and ultrafast shear wave elastography, have further enhanced the ability to measure tissue deformation and stiffness, providing insight into contractility and respiratory muscle health [68]. Ultrasound imaging has also been integrated with real-time motion tracking systems to evaluate dynamic changes in muscle activity during both voluntary and non-voluntary breathing. These advancements provide a comprehensive view of muscle coordination, which is especially valuable for assessing conditions like diaphragmatic paralysis and COPD [69, 70].

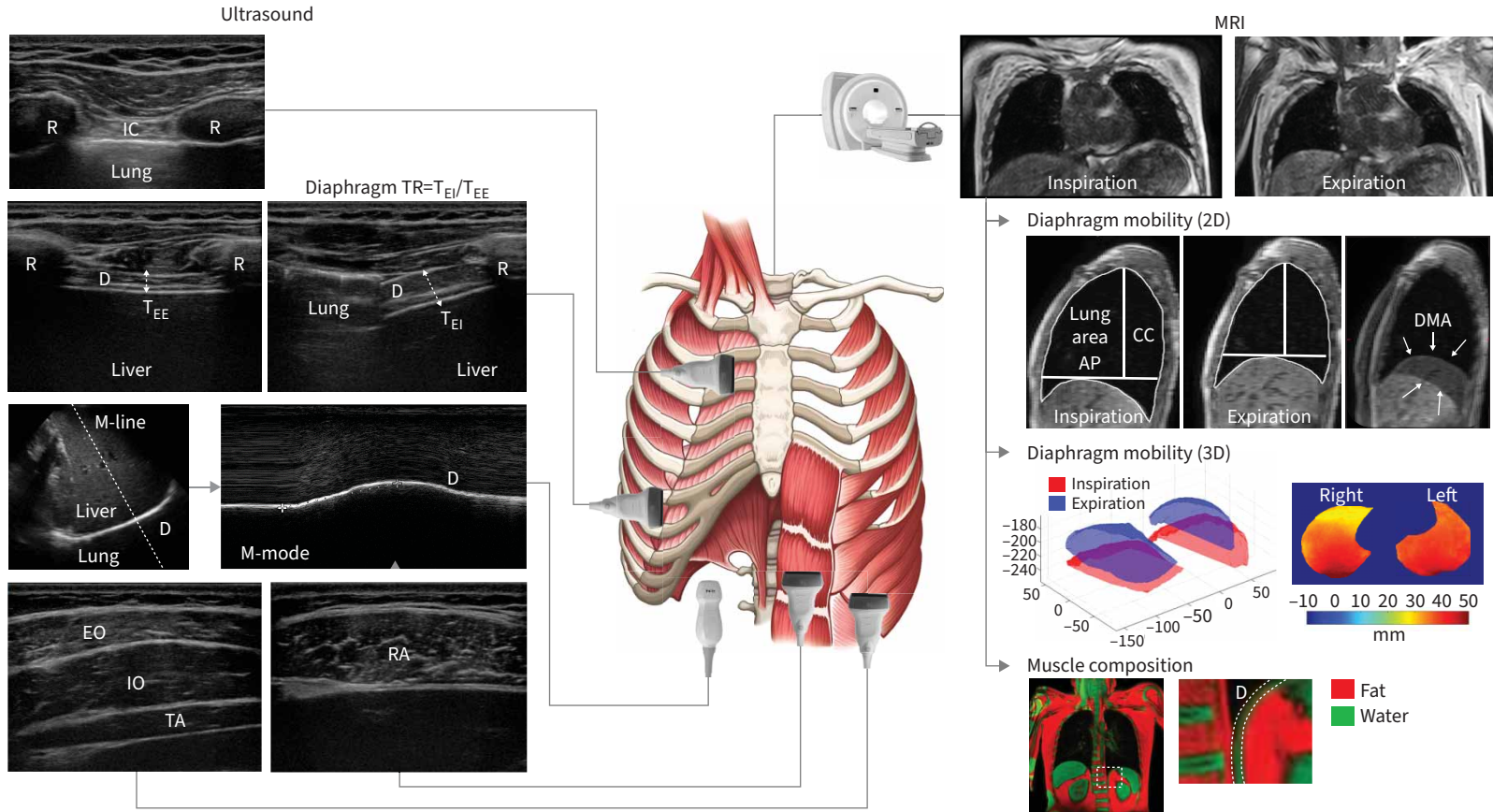


FIGURE 2 Overview of currently used respiratory muscle imaging techniques: ultrasound and MRI. R: rib; IC: intercostal muscles; TR: thickening ratio; T_{EI} : end-inspiratory thickness; T_{EE} : end-expiratory thickness; D: diaphragm; EO: external oblique muscle; IO: internal oblique muscle; TA: transverse abdominal muscle; RA: rectus abdominis muscle; 2D: two-dimensional; CC: cranio-caudal; AP: antero-posterior; DMA: diaphragmatic movement area; 3D: three-dimensional. Reproduced and modified from [45] with permission.

Portable ultrasound systems have expanded the use of this technology to outpatient and bedside settings, increasing accessibility to clinical assessment and follow-up monitoring [36].

Emerging imaging modalities

In addition to established techniques, novel imaging modalities are being developed to provide new insight into respiratory muscle function. Techniques like electrical impedance tomography are gaining traction for their ability to map regional ventilation distribution and assess diaphragmatic activity in real-time. Electrical impedance tomography has been especially valuable in monitoring patients during mechanical ventilation, offering a noninvasive means of optimising ventilatory support and reducing the risk of lung injury. Another promising technology is contrast-enhanced ultrasound, which can assess microvascular perfusion in the respiratory muscles, providing a window into their metabolic demands and adaptations during exercise or disease progression.

OEP

OEP is an advanced technique used to measure dynamic movement of the chest wall and to quantify its volume change during breathing (figure 3) [71, 72]. This noninvasive method relies on optical motion analysis to track the three-dimensional (3D) movement of the thoracoabdominal surface.

The technique involves placing reflective markers on specific anatomical landmarks on the chest wall [73]. Infrared cameras detect the displacement of these markers during breathing, capturing detailed data on the geometry and volume of the chest wall. The reflective markers are strategically positioned along horizontal rows at key anatomical reference points, such as the clavicular line, nipples, xiphoid process and anterior superior iliac crest. Vertical alignment is

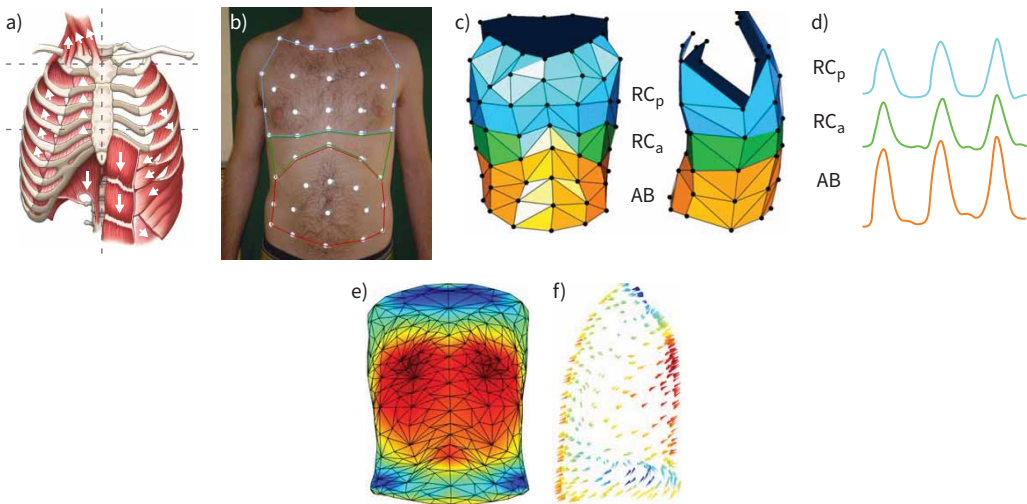


FIGURE 3 a) Respiratory muscles. b) Marker positioning on the anterior chest wall surface for optoelectronic plethysmography (OEP). c) Triangulation of thoraco-abdominal surface, *i.e.* connection of the three-dimensional coordinated markers to define chest wall surface and to calculate total and compartmental volumes using OEP. d) An example of volume variations of three chest wall compartments. e) Colourmap showing magnitude during spontaneous breathing at rest in a healthy subject. f) Vector showing the direction of trunk motion during spontaneous breathing at rest in a healthy subject. RC_p: pulmonary rib cage; RC_a: abdominal rib cage; AB: abdomen.

ensured through midline placement and other significant anatomical divisions. The recorded marker displacements are used to reconstruct a 3D mesh of the chest wall surface. The enclosed volume of the chest wall is calculated using Gauss's theorem, and changes in lung volume are inferred. The chest wall is divided into three main compartments: the pulmonary rib cage, the abdominal rib cage and the abdomen. This segmentation allows researchers to study the independent movements of these regions, which are influenced by different muscle groups and pressures during respiration.

OEP analysis has recently been further developed for the study of both global and local motion [74, 75]. The original anatomical markers placed on the chest wall are supplemented with virtual markers to complete the trunk's shape. Data is processed to create triangulation matrices and increase the mesh's precision. The method allows quantification of key geometrical parameters, such as trunk volumes, surface areas, perimeters and derived shape factors, like surface-to-volume ratios. Local motion is analysed through vector field computation, which provides a detailed view of the magnitude and direction of movement at individual points on the thoraco-abdominal surface. Heatmaps and vector field plots visually represent this data, with heatmaps showing motion magnitude through colour gradients, and vector field plots illustrating both magnitude and direction with colour-coded arrows. The approach enables intra-subject analysis (*e.g.* comparison of motion under different respiratory states) and inter-subject comparison (*e.g.* assessment of variation between genders or between healthy and pathological subjects).

OEP studies in healthy individuals have highlighted distinct kinematic patterns during different activities and conditions. During exercise, the end-expiratory lung volume decreases due to the recruitment of expiratory muscles. This reduction becomes more pronounced with increasing exercise intensity, with the abdominal compartment primarily contributing to tidal volume variations [18]. In phonation studies, OEP has demonstrated that expiration control involves all three chest wall compartments, showcasing the complexity of respiratory muscle coordination during speech and singing [76–78].

OEP has shown how posture significantly influences thoraco-abdominal kinematics. Increasing trunk inclination – from seated without back support to supine – reduces rib cage displacement, tidal volume and minute ventilation [79]. Simultaneously, abdominal contribution to tidal volume progressively increases. This shift highlights the role of gravitational forces and mechanical properties of the respiratory system. The rib cage's elastic properties contribute more in upright positions, while in supine position, increased abdominal compliance favours abdominal motion. Specific postural findings include reduced antero-posterior rib cage diameters and cross-sectional areas in the supine *versus* the upright position, and a significant increase in abdominal volume changes in the supine position, reflecting enhanced abdominal contributions to ventilation. Use of a back support during seated breathing increases abdominal contributions and decreases rib cage displacement, probably due to reduced abdominal compliance and altered diaphragmatic activity for postural stability.

Sex also significantly affects thoraco-abdominal kinematics. Males exhibit larger thoraco-abdominal dimensions (rib cage and abdomen) and greater tidal volumes during quiet breathing. Higher abdominal contributions to tidal volume across all postures reflect anatomical differences, such as larger lung and rib cage sizes, in males. In contrast, females display predominantly rib cage-based breathing patterns, with consistently smaller abdominal contributions, and significant variations in abdominal kinematics with posture changes, particularly in supine position, suggesting greater influence of gravity on abdominal compliance

in females. Normalised measurements for body weight eliminate differences in tidal volume and minute ventilation between genders, suggesting size as the primary driver of the observed differences.

OEP has provided invaluable insight into the respiratory mechanics of various diseases. It has revealed distinct patterns of dynamic hyperinflation and asynchronous chest wall motion in COPD, pulmonary fibrosis and CF [70, 80, 81]. It has also demonstrated that certain patients develop paradoxical movements in specific chest wall compartments during exercise [82]. In conditions like Duchenne muscular dystrophy, OEP has been used to identify early signs of diaphragm weakness [83] and to evaluate coughing efficiency [84]. Reduced abdominal contribution during breathing has been linked to respiratory impairment in these patients [85]. Post-surgical adaptations in chest wall kinematics, including changes in diaphragm positioning and abdominal volume, have been effectively assessed using OEP [86–89]. In mechanically ventilated patients, OEP has revealed differences in chest wall volume distribution under various ventilation modes [90, 91]. It also helps monitor patient–ventilator synchronisation, offering critical insight for the optimisation of care [92]. Respiratory kinematics is also strongly affected by the presence of obesity [93, 94].

OEP has undergone rigorous validation through comparison with spirometry and pneumotachography. These studies confirm its accuracy in measuring tidal volume and compartmental chest wall volume variations, with discrepancies consistently <5% [72, 90]. Recent applications of OEP in newborns and patients with severe respiratory diseases have further demonstrated its reliability across diverse populations [94–97].

DBP

OEP has been integrated with whole-body plethysmography (WBP) to study blood shifts between the trunk and extremities during breathing and exercise. This “double plethysmography” approach provides a comprehensive view of the interactions between respiratory and circulatory systems, shedding light on the diaphragm’s dual role as a ventilatory and circulatory pump [98].

WBP measures changes in overall body volume (ΔV_b), which occur due to lung ventilation and the compression or expansion of thoracic gas. Simultaneously, the OEP system uses infrared cameras to track the movement of reflective markers placed on the chest wall, allowing for the calculation of trunk volume changes (ΔV_{tr}). The difference between ΔV_b and ΔV_{tr} represents the volume of blood shifting (V_{bs}) between the trunk and the extremities. This relationship is expressed mathematically as $V_{bs} = \Delta V_{tr} - \Delta V_b$, isolating blood redistribution from other volume changes such as ventilation. During the procedure, participants are seated in a transparent WBP chamber equipped with a pneumotachograph for airflow measurement. The chamber is designed to control temperature and humidity, ensuring minimal signal drift caused by thermodynamic changes. The OEP system, with its 3D calibrated cameras, simultaneously captures chest wall movements by tracking 89 retro-reflective markers placed on the front and back of the torso. These combined measurements allow researchers to analyse the dynamics of blood redistribution in real time.

DBP has been applied in various contexts to study physiological responses, including exercise-induced blood shifts [23, 99] and the effects of EFLe. For example, research has shown that EFLe amplifies the respiratory pump mechanism, leading to increased intrabreath blood shifts and progressive blood pooling in the trunk. These findings highlight the significance of respiratory mechanics in venous return and circulatory function.

Conclusion

Recent advancements in respiratory muscle physiology and assessment have significantly expanded our understanding of how these muscles function in health and disease. The integration of new imaging techniques, metabolic analyses and neurophysiological tools has provided novel insight into muscle structure, function and adaptation. Moreover, the development of more accurate and noninvasive methods for assessing respiratory muscle strength, endurance and activation promises to enhance the diagnosis and management of respiratory diseases. As these technologies continue to evolve, they will undoubtedly lead to better patient care and improved outcomes for individuals suffering from chronic respiratory conditions.

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Breath analysis for health monitoring and clinical applications

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Breath composition mirrors real-time physio-metabolic processes in the human body by producing volatile organics. Its analysis offers opportunities to monitor pathophysiological processes, with potential applications in healthcare. <https://bit.ly/ERSM107>

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Breath composition can now be measured in real time, offering a dynamic and noninvasive view of physiological and biochemical processes occurring in the human body. However, despite many decades of research in the field, clinical use of breath analysis remains limited. Within the breath community, there is no consensus on the exact reasons, but the lack of standardised protocols for breath sampling and analysis, the influence of extrinsic and intrinsic confounders as well as misconceptions in appropriately designing and conducting studies are thought to play a major role. Here, we present a snapshot of the research field, starting with the rationale and origins of breath components, followed by a discussion of the state-of-the-art technologies for breath sampling and analysis, and concluding with present clinical applications, limitations and perspectives.

Introduction

Breath analysis has gained interest as a valuable noninvasive approach with wide-ranging applications in various medical domains. The chemical profiling of exhaled breath offers distinct advantages over traditional diagnostic methods such as blood tests or tissue biopsies. Unlike these invasive tools, breath analysis enables real-time and repeatable noninvasive monitoring of metabolic, physiological and pathophysiological states without placing any physical burden on the patient. For these reasons, it promises significant opportunities for early disease detection, monitoring disease progression, and assessing treatment efficacy.

Recent advancements in analytical techniques, most notably gas chromatography-mass spectrometry (GC/MS) and real-time mass spectrometry, have enhanced the precision and reliability of breath analysis. Emerging technologies, such as point-of-care (PoC) smart sensor techniques, offer the potential to expand the application of breath analysis to broader

multicentre applications once key marker compounds are identified. The clinical potential of this approach has been extensively explored in pilot investigations across diverse medical contexts, including respiratory diseases such as asthma and COPD, as well as metabolic disorders, infectious diseases and cardiovascular conditions. However, a fundamental understanding of the origin, distribution, metabolic transitions and exhalation pathways of most exhaled biomarker candidates remains insufficient, limiting further progress.

This chapter delves into the key technical challenges that currently limit the routine adoption of breath analysis in clinical practice. It reviews the state of the field, examines hurdles related to standardising sampling and analytical protocols, and evaluates the potential of breath biomarkers to advance personalised medicine. By showcasing recent technological innovations and identifying critical areas for future research, this chapter underscores the transformative potential of breath analysis as a noninvasive tool in personalised healthcare.

Origin and transport of breath biomarkers: exploring lung physiology and the blood–lung interface

The human exhalation consists of a mixture of permanent gases (*e.g.* N₂, O₂, CO₂, NO and CO), water, volatile organic compounds (VOCs; *e.g.* isoprene, pentane, dimethyl sulfide and acetone), and other typically nonvolatile, aerosol-bound substances (*e.g.* cytokines, isoprostanes, H₂O₂ and peroxynitrite). While the concentration of the major permanent gas, N₂, remains constant, O₂ is mainly taken up by haemoglobin in the red blood cells, transported into different body compartments through the vascular system and utilised by cells in metabolic processes [1]. This metabolic activity produces CO₂, which is transported back *via* the blood to the lungs, where CO₂ and O₂ are exchanged in the alveoli and exhaled in the alveolar breath fraction. Given the well-documented nature of these processes, both CO₂ and O₂ are routinely monitored in clinical settings. Peripheral O₂ saturation, which provides a straightforward indication of tissue oxygenation, is largely unaffected by the complexities of pulmonary gas exchange. By contrast, the estimation of arterial CO₂ pressure (P_{aCO_2}) through exhaled end-tidal CO₂ tension (P_{ETCO_2}) or transcutaneous CO₂ measurements is influenced by factors such as mixed venous blood composition, response time and the gradient of alveolar dead space gradients. Nevertheless, P_{ETCO_2} remains a reliable target for PoC devices determining clinically significant ventilatory changes, such as hyperventilation or hypoventilation.

Other exhaled components, such as NO, are predominantly generated in the airways or nasal passages [2]. Research has shown that NO synthesis occurs *via* three distinct isoforms of nitric oxide synthase, which are encoded by three genes: nitric oxide synthase 1, nitric oxide synthase 2A and nitric oxide synthase 3 [3]. In patients with asthma, exhaled NO (eNO) levels have been linked to airway inflammation and epithelial cell damage, primarily because of the activation of nitric oxide synthase 2A [4, 5]. This relationship enables the differentiation of asthma from other causes of chronic cough based on changes in eNO levels [6].

Technical standards for measuring NO in clinical settings are well-established [7], and equipment manufacturers are required to comply with these standards when marketing their products. Owing to the extensive knowledge on NO generation during airway inflammation and the availability of technical standards, fractional exhaled nitric oxide (F_{ENO}), which occurs in parts per billion by volume (ppbV, equivalent to nmol·L⁻¹), can be used for monitoring asthma and other pulmonary conditions like pulmonary hypertension. The US National Asthma Education and Prevention Programme updated its guidelines in 2020 to advocate for eNO monitoring of unstable asthma, while paediatricians find F_{ENO} measurement useful in children

who cannot perform spirometry. The relevance of F_{ENO} for therapeutic monitoring and treatment success in asthma (*e.g.* in children [8]) has been recently demonstrated in larger scale clinical trials [9, 10]. As a limitation to eNO, it is mainly generated locally in the airways, in contrast to most VOCs that are blood-borne and can provide insights into metabolic processes occurring throughout the entire body [11]. Although over 500 VOCs have been identified in human breath [12], the endogenous origins and metabolic pathways of many remain largely unclear and putative. Endogenous VOCs are thought to originate from various locations within the body, ranging from the proximal airways to internal organs, including contributions from the gut microbiome (figure 1). A significant proportion of exhaled VOCs, however, stem from exogenous sources, such as smoking, environmental contaminants, perfumes and plastic additives. These compounds can be absorbed through the lungs or skin, distributed across body compartments, and are subsequently exhaled minutes to months after exposure. For instance, smoking-related compounds such as acetonitrile, benzene or toluene can be detected in breath both immediately and weeks after inhalation. Similarly, fuel or combustion-related compounds have potential applications in biomonitoring for previous environmental exposures [15].

The origins and pathways of endogenous VOC biomarkers have been investigated only for a limited number of compounds. Even for a prominent breath constituent such as isoprene – an unsaturated branched hydrocarbon found in nearly everybody’s breath – recent investigations have disputed the longstanding assumptions about its origin, which had been accepted for more than four decades [16], and identified alternative sources *via* multi-omic investigations [17]. A thorough understanding of origins, pathways, and the physiological and metabolic factors influencing exhaled concentrations – such as haemodynamics, ventilation, endocrine status, and the dynamic behaviour of breath biomarkers – is essential to translate VOC profiling into reliable clinical applications. In addition, technical standards concerning the measurement of VOC profiles in clinical settings are still lacking.

The expectation that unique VOC biomarkers or profiles derived from cross-sectional, single-point measurements could serve as early and definitive indicators for diseases such as cancer, diabetes or organ failure has not been consistently validated in independent studies [18]. While pathophysiological processes such as inflammation or infection are known to alter the concentrations of certain VOCs (*e.g.* hydrocarbons or aldehydes), the fast concentration changes in exhaled VOC profiles often mirror a broad spectrum of overlapping and sometimes conflicting influences. These influences include normal physiological variations (*e.g.* changes in haemodynamics and ventilation), regular or disrupted metabolism, biomarker origins and distribution, medication or supplement intake, lifestyle habits and prior environmental exposures. Owing to the inherent “volatile” nature of these factors, VOC profiles are often unstable over extended periods and can exhibit rapid and significant changes – within seconds and by orders of magnitude [19]. While some VOC concentration changes occur over seconds, minutes or hours, others evolve over weeks, months or even across an individual’s lifetime. This variability underscores the importance of monitoring concentration changes over time, rather than relying on single-point absolute values, even in clinically established breath tests (see section entitled “Main clinical application and perspectives”) [14].

Breath sampling, collection and analysis

Breath phases

At rest, a typical respiratory cycle lasts ~5 s and involves the exchange of about 300–500 mL of air through the lungs, known as the tidal volume. This contributes to the total volume of air moved in and out of the lungs per minute, referred to as minute ventilation ($\dot{V}_E$), which

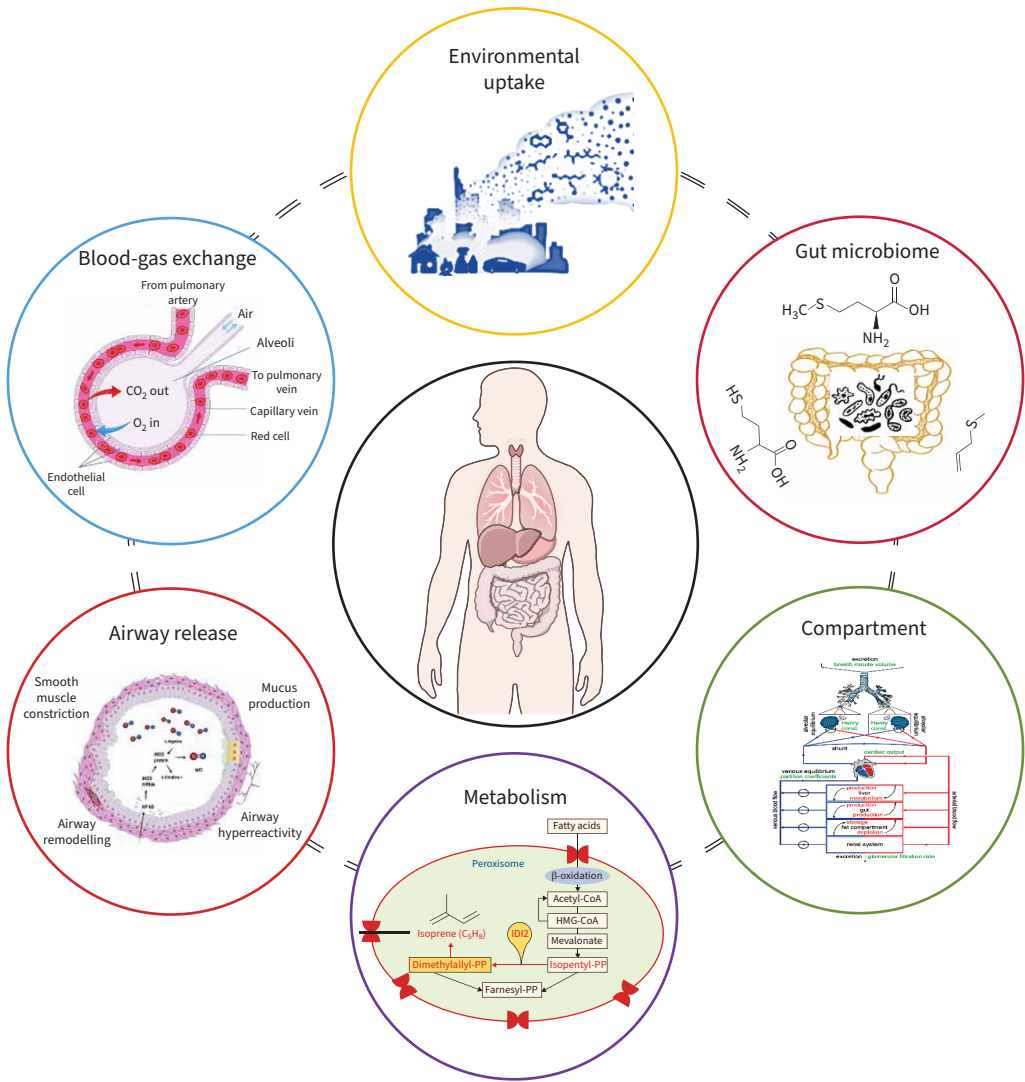


FIGURE 1 Overview of the main sources of volatile organic compounds (VOCs) from different body compartments. Light blue circle: VOC exchange of blood-borne compounds occurs at the alveolar phase. Brown circle: nitric oxide (NO) is generated and released in the airways. Purple circle: isoprene is produced *via* peroxisomal *ID12*-mediated skeletal-muscular lipolytic cholesterol metabolism. Green circle: generation, distribution and elimination of endogenous VOCs in the human body. Red circle: VOCs are generated from the microbiome in the gut and oral cavity. Yellow circle: VOCs are taken up from the environment. CoA: coenzyme A; HMG-CoA: 3-hydroxy-3-methylglutaryl CoA; NF- κ B: nuclear factor- κ B; iNOS: inhaled nitric oxide synthase. Reproduced and modified from [13] and [14] with permission.

averages around $6 \text{ L} \cdot \text{min}^{-1}$ [20]. The phases of the respiratory cycle can be noninvasively monitored by analysing the P_{aCO_2} in exhaled breath. At the start of exhalation, P_{aCO_2} remains close to ambient levels ($\sim 0.5 \text{ mmHg}$) because the initial portion of exhaled air originates from the anatomical dead space ($\sim 150 \text{ mL}$), where no gas exchange occurs (phase I). This is followed by a steep rise in P_{aCO_2} as the expelled air becomes a mixture of dead space air and alveolar air (phase II). The relatively flat portion of the waveform, known as the alveolar

plateau (~ 350 mL), indicates that most of the exhaled air is derived directly from the alveoli, where gas exchange predominantly occurs (phase III). This phase continues until the next inhalation, marked by a sharp downward slope in the waveform [7]. P_{ETCO_2} is defined as the maximum P_{aCO_2} level at the end of exhalation. Two characteristic angles, α and β , typically measuring approximately 110° and 90° in healthy individuals, define the transitions between these phases and provide valuable insights into the alveolar slope and the respiratory dynamics of respiratory gas exchange. Deviations from the typical square-shaped capnogram may indicate pathological conditions [21].

Sample collection is the most crucial step in breath analysis, as it directly affects the reliability and accuracy of the data. The principal goal of sampling procedures is to obtain a representative sample that accurately reflects the systemic fraction of the VOCs of interest, enabling reliable assessments of physiology, metabolism, pathophysiology and therapeutic responses. Breath sampling methods can be broadly categorised into mixed breath collection and end-tidal sampling, each tailored to meet specific research or clinical requirements.

Mixed breath collection involves the collection of the entire exhalation, including air from both the dead space and the alveolar fraction, whereas end-tidal sampling specifically targets the alveolar fraction involved in gas exchange [22]. Both samples can be obtained over one or more exhalations, with the last approach minimising issues resulting from dead-space-driven dilution and contaminations from the upper airways (including the oropharyngeal and nasopharyngeal cavities). For end-tidal sampling, real-time CO_2 and flow sensors can be used to identify and separate different phases of exhalation, allowing accurate collection of the systemic fraction of the exhaled VOCs [23]. Figure 2 shows a representative profile of P_{aCO_2} of a nominally healthy volunteer.

Samples are often collected into silco-steel canisters or polymeric bags, such as Tedlar [24] or Nalophan bags [25], before being transferred to extraction devices for instrumental analysis. Storing polymeric bags at 40°C facilitates water diffusion through the bag membranes, reducing relative humidity in the samples and minimising the loss of polar compounds, such as alcohols [24, 25]. However, challenges remain with the limited stability of VOCs [26], the release of chemicals from bag materials and the labour-intensive preparation protocols, including the cleaning step for Tedlar bags [27] and manual assembly for Nalophan bags [25].

To streamline the analytical workflow and address these limitations, breath samples can be directly collected into extraction devices such as sorbent tubes [28] or needle traps [15, 29–31]. These devices can be integrated into lab-made or commercially available samplers equipped with vacuum pumps, mass flow meters and electronic valves [32]. This approach improves the stability of VOCs by retaining analytes on sorbent materials, although some compounds, such as aldehydes, may require low storage temperatures to prevent oxidative degradation into carboxylic acids [33, 34]. Additionally, sorbent tubes and needle traps enable the addition of internal standards during sample collection, further improving the reliability of the analytical workflow [35, 36].

Physio-metabolic effectors during breath sampling

Minimising intra-individual physiological and metabolic effects during breath sampling is essential to ensure the accuracy of VOC measurements. These effects, if not properly managed, can easily override the pathophysiological changes of interest in VOC profiles [37]. Investigations in healthy adults using real-time mass spectrometry for continuous and breath-resolved end-tidal VOC analysis, alongside respiratory and haemodynamic monitoring, have identified subtle yet impactful physiological effectors. Although inseparable from normal physiology, these factors can be mitigated through well-defined protocols involving controlled

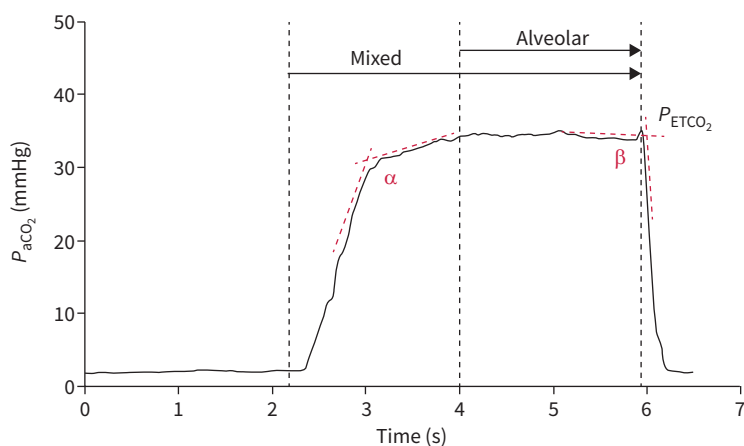


FIGURE 2 Typical arterial CO_2 pressure (P_{aCO_2}) values measured in exhaled breath during a single respiratory cycle. α and β are the characteristic angles typically measuring $\sim 110^\circ$ and $\sim 90^\circ$ in healthy individuals. Mixed breath refers to the collection of the entire exhalation while alveolar to the last portion when P_{aCO_2} reaches a plateau value. P_{ETCO_2} : end-tidal partial pressure of CO_2 .

breathing patterns [38], postures [39], breathing kinetics [40], breathing routes [41], upper-airway resistances against breathing [41] and respiratory rhythms [42, 43]. By adhering to such protocols, it is possible to achieve a steady state of exhaled end-tidal VOC concentrations, reducing the variability caused by physiological noise. For instance, spontaneously breathing subjects tend to hyperventilate when instructed to “breathe normally”, as the automatic, involuntary regulation of respiration is overridden by conscious effort. A customised respiratory rhythm has recently overcome such challenges and offered a state-of-the-art breath sampling protocol with minimal physiological interferences [42].

In addition to physiological effects, studies have shown that metabolic adaptations (*e.g.* diurnal response and food intake) [44, 45], endocrine status during menstrual cycle phases, intake of daily oral contraceptive pills [46], menopause, sexual orientations as well as the subject’s biological age [47] have profound effects on exhaled VOC profiles. Such metabolic denominators are extremely important to be recorded during sampling and afterwards considered for data interpretations.

Analytical tools in breath research

While real-time breath analysis in the clinic remains the ultimate goal, breath research has so far primarily relied on off-line analysis. A wide range of analytical chemistry instrumentation has supported this research, but GC/MS stands out as one of the most widely used technologies. Initially developed in the late 1950s and refined over subsequent decades, GC/MS has become a gold standard for the off-line chemical analysis of complex gas mixtures.

The GC/MS is a hyphenated analytical technique combining two instruments to identify and quantify trace chemical constituents. The gas chromatograph separates the mixture components by using a thin, sub-millimetre diameter capillary column, which is often several metres long and coiled for compact assembly. The column is coated internally with a thin film of chemically active material (stationary phase) that interacts differentially with the sample components based on chemical properties such as polarity and volatility. These molecular interactions make

mixture components travel through the column at varying speeds until separation is achieved. The column temperature is typically increased according to specific programmes to speed up measurements and enhance separation.

As chemicals sequentially exit the GC column, they are fragmented into ions by an electron beam and routed into the secondary MS instrument. There are several types of MS instruments that detect fragment ions in slightly different ways. In a quadrupole MS, ions are filtered based on their mass-to-charge ratio using an radio frequency electric field and quantified by an electrometer. Each molecule produces a specific pattern of ion abundances (mass spectrum) that allows its identification by comparison with a mass spectra database. Upon calibration with known chemical standards, the instrument can accurately quantify mixture components. Alternatively, a time-of-flight (TOF) mass spectrometer (TOF-MS) accelerates an ionised chemical in an electric field, and the velocity of the ion is proportional to its mass. A sophisticated system then measures the time needed to traverse a fixed distance, providing an accurate estimation of the ion mass. In this approach, the separation, which is needed not to confuse fragments originated from different chemicals, is the slow step (tenths of minutes). In summary, in the past 20 years, instruments have become commercially available for the real-time monitoring of multiple volatile chemicals in breath [48].

Ion mobility spectrometer

An ion mobility spectrometer (IMS) separates individual chemical species within complex mixtures based on their difference in mobility when subjected to varying electrical field strengths [49]. This separation is achieved by exploiting the unique movement trajectories of charged ions as they pass through a drift tube under a nonlinear electric field. IMS can also be coupled with GC to obtain a two-dimensional separation approach. In this setup, GC is used to pre-separate chemicals and enhance the resolution and detection capabilities of the mobility-based separation of IMS in the case of complex samples. A major advantage of IMS technology is that it can operate in real-time for rapid chemical detection, with high sensitivity and specificity. There are different IMS instruments available from major manufacturers, and several have been explored for breath biomarker detection [49].

Proton-transfer-reaction mass spectrometry

Proton-transfer-reaction (PTR) MS (PTR-MS) and PTR/TOF operate by introducing hydronium ions (H_3O^+), which transfer a proton to analytes in the gas phase with sufficient proton affinity. The resulting protonated analytes (molecular ions) are then directed into a coupled MS instrument for detection and analysis. Since a limited number of molecular ions are generated, mass spectra are easily interpreted. Furthermore, this approach makes the bottleneck of separation unnecessary, as it would be in the case of fragmentation, and allows real-time measurements. However, only analytes with a proton affinity higher than of water can be protonated, but such limitation is partially reduced with the possibility of using other reacting ions (e.g. NH_4^+ , NO^+ , O_2^+ and I^-).

Selected-ion flow-tube mass spectrometry

Selected-ion flow-tube mass spectrometry (SIFT-MS) is another tool for real-time breath analysis that shares similarities with PTR-MS but differs in its design. Unlike SIFT-MS, PTR-MS, does not incorporate a mass filter between the ion source and mass analyser. Instead, it directly introduces precursor ions into a flow tube, where they react with analyte molecules. Owing to the different pre-selection (mass filtering) ions, SIFT-MS has more defined primary ions while the amount in the PTR is higher but less controlled. Both instruments can work with

multiple precursor ions for an expanded variety of detected compounds. SIFT-MS has also been effectively used to analyse breath biomarkers [50]. Figure 3 shows an illustrative example of real-time breath analysis by SIFT-MS combined with CPET and exercise stress echocardiography.

Direct analysis in real time

Finally, direct analysis in real time (DART) is a further advanced ionisation technique that can be coupled with MS detectors for breath analysis. In this method, inert gases such as helium, argon or nitrogen are excited in a plasma source and used to transfer charge to analytes in complex gas phase reactions. These reactions can produce either positive or negative ion spectra, depending on the chemical nature of the analytes and the selected ionisation mode. This approach has recently been used to analyse breath biomarker compounds [51].

Translational technologies for clinical breath analysis

Methods for routine breath analysis in clinical settings must prioritise reliability, cost-effectiveness, ease of use and high throughput. In addition, they must comply with official regulations and be approved by the European Medicines Agency before they can be used in clinical practice. While GC/MS remains the gold standard for identifying breath biomarkers associated with pathology, its limited throughput and complexity make it unsuitable for routine use. Among current mass spectrometric technologies, PTR-TOF shows potential in this field, provided a dedicated user interface is developed to simplify its operation for specific tasks [52]. Although PTR-TOF systems require a relatively high initial investment, this cost could be offset by their high throughput, which enables low per-measurement costs when employed at scale.

Other emerging technologies are available or under development to enable fast, low-cost breath testing. For instance, sensors are compact devices that measure specific vapours or gases thanks to interactions between the analyte and the sensing material. Numerous sensor types exist for detecting concentrations in the range of 0.1–10 parts per million by volume (ppmV) [53]. This method is only suitable for detecting the most abundant breath components. However, the



FIGURE 3 Experimental session combining breath analysis by selected-ion flow-tube mass spectrometry with CPET and exercise stress echocardiography. In this setup, the CPET mask for O₂ and CO₂ analysis was connected to a custom 3D-printed polycarbonate adapter, enabling breath collection into a needle trap device for off-line analysis. The end-tidal breath fraction was selected based on CO₂ tension levels [24].

primary limitation is specificity, as sensors typically rely on reversible affinity interactions (*e.g.* van der Waals forces) to allow repeated measurements using the same device within a short timeframe. The high humidity of breath exacerbates these specificity challenges, as many sensing materials are sensitive to moisture.

Electronic noses (e-noses) utilise sensor arrays with partially overlapping sensitivities and pattern recognition algorithms to analyse breath samples [54]. Unlike traditional analytical devices, which identify and quantify specific components, e-noses produce multivariate fingerprints of samples. Classification is based on comparisons with a reference measurement database, requiring a comprehensive and well-balanced dataset. Effective training of the algorithm demands a sufficient number of representative examples – at least 5–10 times greater than the number of signal features – to avoid overfitting, *i.e.* a model that may fail to predict future observations reliably for corresponding too closely to a particular set of data. However, classification criteria are often opaque, and collecting enough data for training is labour intensive. Additionally, sensor signals are prone to drift over time, necessitating compensation strategies to maintain classification performance [55]. Despite advancements, e-noses remain insufficiently reliable for clinical applications.

In the past decade, advances in laser technology have enabled the development of new spectroscopic instruments [56]. In particular, photoacoustic spectroscopy detects pressure waves generated in a measurement chamber by the absorption of laser pulses by target analytes. The amplitude of the pressure fluctuations is proportional to the analyte concentration, while specificity is achieved through careful selection of absorption wavelengths in spectral regions with minimal interference [57]. These devices are particularly suited for detecting small molecules down to concentrations of a few ppbV and are more compact than other laser spectroscopic techniques. However, the high cost of lasers currently limits their feasibility for widespread PoC use.

Main clinical application and perspectives

Current clinical applications

Current clinical applied breath tests include procedures based on the main exhaled breath constituents such as CO₂ and O₂ as well as tests with external administered substances such as anaesthetic drugs and (isotopic labelled) substrates.

Noninvasive monitoring of O₂ and CO₂ has become a part of the clinical routine. Capnography is a method of monitoring the concentration or partial pressure of CO₂ in the exhaled breath against time. Capnographic monitoring is essential at all times in patients with tracheal tubes, supraglottic airway devices and those who are sedated to a level unresponsive to verbal commands. It is applied for confirmation of tracheal intubation, to assess tracheal tubes and tracheostomy patency and position, to monitor patients with raised intracranial pressure, to monitor response to treatment of bronchospasm, to confirm adequate ventilator support, to estimate the cardiac output and also during cardiac arrest and to measure breath-by-breath kinetics of CO₂ elimination.

The indirect calorimetry (IC) technique has been developed through the use of respiratory gas exchange measurements. By measuring the averages of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ from exhaled breath, IC is used to estimate the resting energy expenditure and the metabolic substrate utilisation in humans [57, 58]. Monitoring of inhaled and exhaled O₂ and exhaled CO₂ in combination with other parameters (heart rate, electrocardiogram, blood pressure, O₂ saturation, respiratory rate and

minute ventilation) is applied for CPET under exhaustive exercise on the ergometer. This test is often used to determine if a patient can tolerate surgery or lung resection or to define the cause of a patient's dyspnoea [59].

Apart from the ethanol breath test used for law enforcement, monitoring of anaesthetic drugs by a vapour analyser is essential during anaesthesia whenever a volatile anaesthetic agent or nitrous oxide is in use to avoid overdosing and/or awareness episodes. The end-tidal concentrations are documented on the anaesthetic records.

Substrate-based breath tests such as hydrogen breath tests are used for 1) the assessment of carbohydrate malabsorption of sugars such as lactose and fructose that are variably absorbed in the small bowel, 2) measurement of the time interval between ingestion of an absorbable carbohydrate, such as lactulose, and its contact with colonic bacteria in the cecum, and 3) contact of a test carbohydrate, such as glucose or lactulose, with abnormally high concentrations of bacteria in the small bowel. Measurement of H_2 excretion from tidal breath for the assessment of carbohydrate malabsorption was introduced into clinical practice in the 1970s and has been recommended and widely used for diagnostic purposes in adults and children. Additional measurement of CH_4 concentrations has been proposed to improve test accuracy, especially in patients who do not excrete measurable quantities of H_2 in breath.

The urea breath test is applied for the initial diagnosis of *Helicobacter pylori* and the evaluation of post-treatment eradication of infection. As urease is not present in mammalian cells, its presence indicates urease-containing microbes. *H. pylori* utilises urease to break down urea into ammonia and CO_2 . This CO_2 enters the bloodstream and is later exhaled via the lungs. Use of urea as a substrate with a labelled carbon (C) isotope, the exhaled CO_2 with the labelled C can be measured and quantified to diagnose *H. pylori*. Testing commonly uses two isotopes of carbon for the urea breath test: ^{14}C (a radioactive isotope) and ^{13}C (a naturally occurring, stable nonradioactive isotope). The performance of both tests is similar, with sensitivities >95% and specificities >93%.

Other officially approved methods

Recently, the analysis of exhaled NO has been approved for clinical testing. F_{ENO} is elevated in people with eosinophilic airway inflammation, typically found in asthma, and F_{ENO} has been linked to inflammation and oxidative stress in several chronic lung disorders. With a defined set of guidelines for sampling and measurement parameters, the F_{ENO} test represents the level of detail required of a standardised breath test [2]. This kind of test may be of special interest for patients with asthma because of home care settings or m-health applications. In the clinical routine these tests are, however, rarely used for diagnoses and monitoring as other, more specific or simpler methods to assess patient status exist.

The clinical deployment of exhaled VOC analyses has been limited to exploratory studies for biomarker discovery. Owing to the pure number of detectable breath VOCs, the effect of external confounders and the highly dynamic behaviour of exhaled VOC profiles, differences between single points cross-sectional measured test groups are mostly always detectable but often cannot be reproduced. Most breathomics studies have not included external validation, with fewer still reporting cross-site validation. Nevertheless, some VOC-based tests have also (temporarily) been approved for clinical use in the USA based on preliminary experimental data: 1) VOC markers of oxidative stress that can predict the probability of a grade 3 heart transplant rejection (Menssana US), 2) assessment of VOCs associated with tuberculosis infection to detect acute pulmonary TB (Menssana US) [60], and 3) COVID-19 detection VOC test [61].

Sadly, none of these approved tests have been applied or proved in clinical routine practice or in larger scale clinical studies to validate the preliminary experimental differences in VOC profiles described so far.

Breath VOCs and diseases: an elusive connection

To date, many pilot studies have been conducted to investigate the potential relationship between breath composition and a certain disease. Table 1 provides an overview of the most investigated VOCs and the potential link with diseases and pathophysiological conditions. The presence of various VOCs in exhaled breath reflects the complex interaction among metabolic, microbial and environmental factors. For example, ethane, pentane, pentanal and hexanal are products of specific metabolic pathways involving fatty acid oxidation. Similarly, cholesterol biosynthesis contributes to the presence of isoprene, while ethanol metabolism affects acetaldehyde and acetone levels. Protein metabolism also contributes to the release of methylamines in exhaled breath. Beyond metabolic processes, the gut and oral microbes play a

TABLE 1 Key volatile organic compounds (VOCs) investigated so far and their potential associations with diseases and pathophysiological conditions		
Exhaled VOC	Suggested origin	Linked disease/conditions
Ethane and pentane	Oxidative degradation of omega 3 and omega 6 fatty acids	Oxidative stress [62], ischaemia [63], lung disease [64]
Isoprene	Lipolytic cholesterol metabolisms	Cancer [65], ageing [66], exercise [67], compartments [68]
Benzene, xylenes and toluene	Smoking and environment	Exposure [69]
1,3-cyclopentadiene	Smoking	Smoking [70]
2-propanol	Disinfectant	Clinical exposure [71]
Ethanol	Metabolism	Intestinal and oral microbiota [43]
Formaldehyde	By-product of disinfectants and smoking	Cancer [72], environment [73]
Acetaldehyde	Ethanol metabolism	Metabolism [74]
Pentanal and hexanal	Oxidative degradation of fatty acids	Cancer [72]
Acetone	Energy metabolism	Diabetes [75], metabolic stress [76], heart failure [28, 77, 78]
Ethyl acetate	Metabolism	Bacterial metabolism [79]
Furan, 2,5-dimethyl	Smoking	Smoking [80]
Furan, 2-ethyl	Bacteria metabolism	Bacteria [70]
Methylamines (e.g. dimethylamine and trimethylamine)	Protein metabolism	Kidney disease [81]
Nitric oxide	Airway inflammation	Asthma [5, 6], COPD [82]
Formamide, N,N-dimethyl-amide and 2-methyl-1,3-dioxolane	Sampling bags	Contamination [24, 25]
Methyl nitrate	Metabolism	Diabetes [83]
Carbon disulfide, carbonyl sulfide, allyl-methyl-sulfide, methyl-propyl-sulfide	Metabolism	Coronary artery disease [84], organ rejection [85], oral contamination [86]
Dimethyl sulphide	Bacteria	Oral cavity [86], airways [42], methylation in gut [87]
1,2-dichloroethane and trichloroethene	Clinical environment	Contamination and exposure [88]
Reproduced from [89] with permission.		

significant role in shaping breath composition. VOCs linked to bacterial metabolism, such as 2-ethyl furan and dimethyl sulfide, highlight the influence of these microbial communities. Environmental and lifestyle factors further impact the VOC profile. For instance, smoking significantly increases breath levels of compounds like benzene, xylenes, toluene, 1,3-cyclopentadiene and 2,5-dimethyl furan, which can serve as biomarkers for smoking exposure. Table 1 suggests that exhaled breath analysis may lead to promising clinical applications. For instance, elevated acetone levels have been associated with acute heart failure and uncontrolled diabetes, while monitoring methylamines may be useful in assessing kidney function. However, as discussed below (in the section entitled “Limitations and current open areas of development”), current research faces significant limitations. At this stage, the lack of a clear causal relationship between pathophysiological mechanisms of diseases and VOC exhalation is a main limitation. Further investigation is required to address this gap and advance the field. Longitudinal monitoring of breath components is a more promising approach, as patients may act as their own controls, improving the reliability of VOC-based tests.

Limitations and current open areas of development

Major limitations in the fields of biomarker research and omics often lie in investigating the wrong research questions. Similarly, in the field of clinical breathomics, we are often asking inappropriate questions. Since the dawn of modern breath VOC analysis, we have been urged to find unique and specific biomarkers that can primarily define a given disease/condition of interest. However, to date, the discovery of such disease markers remains only as random and nonreproducible outcomes from cross-sectional pilot studies conducted on a handful of subjects and *via* single-point measurements. Such limitations will remain unsolvable, unless we start to realise the actual strength of breath VOC markers and direct ourselves toward the correct research aims. As is the case for the hundreds of parameters measured in conventional blood and/or urine tests, the VOCs detected in our breath also represent an extremely dynamic nature and range [19]. Until now, the normal ranges for breath VOCs are neither well-defined nor separable *via* interindividual health status. Eventually, we have realised that differences and/or changes in VOC concentrations are more realistic as biomarkers than hoping for the presence or absence of any specific VOC under any condition.

As described above (in the section entitled “Physio-metabolic effectors during breath sampling”), such dynamic nature is predominantly driven by the subject’s own physio-metabolic status during sampling. Therefore, standardisation of sampling protocols is unavoidable to overcome ubiquitous challenges in achieving the individual steady-state of breath. Therefore, reliable clinical breath sampling must control the subject’s respiratory and haemodynamic fluctuations as well as account for their metabolic attributes. In general, for sampling at rest, one should conduct alveolar/end-tidal sampling; maintain a defined posture and avoid unnecessary muscle movements; control breathing patterns, routes and kinetics, and resistance against breathing; and avoid ventilatory variations [37]. As for sampling procedures, analysis and interpretation of VOCs should consider influences from the immediate surroundings and the long-term effects from subjects’ ethnicity, age, gender, habitat, lifestyle habits, any special diet, medication/therapy, and acute/chronic comorbidity. Determination of VOC concentrations in the inspired air are crucial to realise immediate confounding factors from the environment, such as clinical contaminants, disinfectants and instrumental emissions [90]. Similarly, analytical standards must be maintained by reporting detailed methodological and technological parameters including instrumental detection and quantification limits along with quantified data wherever possible, such as in discovery studies and based on repeated measures. These are basic prerequisites to control the quality of measured breath data in independent studies and thereby, to enhance reproducibility and validation.

While state-of-the-art paths are gradually being paved to overcome physio-metabolic and analytical challenges, there are more fundamental problems yet to be addressed regarding our present knowledge and understanding of breath biomarkers as well as in relation to our research practice involving study designs, conducts, data analysis and interpretations.

As discussed above (in the section entitled “Origin and transport of breath biomarkers”), there is a profound knowledge gap regarding the definite origins of endogenous VOCs in human breath. During the past four decades, a few sources have been investigated *via in vitro* (e.g. cellular, systemic-microbial pathogenic, culture and cross-matrices), *ex vivo* (e.g. extracted tissue and organoids) and pre-clinical experiments; however, system-wide *in vivo* clinical investigations have not been performed for potentially endogenous VOCs. Consequently, in 2021 an *in vivo* clinical multi-omic study failed to validate the putative source of isoprene (C₅H₈), the second most abundant breath VOC. Based on the outcome of an *in vitro* pre-clinical study from 1984, hepatic cholesterogenesis was erroneously proposed to be the principal source of isoprene in humans [16]. Since 1984, different cross-sectional studies have proposed isoprene as a breath biomarker for diseases including cancers, cardiopulmonary complications and hypercholesterolemia, and the pathological effects were attributed to the erroneous origin. As a result, none of these observations were ever validated or reproduced in independent studies. In 2023, a large *in vivo* clinical multi-omic study finally discovered that the true origin of isoprene in human breath is peroxisomal *IDL2* mediated skeletal-muscular lipolytic cholesterol metabolism [17]. Thus, with the exception of exhaled isoprene, the principal metabolic origin of other breath VOCs is yet putative. Therefore, if the actual origin and metabolic pathways are uncertain, clinical interpretations of VOCs may easily become confused, as was the case for isoprene. In this context, breathomics does offer a vast area of fundamental, experimental and translational investigations to discover the principal metabolic source(s) of other endogenous VOCs in humans. Such developments will elucidate large scopes and novel avenues towards clinical applications of breath research. From the discovery of isoprene’s origin, we have also learned that in 2000 recruited subjects the normal range of breath isoprene (in subjects without any acute/chronic disease or therapy) is below the limit of detection (*i.e.* at mid pptV (parts per trillion volume) range to >450 ppbV (parts per billion volume)) which incorporates any concentration ranges published earlier as disease-assigned values for isoprene in cross-sectional studies.

In line with the above, we need to rethink our regular research practice in breathomics and should be able to adopt study designs suitable for exploratory research to avoid conventional problems of pure mathematical correlations, statistical misconceptions and overfitting, and the fallacy of breath-based early diagnosis of diseases. As of the infamous dead salmon experiment by BENNETT *et al.* [91], mathematical prophecies based on small sample sizes *versus* large numbers of partly dependent and independent variables in cross-sectional pilot breath studies have repeatedly produced artificially high diagnostic values based on multiple comparisons – without having any correction for nonindependent errors. Not surprisingly, such results have always failed the basic requirements for validations and reproducibility in independent studies and, therefore, fail to enter routine practice for diagnosis [92]. Besides, the term early diagnosis is rather used without having a clear notion of its true clinical sense. Thus, early diagnostic breath markers are readily proposed based on comparisons between healthy subjects *versus* sick patients suffering from the mid or late stages of a disease.

Considering the dynamic nature of breath markers, it is now perceivable that VOC-based breathomics is rather more suitable for monitoring of physiology, metabolism, diseases/conditions and corresponding therapeutic responses if a subject is treated as his/her own control. Therefore, it is important to conduct longitudinal studies aiming beyond conventional knowledge

and understanding of *in vivo* biochemical and pathophysiological processes. Nevertheless, cross-sectional studies are important for the biomarker discovery phase, and they should be conducted in a prospective manner and without having any pre-selection bias *e.g.* under a real-life clinical screening scenario [93, 94]. Validation of such outcomes in independent cohorts (*i.e.* recruited in a similar screening manner) and/or setup are unavoidable if/once clinical translation is the aim [95]. Multicentre studies with randomisation are desirable in such a context to enhance robustness and reproducibility.

While O₂, CO₂ and NO provide a conventional respiratory outlook at systemic and local levels, VOCs offer more than a conventional understanding of *in vivo* physio-metabolic processes. For example, a recent study investigated the continuous cardio-respiratory effects of medical face masks in real-time, where P_{ETCO_2} , peripheral O₂ saturation and haemodynamic parameters were measured in parallel to exhaled VOCs [96]. Here, exhaled VOCs provided unique information on cross-talk between transient hypoxaemia, oxidative stress, hypercarbia and vasoconstriction, as well as depicting the immediate changes in systemic microbial activity, energy homeostasis, compartmental storage and washout mechanisms. Thus, exhaled breath is indeed an unconventional and noninvasive window to continuously investigate organ-level, cellular, and even subcellular activities without imposing any additional burden to the subject. Herewith, it is foreseeable that the present limitations are substantial links and clues for further advancements and optimisation of our present approaches in clinical breathomics. Therefore, by adapting our research practices, embracing longitudinal and multicentre studies, and addressing knowledge gaps in VOC origins, we can progress the field of breathomics toward reliable and impactful clinical applications.

Concluding remarks

Breath composition reflects real-time biochemical processes in the body producing volatile chemicals, food and beverage consumption and exposure to xenobiotic. Its analysis enables dynamic, noninvasive insights into physiology, metabolism and pathophysiology, with significant potential for healthcare monitoring provided that the effects of confounding factors are properly addressed. At present, clinical applications include noninvasive monitoring of CO₂ and O₂ *via* capnography for respiratory and metabolic assessments, and IC for estimating energy expenditure and substrate utilisation. Substrate-based tests, such as hydrogen and urea breath tests, are widely used for diagnosing conditions like carbohydrate malabsorption and *H. pylori* infection. Other applications include monitoring anaesthetic drugs, CPET and exhaled nitric oxide (F_{ENO}).

Despite the efforts, the application of VOC analysis for diagnosis and for personal healthcare monitoring remains largely experimental with limited clinical validation. Reasons behind this are multiple, and there is no consensus among researchers. The gaseous nature of the breath sample and the limited stability over time makes sample exchange among groups difficult, and this hampers establishing standardised protocols for sampling and analysis. For similar reasons, while many small single-measurement cross-sectional studies have tried to identify breath biomarkers to diagnose many kinds of pathology, large clinical screening studies, longitudinal studies and randomised controlled trials are scarce. This makes the effect of confounding factors like diet, lifestyle or comorbidities complex to be removed or accounted for. In addition, a detailed understanding of the origins of breath VOCs is still largely missing, and this prevents establishing clear connections with the aetiology and pathophysiology of diseases.

However, the breath community is actively engaged in overcoming present limitations. The peppermint experiment represented a first attempt to assess the variability of breath sampling

and analysis procedures, and to compare them to the interindividual variability in the metabolism of an oil capsule [94, 97, 98]. Awareness of the need to design large-scale clinical studies while testing clearly defined and sound clinical hypotheses is increasing, and a higher impact is keenly expected as clinicians get more involved in this field. At the same time, technologies are being developed to allow easy and low-cost analyses at the point of care.

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Cardiopulmonary exercise testing

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The utility of cardiopulmonary exercise testing is multifactorial and crosses specialisms. It provides essential information on the reasons for the presented symptoms, and offers prognostic and risk stratification information. <https://bit.ly/ERSM107>

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CPET evaluates cardiovascular, respiratory and metabolic responses during exercise, and is crucial to the diagnosis and management of cardiopulmonary diseases and nonpathological conditions. CPET involves stress testing using treadmills or cycle ergometry, with protocols tailored to patient tolerance and disease severity. It measures parameters such as O_2 consumption, $\dot{V}_{CO_2}$, heart rate (HR), blood pressure and ventilation to pinpoint functional impairments and underlying mechanisms of symptoms. CPET assists with differential diagnosis in unexplained exertional dyspnoea, fatigue and exercise intolerance, and assesses known or suspected cardiovascular or pulmonary conditions. CPET can reveal subtle abnormalities that cannot be detected at rest, aiding in risk stratification and individualised therapy. It can also distinguish between cardiac and pulmonary causes of dyspnoea, assess the long-term effects of COVID-19, identify dysfunctional breathing and detect exercise oscillatory ventilation. CPET is, therefore, an essential physiological procedure for any respiratory physiology laboratory.

Introduction

CPET is a cornerstone in the diagnosis and management of various cardiopulmonary diseases, providing invaluable insight into an individual's physiological response to exertion. Through integration of the measurement of cardiovascular, respiratory and metabolic parameters into exercise, CPET offers a comprehensive evaluation of an individual's functional capacity and can aid in the diagnosis, prognosis and optimisation of treatment strategies for a spectrum of conditions.

In clinical practice, CPET is frequently employed to assess patients with unexplained exertional dyspnoea, fatigue or exercise intolerance, as well as those with known or suspected cardiovascular or pulmonary diseases. Through continuous monitoring of variables such as O_2 consumption, $\dot{V}_{CO_2}$, HR, blood pressure and ventilatory parameters, CPET enables clinicians to identify and quantify impairments in cardiopulmonary function, pinpointing the underlying mechanisms contributing to symptoms. Moreover, CPET can unmask subtle abnormalities that may remain undetected at rest, offering a dynamic perspective on cardiovascular and pulmonary reserve capacities and facilitating risk stratification and individualised therapeutic interventions.

In this chapter we aim to outline how CPET is conducted, the essential equipment needed to run a quality-assured and safe service, the right protocols to elicit the right responses to answer the question being asked by the referrer, and the parameters that can be measured. The patterns of responses seen in both pathological and nonpathological abnormalities will be described to allow a differential diagnosis to be made or for reassurance that there is no pathological cause for presenting symptoms.

Exercise testing

If baseline physiological assessments (*e.g.* pulmonary function testing and echocardiography) have not identified the specific aetiology of presenting symptoms (dyspnoea, shortness of breath, exercise intolerance, *etc.*), a natural next step in the diagnostic pathway is to perform standardised CPET. CPET is useful for diagnosing the causes of exercise limitation, for assessing the severity of the underlying disease and prognosis, and for monitoring the response to therapy. Indeed, baseline routine assessments completed at rest, including spirometry, cardiac ultrasound, biochemical measurements or more advanced imaging techniques, have all been demonstrated to be poor predictors of exercise performance [1–6]. The way in which CPET should be performed according to the underlying disease is described in detail elsewhere, and we would refer the reader to those documents [7–11]. Below we provide a summary of the salient points in terms of equipment, protocols and most useful parameters to measure.

Equipment

Generally, there are two possible methods of stressing the system and delivering a load against which the exercising individual is required to make an effort. These are treadmill ergometry and cycle ergometry. However, use of arm ergometry is gaining popularity [12–14], especially in individuals who may have limitations to exercises that use the lower limbs, as a result of hip/knee replacements or paralysis, for example. There are also other ways of providing an exercise-specific option, such as swimming, but these are outside the realms of patient testing. Other pieces of equipment that are important from a measurement perspective and that help ensure the tests are conducted safely include blood pressure monitors, ECGs and pulse oximeters.

During CPET, both ventilation and exchange of respiratory gases need to be collected. There must be a means of recording ventilation, using either a volume- or flow-measuring device that the individual breathes through. Inspired O₂ and expired CO₂ also need to be measured, which requires some form of gas-analysis equipment. The usual method is for the individual to breathe via a mouthpiece or a face mask.

Protocols

There are specific protocols for particular ergometers and patient populations [15–18]. These should be adjusted based on the exercise tolerance and/or disease severity of the patient undertaking the exercise test to ensure that an appropriate, quality-assured assessment is completed. Practitioners should aim for the exercise test to be completed within 8–12 min. A short test may mean the protocol was set too hard, with the patient fatiguing before meaningful information is attained. Too long and it is likely the patient may stop due to boredom or, in cycle ergometry, discomfort from the saddle.

CPET should be separated into specific phases. A rest phase allows for assurance that data is being recorded appropriately, although some systems have a check phase that ensures accuracy of data collection. The rest phase, which should be a minimum of 2 min in duration, is also used to: monitor response variables during quiet normal breathing; allow collection of other

resting variables, such as those from blood gas analysis; and ascertain resting symptoms, such as breathlessness or muscle fatigue. Specifically in cycle ergometry, there should be a warmup phase lasting 3 min. During this phase, the individual will be cycling at a set cadence but there will be no load applied to the system. It is then possible to record the metabolic demands required to just turn the lower limbs. A duration of 3 min usually allows the individual to reach a steady state from which loaded exercise can be introduced. It is difficult to introduce a warmup phase on the treadmill as a load is applied as soon as the patient begins to walk. During the loaded phase of cycle ergometry, an increasing resistance (or load) is introduced against which the patient is required to pedal at the same set cadence as the warmup phase. The optimal protocol is one in which an increasing load is introduced over seconds rather than minutes, giving an incrementally ramped response. Clinically, load increments up to 1 min are considered ramp protocols.

During the loaded phase of a treadmill test, either the speed and/or the elevation of the treadmill are increased. The degree by which these change is again based on the underlying perceived ability of the individual by the healthcare professional. As with cycle ergometry, small incremental changes are better than large sudden step changes to allow for detection of submaximal responses and slopes variables.

All tests should be terminated at symptom limitation, not when maximum reference standards, including HR, have been met, as some individuals will exceed their predicted exercise capacity. Tests should also be terminated if pre-established safety protocols have been reached, *e.g.* ST depression or elevation, and hypotension or hypertension.

A period of recovery is useful to ensure the individual is returning to the pre-exercise state as expected and there are no adverse events taking place during this period. It is also possible to detect abnormalities during this timeframe, such as autonomic dysfunction, gas exchange abnormalities and pulmonary oedema, as may happen when testing patients with severe heart failure [19–21].

Parameters

There are a number of different parameters that are either directly measured or calculated during CPET to provide a wealth of information that the healthcare professional uses to interpret the outcome of the test (table 1). The healthcare professional is able to ascertain whether the exercise response is normal or abnormal and whether any abnormality is associated with a cardiac, ventilatory or gas exchange cause, or any combination of all three. The following sections describe a selection of the main parameters recorded during CPET.

TABLE 1 Common exercise responses in specific disease						
	Peak $\dot{V}_{O_2}$	$\dot{V}_{O_2}$ /WR	HR/ $\dot{V}_{O_2}$	O ₂ pulse	AT or V _T	$\dot{V}_E/\dot{V}_{O_2}$
Heart failure	↓	↓	↑	↓ and flat	↓	→
Coronary artery disease	↓	Slope inflection	Slope inflection	↓ and flat	→	→
Lung disease	↓	→	→	→	→ or ↓	→ or ↑
Pulmonary hypertension	↓	→	→	→	→ or ↓	→ or ↑
Obesity	↑	→	↓	→ or ↑	→ or ↓	→
WR: work rate; HR: heart rate; AT: anaerobic threshold (equally known as ventilatory threshold 1); V _T : tidal volume; $\dot{V}_E$: minute ventilation. ↑: increased; ↓: decreased; →: normal.						

$\dot{V}_{O_2}$

CPET allows evaluation of $\dot{V}_{O_2}$, which is, according to the Fick formula:

$$\dot{V}_{O_2} = Qc \times a-v\Delta O_2 \quad (1)$$

where Qc is cardiac output and $a-v\Delta O_2$ is the arterio-venous O_2 content difference. $\dot{V}_{O_2}$ at peak exercise and at the anaerobic threshold is a pivotal parameter used to assess exercise performance. Low values in the presence of a physiologically maximal test often suggest pathophysiology and severity of specific diseases, such as heart failure, irrespective of left ventricle ejection fraction, pulmonary hypertension, congenital heart disease or myopathies. Peak $\dot{V}_{O_2}$ is a major diagnostic tool but most importantly, it is a prognostic tool in heart failure [21]. Although guidelines still suggest use of peak $\dot{V}_{O_2}$ cut-off for prognosis assessment or heart transplant indication, the most efficacious way to detect a reliable prognosis is by using scores that may include exercise parameters [22–24]. At present, the MECKI (Metabolic Exercise test data combined with Cardiac and Kidney Indexes) score is the most efficacious method [25]. The MECKI score is recommended by the guidelines of the European Society of Cardiology (ESC) [26] and includes, in addition to left ventricle ejection fraction, sodium, haemoglobin concentration and kidney function, as well as the exercise-derived parameters of peak $\dot{V}_{O_2}$ and minute ventilation ($\dot{V}_E$) versus $\dot{V}_{CO_2}$ relationship slope.

Work rate efficiency slope

The work rate efficiency slope, or the relationship between $\dot{V}_{O_2}$ and the amount of work delivered in Watts ($\dot{V}_{O_2}/WR$), gives an indication of whether O_2 is being delivered and/or extracted efficiently by the cardiovascular system or exercising muscles. A normal response for cycle ergometry yields a slope of $\sim 10.3 \text{ mL} \cdot \text{min}^{-1} \cdot \text{W}^{-1}$.

In instances of poor cardiovascular delivery and/or O_2 extraction at the exercising muscle, the slope is $< 8.3 \text{ mL} \cdot \text{min}^{-1} \cdot \text{W}^{-1}$. For cardiac performance, the $\dot{V}_{O_2}$ versus work relationship and the O_2 pulse must be considered. The former allows the efficacy of O_2 delivery to the working muscles to be evaluated. A low $\dot{V}_{O_2}$ versus work slope indicates a reduced Qc increase during exercise, while the loss of a linear relationship is suggestive of an abrupt reduction of stroke volume (SV), as seen in cardiac ischaemia or in cases of mitral insufficiency development. The relationship is also low in instances of metabolic myopathy, where there is insufficient capacity within the exercising muscle to use the O_2 available.

The cause of an elevated slope on cycle ergometry is almost certainly due to the recruitment of additional muscles other than leg muscles to exercise (*i.e.* chest, shoulder, biceps) for exaggerated grip on the handlebars and/or cycling out of the saddle.

Anaerobic or ventilatory threshold

In a progressively increasing workload exercise protocol (ramp), energy production shifts from predominantly aerobic metabolism to increasing supplementation of energy production through anaerobic metabolism. The point at where this occurs is named the anaerobic threshold or the ventilatory threshold and can be obtained by so-called V-slope analysis [27].

A higher anaerobic threshold in relation to the peak predicted O_2 consumption will suggest a normal response to exercise and an individual who is relatively well-conditioned. A low value suggests either deconditioning or impact of disease. In heart failure, the absence of an identifiable anaerobic threshold has a relevant negative prognostic meaning [28].

Thereafter, there is an exercise phase when lactic acid is buffered by the bicarbonate system, when additional CO_2 in addition to that from aerobic metabolism is produced, ensuring pH is kept constant. This phase, named the isocapnic buffering period, ends when the availability of bicarbonate diminishes, leading to an increase in circulation of unbuffered hydrogen ions (H^+) resulting in acidosis. Indeed, beyond this point, increasing H^+ levels and limited bicarbonate buffering cause a rise in H^+ concentration, which is detected by the carotid bodies, further driving up ventilation and causing an inflection in the ventilation to CO_2 slope. This second threshold is usually called the respiratory compensation point or ventilatory threshold 2. The absence of this threshold either means the test was of a submaximal nature or there is disease present that prevents the individual being unable to reach sufficient ventilation to produce a respiratory compensation point, such as severe respiratory disease.

Cardiovascular slope

The slope produced by the relationship between $\dot{V}_{\text{O}_2}$ on the x-axis and HR on the y-axis during CPET is an important measure, which reflects the cardiovascular system's response to exercise (figure 1). This relationship is often referred to as the $\dot{V}_{\text{O}_2}/\text{HR}$ slope and represents how HR rises with increasing $\dot{V}_{\text{O}_2}$ during incremental exercise. It provides insight into the cardiovascular system's efficiency and the body's ability to deliver and utilise O_2 during physical activity.

In healthy individuals, the relationship between $\dot{V}_{\text{O}_2}$ and HR is typically linear during progressive exercise. This linearity indicates that as the body's demand for O_2 increases, the HR increases proportionately to supply the required O_2 .

An abnormal $\dot{V}_{\text{O}_2}/\text{HR}$ slope can either be steeper or flatter than normal, suggesting underlying cardiovascular inefficiency or pathology. A steeper slope (greater increase in HR for a given

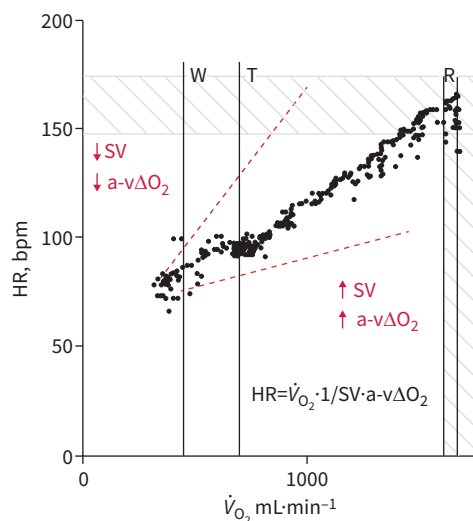


FIGURE 1 Cardiovascular slope demonstrating the relationship between $\dot{V}_{\text{O}_2}$ and heart rate (HR) during an incremental exercise test, and the influence of stroke volume (SV) and arterio-venous O_2 content difference ($a-v\Delta\text{O}_2$) on the slope of the relationship. Hatched areas are limits of normality. W: start of unloaded (warm-up) phase; T: start of loaded (test) phase; R: start of recovery phase.

$\dot{V}_{O_2}$) may indicate a reduced SV or deconditioning, where the heart has to compensate by increasing the rate more significantly. A flatter slope (smaller increase in HR for a given $\dot{V}_{O_2}$) might indicate chronotropic incompetence, where the HR does not rise adequately with increasing O_2 demand.

O₂ pulse

O_2 pulse is defined as the amount of O_2 consumed per heartbeat and is derived from expanding and rearranging the Fick equation:

$$\dot{V}_{O_2} = Q_c \times a-v\Delta O_2 \quad (2)$$

$$\dot{V}_{O_2} = HR \times SV \times a-v\Delta O_2 \quad (3)$$

$$\dot{V}_{O_2}/HR = SV \times a-v\Delta O_2 \quad (4)$$

The key characteristic of a normal O_2 pulse response is a gradual increase with rising exercise intensity. Higher O_2 pulse values at higher workloads indicate a more efficient cardiovascular system capable of delivering and utilising O_2 effectively. For a healthy individual, O_2 pulse values typically range 10–20 mL O_2 per beat during peak exercise, depending on factors like age, sex and fitness level.

Abnormal O_2 pulse responses can indicate various underlying cardiovascular or pulmonary issues. A flattened or low O_2 pulse is seen in a number of conditions:

- 1) Heart failure. A low or flattened O_2 pulse suggests reduced SV, indicating that the heart is unable to increase its output efficiently in response to increased demand during exercise.
- 2) Coronary artery disease. Insufficient blood flow to the myocardium due to blocked arteries can limit the increase in SV, resulting in a lower O_2 pulse.
- 3) Valvular heart disease. Conditions affecting the heart valves can impair SV and reduce O_2 pulse.
- 4) Pulmonary disease. Conditions like COPD or pulmonary fibrosis can lead to impaired $\dot{V}_{O_2}$ in the lungs, limiting the increase in $a-v\Delta O_2$ and thus flattening the O_2 pulse.

An elevated O_2 pulse is usually a sign of an athletic response. Highly trained athletes may exhibit higher O_2 pulse values due to increased SV and efficient O_2 extraction by muscles. Compensatory mechanisms in anaemia might transiently increase SV, potentially leading to an elevated O_2 pulse, though this is less common.

Ventilatory slope

The relationship between $\dot{V}_E$ and $\dot{V}_{CO_2}$ is represented by the $\dot{V}_E/\dot{V}_{CO_2}$ slope (figure 2). This slope is influenced by the following equation:

$$\dot{V}_E/\dot{V}_{CO_2} = 863/P_{aCO_2} \times (1/V_D/V_T) \quad (5)$$

where P_{aCO_2} is arterial CO_2 tension and V_D/V_T is deadspace volume/tidal volume ratio.

In a normal response, the $\dot{V}_E/\dot{V}_{CO_2}$ is linear, with a proportional increase in ventilation as $\dot{V}_{CO_2}$ increases. The slope of the relationship usually ranges 25–30. The $\dot{V}_E/\dot{V}_{CO_2}$ slope increases with age and it is a little higher in women than men.

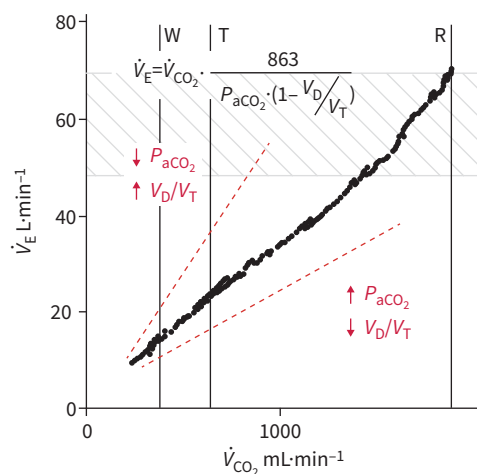


FIGURE 2 Ventilatory slope demonstrating the relationship between $\dot{V}_{CO_2}$ and minute ventilation ($\dot{V}_E$) during an incremental exercise test, and the influence of arterial CO_2 tension (P_{aCO_2}) and deadspace volume/tidal volume (V_D/V_T) ratio on the slope of the relationship. Hatched area is limit of normality. W: start of unloaded (warm-up) phase; T: start of loaded (test) phase; R: start of recovery phase.

Abnormal responses are characterised by deviations from the typical linear relationship and normal slope values. As per the above equation, these are driven by changes in P_{aCO_2} and/or V_D/V_T . These can indicate various cardiopulmonary conditions.

An increased $\dot{V}_E/\dot{V}_{CO_2}$ slope can be seen in:

- 1) Heart failure. An elevated $\dot{V}_E/\dot{V}_{CO_2}$ slope, often >34 , can indicate heart failure, reflecting inefficient ventilation and poor Qc.
- 2) Pulmonary hypertension. A significantly steep slope suggests pulmonary hypertension, indicating an increased ventilatory response to exercise due to ventilation/perfusion mismatch.
- 3) COPD. In COPD, the slope may be increased due to airway obstruction and ventilation inefficiency.

A decreased $\dot{V}_E/\dot{V}_{CO_2}$ slope can be suggestive of respiratory muscle weakness, where ventilatory constraints from weak muscles result in ventilation not increasing appropriately with carbon [12–14]. Positive y-intercept values are suggestive of deadspace development during exercise, as seen in pulmonary hypertension.

Examples of the utility of CPET with respect to disease

Unexplained dyspnoea

Breathlessness associated with exercise can be multifactorial and linked with cardiovascular, ventilatory, gas exchange or muscular/metabolic dysfunction [29]. It is also important to note that these are not always distinct from each other and individuals can present with comorbidities. However, CPET is uniquely placed to be able to discern and distinguish the origin of any associated pathophysiology.

Separating cardiac from pulmonary aetiologies of dyspnoea

As explained above, it is not uncommon for individuals to present with comorbidities of cardiac and respiratory disease [6]. CPET can be useful in identifying the primary cause for symptoms

of breathlessness associated with disease, determining which one is predominant and then allowing targeted therapy to improve symptoms.

Long-term effects of COVID-19

As we move into the endemic phase of severe acute respiratory syndrome coronavirus 2, there is increasing literature published on the acute and long-term effects of the virus on breathlessness and exercise tolerance [30–32]. There is heterogeneity in the response patterns to exercise in patients recovering from the virus. CPET, however, with its ability to distinguish between cardiac and respiratory disease, allows identification of any associated pathophysiology, allowing targeted therapy to be used to assist in alleviating any presenting symptoms.

There is evidence that in some individuals, prolonged symptoms of breathlessness from COVID-19 are not associated with any pathophysiology but with breathing pattern disorder [33, 34].

Dysfunctional breathing/hyperventilation/psychogenic dyspnoea

Breathlessness is not always associated with the presence of pathophysiology but can be psychosomatic in nature. The term dysfunctional breathing is a collective term used to describe a collection of conditions in which the normal biomechanical pattern of breathing is disrupted, resulting in breathlessness and associated non-respiratory symptoms that cannot be fully explained by organic pathology [35]. Although there are non-physiological methods that determine the presence of such dysfunctional breathing, *e.g.* questionnaires, these do not identify whether this is truly non-pathological. The performance of CPET with concomitant blood gases allows true determination of a dysfunctional breathing pattern that is not associated with pathophysiology.

Exercise oscillatory ventilation

Exercise oscillatory ventilation (EOV) is an abnormal breathing pattern characterised by cyclic fluctuations in ventilation during exercise. The cycles usually last between 40 s and 2 min. Fluctuations can range from subtle to very pronounced and are typically seen as alternating periods of hyperventilation and hypoventilation. EOV is often associated with various cardiovascular and pulmonary conditions, including heart failure, COPD, and other disorders that affect respiratory control and gas exchange.

The presence of EOV on CPET is clinically significant because it is often associated with worse outcomes in patients with heart failure and other chronic diseases [36]. It indicates poor ventilatory efficiency and underlying pathophysiological issues that may require targeted treatment. Recognising EOV can help guide management strategies, including optimising medical therapy, considering advanced heart failure therapies, or addressing any contributing respiratory issues.

Conclusion

CPET is an extremely useful tool that supports the identification of pathophysiology where baseline tests have failed to highlight any abnormality. Equally CPET is able to provide reassurance to patients where presenting symptoms are not associated with any significant pathology. The performance of these tests, whether on a treadmill, cycle ergometer or other means of stressing the system, have been well standardised through many available published guidelines from various international organisations. The plethora of parameters that can be measured during CPET allows interpretation of the cardiovascular, ventilatory, gas exchange and metabolic responses to exercise. CPET can be used to aid differential diagnosis of disease, provide suggestions for next steps in the treatment pathway, assist in risk stratification for surgical interventions, and help monitor the response to appropriate therapies.

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Monitoring respiratory physiology during noninvasive ventilatory support

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Monitoring respiratory physiology during acute and chronic NIV is challenging but key to optimising treatment and predicting patient response. The ideal approach uses instruments that offer the most accurate assessment tailored to the patient's condition. <https://bit.ly/ERSM107>

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Effective monitoring is crucial to optimise noninvasive ventilation (NIV) and prevent complications. Built-in software in ventilators provides real-time data on adherence, leaks, tidal volume and apnoea–hypopnoea index, supporting therapy adjustments. Respiratory polygraphy remains the gold standard for detecting patient–ventilator asynchronies and upper airway obstruction in the chronic setting, although its use is often reserved for complex cases. In the acute setting, oesophageal manometry offers detailed insights into respiratory mechanics and patient effort, but it is invasive and has limited application. Ultrasound and surface electromyography are emerging tools to assess diaphragm and respiratory muscle function, with potential applications in NIV monitoring. Innovative approaches like nasal pressure swings, electrical impedance tomography and forced oscillation techniques show promise but require further validation. These methods help address key challenges such as unintentional leaks and variability in ventilator algorithms that have an impact on the accuracy of data interpretation. Integrating advanced monitoring tools with clinical expertise ensures more personalised care and improved outcomes for NIV patients in acute and chronic settings.

Introduction

Noninvasive ventilation (NIV) is a technique for respiratory support that avoids endotracheal intubation by delivering positive airway pressure through noninvasive interfaces. It is widely used in hospitals, intensive care units (ICUs), general wards and at home to treat acute and chronic respiratory failure [1]. In acute respiratory failure (ARF), its primary goal is to reverse respiratory acidosis, support respiratory muscles and improve oxygenation. The strongest evidence is in COPD exacerbations and cardiogenic pulmonary oedema [2]. For chronic respiratory failure (CRF), NIV helps reverse hypercapnia, prevent exacerbations and enhance quality of life by improving symptoms and sleep quality [3]. Ventilators for NIV vary by setting and include ICU ventilators with NIV algorithms, and turbine-based ventilators, categorised as life support and

bilevel ventilators according to some behaviours such as alarms, internal batteries, ventilatory modes and compatibility with different circuit types. A key distinction is performance, as some ventilators can better reduce muscle workload, support effort and minimise asynchrony [4, 5].

Monitoring NIV is crucial for its effectiveness and to prevent complications. In acute care, it helps to avoid intubation and reduce mortality [6]. In chronic care, it optimises benefits, enhances adherence and, consequently, improves long-term outcomes [7, 8]. Various monitoring methods are available, each aimed at optimising NIV settings.

Monitoring with built-in software

Advancements in portable ventilators for NIV include integrated software for monitoring patient compliance, mask leaks, respiratory events and ventilatory trends, with some devices analysing flow and pressure data for deeper insights. Data from ventilators, accessible *via* built-in software (BIS), support the titration and follow-up of home NIV in CRF [7, 8]. A proposed algorithm for monitoring home NIV using BIS is presented in figure 1. In the following sections, we will discuss the most important parameters provided by the BIS.

Adherence

Effective NIV in CRF requires at least 4 h use per night during sleep, with higher usage linked to better survival, especially in COPD patients [9]. Consistent night use without fragmentation is ideal, as interruptions (figure 2a) may signal issues like sleep fragmentation, nocturia or worsening conditions. Increased usage might indicate disease progression or exacerbation, while suboptimal or fragmented use may also reveal problems like leaks, obstructions or patient–ventilator asynchrony [7].

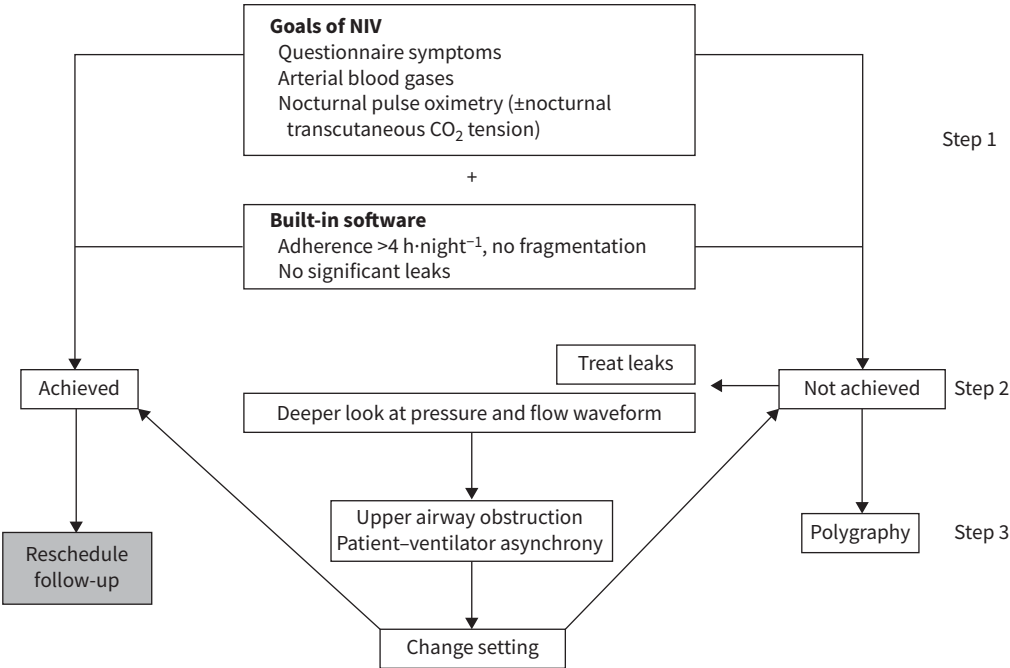


FIGURE 1 Proposed algorithm for systematically monitoring patients on home noninvasive ventilation (NIV) with the assistance of built-in software.

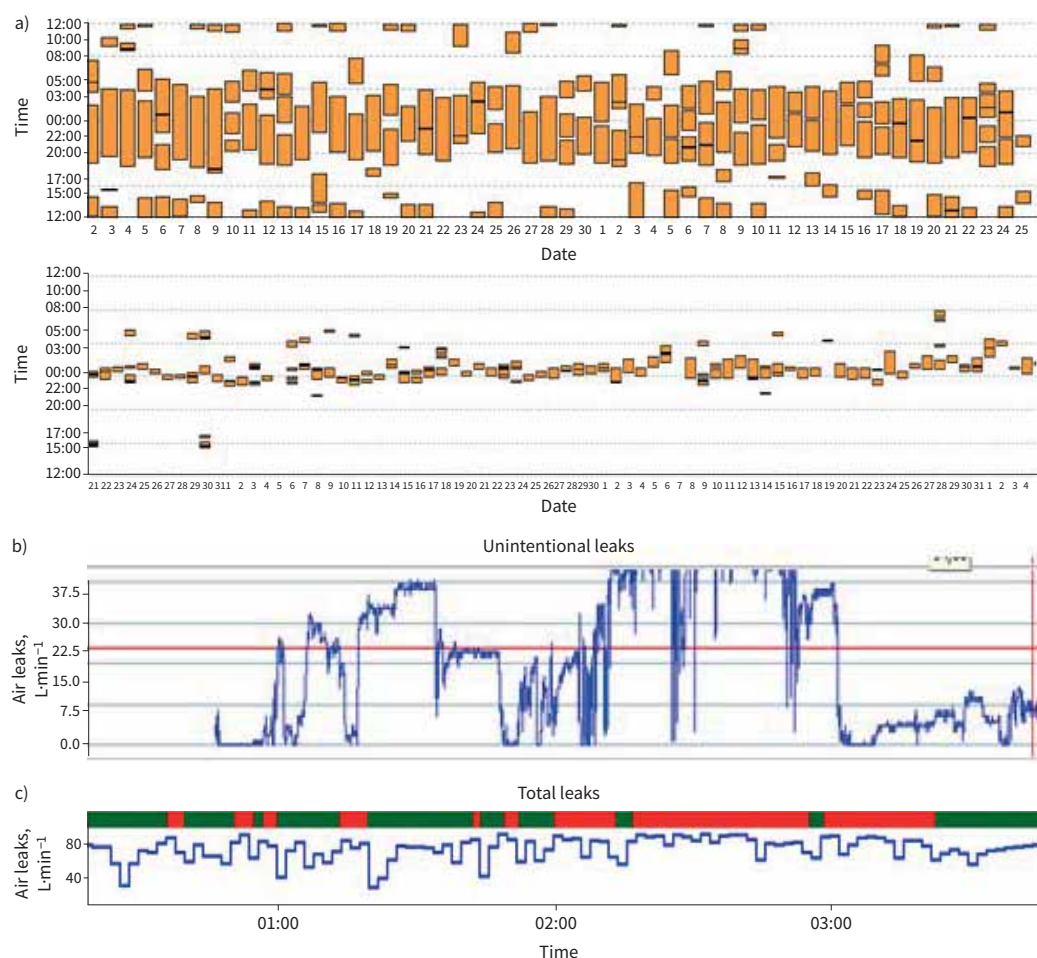


FIGURE 2 a) Adherence to home noninvasive ventilation (NIV): an example of nocturnal fragmentation and irregular NIV use. Graphic transcription of ventilator use provided by built-in software. Each bar represents time spent using the ventilator for a given day. b and c) Built-in software data from two different manufacturers displaying b) unintentional leaks and c) total leaks. The acceptable leak thresholds differ between the two systems: b) 24 L·min⁻¹ (red line) and c) 80 L·min⁻¹ (green bars: total leaks lower than this; red bars: total leaks higher than this).

Leaks

Leaks during NIV may be intentional (to prevent rebreathing in single-limb circuits) or unintentional (due to poor mask fit or mouth leaks) [10]. Turbine-based ventilators can compensate for leaks, within limits, to maintain prescribed pressures. Manufacturers may report two different types of leaks: the more accurate “total leaks” (with acceptable medians being <60–80 L·min⁻¹), or less precise “unintentional leaks” (with acceptable medians being <25 L·min⁻¹) (figure 2b and c) [8]. Leaks can impair ventilator triggers, cycling, pressurisation and data reliability, causing asynchrony, desaturation and poor therapy tolerance [11]. Asymmetric leaks (varying between inspiration and expiration) have been shown to further reduce BIS accuracy [12]. Therefore, examining detailed flow waveforms is recommended, especially in cases of desaturation, asynchronies or elevated CO₂ tension without a noticeable increase in leak trends.

Tidal volume

Tidal volume (V_T) is a key variable in ventilator monitoring, displayed either breath by breath or as trends *via* BIS. In hypoxaemic ARF, $V_T > 9$ mL per kg of ideal body weight predicts NIV failure due to alveolar overdistension [13], and a target range of 6–8 mL·kg⁻¹ is recommended. For hypercapnic ARF and CRF, a target V_T of 8–10 mL·kg⁻¹ is often suggested to reduce hypercapnia, although no specific threshold is tied to outcomes. V_T accuracy varies by ventilator type: ICU ventilators with dual-limb circuits measure expiratory V_T reliably, while single-limb turbine ventilators estimate V_T based on leak and pressure calculations, often showing errors under significant leaks [11].

Apnoea–hypopnoea index

Unlike ICU ventilators, turbine-based ventilators (regardless of their complexity) can generate reports *via* BIS that include the apnoea–hypopnoea index (AHI), a marker of residual upper airway obstruction (UAO). The methods used to detect AHI vary by manufacturer. Some devices rely on the shape of the flow waveform to define hypopnoeas and pressure spikes to differentiate central from obstructive apnoeas. Others incorporate newer technologies, such as the forced oscillation technique (FOT), either alone or in combination with flow curve analysis, to detect and classify apnoeas [7]. The reliability of BIS in identifying these events has been assessed in only a few studies, mostly focused on specific devices used in obesity hypoventilation syndrome and restrictive diseases [14, 15]. They found an acceptable correlation between BIS-detected events and those manually scored *via* polygraphy. However, it is important to note that BIS cannot determine the level of UAO, making it challenging to differentiate classic pharyngeal obstruction (typically associated with active respiratory drive) from laryngeal obstruction (more often linked to blunted respiratory drive). A polygraph remains necessary for this distinction [16]. Irrespective of the ventilator’s ability to provide an AHI, examining the flow waveform (whether in real time or through BIS) is valuable for identifying UAO in both acute and chronic settings, as will be described later in this chapter. Unlike during invasive ventilation, UAO and unintentional leaks are particularly common during NIV. In a small cohort of amyotrophic lateral sclerosis patients, the persistence of nocturnal events during NIV was significantly associated with higher mortality rates [17]. The authors determined that >70% of these events were attributed to leaks and UAO. This underscores the importance of recognising and addressing these factors to improve the efficiency of NIV and patient outcomes in CRF.

Limitations

The integration of BIS with portable ventilators has revolutionised the monitoring of home NIV in CRF, offering detailed insights into key parameters essential for NIV success. However, caution is warranted when interpreting data from device-specific algorithms, as their accuracy may be compromised by factors such as leaks and differences in algorithm design. Many of these algorithms lack independent validation in physiological studies, underscoring the need for clinical staff to critically evaluate their outputs. By combining advanced technologies with clinical expertise, it is possible to optimise NIV management, address therapy limitations and improve patient outcomes effectively.

Monitoring patient–ventilator interaction

Patient–ventilator interaction is crucial for effective NIV. Poor interaction correlates with reduced tolerance in both acute [18] and chronic [19] settings, increasing the risk of NIV failure [20]. In chronic cases, asynchronies can reduce adherence and, in neuromuscular patients, significantly affect mortality [17]. Detecting asynchronies is essential to adjust ventilator settings and minimise

their occurrence. Compared to invasive ventilation, NIV poses a higher risk of asynchronies due to leaks, UAO and the choice of interface. For example, helmets worsen interaction compared to masks, negatively affecting trigger, pressurisation and cycling phase [21].

Data from the ventilator

In critically ill patients receiving NIV, visual inspection of pressure and flow waveforms on the ventilator screen is the most common method for detecting patient–ventilator asynchronies. ICU ventilators and life-support turbine-based ventilators feature screens of varying sizes for real-time monitoring. Additionally, turbine-based ventilators, both life-support and bilevel types frequently used in chronic settings, provide detailed breath-by-breath pressure and flow curves through BIS for more accurate analysis. However, clinicians should be aware that ventilators do not display raw flow signals; instead, the data are processed by BIS using algorithms. These algorithms produce a “readable” signal (filtered, auto-calibrated and scaled) while estimating total leakage [22]. Inaccuracies in this estimation can alter the interpretation of tracings, leading to potential misinterpretation of events. This may represent a major limitation in the application of automatic detection software for patient–ventilator asynchronies. The variability in signal-processing algorithms across manufacturers further contributes to differences in the presentation and reliability of monitored parameters [23]. Patient–ventilator asynchronies have been differently classified based on the underlying physiological mechanisms, the tools used to identify them, or the phase of pressurisation affected [8]. There are no fundamental differences in the types of asynchronies occurring between acute and chronic settings or between invasive ventilation (in pressure support mode) and NIV, aside from their underlying causes (more frequently due to leaks and UAO for NIV). Figure 3 illustrates examples of the most frequent patient–ventilator asynchronies detected by looking at flow and pressure waveforms on the ventilator screen or BIS. While seemingly straightforward, recognising patient–ventilator asynchronies during NIV in this way is quite challenging. A multicentre study showed that the overall ability of ICU physicians to detect an asynchrony index higher than 10% is low, regardless of the clinician’s level of expertise [24]. To improve asynchrony management, it is crucial to invest in education and training programmes to equip healthcare professionals with the necessary expertise. However, the varying levels of agreement, even among experts, can be improved by incorporating additional tools, such as belts in chronic settings or oesophageal pressure monitoring in critical care.

Polygraphy

Respiratory polygraphy, particularly the use of respiratory inductance plethysmography, along with an external flow sensor (to overcome the already described limits of flow treated by BIS), remains the gold standard for detecting undesired respiratory events during NIV in chronic settings. Respiratory inductance plethysmography is a technique based on Faraday’s and Lenz’s laws that allows the qualitative or quantitative study of the individual’s thoracic and abdominal movements in different clinical situations (sleep, exercise, rehabilitation) using two belts attached to the thoracic and abdominal surfaces. While in the critical care setting its use has been very limited, this technique has been validated by the American Academy of Sleep Medicine (AASM) as a surrogate for effort in sleep studies [25]. The SomnoNIV group has provided detailed classification and iconographic descriptions of patient–ventilator asynchronies during NIV with polygraphy [26]. Even if belts can serve as surrogates for patient effort, it is important to note that with open upper airways, the signal from thoracic and abdominal belts reflects not only patient effort but also mechanical insufflation. Although recommended by the AASM for NIV titration, polygraphy is not widely adopted as standard practice during NIV. The manual interpretation of recordings remains time-consuming and requires specialised expertise. Consequently, most experts suggest reserving polygraphy for selected cases where

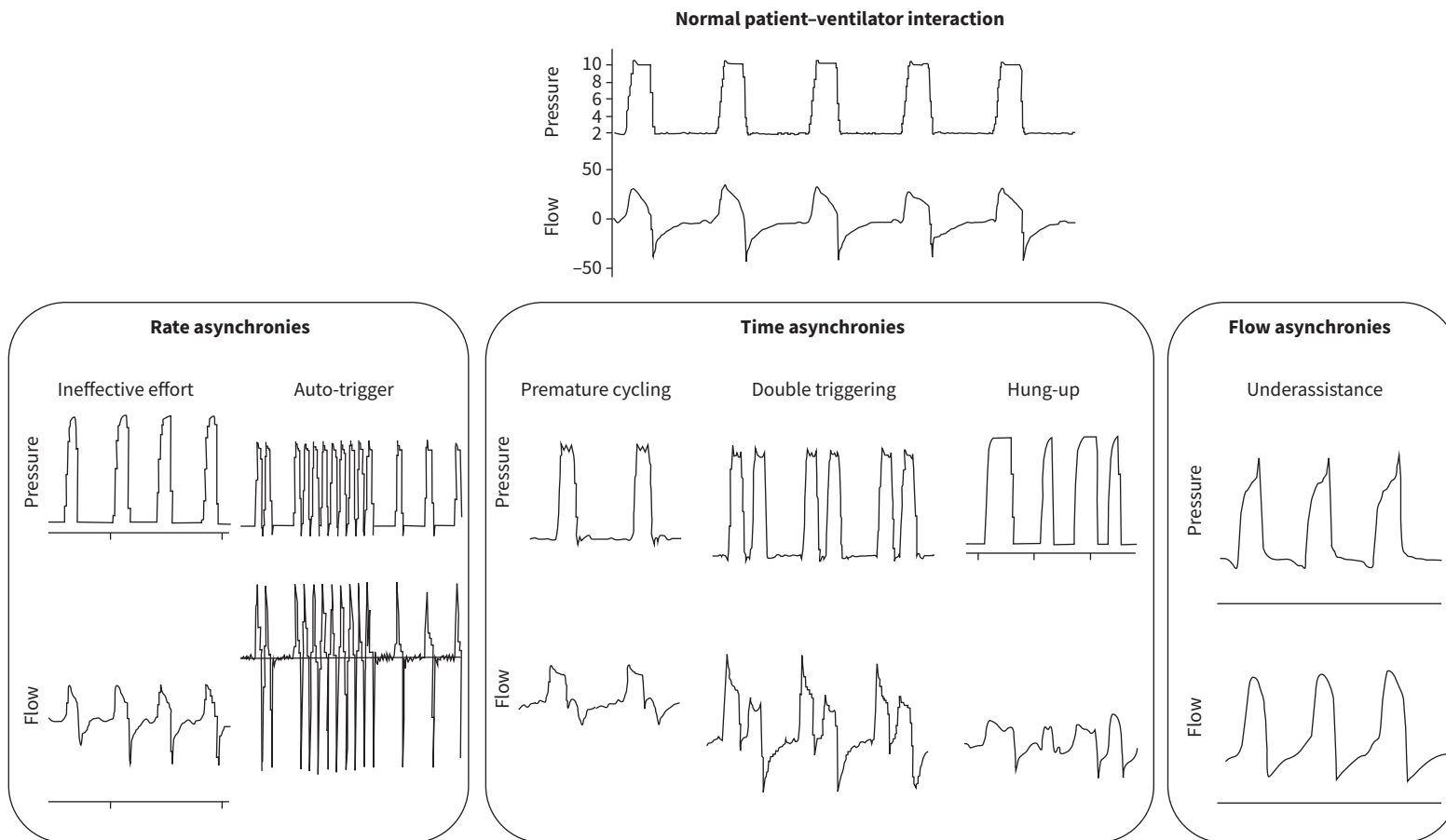


FIGURE 3 Examples of normal interaction and patient-ventilator asynchronies, classified based on type of mismatch between patient and ventilator. Rate asynchronies occur when the patient's respiratory rate differs from the ventilator's rate; time asynchronies occur when the patient's neural drive does not align with the ventilator's inspiratory time; flow asynchronies happen when the ventilator's delivered flow does not match the patient's instantaneous inspiratory flow.

BIS data are insufficient to manage significant events effectively (figure 1). Modern ventilators now offer modules that allow direct connection of belts to the device. This integration simplifies recording by enabling simultaneous display of airway pressure (P_{aw}), flow, effort belts and peripheral O_2 saturation (S_{pO_2}) on a single interface. However, a current limitation is the inability to manually score events during interpretation, as is done in standard polygraphy.

Electromyography

The ability to measure or estimate a patient's inspiratory effort during assisted mechanical ventilation is crucial for evaluating patient–ventilator interaction and identifying signs of overassistance or underassistance. The gold standard for this measurement is the oesophageal pressure (P_{oes}). Electromyography (EMG) of the respiratory muscles offers a promising, less invasive alternative, allowing clinicians to monitor patient activity as a surrogate for respiratory drive [27]. This can be achieved using either 1) an EA_{di} (diaphragmatic electrical activity) catheter, which is a specialised nasogastric catheter embedded with electrodes to measure EA_{di} , or 2) surface EMG, which is a noninvasive method that measures diaphragmatic and intercostal muscle activity using surface electrodes [28]. While EMG is a less invasive alternative to P_{oes} monitoring, its use requires specialised technology and expertise from trained physicians. This limits its accessibility and current applicability in clinical practice. Moreover, variability between and within subjects complicates the estimation of effort solely from EMG amplitudes. Although a conversion factor has been proposed, it is not yet considered reliable enough for precise computation of inspiratory effort. Instead, it serves as a qualitative tool for monitoring respiratory effort and patient–ventilator interaction. To address these challenges, parasternal EMG has been explored, particularly in critical care, for automatic quantification of patient–ventilator asynchronies [29]. This method has also been combined with thoracoabdominal belts and mask pressure monitoring to classify and quantify the type and frequency of asynchronies during NIV initiation or follow-up in patients with CRF [30]. However, surface EMG faces significant limitations, especially in chronic and overnight settings. These include susceptibility to postural artefacts and potential contamination of the signal by activity from other chest wall muscles, reducing its reliability in such contexts.

P_{oes} and respiratory mechanics measurements during NIV

The oesophageal balloon is a catheter used to measure P_{oes} via a solid-state transducer connected to an air-filled pressure-sensing line. When inflated, it indirectly estimates pleural pressure (P_{pl}). Gastric pressure (P_{ga}) can also be monitored with a double-balloon catheter, which approximates abdominal pressure. Despite its invasiveness, this technique is the gold standard for detecting patient effort during NIV, offering key insights into respiratory mechanics and patient–ventilator interaction. Therefore, we will describe it in detail here.

The oesophageal balloon catheter is inserted through the nose and advanced to the hypopharynx while the patient is in a semi-sitting position. Topical lidocaine anaesthesia is recommended to minimise discomfort. The catheter is passed into the oesophagus and to the stomach. Cooperative patients with preserved swallowing function are instructed to swallow, helped with small sips of liquid. If the catheter has a single balloon, a positive pressure deflection during inspiration indicates proper stomach placement in patients with proper diaphragm function. A gentle compression of the stomach can be applied to generate a positive pressure reading in the measurements. At this point, the balloon is gradually withdrawn into the oesophagus, where pressure should become negative. If the catheter also has a gastric balloon, the same procedure is followed monitoring the gastric balloon, but the catheter is not withdrawn [31]. The tip of the oesophageal balloon should normally be 35 cm from the nostrils, in the middle third of the oesophagus [32].

Correct oesophageal balloon position is confirmed using Baydur's occlusion test, which measures simultaneous changes in P_{aw} and P_{oes} during an inspiratory effort against an occluded airway. A P_{aw}/P_{oes} ratio between 0.8 and 1.2 confirms the accuracy of the P_{oes} measurement [33]. When a hold manoeuvre is not available on the NIV ventilator, airway occlusion can be achieved using a unidirectional valve at the level of the mask or a mouthpiece with a nose hook. In this case, P_{aw} should be monitored with an external monitor, and it is crucial to ensure a proper mask seal to prevent pressure drops that could alter P_{aw} readings. Various catheters are commercially available: smaller sizes (8 Fr) are preferred for NIV patients due to minimal sedation requirements, while larger sizes, also used for feeding, allow for more prolonged monitoring.

The same considerations for balloon positioning in spontaneously breathing patients undergoing invasive mechanical ventilation also apply to those on NIV [34]. However, it is important to highlight some key differences: although the procedure is minimally invasive, it should be clearly explained to the patient that, in most cases during NIV, its sole purpose will be respiratory monitoring. This contrasts with intubated patients, where the catheter is typically positioned also for feeding purposes.

Monitoring dynamic compliance of the respiratory system and the lungs during NIV

Oesophageal manometry for respiratory monitoring has provided a detailed understanding of pulmonary pathophysiology, although it is not routinely used in patients undergoing NIV. This technique helps differentiate between the chest wall and lung components by indirectly estimating P_{pl} . Additionally, assessing oesophageal swings (ΔP_{oes}) and the morphology of the P_{oes} curve allows for quantifying inspiratory effort and analysing patient-ventilator interactions.

Dynamic compliance of the respiratory system

The respiratory system includes the airways, lung parenchyma, pleural space, chest wall and respiratory muscles. A simple model of the respiratory system is the linear, one-compartment model, where the airway is linked to an elastic balloon representing the alveoli and chest wall. In this model, airway resistance and balloon compliance are constant. The equation of motion for the entire respiratory system is the mathematical model that describes how pressure, volume and flow interact during ventilation. Actually, the ventilator's pressure, volume and flow waveforms are merely graphic representations of the equation of motion. This equation describes the relationship between total respiratory system pressure (P_{tot}) and the pressures caused by elasticity (P_{el}) and resistance (P_{res}) (figure 4a):

$$P_{tot} = \text{resistive load} + \text{elastic load} + k \quad (1)$$

The resistive load is the product of instantaneous flow and total resistance, while the elastic load is the ratio of the volume above end-expiratory volume to total compliance. The constant (k) represents the pressure at the end of expiration. During mechanical ventilation, k is influenced by both the positive end-expiratory pressure (PEEP) set on the ventilator and the degree to which the lungs have emptied before the next inspiration ($PEEP_{tot}$). Thus:

$$P_{tot} = (\text{flow} \times \text{resistance}) + (\text{volume} \times \text{elastance}) + PEEP_{tot} \quad (2)$$

During NIV, the force responsible for lung inflation and chest wall expansion results from the combined pressures generated by the ventilator (P_{vent}) and the spontaneous breathing effort

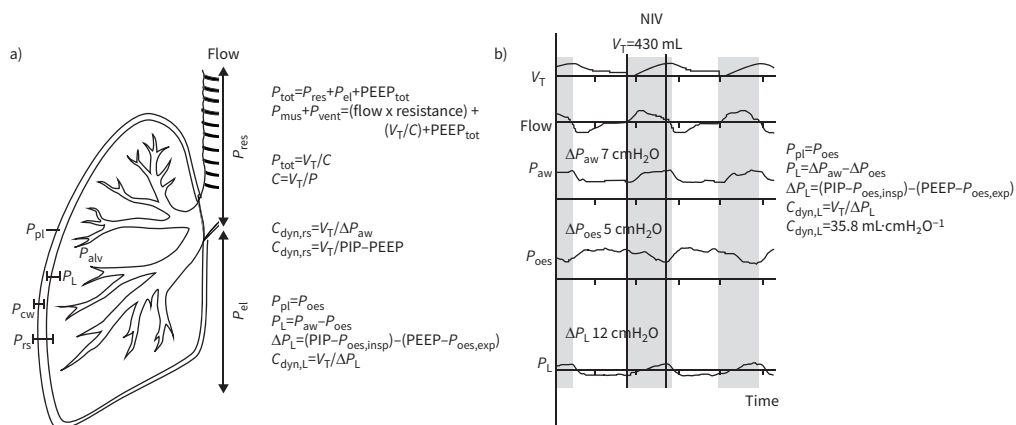


FIGURE 4 a) Diagram of the respiratory system with one-compartment lung and chest wall. The chest wall is subdivided into rib cage and diaphragmatic and abdominal wall components. The diagram shows the pressure differences across the different components of the respiratory system (see main text for better understanding of the terms). b) Calculation of mechanics during noninvasive ventilation (NIV) using data provided by the ventilator and oesophageal manometry. P_{tot} : total respiratory system pressure; P_{res} : pressure caused by resistance; P_{el} : pressure caused by elasticity; $PEEP_{tot}$: total positive end-expiratory pressure; P_{mus} : muscular pressure; P_{vent} : positive pressure from the ventilator; V_T : tidal volume; C : compliance; P : pressure; $C_{dyn,rs}$: dynamic compliance of the respiratory system; P_{aw} : airway pressure; Δ : change in; PIP: peak inspiratory pressure; P_{pl} : pleural pressure; P_{oes} : oesophageal pressure; P_{alv} : alveolar pressure; P_L : transpulmonary pressure; P_{cw} : chest wall pressure; P_{rs} : respiratory system pressure; $P_{oes,insp}$: peak inspiratory P_{oes} ; $P_{oes,exp}$: P_{oes} during expiration; $C_{dyn,L}$: dynamic compliance of the lungs.

(P_{mus}). P_{tot} equals P_{vent} plus P_{mus} :

$$P_{mus} + P_{vent} = (\text{elastance} \times \text{volume}) + (\text{resistance} \times \text{flow}) + PEEP_{tot} \quad (3)$$

This equation is sometimes written in terms of compliance (C) rather than elastance (E). Compliance is the inverse of elastance ($E=1/C$) and is a measure of the entire distensibility of the respiratory system (C_{rs}) between two points in time when the flow at the airway opening is zero:

$$P_{mus} + P_{vent} = (\text{volume}/C_{rs}) + (\text{resistance} \times \text{flow}) + PEEP_{tot} \quad (4)$$

During NIV, static elastance/compliance of the respiratory system cannot be calculated, due to the inability to perform inspiratory/expiratory occlusion manoeuvres in spontaneously breathing patients, and the inability of some ventilators specifically designed for NIV (e.g. turbine-driven ventilators) to perform hold manoeuvres. Static compliance, measured using an occlusion manoeuvre (at zero flow), eliminates the pressure caused by airflow and isolates only the elastic load that contributes to the pressure. In contrast, dynamic compliance of the respiratory system ($C_{dyn,rs}$) is a basic indicator of mechanics that can be calculated without requiring a hold manoeuvre. However, clearly this metric is not a form of compliance as it reflects both elastic and resistive loads, making its interpretation challenging in clinical practice. For example, changes in $C_{dyn,rs}$ can result from variations in airway resistance, lung elasticity or patient effort, so it should always be interpreted in the clinical context. Moreover, the linear, one-compartment model can be applied to normal subjects and in restrictive syndromes, while its application in obstructive syndromes may be more critical, as the response to pressure changes might be asymmetrical due to the heterogeneous nature of the respiratory system.

In any case, the calculation of the $C_{\text{dyn,rs}}$ is described as the ratio between V_T and the difference between the peak inspiratory pressure (PIP) and the PEEP_{tot} :

$$C_{\text{dyn,rs}} = V_T / (\text{PIP} - \text{PEEP}_{\text{tot}}) \quad (5)$$

Some new turbine-based ventilators are equipped to measure dynamic compliance during NIV, but the accuracy of these measurements remains low due to the highly dynamic respiratory pattern in ARF and the difficulty in sealing the mask properly to prevent unintentional leaks.

Dynamic compliance of the lungs

Thanks to oesophageal manometry, pressure can be divided into the transpulmonary pressure (P_L) and the chest wall pressure (P_{cw}), which act in series. P_L is the elastic recoil pressure of the lung, calculated as the P_{aw} minus pressure in the pleural space (P_{pl}). Transpulmonary driving pressure (ΔP_L) is the elastic pressure required to inflate the lung with the V_T . Experimental research demonstrated that P_{pl} can be accurately reflected by P_{oes} close to the measurement site. ΔP_L is often calculated at end-inspiration and end-expiration by means of end-inspiratory and end-expiratory occlusions (when alveolar pressure equals P_{aw}), and may reflect the stress on the non-dependent lung [35]. As stated before, during NIV, it is not possible to perform inspiratory and expiratory hold manoeuvres. For this reason, inspiratory change in dynamic P_L ($P_{L,\text{dyn}}$) can estimate dynamic lung stress on the dependent lung and is computed as $\Delta P_{L,\text{dyn}} = \text{peak } P_L - \text{end-expiratory } P_L$ [35]. In this way, the use of the oesophageal balloon catheter allows us to divide the $C_{\text{dyn,rs}}$ into its components of the lung and chest wall dynamic compliance ($C_{\text{dyn,L}}$ and $C_{\text{dyn,cw}}$, respectively). The calculation to measure $C_{\text{dyn,L}}$ is:

$$C_{\text{dyn,L}} = V_T / \Delta P_{L,\text{dyn}} \quad (6)$$

$$C_{\text{dyn,L}} = V_T / (\text{peak inspiratory } P_L - P_L \text{ during expiration})$$

or

$$C_{\text{dyn,L}} = V_T / ((\text{PIP} - \text{peak inspiratory } P_{\text{oes}}) - (\text{PEEP} - P_{\text{oes}} \text{ during expiration})) \quad (7)$$

During NIV, as during invasive ventilation in assisted-controlled modes, the PIP set on the ventilator reflects only a portion of the total pressure applied to the lungs, and is better accounted for by P_L resulting from PIP and P_{oes} (figure 4b) [36]. Leaks during NIV can affect P_{aw} and V_T monitoring, potentially compromising the accuracy of $C_{\text{dyn,L}}$ and $C_{\text{dyn,rs}}$. For this reason, some studies have used bioelectrical impedance monitors and sensors placed on the chest wall to improve V_T monitoring accuracy during NIV [37, 38].

Monitoring P_{oes} morphology and dynamic changes of P_{oes} during NIV

In spontaneously breathing patients, P_{oes} decreases as the inspiratory effort begins and reaches its lowest point at the end of inspiration, returning to baseline during the relaxation phase. During NIV, the initial drop in P_{oes} reflects the patient's effort before triggering the ventilator (figure 5) [39]. The delay between the patient's effort and the ventilator's response (onset of inspiratory pressurisation) should be brief (<100 ms). As the ventilator delivers air, P_{oes} changes slope, and lung inflation starts, with P_{oes} gradually returning to baseline with the relaxation of the inspiratory muscles. If the inspiratory mechanical time is too long, or in cases of expiratory flow limitation, active expiration can occur, indicated by a sudden rise in P_{oes} above baseline (figure 6). High breathing efforts during assisted ventilation ($\Delta P_{\text{oes}} > 12\text{--}15 \text{ cmH}_2\text{O}$) are likely to be unsustainable over long periods. Moreover, a reduction in ΔP_{oes} after 2 h of NIV is a

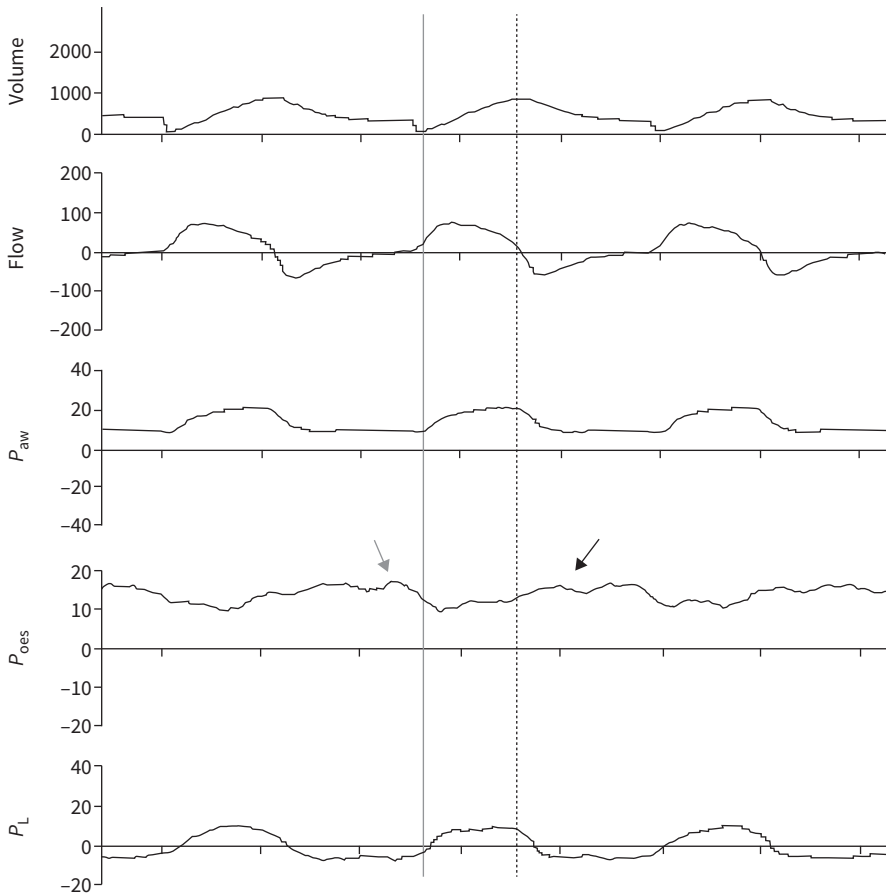


FIGURE 5 The negative deflection of oesophageal pressure (P_{oes}) is evident (grey arrow), approximately simultaneous with the positive pressure and flow applied by the ventilator (solid vertical line). This indicates that inspiration is driven by the combined action of the inspiratory muscles and the mechanical ventilator, both working together to expand the lungs and chest wall. P_{oes} gradually returns to baseline with the relaxation of the inspiratory muscles (black arrow). Ventilator cycling (dotted vertical line) approximately coincides with the midpoint of the relaxation phase. P_{aw} : airway pressure; P_L : transpulmonary pressure.

predictor of treatment success [40]. However, determining a “safe” range, particularly under NIV, requires further research [36, 41, 42]. The morphology of the P_{oes} at the beginning of the patient’s inspiratory effort provides insight into the patient’s respiratory drive and neuromuscular function. Dynamic changes in P_{oes} can provide an accurate measure of inspiratory effort. A rapid and steep drop in P_{oes} is characteristic of a strong inspiratory effort or underassistance. Conversely, especially in conditions of overassistance, a weak effort results in a small, gradual decrease. P_{oes} presents some limitations in computing inspiratory muscle effort: ΔP_{oes} underestimates P_{mus} , which reflects the total pressure generated by inspiratory muscles to expand the lungs and the chest wall. During active breathing, calculating P_{mus} is not possible, as it requires determining the elastance of the chest wall (E_{cw}) during passive lung inflation or roughly estimating E_{cw} as 4% of the predicted vital capacity [43]. Moreover, during NIV in acute care, activation of the expiratory muscles often occurs, leading to a rise in P_{ga} and, consequently, P_{oes} , followed by a decrease in both pressures as the expiratory muscles relax and

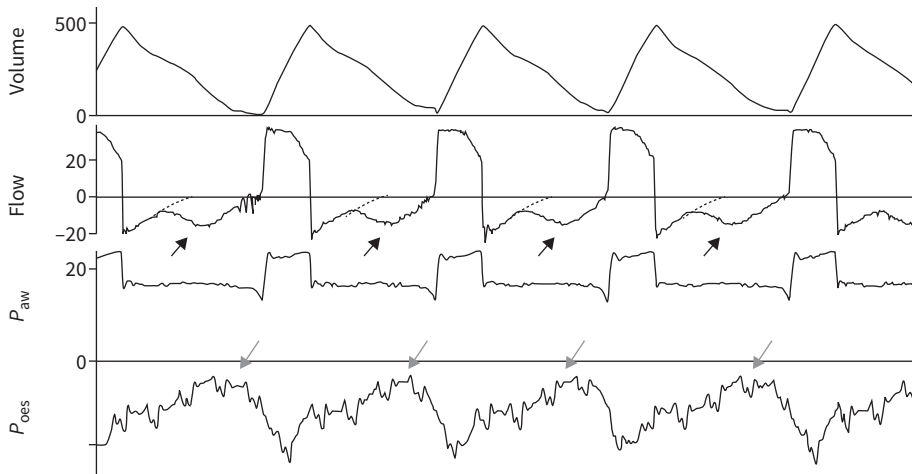


FIGURE 6 Delayed cycling with expiratory effort. If, following an initial peak, the expiratory flow does not decline exponentially (dotted lines) but instead increases (black arrows), active expiration should be suspected. Forced expiration increases intrapleural pressure, which is observed as a rise in the oesophageal pressure (P_{oes}) curve (grey arrows). P_{aw} : airway pressure.

the inspiratory muscles initiate inspiration (figure 6). To accurately calculate ΔP_{oes} , it is preferable to quantify expiratory muscle effort by measuring the rise of P_{ga} during expiration and subtracting it from P_{oes} during the subsequent inspiration. The magnitude of P_{ga} increase, in fact, reflects the effort of the expiratory muscles [44, 45]. Moreover, P_{ga} allows for the calculation of transdiaphragm pressure ($P_{di}=P_{ga}-P_{oes}$), which reflects diaphragm activity. ΔP_{di} normal values range from 5 to 15 cmH₂O.

Intrinsic PEEP: qualitative and quantitative assessment

Another common issue during NIV is dynamic hyperinflation or expiratory flow limitation that leads to intrinsic PEEP ($PEEP_i$). Dynamic $PEEP_i$ is qualitatively assessed by inspecting the real-time flow waveform on the ventilator screen, without the need for an occlusion manoeuvre. This method relies on analysing the shape of the expiratory flow.

In normal expiration, the flow waveform shows a smooth, exponential decay as the lungs empty passively. In cases of flow limitation, the expiratory flow may plateau without reaching zero flow (figure 7). However, positioning of an oesophageal balloon is essential for the quantitative measurement of $PEEP_i$. The measurement is based on the concept that, in a dynamically hyperinflated, actively breathing patient, inspiration (or the increase in the inspiratory volume) can only occur once the inspiratory muscles generate a drop in P_{oes} , which counteracts the respiratory system's elastic recoil pressure. This means that, after initiating an inspiratory effort, the gas flow shifts from exhalation to inhalation only after P_{oes} decreases by an amount equal to the $PEEP_i$. Consequently, during NIV, $PEEP_i$ can be calculated as the difference between the P_{oes} at the start of an inspiratory effort and the P_{oes} at the actual onset of inspiration. The onset of inspiration is defined as the point at which flow transitions from expiration to inspiration, which corresponds to when the ventilator begins to deliver air to the patient (figure 7). In this context, part of the total ΔP_{oes} represents the pressure needed to overcome $PEEP_i$ without generating a change in V_T . When P_{oes} fails to overcome the $PEEP_i$, preventing a sufficient change in P_{aw} or flow to trigger inspiration, a common asynchrony known as “ineffective effort” occurs.

Monitoring inspiratory resistive loads during NIV

Quantifying resistive loads during NIV is challenging, due to the inability to perform inspiratory occlusion manoeuvres and the use of pressure-targeted modes with variable flows. However, the inspiratory resistive component of the upper airways can be visually estimated by examining the flow–time waveform on the ventilator screen or with BIS. Normally, the inspiratory flow curve

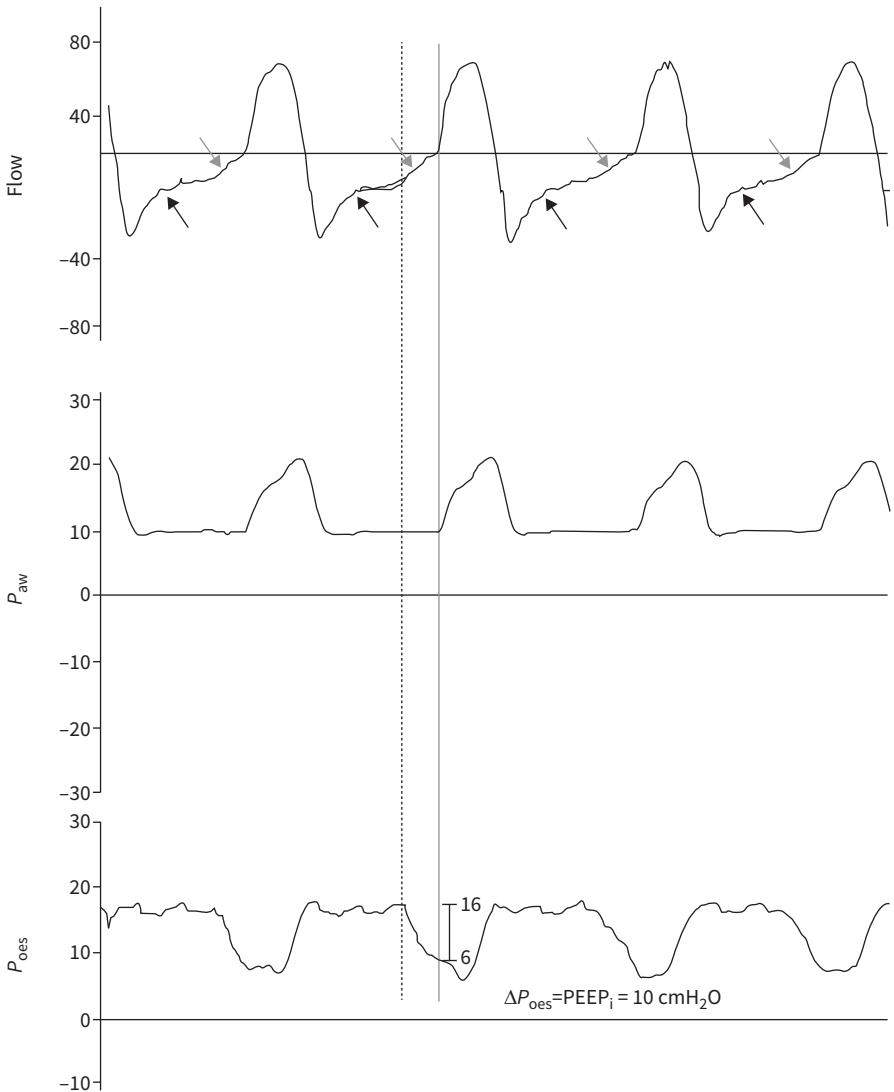


FIGURE 7 Observing the flow–time curve, expiratory flow does not reach zero before the next mechanical insufflation. The homogeneous downward concavity of the expiratory flow profile (black arrows) indicates that most of the exhalation is passive. Just before the end of exhalation, the flow curve shows an upward inflection (grey arrows). This end-expiratory upward inflection is typically caused by the patient’s inspiratory effort, as evidenced by the simultaneous drop in the oesophageal pressure (P_{oes}) curve, where the dotted line marks the start of the effort. Only when the magnitude of the P_{oes} reduction is sufficient to overcome intrinsic positive end-expiratory pressure ($PEEP_i$) does mechanical insufflation begin, although with some delay. P_{aw} : airway pressure; Δ : change in.

features a peak followed by a decline. Peak inspiratory flow is determined by driving pressure (pressure support above PEEP plus inspiratory effort) and inspiratory resistance. During NIV, some patients are more prone to UAO, influenced by factors such as comorbidities (*e.g.* obesity or neuromuscular disease), medications, patient position, anatomical features like epiglottic collapse and use of an oro-nasal mask. UAO during NIV is typically characterised by a periodic reduction in flow, accompanied by a progressively longer inspiratory time and a flatter waveform shape, followed by a sudden resumption of the flow waveform [46]. Adjusting the PEEP can improve upper airway patency (figure 8) [47–49].

Interestingly, the “Delta-inst” method has been proposed as a noninvasive tool for monitoring the airway obstruction during nasal NIV. It works by increasing or decreasing the inspiratory pressure for a single respiratory cycle. This allows the computation of respiratory resistance and elastance. Delta-inst utilises the relationships between the change of P_{aw} over time and the linear regression analysis of pressure and flow signals. It was tested in a bilevel device with minimal software modifications. In addition, it has been shown to be comparable in performance to P_{oes} as well as to FOT [50].

Efficacy of NIV assessment

Peripheral O_2 saturation

S_{pO_2} , measured noninvasively using a pulse oximeter, is used as standard practice for monitoring oxygenation during NIV [6]. The advantages and the limitations of pulse oximetry are summarised in table 1.

Transcutaneous CO_2

Transcutaneous CO_2 tension (P_{tcCO_2}) monitoring has become popular in the context of both acute and chronic NIV [6]. P_{tcCO_2} measurement is based on an electrochemical method. The

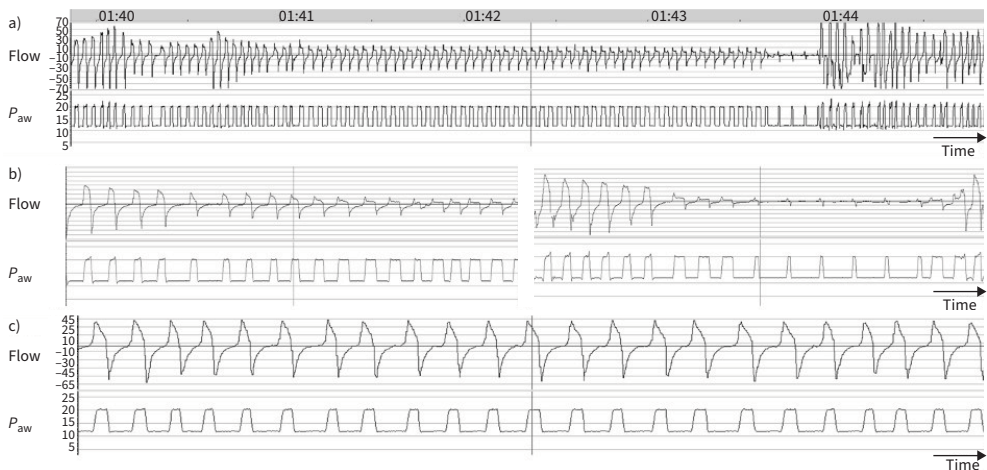


FIGURE 8 a) Sequences of inspiratory flow curve limitation, followed by a complete absence of flow. The event ends with an arousal that opens the upper airway again, resulting in high peak inspiratory and expiratory flow. b) Progressive flattening of the inspiratory portion of the flow-time waveform and even absence of flow (obstructive apnoeas). Reductions in both peak inspiratory and expiratory flow are also observed. c) A flow-time curve with normal inspiratory morphology that rules out upper airway obstruction. P_{aw} : airway pressure.

TABLE 1 Advantages and limitations of pulse oximetry monitoring during noninvasive ventilation (NIV)

Advantages	Limitations
Cheap	Low accuracy with high arterial O ₂ levels
Easy to use	Inaccurate readings in cases of:
Sensitive tool for detecting a problem	CO poisoning and other haemoglobin variants
High positive predictive value	Low perfusion state
Possible to connect to the ventilator	Dark skin pigmentation
Possible to monitor in telemedicine	Anaemia
	Nail polish or artificial nails
	Body movement or tremors
	Misinterpretation in cases of unintentional leaks
	Insufficient for detecting hypoventilation
	Not sensitive in defining the type of sleep disorder
	Low accuracy, especially when O ₂ is added to NIV

skin is typically heated, increasing the diffusion of CO₂ from the capillaries into the electrolyte solution. The decrease in pH due to the carbonic acid formation is proportional to the logarithm of arterial CO₂ tension change [51]. Table 2 describes the main advantages and limitations of P_{tcCO_2} monitoring.

Clinical scores and questionnaires

Various clinical scores have been proposed and validated for acute patients undergoing NIV. The HACOR (heart rate, acidosis, consciousness, oxygenation, and respiratory rate) scale and its updated version have been developed for prediction of NIV failure in patients with hypoxaemic respiratory failure [52, 53]. More recently, the Noninvasive Ventilation Outcomes (NIVO) score, assessed before mechanical ventilation initiation, demonstrated a good performance in predicting NIV failure, hospital mortality and 1-year mortality in COPD patients with acute exacerbation [54, 55]. In chronic settings, monitoring symptoms, sleep quality and the effectiveness of therapy is crucial to ensuring long-term NIV success [51]. The main questionnaires relating to home ventilation outcomes are listed in table 3.

New frontiers

Airway occlusion pressure

Airway occlusion pressure ($P_{0.1}$) was proposed in 1975 by WHITELAW *et al.* [56] as a noninvasive measure of respiratory drive. It is possible to assess the $P_{0.1}$ during an end-expiratory occlusion from the P_{aw} signal. In fact, $P_{0.1}$ is the negative deflection of P_{aw} generated after the onset of an

TABLE 2 Advantages and limitations of transcutaneous CO₂ monitoring during noninvasive ventilation (NIV)

Advantages	Limitations
Integration of transcutaneous CO ₂ tension and peripheral O ₂ saturation measurements	Expensive
Essential for the diagnosis of nocturnal hypoventilation (useful when O ₂ is added to NIV)	Not sensitive in periodic short changes of arterial CO ₂ tension
Possible to connect to the ventilator	Possible technical issues (<i>i.e.</i> the electrode needs baseline calibration; trapped air bubbles between the skin and the sensor; damage to the sensor membrane)
Possible to monitor in telemedicine	

TABLE 3 Questionnaires commonly used in noninvasive ventilation trials to assess patient-reported outcomes

Questionnaire	Aim	Scoring
Epworth Sleepiness Scale	To assess sleepiness	0–24
Pittsburgh Sleep Quality Index	To assess sleep quality	0–21
Functional Outcomes of Sleep Questionnaire-10	To assess the impact of daytime sleepiness on activities of daily living	5–20
Severe Respiratory Insufficiency questionnaire	To assess health-related quality of life	0–100
Maugeri Respiratory Failure Questionnaire-28	To assess health-related quality of life	0–42
S³-NIV questionnaire	To assess respiratory symptoms, sleep and comfort	0–10
Data from [51].		

inspiratory effort during the first 100 ms of occlusion. In healthy adults, normal $P_{0.1}$ values range between 0.5 and 1.5 cmH₂O [57]. Although it is an old technique, $P_{0.1}$ has gained renewed attention, especially in mechanically ventilated patients with ARF [57]. In mechanically ventilated patients, a threshold of $P_{0.1} > 3.5$ cmH₂O is an accurate cut-off point associating with high drive and effort, despite frequent respiratory muscle dysfunction [57]. Modern ICU ventilators can measure or estimate $P_{0.1}$. In the first case, it is recommended to take three consecutive measurements with 10 s pause in between, and to compute the average of the three $P_{0.1}$ values. It should be noted that the accuracy of $P_{0.1}$ can be influenced by various factors, such as higher compressible volume, the presence of humidity in the circuits, or leaks [57].

Recently, LE MAREC *et al.* [58] demonstrated a correlation between high $P_{0.1}$ values and dyspnoea, as well as a higher mortality among intubated patients. $P_{0.1}$ was also independently associated with a longer duration of mechanical ventilation [58]. Although $P_{0.1}$ has shown value in critically ill patients to discover an excessive or low respiratory drive, the evidence for the role of $P_{0.1}$ in monitoring subjects receiving NIV is more limited. The accuracy of $P_{0.1}$ measurement during NIV is challenging, due to various factors that include the presence of leaks, patient discomfort and technical limitations of the monitoring capabilities. In a cohort of patients with hypercapnic CRF due to various diseases, the value of $P_{0.1}$ obtained before home NIV initiation was associated with poor long-term survival [59]. More recently, FERNÁNDEZ ÁLVAREZ *et al.* [60] assessed the utility of $P_{0.1}$ in the follow-up of patients with COPD and obesity hypoventilation syndrome receiving long-term positive pressure treatment. Interestingly, baseline $P_{0.1}$ measurements were increased in patients with COPD and/or air trapping, and these values decreased after 6 months of treatment with continuous positive airway pressure or NIV. Considering all these factors, application of $P_{0.1}$ in NIV in both acute and chronic settings is still under investigation.

Nasal pressure swings

Sniff nasal inspiratory pressure is a validated test used for assessing and monitoring respiratory muscle strength, especially in patients with neuromuscular diseases and COPD [61]. The manoeuvre is performed by encouraging the subject to perform a quick, forceful voluntary inspiratory effort from FRC through the nose with the mouth closed and without a nose clip [61]. An old physiological study showed a linear relationship between the ΔP_{oes} and nasal and mouth pressure swings during incremental inspiratory effort in both normal subjects and patients with inspiratory muscle weakness [62]. More recently, in a proof-of-concept study, TONELLI *et al.* [63] confirmed a significant correlation between nasal pressure swings (ΔP_{nos}) and ΔP_{oes} in a

population of patients with *de novo* ARF due primarily to COVID-19 pneumonia. After 24 h, this correlation remained significant, regardless of the noninvasive respiratory support applied (high-flow nasal cannula (HFNC) or NIV) [63]. The same group performed a *post hoc* analysis of this study, demonstrating that a cut-off >5.1 cmH₂O at 2 h has a good diagnostic accuracy for HFNC failure [64]. The equipment for ΔP_{nos} recording consisted of a self-expanding hypoallergenic foam ear plug with a 16-gauge catheter inserted (typically used for intravenous access), connected to a pressure transducer. By placing the nasal plug into the nostril, it is possible to seal off the nasal passage and to continuously measure internal pressure without external interference [63]. Although ΔP_{nos} should be an interesting tool to monitor patients' inspiratory effort, because it does not require the passage of an oesophageal balloon, its role and applicability during NIV should be confirmed and validated in larger multicentric studies.

Ultrasound for NIV monitoring

Lung sonography has become an indispensable tool in clinical practice, allowing an accurate, continuous, real-time assessment of lungs, diaphragm and pleural space. It can be performed quickly without contraindications, making this technique suitable for a wide range of patients [6]. The main potential clinical indications for ultrasound to monitor patients undergoing NIV are briefly described in the following sections.

Prediction of NIV outcomes

Several small, observational, prospective studies have explored the use of ultrasonographic techniques to predict outcomes for patients requiring NIV, particularly in the context of COVID-19 [65–68]. The lung ultrasound score (LUS; an aeration score) and its updated version, known as the integrated lung ultrasound score (i-LUS), were applied together with other clinical scores and oxygenation parameters to predict NIV failure in acute hypoxaemic COVID-19 patients [65–68]. Different cut-off values have been proposed with different accuracy in terms of sensitivity and specificity. However, higher scores, both at baseline and during NIV attempts, can assist clinicians in making decisions regarding NIV prolongation or the escalation to invasive mechanical ventilation [68].

Evaluation of diaphragmatic and accessory respiratory muscle function

The diaphragm's function can be assessed with ultrasound by measuring two key parameters: the estimation of the diaphragmatic displacement and the measure of the diaphragm thickening fraction (TF_{di}). This latter refers to the change in the diaphragm's thickness during the breathing cycle, and it can be calculated as the percentage change between the diaphragm thickness at the end of inspiration and at the end of expiration. A TF_{di} $>20\%$ is generally considered normal [6].

According to the most current evidence, diaphragm ultrasound is a useful tool in the management of critically ill patients, especially for monitoring effort, guiding diaphragm-protective ventilation and predicting weaning success from invasive mechanical ventilation [69]. However, evidence regarding its use for noninvasive respiratory support monitoring has shown mixed results, particularly in COPD patients with acute hypercapnic respiratory failure needing NIV treatment. In a preliminary study, CAMMAROTA *et al.* [70] showed that the diaphragm excursion had a better accuracy in predicting NIV failure than TF_{di}, which did not change over time. These results are in contrast with a previous study, in which the presence of TF_{di} $<20\%$, assessed before starting NIV, correlated with NIV failure, mortality, prolonged mechanical ventilation, and higher tracheostomy rate [71]. More recently, a larger study including 158 COPD patients with acute exacerbation showed that the intercostal muscle thickening fraction was associated with NIV failure, even after adjusting for Acute Physiology And Chronic Health Evaluation II (APACHE II) and Sequential Organ Failure Assessment (SOFA) scores [72].

Detection of patient-ventilator interaction

A new method for easy detection and monitoring of asynchronies has been proposed [73]. In this study, VIVIER *et al.* [73] artificially generated unintentional leaks in 15 healthy volunteers undergoing NIV, inducing respiratory asynchronies such as auto-triggering and delayed cycling. By combining the diaphragm ultrasound signals (both excursion and thickening) with ventilator waveforms, the authors demonstrated a greater accuracy in detecting asynchronies compared with the assessment of respiratory flow/pressure tracings. However, this method is not yet suitable for clinical use due to the need for technical improvements. Specifically, it requires the integration of ventilator curves and ultrasound signals on a single screen.

Limitations

Besides these potential clinical advantages, the application of ultrasound for NIV monitoring has some limitations that include the operator dependency, the poor quality of images (for example in obese patients), the difficulty in achieving reproducible results, particularly when assessing respiratory muscle thickness, as well as the lack of standardised protocols. To conclude, lung ultrasound is a complementary technique that offers valuable information alongside clinical and functional assessment and enhances patient care when used in conjunction with other monitoring tools.

Forced oscillation technique

FOT uses small oscillations or pressure pulses continuously delivered by the ventilator, and the technique measures the resistance and reactance at different lung volumes or end-expiratory pressures [74]. As mentioned earlier, FOT, alone or combined with flow curve analysis, has been used to detect and classify apnoeas [7]. In addition, FOT has been proposed as a promising alternative to traditional methods for diagnosing expiratory flow limitation, which is a phenomenon mainly observed in COPD patients [74, 75]. However, it is still in the research phase, and the technique may require further clinical validation to become a standard tool in this area [76].

Electrical impedance tomography

Electrical impedance tomography (EIT) has made significant strides from its initial research applications to become a valuable tool in clinical settings, particularly in the management of mechanical ventilation in the ICU [69, 77]. EIT provides continuous noninvasive monitoring of global and regional lung ventilation as well as perfusion of the lung. By measuring impedance variations across the chest, it creates a continuous image of how air is distributed throughout the lungs, providing a real-time feedback on therapeutic interventions (*i.e.* PEEP optimisation in patients with acute respiratory distress syndrome).

EIT offers several advantages, but it also faces notable limitations that include limited depth penetration and spatial resolution, impedance variability and lack of standardisation. Additionally, EIT is susceptible to noise and artefacts, which can complicate interpretation [69]. Its clinical application in patients undergoing noninvasive respiratory support requires further studies.

Need for education

A recent study highlighted a concerning decay in pulmonary physiology knowledge among medical trainees, including those specialising in critical care, despite its fundamental role in clinical practice [78]. This decay challenges the assumption that core knowledge acquired in medical school is consistently reinforced throughout training. Given the complexity of

monitoring technologies for NIV, particularly those providing insights into respiratory mechanics, this underscores the critical need for continuous education and updates.

Conclusion

Monitoring in acute and chronic settings is a cornerstone of effective clinical management, enabling timely interventions and optimising patient outcomes. In acute settings, rapid and precise monitoring is crucial to address life-threatening conditions and improve the success rate of NIV. Conversely, chronic care settings focus on long-term monitoring to assess therapy adherence, identify causes of reduced efficacy, track disease progression, and refine treatment plans. Technological advancements have greatly improved our capability to gather real-time data, effectively bridging the gap between acute care and chronic management. A personalised, patient-centred monitoring approach that combines clinical expertise with innovative technologies ultimately provides optimal care in both contexts.

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Measurement of pulmonary structure and function using X-ray imaging

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Quantitative structural and functional X-ray imaging allows detection of early stage diseases, enables patient phenotyping, provides surrogate biomarkers and gives valuable insight into the mechanisms of respiratory diseases down to the microscale <https://bit.ly/ERSM107>

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Lung diseases are characterised by structural and functional heterogeneity that is not directly appreciable with traditional physiological measurements. Quantitative characterisation of the lung's anatomy and microstructure is necessary to understand how it affects function. This is a challenging task due to the small size and complex architecture of pulmonary airspaces, terminal airways and vessels, as well as their constant motion and deformation. X-ray imaging is the reference method for non-destructive assessment of lung morphology. In recent decades, quantitative techniques have been developed to measure regional lung function, both in the clinical and the experimental field. In this chapter we will discuss the quantitative measurements that can be derived, the pulmonary diseases in which structural and functional X-ray imaging have demonstrated utility, some of the strengths and limitations specific to each field, and key areas for future research.

Introduction

Regional changes in lung structure and function can precede detectable changes in global functional parameters, particularly in the early stages of airway or parenchymal diseases. In virtually all lung diseases, local structural and functional alterations are heterogeneous, while traditional lung function measurements can only provide global measurements. For example, small airways, which have been called the lung's "silent zone", contribute very little to overall airflow resistance, and local small airway disease can evolve long before it is detected by global pulmonary function tests, whereas it can be detected at an earlier stage with imaging [1].

Micro-CT allows three-dimensional (3D) assessment of lung structure, and is a non-destructive method that offers an advantage over classical histological techniques, which only provide two-dimensional (2D) data. In functional CT imaging, quantitative measurement of the dynamic changes in inhaled or infused contrast over short periods of time or processing of dual volume images, enables regional parametric mapping of physiological functions, such as ventilation, perfusion or tissue deformation.

Besides detecting disease processes early on, quantitative CT imaging can also allow the precise description of the lung's structural and functional phenotype. Such precise phenotyping is valuable in describing individual patient characteristics and disease trajectories, and in providing surrogate outcomes as imaging biomarkers for the assessment of new therapeutic interventions. These capabilities of CT imaging are integral parts of precision and personalised medicine [2].

Quantitative structural and functional imaging also provides unique insight into disease processes and a better understanding of the mechanisms involved in airway and parenchymal diseases, whilst also offering unique regional insight into the signals obtained with global lung function tests.

The goal of this chapter is to describe the functional parameters that can be quantified using different X-ray imaging techniques, and to provide examples of their preclinical and clinical applications.

Airway and parenchymal morphology

Micro-CT

Histology is the gold standard technique for examining lung tissue on a microscopic scale, and quantitative stereological measurements have deepened our understanding of normal lung tissue and pathology. However, histological analysis is a labour-intensive and terminal procedure in preclinical studies, which often only provides 2D information for specific regions. This may lead to underestimation or overestimation of lung disease based on sampling. Tissue distortion may also occur during paraffin inclusion and mechanical cutting, potentially introducing further bias. Due to the ability of X-rays to penetrate deep into tissue, micro-CT combined with image processing allows the rapid quantitative assessment of 3D microstructures, enabling virtual histology [3].

Morphometric studies have analysed the lung acinar structure in terms of alveolar volume, diameter, surface/volume ratio and porosity [4–6]. Porosity and alveolar surface density appear to be meaningful parameters in the study of diseases such as emphysema [7]. LITZLBAUER *et al.* [8] used micro-CT to quantify alveolar volume and alveolar surface/volume ratio in *post mortem* fixed and stained normal pig lung. This approach has also been used to assess airway length, diameter and compliance in *post mortem* rat lung [9]. With micro-CT, acinar morphometry has been characterised in unstained perfusion-fixed normal mouse lung [5, 10], with longitudinal follow-up of lung fibrosis in mouse [11] and in endotoxin-injured rat lung [12].

Respiratory gating methods have been introduced for *in vivo* micro-CT [13]. Although respiratory gating improves image quality by mitigating motion blurring, its spatial resolution (50–80 μm) is insufficient to resolve acinar structures *in vivo* with scan times in the order of several minutes [14].

Micro-CT of human lung samples *post mortem* has provided novel insight into the 3D structural alterations that occur in COPD [15, 16]. The centrilobular emphysematous phenotype of COPD has been found to be associated with narrowing and obliteration of the terminal bronchioles, which begins prior to the onset of emphysematous destruction [15].

Conventional micro-CT generates contrast that relies on the attenuation of X-rays. However, attenuation contrast is inherently low in soft tissue, particularly in the lung due to the large amount of air. This limitation is partially addressed by staining fixed tissue samples [17]. Synchrotron radiation X-ray tomographic microscopy (SRXTM) uses the high flux and

monochromaticity of X-rays produced with a synchrotron to acquire high-resolution ($1\text{--}2\ \mu\text{m}^3$ voxel size) and higher-contrast volumetric images in much shorter acquisition times than laboratory micro-CT. Using SRXTM, the timing of post-natal acinar development has been studied in *post mortem* fixed and stained rat lung, in terms of acinar volume and number of alveoli [18]. Individual acini can be identified more easily in 3D images than 2D histological lung sections, allowing their structural analysis [19]. Recently, respiration-gated *in vivo* SRXTM at $\sim 1\ \mu\text{m}$ resolution has shown more spherical and larger murine alveolar diameters than in *post mortem* samples (figure 1d and e) [21].

Synchrotron radiation X-ray phase contrast imaging (SRXPC) on the other hand, addresses the low attenuation of the lung by relying on phase change and refraction within the lung tissue to enhance contrast without the need for staining or contrast agents [21, 22]. Synchrotron radiation offers advantages over standard X-ray sources for phase-contrast imaging due to its high degree of spatial coherence, flux, monochromaticity and small source size [23]. For biological tissues, at higher X-ray energies, refraction is orders of magnitude larger than attenuation [24]. Moreover, the numerous air–tissue interfaces in the lung enhance the refraction of X-rays [25]. Lung and airway morphology have been assessed in small animals using this technique, both in normal lung [26–29] and in models of asthma or pulmonary fibrosis [30, 31]. Anatomic assessment of fresh *post mortem* lungs of large mammals can be performed using an anthropomorphic chest phantom [32]. As well as the morphological study of the airspaces, SRXPC can be used for the 3D assessment of pulmonary vascular microanatomy in both human [33] and animal models [34] of

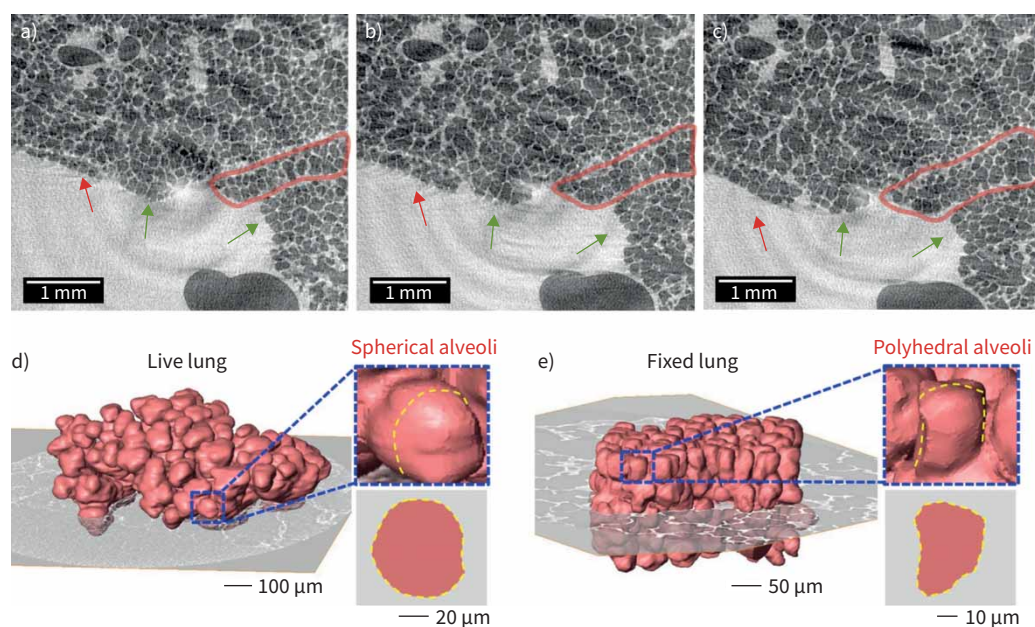


FIGURE 1 Alveolar morphology at different pressures. a) 5 cmH₂O, b) 10 cmH₂O and c) 15 cmH₂O, in neonatal rats imaged *in vivo* with synchrotron radiation X-ray phase contrast imaging. The green arrows indicate matching lung structures, while the red lines encircle regions that exhibit a stronger volume increase compared with the surrounding structures. The red arrows show areas in which different alveolar structures appear into the image plane with increasing pressure. d and e) Three-dimensional rendering of synchrotron radiation X-ray tomography of the alveoli in a mouse, showing d) spherical alveoli at end-expiration *in vivo*, and e) polyhedral alveoli *post mortem* fixed lung. Reproduced and modified from [20] (a–c) and [21] (d and e) with permission.

pulmonary hypertension. Such studies have identified distinct types of plexiform lesions in pulmonary hypertension, providing insight into microvascular remodelling and its haemodynamic consequences [33]. With SRXPC microscopy, multiscale ($\sim 25\ \mu\text{m}$ down to sub- μm) 3D virtual histology of human lung samples can be performed [35]. Multiscale SRXPC imaging has recently been used in a whole *post mortem* human lung, locally imaged down to $2.5\ \mu\text{m}$ voxel resolution within regions of interest [36], and in the assessment of airway morphology in congenital pulmonary airway malformation [37]. Recently, X-ray ptychographic tomography, a quantitative microscopy method, has been developed to enable spatial resolution of a few nanometres [38]. This technique allows the resolution of features at the cellular and sub-cellular level, such as neuronal dendrites [39], although data in the lung are very limited.

SRXPC has also been adapted to more widely available laboratory X-ray sources at the cost of much longer image acquisition times than with a synchrotron source [40]. Recent developments in SRXPC setups [41, 42] and X-ray sources [43, 44] have accelerated data acquisition. Phase-contrast imaging allows dark field image contrast, due to the local small angle scattering of X-rays by sub-resolution microscopic structures on the nanometre to hundreds-of-micrometres scale [43, 44]; this is inaccessible in attenuation or phase-contrast images [45]. Using this technique, the dark field signal intensity in 2D radiographs was found to reduce with the increasing severity of emphysema in mice, correlating with histological mean cord length [46–48]. It has similarly been shown to decrease in bleomycin-induced fibrosis [49, 50] and ventilator-induced lung injury [51], suggesting that the dark field imaging signal is related to the density of air–tissue interfaces, and may indirectly reflect alveolar size. Recently, 2D dark field radiography has shown promise in detecting emphysema in patients with COPD [52]. Efforts are under way to translate laboratory dark field CT imaging to the human scale [53]. However, evidence on the added value of dark field imaging compared to standard clinical CT for detecting emphysema is still lacking.

Clinical CT

Clinical CT is the gold standard method for quantitative characterisation of lung morphology. Structural pulmonary measurements using X-ray CT involve parenchymal assessment and airway measurement [54].

Parenchymal assessment

CT imaging allows quantification of emphysema through the assessment of lung tissue density. Each voxel in a CT image is assigned a Hounsfield unit (HU) based on X-ray attenuation, with air at -1000 HU, water at 0 HU, and denser materials >0 HU. A threshold of -950 HU is generally used for emphysema [55].

Another approach involves analysing the lung density at specific percentiles on a distribution curve, particularly the 15th percentile, which is sensitive to emphysema progression in patients with α_1 -antitrypsin deficiency [54, 56].

Densitometric emphysema measurements have been correlated with lung histology [57], and precede abnormal spirometry [58].

However, quantitative CT densitometry and both emphysema and airway measurements are impacted by image quality. Factors relating to image acquisition, such as the CT imaging device used, the radiation dose applied, the reconstruction kernel, the field of view and the voxel size, as well subject-related factors, such as body mass index, positioning within the scanner and breath-hold volume, affect densitometric measurements [59, 60]. Sharper image reconstruction

kernels and thinner slices have been demonstrated to overestimate the amount of emphysema. It is also important that normal variations are accounted for in nonsmokers in order to accurately interpret emphysema extent [54, 61].

CT measurement of emphysema would benefit from methodological standardisation, which is crucially lacking [62].

Airway dimensions

Segmentation of the airway tree from multidetector CT images can be performed using commercial as well as open access software [63]. Several morphometric measurements can be derived from the segmented images, including airway lumen and outer wall area and diameter, the wall area or thickness, and the percentage of the airway that is airway wall (figure 2) [64]. CT-based airway measurement is a challenging task because airway branches have different orientations and are not always perpendicular to the image plane, and airway dimensions range from 20 mm down to sub-millimetre [60].

The total number of airways detected within the lung CT, or total airway count (TAC), is another derived metric (figure 3, upper panel) [60, 65]. A reduced TAC is associated with the severity of COPD and the total number of terminal bronchioles [65]. TAC is also significantly decreased in patients with greater asthma severity and is related to airway wall thickness and ventilation defects on helium-3 MRI [66]. The decreased number of detected airways may be due to narrowing or obliteration of the airway lumen by remodelling or mucus plugging, or thinning and destruction of the airway wall [60].

Terminal bronchioles are prone to collapse during expiration as lung volume decreases due to surface fluid tension. In the normal lung, airway closure starts at a level above RV, which

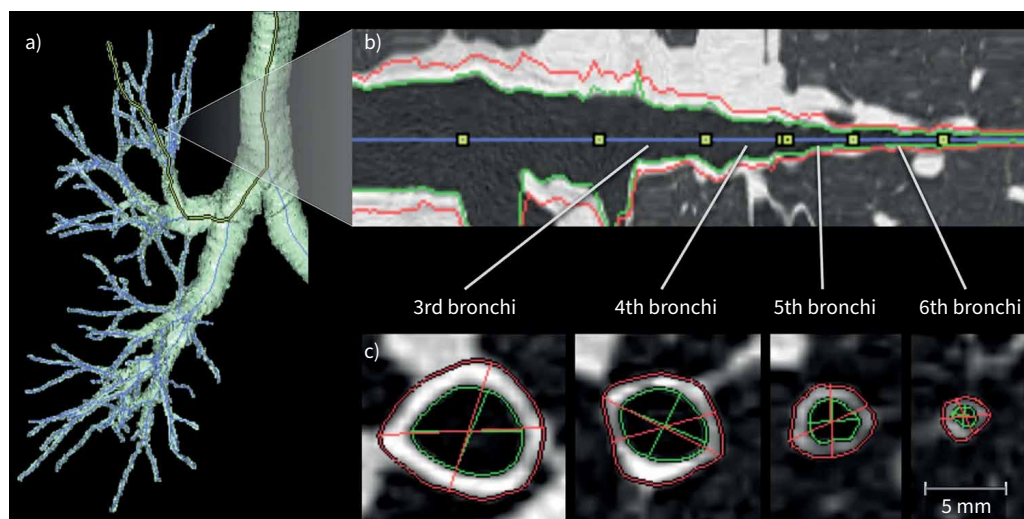


FIGURE 2 Three-dimensional (3D) CT analysis of the airways. a) 3D image of the airway trees. b) Longitudinal image of the reformatted airway. c) Short axis image of the airway. The red and green lines indicate the outer and inner edges of the airways, respectively. The areas within the green lines and between the red and green lines are the airway inner luminal area and the wall area, respectively. Reproduced and modified from [64] with permission.

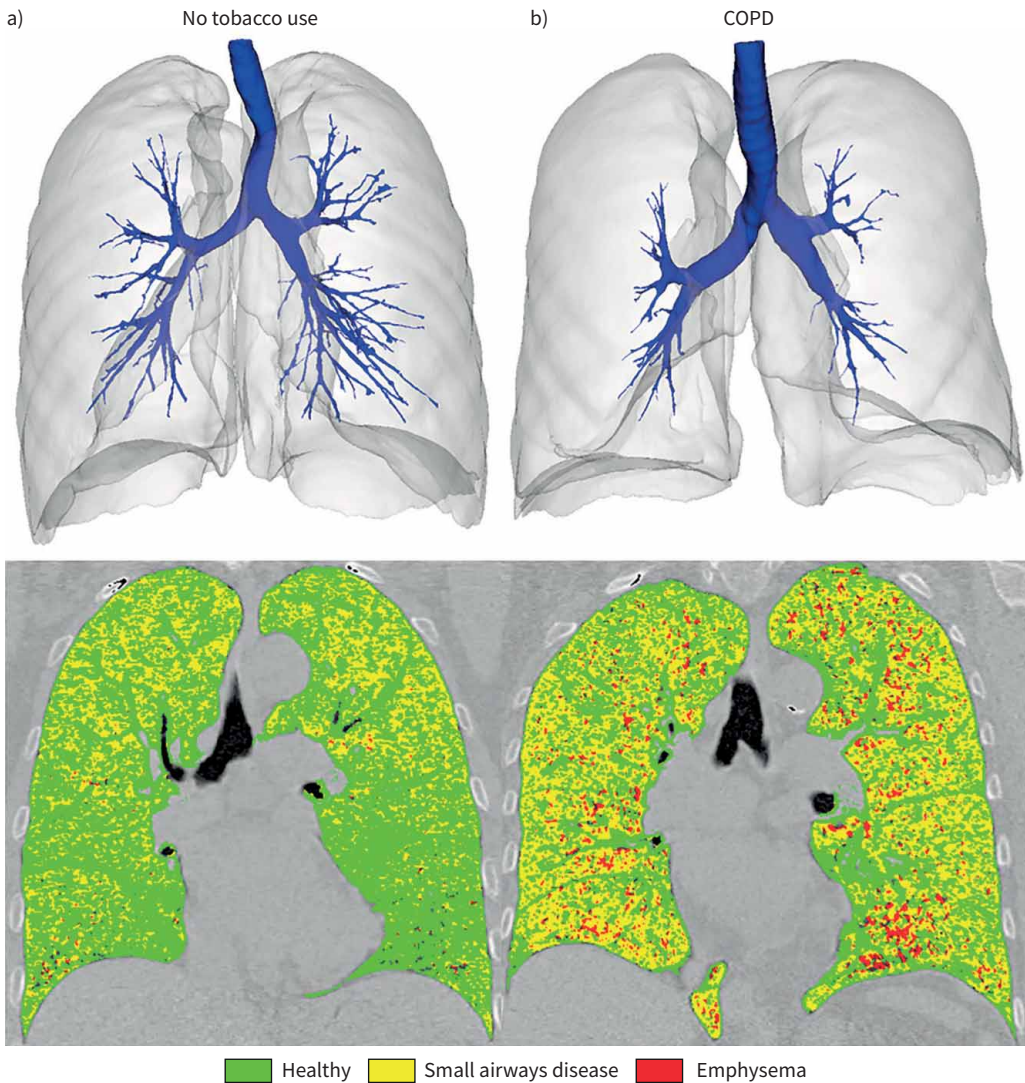


FIGURE 3 CT three-dimensional (3D) airway tree (top panel) and parametric response mapping (PRM) (bottom panel) for a) a patient who had never used tobacco and b) a patient with COPD. The participant who had never used tobacco was a 71-year-old woman with an FEV_1 of 115.3% predicted and an FEV_1/FVC ratio of 74.8%. The participant with COPD was a 64-year-old woman with an FEV_1 of 46.9% pred and an FEV_1/FVC ratio of 52.9%. Compared with the participant who had never used tobacco, the participant with COPD showed a visually and quantitatively reduced number of CT scan airways as measured by total airway count (TAC) (patient who had never used tobacco: TAC = 300; patient with COPD: TAC = 128) and an increased extent of small airways disease, measured using PRM of functional small airway disease (patient who had never used tobacco: CT PRM functional small airway disease = 28.8%; patient with COPD: CT PRM functional small airway disease = 49.6%). Reproduced and modified from [60] with permission.

increases with age [67]. In obstructive diseases, peripheral airway narrowing enhances small airway closure, leading to gas trapping, which in turn leads to lower lung CT attenuations within expiratory images. Although small airways cannot be directly measured due to the limited spatial resolution of clinical CT, areas of low attenuation in expiratory CT images are

taken as an indirect sign of gas trapping due to small airway closure. A threshold of -856 HU is usually used on expiratory CT images to identify gas trapping [68]. CT gas trapping has been shown to correlate with lung function and disease severity in COPD [69] and asthma [70, 71]. However, a single attenuation cut-off may not distinguish gas trapping caused by emphysema from gas trapping caused by small airway closure. Co-registration of inspiratory and expiratory CT images has been proposed as a way to quantify air trapping (>-950 HU on inspiration but <-856 HU on expiration) and to distinguish functional small airway disease (PRM^{fSAD}) from emphysema (PRM^{Emph}) (both <-950 HU on inspiration and <-856 HU on expiration) represented as parametric response mapping (PRM) (figure 3, lower panel) [72].

Follow-up data from the COPDGene study have shown that PRM^{fSAD} at baseline is associated with subsequent FEV_1 decline [73] and emphysema development [74], suggesting it can serve as a surrogate imaging biomarker.

Functional lung imaging

Ventilation

Alveolar ventilation ($\dot{V}_A$) ($\text{mL}\cdot\text{min}^{-1}$) is the volume (V) of fresh or tracer gas delivered to the alveolar space per unit of time. Specific ventilation ($s\dot{V}$) is defined as the fraction of regional gas V that is renewed by fresh gas per unit of time. In other words, $\dot{V}_A/V_g$ (min^{-1}), where V_g is the end-expiratory gas V within the considered region (table 1). Alternatively, $\dot{V}_A$ can be normalised to tissue mass, measured based on attenuation value [75]. These definitions apply to dynamic image acquisition regimen incorporating time. $\dot{V}_A/V$ distributions can visualise mismatches between regional $\dot{V}$ and gas V [75].

TABLE 1 Definition of functional imaging variables

Variable	Expressed as	Definition	Unit
Alveolar ventilation	$\dot{V}_A = \Delta V/t$	Volume of gas renewed per unit of time in volume of interest	$\text{mL}\cdot\text{min}^{-1}$
Specific alveolar ventilation	$s\dot{V}_A = \dot{V}_A/V_g$	Ventilation per unit of gas volume in volume of interest	min^{-1}
Specific volume change	$s\Delta V = \Delta V_g/V_{g0}$	Gas volume change per unit of initial gas volume in volume of interest	Dimensionless
	$s\Delta V = \Delta V/m_{\text{tissue}}$	Gas volume change per unit of tissue mass in volume of interest	$\text{mL}\cdot\text{g}^{-1}$
Perfusion	$\dot{Q} = \Delta V_B/t$	Volume of blood replaced per unit of time in volume of interest	$\text{mL}\cdot\text{min}^{-1}$, $\text{mL}\cdot 100\text{ mL}^{-1}\cdot\text{min}^{-1\#}$
Mean transit time	$\text{MTT} = V_B/\dot{Q}$	Average time it takes for blood to flow through the pulmonary vessels	
Ventilation/perfusion ratio	$\dot{V}_A/\dot{Q}$	Ratio of ventilation to perfusion in volume of interest	Dimensionless
Blood volume	V_B or PBV	Volume of blood in volume of interest	mL
Biomechanics			
Strain	$\epsilon = \Delta V_g/V_{g0}$	Gas volume change relative to initial gas volume in volume of interest, or: $J - 1$	Dimensionless
Elastance	$E = \Delta P_{rs}/\Delta V_g$	Pressure change required to induce gas volume change in volume of interest	$\text{hPa}\cdot\text{mL}^{-1}$, $\text{cmH}_2\text{O}\cdot\text{mL}^{-1}$
Jacobian	J	Determinant of the tissue deformation matrix between two different volumes	Dimensionless
PBV: perfused blood volume. #: further normalised to tissue volume. Reproduced and modified from [75] with permission.			

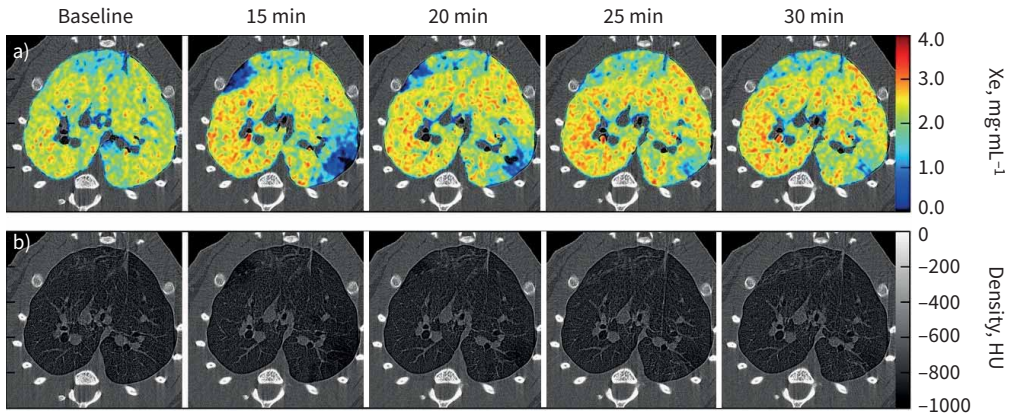


FIGURE 4 Quantitative K-edge subtraction imaging of a) Xenon (Xe) distribution and b) tissue-density at 47- μm resolution in a sensitised Brown Norway rat lung. The transient appearance of defects (shown in blue) in response to inhaled ovalbumin challenge is shown over time. Reproduced and modified from [82] with permission.

Due to its high density, xenon (Xe) enhances X-ray attenuation, which can be quantified and allows measurement of the rate of wash-in or wash-out of an Xe–O₂ mixture; hence, $s\dot{V}$. Clinical CT has been used to measure regional $\dot{V}$ by imaging the kinetics of Xe wash-in or wash-out [76]. Use of the technique is feasible in small animals with pre-clinical micro-CT scanners [77]. Alternatively, krypton can be used, which is a less dense gas than Xe with lower contrast enhancement but with no anaesthetic properties [78–81]. K-edge subtraction (KES) imaging uses two monochromatic X-ray beams produced by a synchrotron source and material decomposition to directly quantify the distribution of Xe in the lung [22]. This imaging modality is free from beam hardening, and is several times more sensitive and spatially resolved than standard CT. However, it is not available for clinical application due to the cost and the scarcity of synchrotron sources. With KES, high-resolution maps of tissue attenuation and inhaled Xe can be obtained from the same CT images (figure 4) [83]. Serial KES CT can be used to compute an $s\dot{V}$ image based on the time constant of Xe wash-in or wash-out [84].

Owing to its high spatial resolution, KES CT has identified a “functional unit” of $\dot{V}$ at which Xe gas distribution becomes homogenous, with a volume close to that of the acinus, in rabbits [85]. KES can be used to investigate the mechanisms of patchy $\dot{V}$ heterogeneity due to bronchoconstriction in rabbits [86]. It has been found that central airways exhibit slower constriction and occasional paradoxical dilation [87], while peripheral airways respond faster and earlier, demonstrating “serial interaction” of the proximal airways with the downstream peripheral airways during bronchoconstriction [88]. In sensitised animals, methacholine has been shown to cause central airway constriction with little peripheral $\dot{V}$ heterogeneity, while allergens, as well as inhaled methacholine, trigger a more heterogeneous peripheral pattern [89]. Uneven aerosol particle and immune cell distribution [90] can be measured with high-resolution KES and correlative histology [82].

Dual-energy clinical CT (DECT) uses two sets of images acquired at different energy spectrum peaks simultaneously or rapidly after each other [91]. This allows for a higher sensitivity to contrast material than standard CT. This technique also uses material decomposition, a method

that enables the extraction and quantification of element-specific contrast. DECT offers simplified $\dot{V}$ imaging with a single Xe mixture inhalation; the inhaled concentration can also be reduced to ~30% in order to lower both ionising radiation dose and the anaesthetic side-effects if the gas is repeatedly inhaled [78]. The development of photon-counting spectral CT techniques using detectors that are sensitive to the energy of incident photons, may have a higher sensitivity, allowing wider use of krypton. Regional lung $\dot{V}$ has been assessed with Xe-enhanced DECT in subjects with COPD, revealing reduced $\dot{V}$ and gas trapping in regions with low attenuation due to emphysema [92]. In asthmatics, the extent of $\dot{V}$ defects on Xe-DECT is associated with decreased lung function [93].

Instead of using an inhaled contrast gas, static lung CT images acquired during breath-hold at two different volumes (total lung capacity and FRC or RV, for example) can be used to measure regional lung gas V change (ΔV_g) or specific gas V change ($s\Delta V_g$) [94]. This is based on measuring the local difference in attenuation, which reflects the change of the air content in a given voxel, assuming the overall lung tissue and blood content remain unchanged. However, because the lung parenchyma moves between the two inflation states, image co-registration algorithms are required to warp one image to match the other (figure 5). In healthy lungs, when $s\Delta V_g$ is normalised to regional lung tissue mass measured based on local attenuation, it becomes more uniform, with little regional difference with respect to gravity, unlike ΔV_g [96]. $s\Delta V_g$ was found to be reduced, with greater heterogeneity in subjects with COPD [96] and positive correlation with GOLD stage, FEV₁, diffusion capacity, arterial blood oxygenation, 6-min walking distance, as well as negative correlation with plethysmographic indices of gas trapping and dyspnoea score [95]. In severe asthmatics, ΔV_g is reduced compared with normal subjects, which is consistent with air trapping [97]. A similar co-registration approach in a previous study utilised dynamic four-dimensional (4D) CT $\dot{V}$, and found increased ΔV_g heterogeneity during tidal breathing in both healthy and asthmatic subjects relative to that of breath-hold, perhaps because of airway resistance present only in dynamic breathing [98].

Perfusion

Perfusion ($\dot{Q}$) ($\text{mL} \cdot \text{min}^{-1}$) is the volume of blood delivered to a given region per unit time. $\dot{Q}$ is sometimes further normalised to tissue volume and expressed as $\text{mL} \cdot 100 \text{ mL}^{-1} \cdot \text{min}^{-1}$. In DECT, it can be measured using dynamic imaging during iodine or gadolinium contrast injection and indicator dilution theory [99], based on the first-pass kinetics of the contrast material. $\dot{Q}$ is calculated as the ratio of peak parenchymal enhancement, based on the attenuation *versus* time curve of a given parenchymal voxel, to the area under time attenuation curve of a reference pulmonary artery [100].

Lung perfused blood volume (V_B or PBV) is the ratio of the late-phase or steady-state parenchymal contrast element mass computed by material decomposition, to the concentration within the main pulmonary arterial lumen: $V_B = q/[C]$. This method allows measurement of the contrast mean transit time as: $V_B/\dot{Q}$ (table 1). Pulmonary V_B may alternatively be assessed during slow contrast injection or at steady concentration contrast infusion.

Previous studies using DECT demonstrated that V_B and $\dot{Q}$ are correlated, which is plausible if the V_B is representative of the vessel diameter that affects its resistance [100]. Lung $\dot{Q}$ imaging has been performed in rodents using cardiac gated micro-CT [101]. Synchrotron KES imaging can be used to map regional V_B distribution with iodine contrast injection [102]. Clinical CT-based $\dot{Q}$ imaging has shown that $\dot{Q}$ heterogeneity is increased in subjects with apical centrilobular emphysema, suggesting that reduced $\dot{Q}$ in regions with smoking-associated inflammation may be a causal factor of emphysema (figure 6) [103].

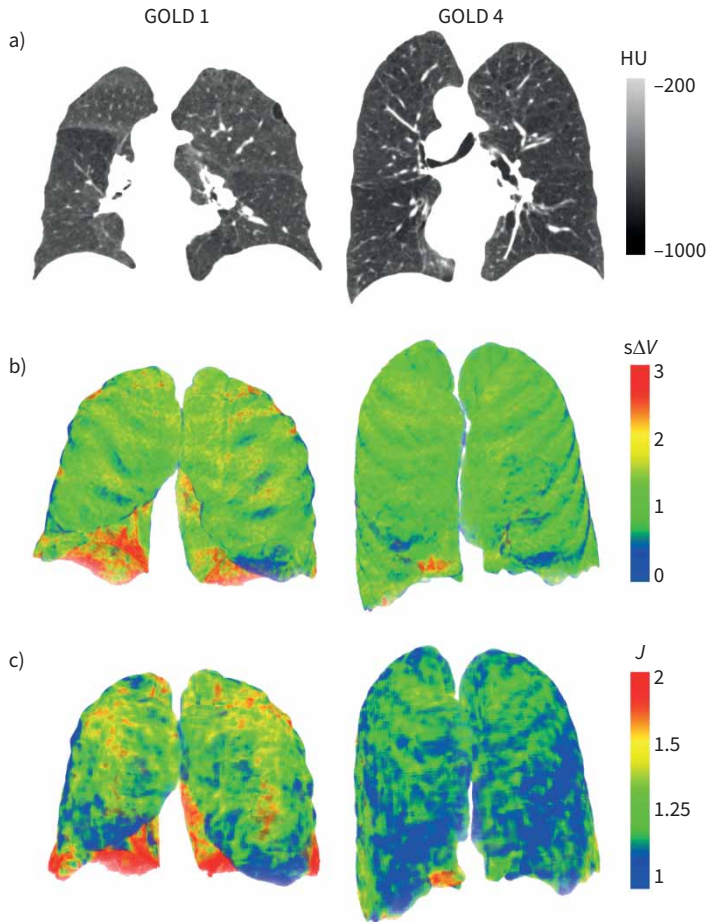


FIGURE 5 a) Coronal expiratory CT and three-dimensional renderings of the b) regional specific volume change ($s\Delta V$) and c) Jacobian (J) in representative Global Initiative for Chronic Obstructive Lung Disease (GOLD)-1 and GOLD-4 participants. Regional values of $s\Delta V$ and J are reduced in the GOLD-4 compared with the GOLD-1 participant. Reproduced and modified from [95] with permission.

$\dot{Q}$ imaging may be more sensitive in detecting peripheral defects due to small emboli [104] and could improve the diagnosis of pulmonary embolism [105]. It may enable differentiation of acute pulmonary embolism and chronic thromboembolic pulmonary hypertension [106].

$\dot{V}/\dot{Q}$ ratio

The $\dot{V}/\dot{Q}$ ratio is a fundamental parameter of gas exchange. $\dot{V}/\dot{Q}$ distributions characterise the contributions of different functional lung units, with pulmonary shunt as the lowest $\dot{V}/\dot{Q}$ ratio and alveolar deadspace as the highest. Using CT imaging with Xe enhancement combined with compartmental modelling, it has been possible for preclinical studies to measure regional lung $\dot{V}$, $\dot{Q}$ and $\dot{V}/\dot{Q}$ [107].

The regional matching of gas and V_B in the lung dynamically changes within the breath in mechanically ventilated animals. These changes and the effect of ventilator settings have been demonstrated with synchrotron 4D KES CT [108].

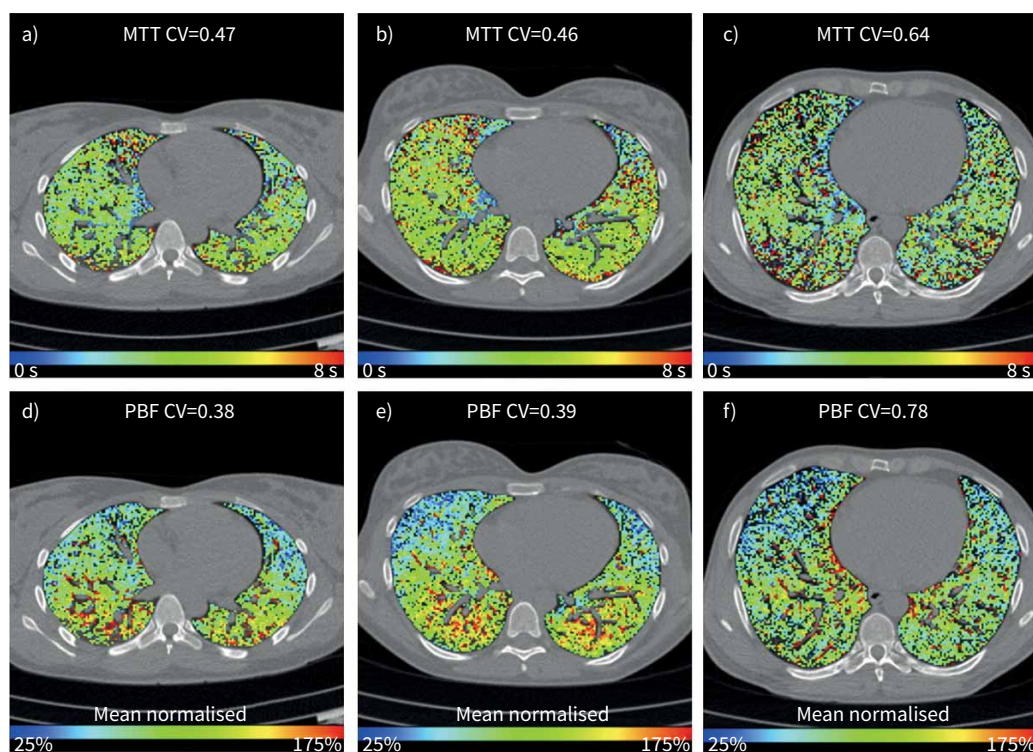


FIGURE 6 Color-coded maps of perfusion parameters overlaid on imaging slices. a–c) Mean transit time (MTT) in a) a nonsmoker, b) a smoker without emphysema and c) a smoker with centrilobular emphysema. The maps show a significant increase in the regional heterogeneity of MTT measurements in the subject with centrilobular emphysema compared with the nonsmoker and the smoker without emphysema. d–f) Pulmonary blood flow (PBF) normalised to the mean PBF in d) a nonsmoker, e) a smoker without emphysema and f) a smoker with centrilobular emphysema. As for MTT, an increased regional heterogeneity is seen in the mean normalised PBF measurement in the subject with centrilobular emphysema compared with the nonsmoker and the smoker without emphysema. CV: coefficient of variation. Reproduced and modified from [103] with permission.

Clinical DECT with Xe and iodine enhancement can provide high-resolution structural and functional images of gas and V_B distribution in pulmonary embolism [109], and their ratio has been used to estimate $\dot{V}/\dot{Q}$ in both normal and emphysematous parenchyma of COPD patients [110]. In a study by HONG *et al.* [80], DECT was used to simultaneously measure the regional distribution of inhaled krypton and perfused iodine in dogs.

Although CT use offers higher resolution maps of regional gas and blood distribution, as well as lung morphological images, radiation-dose considerations have meant that quantitative assessment of $\dot{V}/\dot{Q}$ has been limited. It is possible that the development of photon-counting CT may allow for a decrease in ionising radiation exposure. In a recent study, co-registration of breath-hold images was used to map ΔV_g , while iodine enhancement was used to measure regional V_B with photon-counting CT, allowing lower radiation dose imaging [111].

Biomechanics

Strain is defined by the relative change in gas volume mainly as a result of ventilation. It is a dimensionless parameter and expressed as the change in volume (ΔV) relative to the reference

volume, which may be end-expiratory volume, RV or FRC: $\Delta V/V$. Strain is identical to $s\Delta V_g$ (see Ventilation section). However, it is important to distinguish this metric from $s\dot{V}$ [75]. Local lung stiffness or elastance is expressed as the pressure change (ΔP_{rs}) required to induce a given ΔV_g (or $\Delta P_{rs}/\Delta V_g$), under conditions where regional alveolar pressures are fully equilibrated. Non-rigid co-registration of CT images acquired at two different different lung volumes allows the local lung strain to be mapped based on the 3D transformation matrix between the two volumes. The determinant of this matrix is termed the “Jacobian determinant” or “Jacobian”. The Jacobian is a biomechanical metric that informs on local lung tissue deformation [112]. A Jacobian determinant value of >1 indicates local expansion, whereas a value of <1 indicates local contraction. Imaging local lung elastance requires knowledge of the pressure within the imaged airspaces at any given volume (table 1). This is only feasible in static conditions, reasonably assuming an equal pressure field within communicating airspaces at pressure equilibrium. However, during tidal breathing, the local pressures within the airspace cannot be directly measured.

Lung tissue is viscoelastic, meaning its apparent elastic properties depend on the rate of volume change [113]; because of this, physiology is better characterised by lung deformation in dynamic breathing conditions. However, dynamic breathing and cardiovascular activity limit the spatial resolution of CT imaging due to motion blurring. This makes real-time assessment of regional lung micromechanics very challenging. The study of aeration and deformation within individual alveoli in CT microscopy are particularly difficult. This issue is addressed by 4D CT imaging, which involves gating the acquisition of individual image projections with the cardiac and respiratory motions. These can be prospectively acquired or retrospectively sorted for image reconstruction. The exposure time of each image projection must also be reduced as the microscopic feature of interest becomes smaller, making 4D CT microscopy technically challenging.

In a recent study, lung regional $s\Delta V_g$ was measured in normal and bleomycin-induced lung fibrosis *in vivo* at 50- μm voxel resolution in mice, with 4D CT followed by co-registration of end-inspiratory and end-expiratory volumetric lung images [114]. This made it possible to identify heterogenous local lung stiffening due to inflammation and fibrosis, and to assess the effects of nintedanib treatment. A similar approach with multiple within-breath image reconstruction allows measurement of regional flow–volume curves [115].

In 2D-projection imaging, the numerous air–tissue interfaces crossed by the incident X-rays within the lung produce phase gradient patterns, which resemble random noise or “speckles” [25, 116]. Dynamic changes in regional gas volume can be measured by calibrating these speckles against known lung air volume [117]. This technique has an excellent time resolution; however, it is limited by the fact that only 2D-projection images can be acquired. Therefore, the structure of individual acini cannot be studied directly. Phase-contrast speckle has been used to quantify real-time changes of lung liquid clearance and aeration at birth in rabbits, providing insight into the transition of the respiratory system to life after birth [118].

SRXPC has been used to investigate parenchymal micromechanics of the mechanically ventilated injured lung *in vivo* [119, 120]. Using *in silico* modelling, the dynamic nature of tidal alveolar collapse and recruitment, or “atelectrauma”, and the role of parenchymal interdependence has been demonstrated in animal studies of lung injury [119]. Compliant closure of individual small airways due to increased surface tension of the lining fluid, which is thought to play an important role in downstream atelectasis [121], has been directly visualised using this technique [122]. The progression of lung injury caused by atelectrauma has also been demonstrated and correlated to histology [120].

SERA *et al.* [123] used SRXTM in mice *post mortem* to track acinar deformation during lung inflation. They noted differential expansion of the alveolar ducts and the alveoli. In an earlier study, LOVRIC *et al.* [124] used SRXPC microscopy with cardiac and respiratory gating and a voxel resolution of $1.1\ \mu\text{m}^3$ in new born rats to study alveolar inflation *in vivo* (figure 1a–c). More recently, the use of respiration-gated SRXTM imaging allowed morphometric measurement of the alveoli *in vivo* in mouse lung, at a voxel size of $0.7\ \mu\text{m}$, albeit limited to the very apical alveoli, which could be resolved with sufficient quality at this resolution [125]. Maps of within-breath local acinar and vascular strain (ϵ) have been obtained *in vivo* in rat lungs using 4D SRXPC tomography at $6\ \mu\text{m}^3$ voxel and 10 ms temporal resolution (figure 7) [126]. This makes it possible to quantitatively study the parenchymal dynamics throughout the lungs, showing predominant alveolar rather than ductal expansion over a physiological respiratory pressure range, with no tidal alveolar recruitment.

In clinical CT, co-registration of dual volume images has been used to measure lung deformation (Jacobian) [94] in patients with COPD [95, 112, 127, 128] and asthma [97, 98, 129].

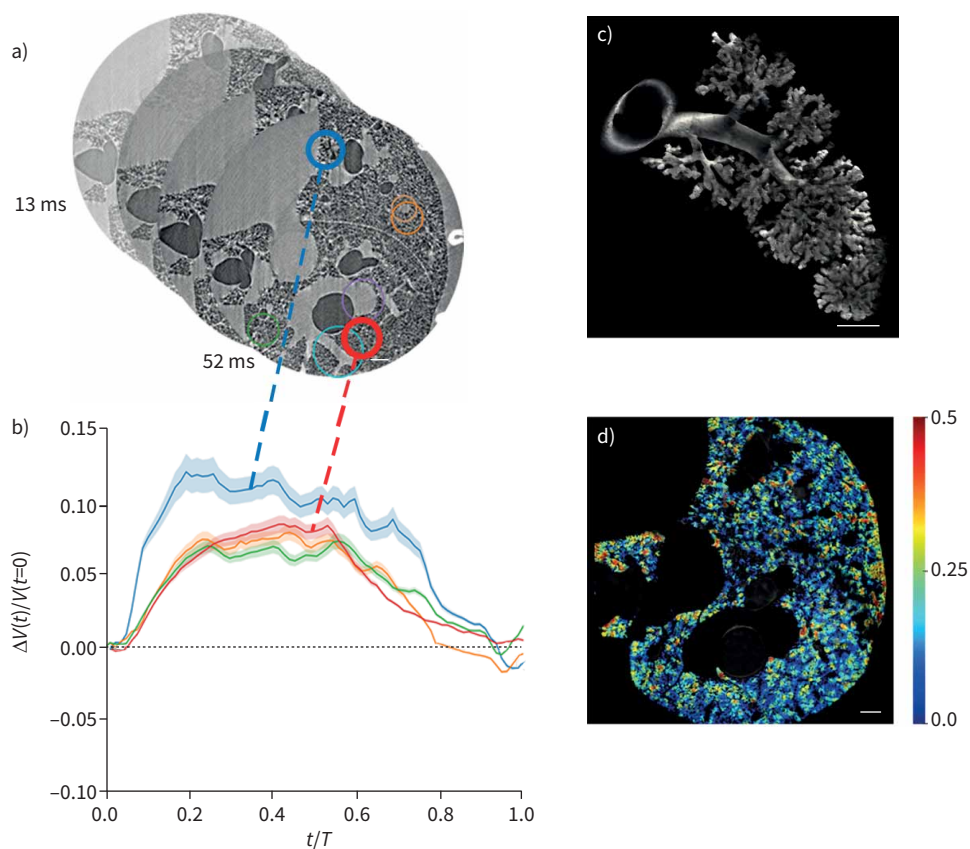


FIGURE 7 Quantitative mapping of local lung tissue deformation in a live rat using four-dimensional synchrotron radiation X-ray phase contrast imaging (SRXPC) with cardiac and respiratory gating. a) SRXPC at successive time-points. b) Regional strain as a function of time (t) expressed relative to the total breath duration (T), computed within airspaces in the regions of interest (ROIs) of the same colour as in a). The shaded area represents the within-ROI standard deviation. c) A segmented airway with subterminal acinar structures at end-expiration. d) Sample regional strain map of airspaces *in vivo*. Coloured bars indicate strain. V : volume; ΔV : change in V . Scale bar: 1 mm. Reproduced and modified from [126] with permission.

In COPD, the Jacobian metric has been found to be associated with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage [130], spirometry [128] and clinical outcome [112, 127]. Lower Jacobian determinant values in the penumbra surrounding emphysematous areas of the lung have been found to be prognostic of future lung function decline [112]. In asthmatic subjects, spatial heterogeneity of the Jacobian has been shown to be associated with a higher risk of future lung function decline and exacerbation [129]. In asthma, low Jacobian determinant values are associated with disease severity and believed to reflect a reduced lung expansion, possibly secondary to gas trapping and hyperinflation [97, 127].

Strengths, limitations and future research

The main limitation of clinical CT imaging is exposure to ionising radiation. CT is the imaging modality that offers the highest quality for structural lung imaging, yet the spatial resolution of clinical CT is still too limited to assess small airways directly. Technical advances may allow for an improvement in image quality with an acceptable radiation dose [131]. While most laboratory micro-CT scanners can reach spatial resolutions of a few micrometres, state-of-the-art phase contrast scanners achieve resolutions of $<1\ \mu\text{m}^3$, enabling the visualisation of cellular structures [132]. Synchrotron radiation with recently developed techniques such as X-ray holography or ptychography have spatial resolutions for biological tissue imaging that are limited only by the incident beam wavelength and have much shorter acquisition times compared to micro-CT [38]. Although synchrotron facilities allow researchers to apply for access, they are generally less accessible than laboratory X-ray sources. *In vivo* micro-CT is hampered by motion artefacts, and image quality is typically lower than in *post mortem* samples, even with respiratory and cardiac gating. Use of novel, high-flux X-ray sources may allow *in vivo* 4D SRXPC imaging in the laboratory [43].

In *post mortem* studies, lung tissue fixation methods can cause shrinkage. While corrections can be applied for homogenous shrinkage, they cannot be applied for inhomogeneous shrinkage [133]. The size and configuration of airspaces also strongly depends on the fixation method and pressure used [133]. In the absence of tissue staining or contrast materials, X-ray imaging methods do not usually distinguish tissue from water or specific cells or molecules [84]. Recent technical developments have allowed examination of specific biomolecules in *post mortem* tissue samples [134] and *in vivo* in rodents [135], paving the way for molecular X-ray imaging.

Quantitative measurements are affected by the methodological differences in image acquisition, reconstruction and segmentation; in addition, co-registration of 3D pulmonary images uses sophisticated image processing methodology and can be computationally challenging. The increasing application of deep learning can significantly accelerate and improve image reconstruction, denoising and processing, as well as the extraction of quantitative metrics. Finally, standardised protocols for acquisition, the definition of quantitative structural and functional metrics, and image post-processing methodologies and algorithms are evolving concerns in clinical studies [136] and are urgently needed for pre-clinical investigations. These protocols are essential to ensure consistency, accuracy and comparability of results across studies.

Conclusion

There is growing evidence that quantitative structural and functional X-ray imaging: allows sensitive detection of early stage diseases; offers precise phenotyping of patient subtypes; provides surrogate biomarkers that can serve as intermediate end-points in clinical drug or intervention trials; and gives valuable insight into the mechanisms of respiratory diseases down to the microscale. Ongoing technological developments aim to improve spatial, temporal and

contrast sensitivity, and reduce ionising radiation. The most advanced and technically challenging methods may be primarily used in research or for specific indications that justify the use of resources beyond traditional testing methods. More widespread clinical use will depend on the demonstration of better patient outcomes, population-based normal reference values for key parameters, standardised protocols for quantitative reproducibility across imaging centres, fast and available tools for image post-processing, robustness and cost-effectiveness [2, 137, 138].

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Functional magnetic resonance imaging of chronic lung disease

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Pulmonary functional MRI provides sensitive, noninvasive, radiation-free and reproducible measurements with high spatial-temporal resolution. Utilised across a wide range of ages and patients, it has high potential for research and clinical evaluation. <https://bit.ly/ERSM107>

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Pulmonary functional MRI (PfmRI) provides a way to noninvasively quantify airway, parenchyma and pulmonary vascular abnormalities in chronic pulmonary diseases such as asthma and COPD and in pulmonary fibrosis. PfmRI may be employed in research and clinical settings and the approach is well-tolerated in children, neonates, the elderly and patients with advanced or severe chronic respiratory diseases. PfmRI does not employ ionising radiation and examinations are rapid (8–15 s) while providing high spatial resolution (~mm³) images. This chapter provides an overview of the main applications of PfmRI, which are to evaluate pulmonary ventilation, alveolar microstructure, pulmonary perfusion, and gas exchange from alveoli to pulmonary microvasculature. These evaluations can be quantified using PfmRI methods, including noncontrast proton-based methods (O₂-enhanced, Fourier decomposition and phase-resolved functional lung imaging) and contrast-based methods (fluorinated compounds containing ¹⁹F and hyperpolarised gases such as ³He and ¹²⁹Xe).

Introduction and rationale

X-ray CT remains the clinical mainstay imaging method for chest and pulmonary examinations, providing detailed anatomical views of both the airways and parenchyma with high spatial resolution (~mm²). Large-scale studies, such as the Genetic Epidemiology of COPD (COPDGene) study [1], have utilised chest CT to evaluate COPD phenotypes and their changes over time. Quantitative CT measurements, including the quantitative analysis of the parenchyma for emphysema [2] and gas trapping [3], as well as airway analysis for airway wall thickening [4] and occlusion [5], have certainly helped enable a deeper understanding of COPD and the drivers of disease worsening, symptoms and exercise limitation.

Historically, MRI methods have not been used for pulmonary examinations for several technical reasons. For example, the low proton density of the lungs because they are mainly air-filled makes conventional proton (^1H) anatomical MRI less informative than x-ray-based methods. In addition, the hundreds of millions of air-filled alveoli pose a unique problem because there are discrete differences in the magnetic resonance properties at the alveolar air-tissue interfaces, which result in magnetic field inhomogeneities and image artefacts, especially at the higher field strengths commonly found in clinical units (1.5 T, 3 T). These fundamental issues pose challenges when using conventional ^1H MRI methods [6] in patients for pulmonary examinations. In this regard, reducing the echo time or the time between the applied excitation radiofrequency pulse and the peak of the acquired signal (or echo) [7] can be very helpful. In fact, ^1H MRI methods that employ short [8, 9], ultra-short [10, 11] and zero echo times [12] have addressed some of the limitations of ^1H MRI for pulmonary investigations of anatomic and lung structural measurements.

Pulmonary functional MRI (PfmRI) methods provide distinct advantages in terms of high spatial ($\sim\text{mm}^3$) and temporal resolution ($\sim 8\text{--}15$ s breath-hold or ~ 5 min free breathing) without ionising radiation, making it suitable for longitudinal studies in critically ill, pregnant and paediatric patients. PfmRI can uniquely probe the physiology responsible for lung function, which cannot be directly measured through standard pulmonary function tests. For example, three-dimensional (3D) whole-lung maps allow visualisation of ventilation, perfusion, alveolar microstructure and gas exchange abnormalities. In figure 1, the anatomical lung compartments from imaging measurements are shown while table 1 summarises the key quantitative measurements derived from PfmRI methods. As shown in figure 1 and table 1, both noncontrast ^1H and inhaled gas contrast MRI can be used to evaluate ventilation regions and areas with limited or absent airflow, otherwise known as ventilation defects. These values are typically reported as either volumes or percentages of the whole lung measured using anatomic imaging of the thoracic cavity volume.

In patients with asthma, ventilation defects are strongly associated with airway inflammation [13], remodelling [14] and disease severity [15, 16], are predictive of asthma control [17] and sensitively respond to therapy [18]. Similarly, in COPD, ventilation defects are linked to symptoms and predictive of exacerbations [15, 19]. Beyond ventilation, pulmonary perfusion and gas exchange measurements provide vasculature markers. Perfusion and microvascular defects can be evaluated using ^1H free-breathing methods and inhaled hyperpolarised ^{129}Xe gas MRI. Inhaled ^{129}Xe gas MRI may also measure gas transfer from the terminal airways into the alveolar membrane and red blood cells (RBC) due to its modest Ostwald solubility coefficient. As shown in figure 1, CT airway and vessel measurements are highly complementary to PfmRI measurements, which enables investigators to derive and estimate structure–function spatial and quantitative relationships. Experienced observers also quantify airway luminal occlusions exposed using thoracic CT as mucus count and mucus score [5], and further categorise individual plug morphologies based on their length and volume [20].

Figure 2 illustrates the general image acquisition framework for PfmRI methods discussed in the section entitled An introduction to pulmonary functional MRI methods. Depending on the method and modality, the acquisition duration may vary from brief breath-holds of 8 to 20 s to several minutes of free breathing. Two-dimensional (2D) and/or 3D images are acquired to provide axial, coronal and sagittal views, which are then processed *via* registration and segmentation to generate quantitative measurements.

In this chapter, we review the processes and results from proton-based techniques such as O_2 -enhanced MRI (OE-MRI), Fourier decomposition MRI (FD-MRI) and phase-resolved functional lung (PREFUL) MRI, along with contrast-based methods using fluorinated gases

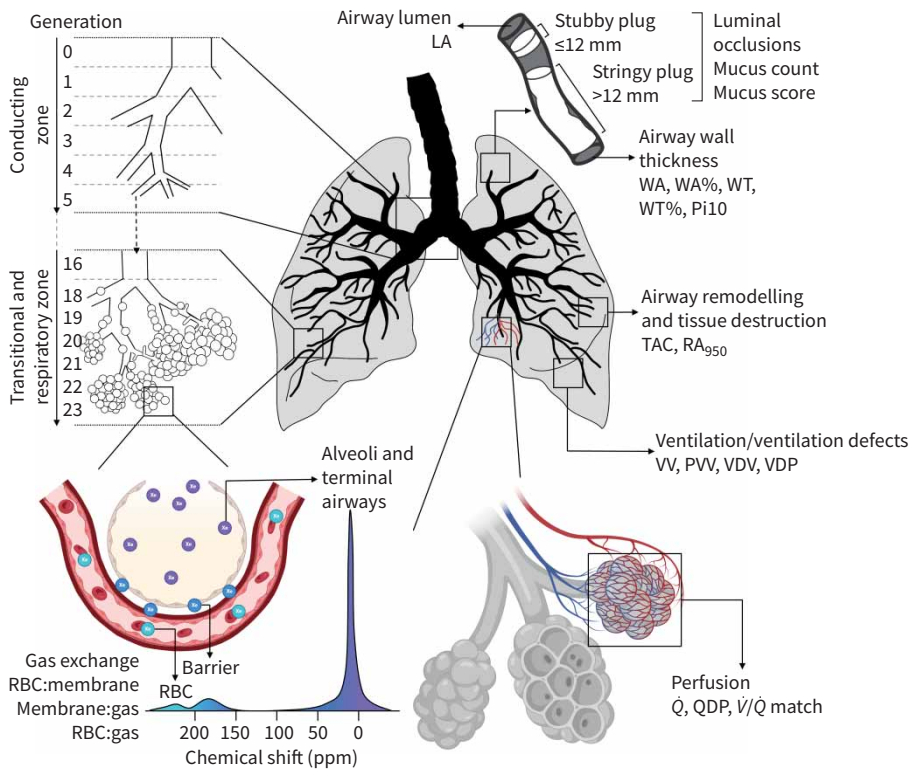


FIGURE 1 Summary of lung measurements acquired using pulmonary imaging. LA: lumen area; WA: wall area; WT: wall thickness; Pi10: square root of the airway wall area for an idealised airway with the lumen perimeter of 10 mm; TAC: total airway count; RA₉₅₀: relative area of lung with attenuation <–950 Hounsfield Units; VDP: ventilation defect per cent; VDV: ventilation defect volume; PVV: percentage ventilation volume; VV: ventilated volume; $\dot{Q}$: perfusion; QDP: perfusion defect per cent; $\dot{V}/\dot{Q}$: ventilation/perfusion; RBC:membrane: red blood cell to alveolar membrane ratio; Membrane:gas: alveolar membrane to gas ratio; RBC:gas: red blood cell to gas ratio.

(¹⁹F) and hyperpolarised noble gases (³He and ¹²⁹Xe). In addition, and as outlined in table 2, we summarise the MRI findings and additional insights from PfMRI. For example, large ventilation defects in COPD, more peripherally localised defects in asthma, and pulmonary fibrosis are characteristic features of these chronic lung diseases. Such findings provide unique insights into disease pathophysiology, even in cases where spirometry results are normal or standard pulmonary function tests do not detect abnormalities. Such insights link structural and functional abnormalities to symptoms, phenotypes, disease severity and progression.

Pulmonary functional MRI methods

Several methods provide static and dynamic imaging of lung microstructure and function and offer quantitative measures of lung function relevant to disease mechanisms and patient outcomes. This section describes these methods, their logistical requirements as well as the quantitative markers or treatable traits that may be estimated using them.

Noncontrast-based ¹H MRI

¹H MRI techniques leverage the abundant hydrogen protons in the body to generate images of the lungs without needing external contrast agents.

TABLE 1 Quantitative pulmonary functional MRI measurements and methods			
Measurement	Utility	Methods	Strengths and challenges
Ventilation volume: total volume of ventilated lung	Measures whole-lung and regional ventilation heterogeneity	^1H free-breathing ^1H PREFUL ^{19}F $^3\text{He}/^{129}\text{Xe}$	Acquired using either contrast or noncontrast MRI
Per cent ventilation volume: normalised fraction of ventilated lung volume	Measures whole-lung and regional ventilation heterogeneity	^1H free-breathing ^1H PREFUL ^{19}F $^3\text{He}/^{129}\text{Xe}$	Acquired using either contrast or noncontrast MRI
Ventilation defect volume: volume of ventilation defects	Measures whole-lung and regional ventilation heterogeneity	^1H free-breathing ^1H PREFUL ^{19}F $^3\text{He}/^{129}\text{Xe}$	Acquired using either contrast or noncontrast MRI
Ventilation defect per cent: percentage of lung with ventilation defects	Measures whole-lung and regional ventilation heterogeneity	^1H free-breathing ^1H PREFUL ^{19}F $^3\text{He}/^{129}\text{Xe}$	Acquired using either contrast or noncontrast MRI
Apparent diffusion coefficient: quantifies terminal airway morphology	Evaluates tissue microstructure and alveolar morphometry	^{19}F $^3\text{He}/^{129}\text{Xe}$	Quantifies morphology and emphysematous tissue destruction Bias towards better functioning lung regions
Ventilation coefficient of variation: quantifies ventilation patchiness	Evaluates ventilation patchiness	^{19}F $^3\text{He}/^{129}\text{Xe}$	Quantifies ventilation patchiness
Perfusion defect per cent: per cent of lung tissue poorly perfused	Quantifies pulmonary perfusion	^1H free-breathing ^1H PREFUL ^{129}Xe	Detects perfusion defects in microvasculature Free-breathing techniques require longer scan times
Ventilation/perfusion match: correspondence between ventilation and blood perfusion	Identifies regions of poorly matched ventilation and perfusion	^1H free-breathing ^1H PREFUL ^{129}Xe	Differentiates if disease is due to ventilation or perfusion Free-breathing techniques require longer scan times
RBC to membrane ratio: ratio of RBC to membrane signal	Ratios provide measurements of gas transfer from airways to alveoli and to microvascular RBC	^{129}Xe	Gas exchange Bias towards better functioning lungs
Membrane to gas transfer ratio: ratio of membrane to gas signal	Ratios provide measurements of gas transfer from airways to alveoli and to microvascular RBC	^{129}Xe	^{129}Xe diffusion into alveolar-capillary membrane Bias towards better functioning lungs
RBC to gas transfer ratio: ratio of red blood cell to gas signal	Ratios provide measurements of gas transfer from airways to alveoli and to microvascular RBC	^{129}Xe	^{129}Xe binding to RBC Bias towards better functioning lungs
PREFUL: phase-resolved functional lung; RBC: red blood cell.			

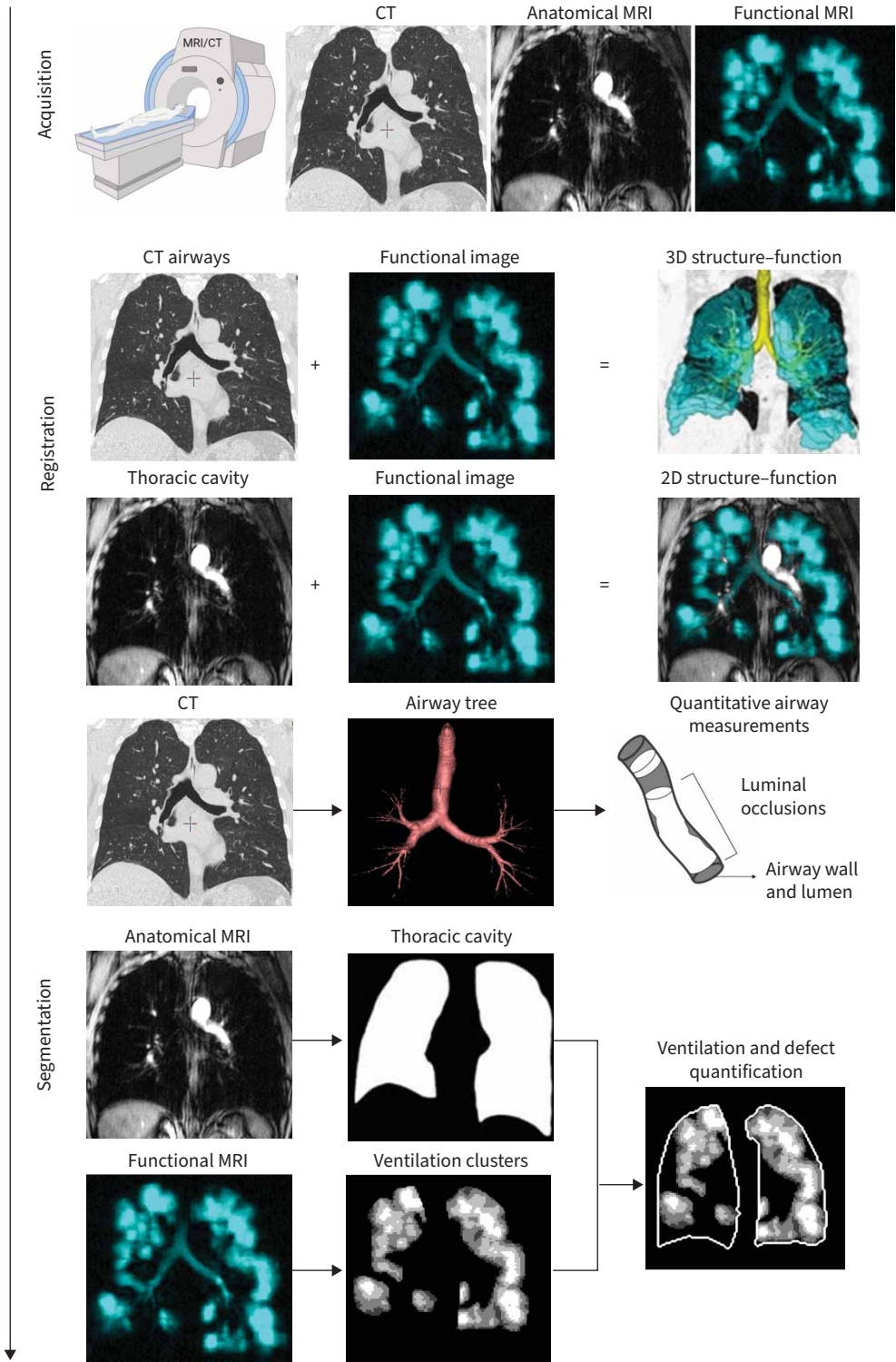


FIGURE 2 Schematic for image analysis towards measurements. Workflow for quantitative image evaluation and measurement. Image acquisition, inter- and intra-modality registration between CT/MRI and MRI/MRI, and segmentation to provide quantitative measurements of structure and function. 2D: two-dimensional; 3D: three-dimensional.

OE-MRI

OE-MRI was first reported in 1996 as an imaging approach to evaluate regional pulmonary ventilation [21]. It utilises the paramagnetic properties of molecular O_2 , which enhances the longitudinal relaxation time (T_1) of nearby water protons (^1H) within surrounding tissues [22–24]. This process uses an O_2 -enhancement ratio comparing the signal intensity between T_1 -weighted ^1H images of hyperoxic (pure O_2) and normoxic (room air) mixture inhalations [25]. The MRI signal enhancement is due to increased oxygenation of lung tissues and alveoli when breathing pure O_2 . Larger ratios indicate a greater increase in signal intensity, reflecting better $\dot{V}_{O_2}$ and diffusion. Conversely, a lower ratio may suggest impaired lung function and compromised $\dot{V}_{O_2}$ [26]. Figure 3 provides examples of raw normoxic and hyperoxic images of a healthy participant undergoing OE-MRI. The change in signal intensity between normoxic and hyperoxic images is calculated voxel-by-voxel to evaluate regional signal enhancement and generate a per cent signal enhancement map used for quantifying ventilation [25, 27–31]. OE-MRI-derived ventilation parameters include regional ventilation, ventilation volume (VV), per cent ventilation volume (PVV) and ventilation defect per cent (VDP). When developed, OE-MRI offered distinct advantages over nuclear medicine approaches, including better spatial resolution and a lack of radioactive gases or radiolabelled aerosols [32, 33]. As outlined in table 3, both 2D slice and 3D volumes can be acquired. OE-MRI is readily accessible and inexpensive in clinical settings compared with other methods reliant on exogenous gas contrast. The method does not require additional equipment. However, OE-MRI has notable limitations, including the extended time needed – approximately 12 min – to acquire normoxic images and subsequent O_2 wash-in to acquire hyperoxic images. In addition, OE-MRI requires indirect interpretations, has a low signal-to-noise ratio (SNR), and reduced sensitivity in detecting subtle ventilation defects, particularly in patients with mild or early-stage lung disease [25, 34, 35].

Over the years, OE-MRI, with the half-Fourier acquisition single-shot turbo spin-echo inversion recovery (IR-HASTE) sequence, has been utilised to evaluate chronic lung diseases, including COPD, asthma, CF, ILD, pulmonary emphysema, pulmonary thromboembolism and lung cancer [36–42]. Dynamic OE-MRI can also reflect the diffusing capacity of the lungs [26], while the O_2 -enhancement ratio is related to disease severity, pulmonary function and therapeutic outcomes in asthma [43, 44]. Recent advances demonstrate the feasibility of OE-MRI at 3T, offering the potential for enhanced imaging capabilities [45].

Fourier decomposition MRI

During continuous MRI acquisition under free-breathing conditions, cyclic breathing and heartbeats induce MRI signal variations in the thorax that can be deconvoluted using Fourier decomposition (FD-MRI [28]). Such acquisitions rely on a balanced steady-state free-precession sequence and constant respiratory and cardiac rates. This method requires high temporal resolution (3.33 images per second) to effectively measure the individual frequency components, as well as a short echo time (0.8 ms) to minimise signal decay.

Given the dynamic nature of lung expansion and contraction during respiration, nonrigid image registration is necessary to correct shape changes. By analysing the time series in the temporal domain, FD-MRI separates – at the voxel level – the higher-frequency signals of blood

TABLE 2 Insights from imaging in addition to clinical lung function tests

Diagnosis	Clinical situation	MRI findings	Additional MRI insights
Asthma	Airway smooth muscle dysfunction with inflammation and occlusions	Ventilation defects localised to peripheral regions	Detects early and mild ventilation heterogeneity and reversible airway obstruction, despite normal spirometry
COPD	Progressive dyspnoea and reduced exercise tolerance	Large ventilation defects, with heterogeneous ventilation distribution	Detects regional gas trapping and emphysematous changes that are difficult to acquire with PFT
ILD	Dry cough and progressive shortness of breath, with normal or near-normal spirometry	Patchy ventilation defects with reduced lung volumes	Visualises early interstitial changes and ventilation defects not detected by PFT
CF	Persistent cough with sputum production and recurrent infections	Widespread ventilation defects, with mucus plugging observed as areas of decreased airflow	Evaluates disease progression by quantifying mucus accumulation and ventilation heterogeneity
Pulmonary fibrosis	Rapid progression of shortness of breath	Small and distributed ventilation defects correlating with fibrotic changes	Evaluates disease progression by mapping regional ventilation changes over time
Bronchiectasis	Chronic productive cough with frequent exacerbations and sputum production	Areas of focal ventilation defects that correlate with dilated airways	Detects airway dilation and mucus-related ventilation changes not reflected in PFT
Pulmonary embolism	Acute chest pain and shortness of breath	Ventilation/perfusion mismatch with segmental defects	Identifies ventilation abnormalities in nonperfused areas, offering detailed localisation
Sarcoidosis	Pulmonary and systemic inflammation causing granulomas in body organs	Ventilation defects associated with granulomas in lung parenchyma	Detects limited or absent airflow based on the extent of granuloma formation
Emphysema	Chronic smoking history with worsening shortness of breath and reduced diffusion capacity	Extensive ventilation defects, with poor gas exchange in affected areas	Quantifies the extent of airflow limitation and regions of low ventilation
Pulmonary hypertension	Dyspnoea with elevated pulmonary and cardiovascular pressures	Ventilation/perfusion mismatch and regional variations in ventilation due to vascular involvement	Evaluates pulmonary perfusion and its effects on ventilation patterns
PFT: pulmonary function test.			

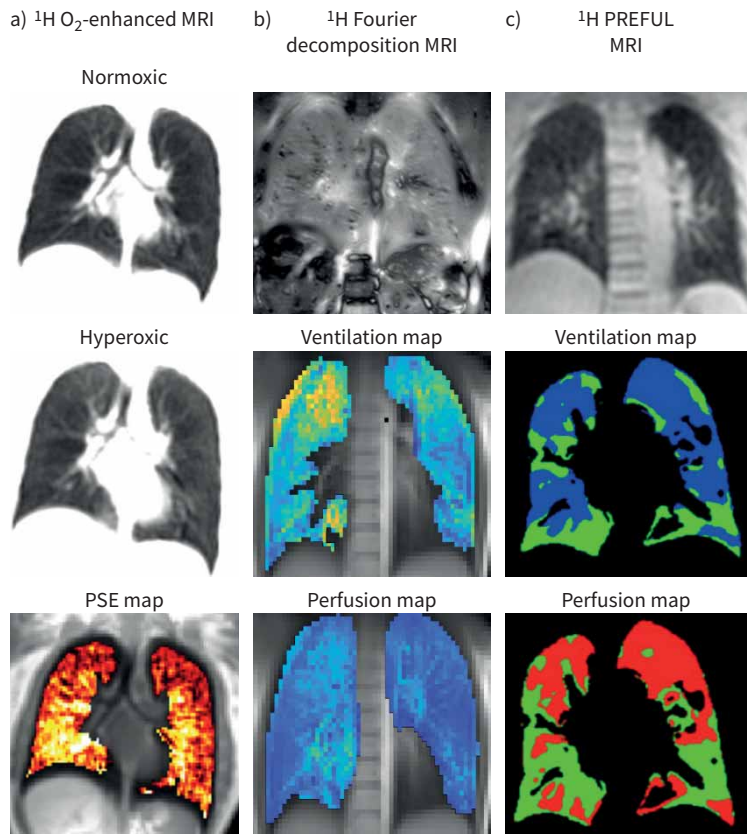


FIGURE 3 Conventional ^1H MRI pulmonary functional methods. a) ^1H O_2 -enhanced MRI of a healthy participant acquired at normoxia and hyperoxia, and regional signal enhancement visualised with a per cent signal enhancement (PSE) map. b) Post-processing of free-breathing Fourier decomposition ^1H MRI yields both ventilation and perfusion maps, which result from cardiac and respiratory frequencies. c) Post-processing of ^1H MRI free-breathing phase-resolved functional lung (PREFUL) images yields ventilation and perfusion maps. Reproduced and modified from [27–31] with permission.

TABLE 3 Overview of pulmonary functional MRI methods

Parameter	OE-MRI	FD-MRI	PREFUL MRI	^{19}F MRI	^3He MRI	^{129}Xe MRI
Total acquisition time	12 min	120 s	5 min	5 min	8–16 s	8–16 s
2D slice	Yes	Yes	Yes	Yes	Yes	Yes
3D volume	Yes	No	Yes	Yes	Yes	Yes
Cost	\$\$	\$	\$	\$\$	\$\$\$	\$\$\$
Free-breathing	No	Yes	Yes	Yes	No	No
Breath-hold	Yes	No	No	Yes	Yes	Yes
^1H MRI	No	No	No	Yes	Yes	Yes
Multi-nuclear	No	No	No	Yes	Yes	Yes
O_2 concentration	Normoxic/hyperoxic	Normoxic	Normoxic	Normoxic	Anoxic	Anoxic

OE-MRI: O_2 -enhanced MRI; FD-MRI: Fourier decomposition MRI; PREFUL MRI: phase-resolved functional lung MRI; 2D: two-dimensional; 3D: three-dimensional; \$, \$\$ and \$\$\$: low, medium and high cost, respectively.

pumping from the lower-frequency signals of breathing. Ventilation- and perfusion-weighted images are then generated by calculating the magnitude of the Fourier spectrum at the frequencies corresponding to respiratory and cardiac cycles. With the advent of 3T MRI, the use of balanced steady-state free-precession sequences has become more challenging due to increased field inhomogeneities in the lung, prompting the development of functional imaging using a spoiled gradient-echo sequence [46–49].

As outlined in figure 3, the FD-MR image acquired requires post-processing to generate ventilation- and perfusion-weighted maps and these may be used to quantify ventilation and perfusion, respectively. Several parameters can be derived from FD-MRI, including regional ventilation, VV, PVV, ventilation defect volume (VDV), VDP, regional perfusion, perfusion defect per cent and ventilation-perfusion match. As outlined in table 3, FD-MRI is dynamic and easily performed during free breathing, has a short acquisition time, and is cost-effective as no extra equipment or packages are required, making it easy to incorporate into clinical workflows. However, interpreting the data requires complex and extensive post-processing. FD-MRI also has a lower resolution, and the quality of the ventilation- and perfusion-weighted images depends on the stability of the patient's breathing and heart rate during acquisition. Irregular rates can broaden spectral lines, necessitating the integration of larger frequency ranges to maintain accuracy [28].

Previous work has shown that ventilation changes in asthma caused by bronchodilator reversibility and methacholine challenge are sensitive and detectable by FD-MRI [48]. Furthermore, ventilation defects identified by FD-MRI in COPD and bronchiectasis patients have shown strong correlations with VDP from ^3He MRI, pulmonary function tests and CT measurements [46]. FD-MRI-derived fractional regional ventilation also strongly correlates with ^{19}F washout in COPD patients [50].

PREFUL MRI

Similar to FD-MRI, PREFUL and self-gated noncontrast-enhanced functional lung (SENCEFUL) MRI [51] provide indirect measurements of ventilation and perfusion by analysing pulmonary signal changes induced by the respiratory and cardiac cycles. However, PREFUL and SENCEFUL enhance this process by incorporating and sorting the phase component of the Fourier spectrum in addition to the peak magnitude. The inclusion of phase information allows for phase-resolved ventilation and perfusion signal cycles with better temporal resolution compared with FD-MRI [52]. The novelty of SENCEFUL is that it separates the respiratory and cardiac cycles using a direct current signal after every signal readout [51]. Alternatively, PREFUL synthesises data through sorting and interpolation to estimate the cycles. The feasibility of PREFUL [52] was first demonstrated at 1.5T using a standard spoiled gradient-echo sequence with a temporal resolution of 288–324 ms. A sine model determined the cardiac and respiratory phases of the acquired images, which were sorted to reconstruct the respective cycles. Image sorting produced PREFUL with an interpolated resolution of 33/35 ms.

As outlined in figure 3, PREFUL images undergo post-processing to generate ventilation- and perfusion-weighted maps used to quantify ventilation and perfusion, respectively. Several ventilation and perfusion parameters can be derived from PREFUL MRI, including regional ventilation, flow volume loops, VV, PVV, VDV, VDP, regional perfusion, perfusion defect per cent, and ventilation-perfusion mismatch maps without requiring respiratory bellows. As outlined in table 3, PREFUL shares several benefits with FD-MRI, being cost-effective, not requiring additional equipment or exogenous inhaled gas contrast and offering higher temporal resolution. However, PREFUL requires complex and substantial post-processing, with much

lower image quality compared with hyperpolarised gas MRI. FD-MRI and PREFUL are typically acquired as 2D images, which can lead to through-plane motion artefacts due to chest wall expansion during respiration. Ongoing and emerging research is focused on developing 3D methods for generating ventilation maps [53–57], which must be validated against established techniques.

Previous work has demonstrated the feasibility of PREFUL-derived ventilation and perfusion data in distinguishing healthy individuals from patients with COPD, chronic thromboembolic pulmonary hypertension and CF [52]. In a double-blind, randomised, crossover drug trial evaluating the efficacy of indacaterol/glycopyrronium in COPD, PREFUL-derived ventilation identified significant changes in regional ventilation [58]. The repeatability of PREFUL for ventilation and perfusion parameters has also been performed in healthy individuals and COPD patients [31]. Additionally, PREFUL can be used to detect early-stage chronic lung allograft dysfunction (CLAD) [59] and predict CLAD-related mortality [60]. It has also identified greater perfusion defects in patients with post-acute COVID-19 syndrome compared with healthy individuals [61]. Moreover, newly developed 3D PREFUL [56] is repeatable in ventilation data and strongly correlates with spirometric data in healthy and COPD patients [56, 62].

More recently, efforts to improve the robustness of amplitude and frequency estimation in FD-MRI [63, 64] have led to the newly proposed technique of dynamic mode decomposition [65]. Briefly, dynamic mode decomposition obtains regional ventilation and perfusion parameters from a series of dynamic acquisitions by extracting dominant spatial-temporal features to analyse underlying system properties, including oscillation frequencies and amplitudes. Dynamic mode decomposition is a novel option for spectral analysis in noncontrast-enhanced dynamic PfMRI, offering improved accuracy in estimating relevant ventilation and perfusion signal changes.

Exogenous inhaled-contrast MRI

A number of MRI methods harness the utility of exogenous contrast agents, such as hyperpolarised gases and fluorinated compounds, to visualise lung structure and function. These inhaled contrast agents whether polarised or in their natural state resonate at different frequencies compared with ^1H , which is used in most conventional MRI. With no background signal from the lung ^1H atoms, an anatomic ^1H MRI is required for co-registration with the functional images to provide regional and lobar measurements.

^{19}F MRI

^{19}F MRI exploits the fact that fluorinated gases, such as sulfur hexafluoride, tetrafluoromethane, hexafluoroethane and perfluoropropane (PFP) have high gyromagnetic ratios (~ 40 MHz/T), with highly suitable magnetic resonance decay parameters. This property has been exploited to quantify static and dynamic ventilation [66–69] in rapid 2D multi-slice and 3D volumetric imaging as an alternative to hyperpolarised gas MRI [70, 71]. Gradient-recalled echo or fast low-angle shot (FLASH) sequences are commonly used to balance SNR with the speed of image acquisition [72]. Fluorinated gases are typically administered as a normoxic mixture because molecular O_2 does not influence the relaxation of the gas, which allows for multi-breath ^{19}F MR imaging without intolerable or unsafe reduction of O_2 levels. In standard protocols, patients inhale several breaths of a PFP- O_2 mixture from a 5 L bag to reach a steady-state gas concentration, followed by a 1 L breath-hold of the same PFP- O_2 mixture for 10–15 s during image acquisition [66, 68]. However, because fluorinated gases are blood-insoluble, ^{19}F MRI cannot provide perfusion measurements.

As outlined in figure 4, pulmonary ^{19}F MRI in a healthy participant can be quantified using image analysis algorithms to quantify regional ventilation, VV, PVV, VDV and VDP [68, 73]. Apparent diffusion coefficients can also be probed in ^{19}F MRI using an ultra-short echo time approach. The use of a room-air O_2 PFP- O_2 mixture makes ^{19}F MRI a radiation-free, noninvasive and well-tolerated method for evaluating regional pulmonary ventilation. As outlined in table 3, ^{19}F MRI is costly compared with the ^1H MRI methods, requiring an exogenous gas, specialised coil and multi-nuclear packages. The ^{19}F MRI signal is directly proportional to gas concentration [74], eliminating the need for additional polarisation equipment. ^{19}F MRI also has lower resolution and SNR compared with other methods. Previous studies have demonstrated the repeatability of ^{19}F MRI [75], as well as its ability to detect ventilation heterogeneity and wedge-shaped ventilation defects in people with asthma [66], COPD [69] and CF [76]. While ^{19}F MRI shows similar VDP, VDV, VV and ventilation coefficient of variation compared with hyperpolarised ^3He and ^{129}Xe MRI, the values may not be equivalent due to differences in signal and resolution [77–79].

Hyperpolarised gas MRI

Ventilation MRI

First discovered in 1994 [80], ^{129}Xe MRI is now the predominant method in pulmonary hyperpolarised gas MRI, with ^3He now phased out due to high costs and the finite global amount of ^3He available. Both ^3He and ^{129}Xe MRI have been used for evaluating and visualising lung ventilation. These noble gases have low natural densities compared with tissue, necessitating an increase in nuclear polarisation through laser-based polarisation devices in a process known as spin-exchange optical pumping [81, 82] to generate an adequate MR signal. Hyperpolarised gas images are acquired with a single breath-hold; therefore, pulse sequences that require multiple high-flip angle pulses, like spin-echo, are ineffective. Instead, low-flip angle gradient-echo sequences are preferred as they consume less magnetisation per pulse, allowing sufficient imaging while minimising signal loss. Although hyperpolarised gases do not recover like thermally polarised magnetisation, the long T_1 relaxation time of the gases in the lungs sustains magnetisation during imaging [83, 84].

Figure 5 provides examples of hyperpolarised ^{129}Xe images co-registered to an anatomical ^1H image to quantify ventilation. Across disease type and severity, there are differences in the characteristic ventilation heterogeneity observed. Asthma and COPD participants have larger

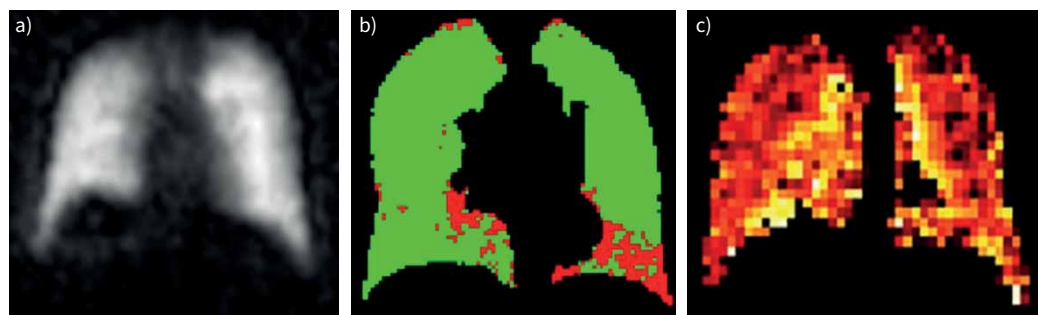


FIGURE 4 Inhaled pulmonary ^{19}F MRI. Segmentation of a) ^{19}F MRI yields b) ventilation and c) apparent diffusion coefficient maps. Reproduced and modified from [68, 73] with permission.

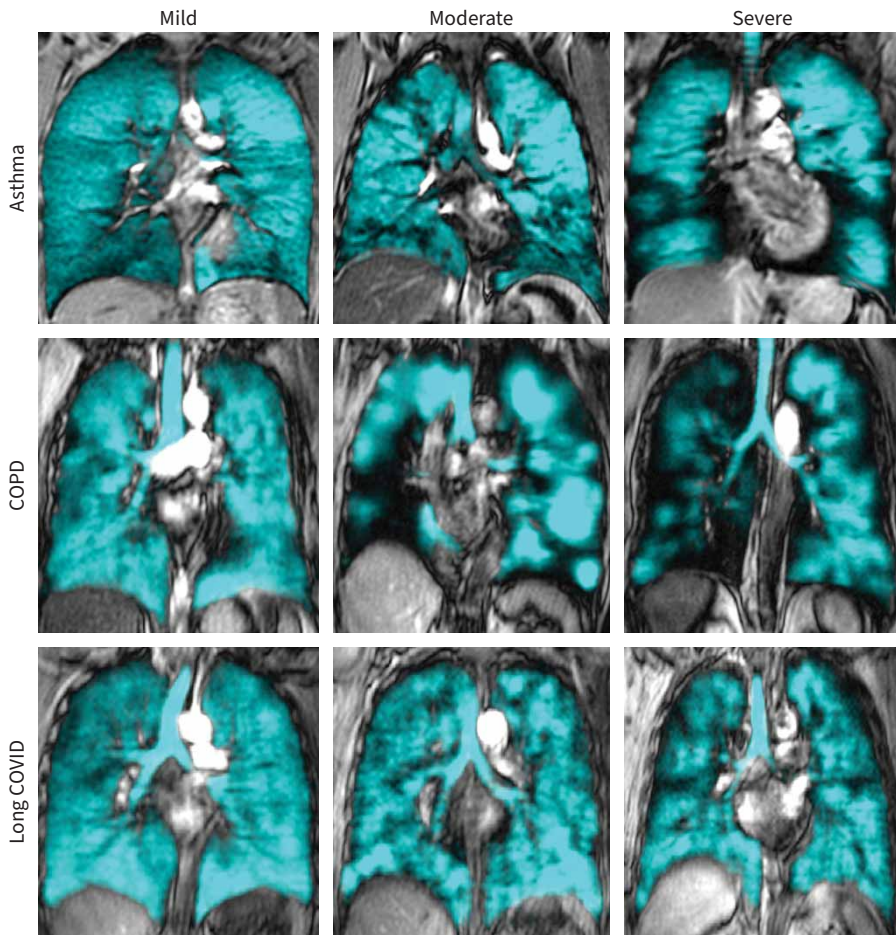


FIGURE 5 ^{129}Xe MRI ventilation in participants with asthma, COPD and long COVID. Coronal ^{129}Xe MRI slices (cyan) co-registered with anatomical ^1H MRI from the thoracic cavity. There are increases in ventilation defects and heterogeneity when comparing mild, moderate and severe asthma, COPD and long COVID participants.

defects while patients with long COVID have patchy ventilation with smaller defects. Ventilation parameters derived from hyperpolarised gas MRI include regional ventilation, VV, PVV, VDV and VDP. As outlined in table 3, ^3He and ^{129}Xe MRI can be acquired in breath-holds in either 2D or 3D. Hyperpolarised gas MRI is very costly due to gas prices, the need for specialised coils, a polariser and multinuclear packages. Although ^3He and ^{129}Xe serve a similar function, the ventilation maps generated are not identical. ^{129}Xe is larger, heavier and less-diffusive than ^3He , and this results in larger ventilation defects and higher sensitivity to airway abnormalities using ^{129}Xe as compared to ^3He MRI [85].

Advantages of hyperpolarised gas MRI include augmented spatial and temporal resolution, no exposure to ionising radiation, minimal invasiveness and the potential for same-day repeatability. However, the disadvantages include using expensive noble gases, the need for specialised polarisation equipment, and training for its operation. Additionally, ^{129}Xe has a lower SNR and polarisation percentage compared with ^3He and also displaces O_2 , which poses

a risk of potential symptoms if administered in high concentrations. The noble gases rapidly lose polarisation when exposed to molecular O₂, necessitating an efficient clinical workflow to acquire high-quality images.

Among the parameters measured, ventilation defect per cent (VDP) is most frequently reported in MRI studies. Previous work has shown that MRI VDP is highly sensitive to airway inflammation and sputum eosinophilia [86], predictive of asthma control and quality of life [16, 17], and responds to therapy in patients with asthma [87, 88]. In COPD, MRI VDP has been predictive of acute exacerbations [19], associated with symptom severity [15, 89], and is sensitive when clinical pulmonary function tests and chest CT remain unchanged [90, 91].

Apparent diffusion coefficient from hyperpolarised MRI

When inhaled, hyperpolarised ³He and ¹²⁹Xe gases are trapped in the terminal alveolar airspaces and move with random Brownian motion. Diffusion-weighted MRI leverages this finding to evaluate alveolar microstructure and morphometry. MRI apparent diffusion coefficients (ADC) [92–94] estimate the distance these gas molecules move within the alveoli by comparing signal intensity with and without applied gradients. Hence, whether the distended alveoli are larger or destroyed, augmented ADC values will be reported because of the greater diffusion path length [93, 95].

Previous work also reported abnormally increased ADC values in patients with emphysema [94], in healthy elderly and COPD participants [95, 96], and in lung fibrosis [97, 98]. In COPD cases in whom the FEV₁ is normal or unchanged over time, ADC is often greater (or worse) [99]. In asthma, regional air-trapping is also associated with augmented ADC values [100] and increases following methacholine challenge [101].

Pulmonary hyperpolarised ¹²⁹Xe gas exchange and microvascular perfusion

Unlike ³He, which is biologically inert, the modest Ostwald solubility of ¹²⁹Xe in biological tissues [102] results in its very limited transmembrane diffusion through biological membranes. When inhaled, ¹²⁹Xe flows through the airways and approximately 1–2% of the gas, referred to as the dissolved phase, participates in transmembrane diffusion into the alveolar-capillary membrane. At the same time, some of the ¹²⁹Xe also moves into RBCs and binds to haem atoms on haemoglobin within the pulmonary microvasculature. As shown in figure 6, ¹²⁹Xe atoms are highly sensitive to the differing environments of these compartments, which results as distinct resonance frequencies that can be measured using MR spectroscopy [103, 104]. The ¹²⁹Xe gas peak is arbitrarily set at 0 parts per million (ppm), and the smaller dissolved phase signals from the alveolar membrane and RBC components appear at 198 ppm and 218 ppm, respectively, from the gas signal [105, 106]. The resonance peaks of each compartment are quantified using the area under the curve and normalised by taking ratios. The presence of ¹²⁹Xe in each of the three compartments – the airways, alveolar membrane and RBC in the microvasculature – can be used to generate representative maps.

Taken together, this method provides functional and physiologic information about all lung compartments, the airways, alveoli and the pulmonary vascular tree [107]. The spectroscopic RBC:membrane ratio was proposed as a measure of gas transfer [108] and depends on alveolar density [109], alveolar membrane thickness [107] and perfusion [110, 111]. CT measurements of the pulmonary vascular tree [112] are correlated with ¹²⁹Xe MRI and MR spectroscopy gas-exchange measurements in COPD [113–115], ILD [116–121] and asthma [122, 123]. Such measurements are reproducible [124] while being dependent on age, sex and haemoglobin [121, 125–127].

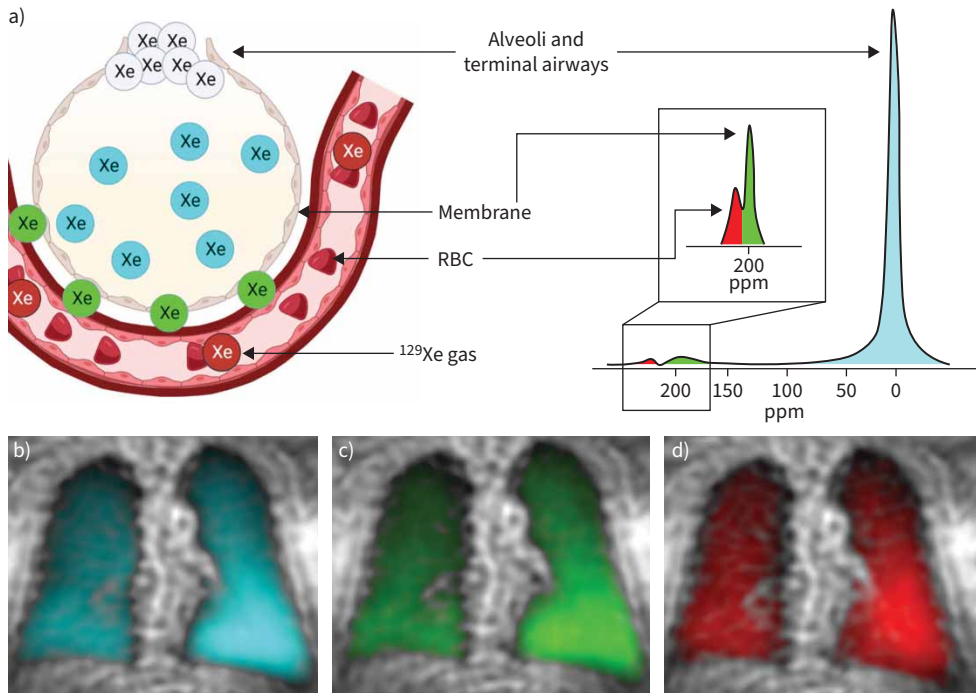


FIGURE 6 Multi-compartment ^{129}Xe magnetic resonance spectroscopy and MRI. a) Inhaled, hyperpolarised ^{129}Xe gas fills the airways and alveoli (cyan peak) and a small amount participates in transmembrane diffusion into the alveolar membrane (green peak) and then diffuses into the red blood cells (RBC) and binds to haem-bound haemoglobin (red peak). Three unique spectroscopic signals may be observed with chemical shifts of gas or cyan signal peak (0 ppm), membrane or green peak (198 ppm) and RBC or red peak (218 ppm). Representative maps for each compartment can be generated, as shown in b) cyan for ^{129}Xe ventilation, c) green for ^{129}Xe in the alveolar membrane, and d) red for ^{129}Xe binding to haem on RBC haemoglobin.

Complementary ^{129}Xe MRI and thoracic CT findings

The structural information from thoracic CT complements the functional data revealed using inhaled gas hyperpolarised MRI. Figure 7 shows the qualitative effect of benralizumab, an interleukin (IL)-5 receptor- α monoclonal antibody, and single-inhaled triple therapy of fluticasone furoate/umeclidinium/vilanterol (FF/UMEC/VI) in representative severe asthma patients. ^{129}Xe MRI ventilation defects were visibly and quantitatively improved for all participants at 2.5 years and 1-year follow-up with benralizumab and FF/UMEC/VI therapy, respectively. The CT airway tree also shows a corresponding increase in total airway counts post-therapy, with similar improvements in FEV₁, and asthma control/quality-of-life questionnaires.

Automated co-registration of MRI and CT datasets also allows for the visual inspection of spatial relationships between structure and function [128], improving our understanding of pulmonary disease pathogenesis and the evaluation of therapies. For instance, CT-MRI co-registration can help evaluate the functional consequences of airway mucus occlusions. Figure 8 shows the qualitative effects of FF/UMEC/VI therapy after 6 weeks in two asthma participants who experienced improvements in FEV₁, VDP and asthma control. These occlusions contribute to ventilation heterogeneity and increased VDP in patients with severe asthma [129–131]. Moreover, ventilation defects are spatially and quantitatively related to CT-derived measurements of thickened airway walls and lumen narrowing resulting from airway remodelling, smooth muscle dysfunction and inflammation [14].

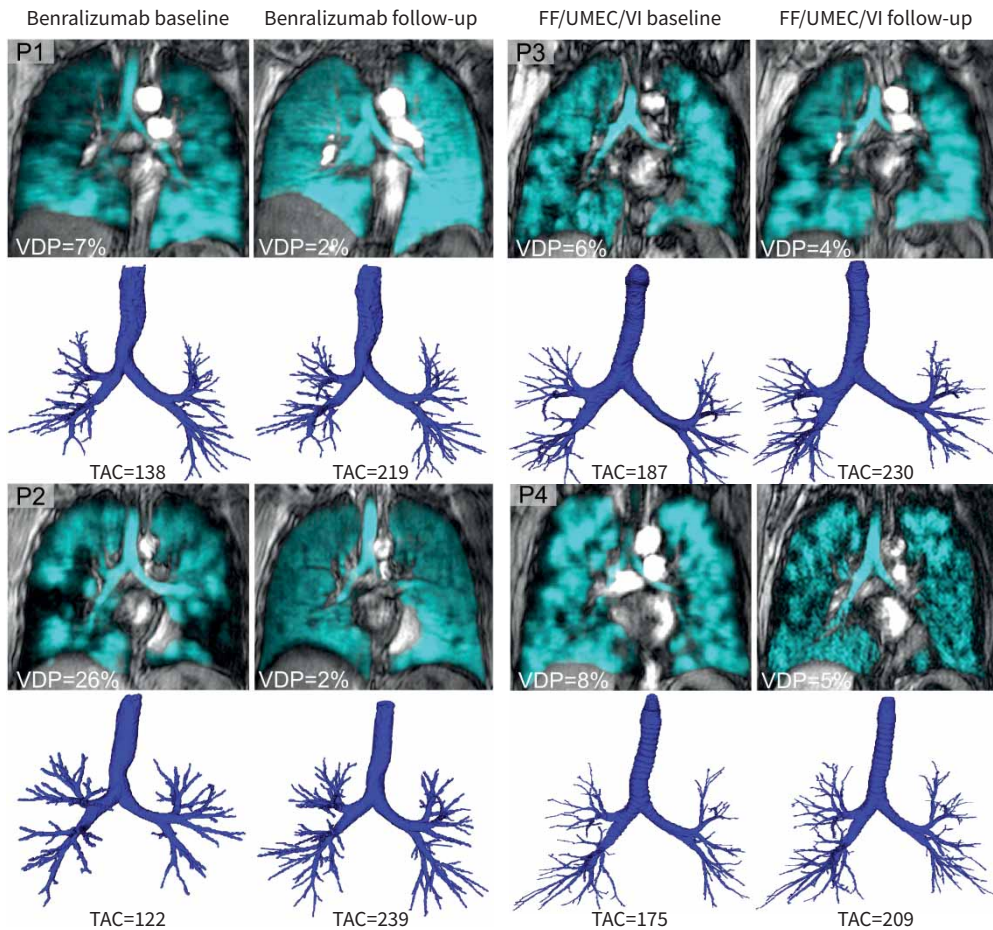


FIGURE 7 Improved MRI ventilation and CT total airway count (TAC) in asthma after treatment. P1 is a 64-year-old female participant with severe asthma treated with benralizumab (an interleukin-5 receptor- α monoclonal antibody) for 2.5 years. Baseline/2.5-year follow-up: FEV₁ % predicted 82/106; Asthma Control Questionnaire (ACQ)-6 2.5/1.5; Asthma Quality of Life Questionnaire (AQLQ) 4.2/6.0; St George's Respiratory Questionnaire (SGRQ) 65/16; ventilation defect per cent (VDP) 7%/2%; mucus score 1/0; and TAC 138/219. P2 is a 43-year-old female participant with severe asthma treated with benralizumab for 2.5 years. Baseline/2.5-year follow-up: FEV₁ % pred 72/114; ACQ-6 4.2/0.3; AQLQ 3.9/6.9; SGRQ 70/6; VDP 26%/2%; mucus score 6/0; and TAC 122/239. P3 is a 60-year-old female participant with severe asthma treated with fluticasone furoate/umeclidinium/vilanterol (FF/UMEC/VI). Baseline/1-year follow-up: FEV₁ % pred 60/70; ACQ-6 1.5/1; AQLQ 6.0/6.8; SGRQ 36/30; VDP 6%/4%; mucus score 1/0; and TAC 187/230. P4 is a 74-year-old female participant with severe asthma treated with FF/UMEC/VI. Baseline/1-year follow-up: FEV₁ % pred 74/81; ACQ-6 0/0; AQLQ 7.0/7.0; SGRQ 4/2; VDP 8%/5%; mucus score 2/1; and TAC 175/209.

Current challenges and limitations

Over the years, PfMRI has made significant progress, such as the approved use of hyperpolarised gas imaging in the UK and the recent US Food and Drug Administration approval of ¹²⁹Xe gas blends and polarisation equipment for evaluating lung ventilation in adults and paediatric patients aged ≥ 12 years [132]. Currently, hyperpolarised ¹²⁹Xe MRI is the only clinically approved method. This approval enables clinical settings to acquire ventilation and perfusion maps, which provide better (10–50 $\times$) spatial resolution compared with nuclear medicine, hybrid CT-SPECT (single-photon emission CT) or CT-PET (positron emission tomography) methods. Despite this,

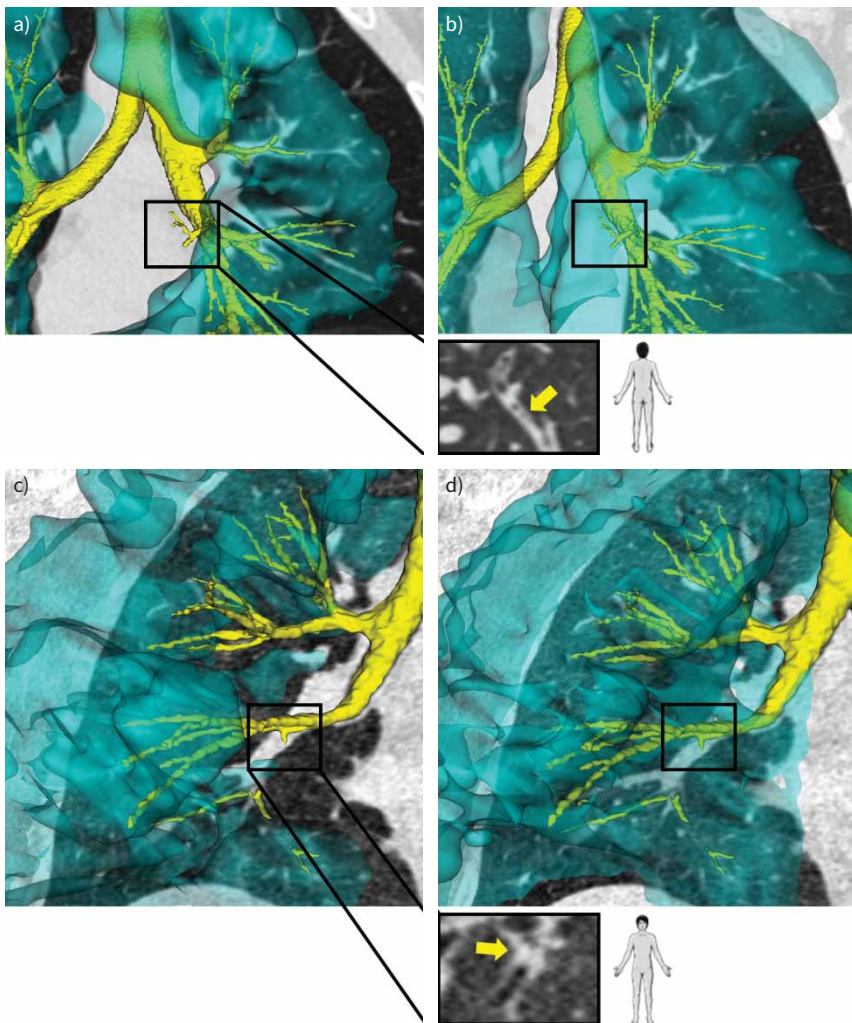


FIGURE 8 Coronal reconstruction of CT with three dimensional airway tree (yellow) and centre coronal slice ^{129}Xe MRI ventilation (cyan) with mucus plug in inset for two asthma patients before and after 6 weeks of therapy. a) Posterior view of a 29-year-old female (mucus score 11; baseline/follow-up: FEV_1 % predicted 59%/69%; Asthma Control Questionnaire (ACQ)-6 3.2/1.8; and ventilation defect per cent (VDP) 33%/12%) with visually obvious ventilation defect in the right lower lobe and mucus plug in the right superior segmental bronchus (yellow arrow). b) After 6 weeks of daily single-dose fluticasone furoate/umeclidinium/vilanterol (FF/UMEC/VI) therapy, ventilation improved proximal to the mucus plug. c) Anterior view of a 26-year-old female (mucus score 10; baseline/follow-up: FEV_1 % pred 65%/97%; ACQ-6 4.3/1.3; and VDP 18%/1%) with visually obvious ventilation defect in the right middle lobe and mucus plug in the medial segmental bronchus (yellow arrow). d) After 6 weeks of daily single-dose FF/UMEC/VI therapy, ventilation improved proximal to the mucus plug.

the adoption of PfmRI in both research and clinical settings has been limited by the availability of MRI equipment with multi-nuclear capabilities, as well as the coils and polarisers required for imaging. Widespread integration into the clinical workflow also requires appropriate image analysis software, training and experience. Further validation and standardisation of imaging protocols and analysis techniques are certainly needed and these are being accelerated by research users who have formed an international consortium to provide support for new sites as well as physics and logistical expertise [133].

Future opportunities

Patients with chronic lung disease often require life-long treatment and monitoring, with clinical evaluations of disease progression, maintenance of symptom control and the monitoring of therapeutic efficiency. In two reports from Canada, the projected cost of integrating annual PfMRI in asthma patients (CAD 600 to 1000) is much lower than the cost of a hospital admission for an exacerbation (~CAD 10 000 [134]), biologic therapy failure (CAD 15 000 to 30 000 annually per patient) and the indirect costs related to lost work and school days (~CAD 10 billion to 14 billion per year in 2014 dollars) [135]. PfMRI also provides information about treatable traits, which offers insight into the pathophysiology of chronic lung diseases.

Although more commonly used for evaluating ventilation, the use of hyperpolarised ^{129}Xe MRI and MR spectroscopy for evaluating pulmonary gas exchange and perfusion in chronic lung disease is relatively unexplored. Current research has concentrated on establishing baseline reference data for healthy individuals and ILD patients by analysing lower-magnitude RBC and membrane peaks, often affected by gas signal contamination, with the largest cohort including 62 healthy participants [127]. Future work, including clinical trials and larger sample sizes, is essential for supplementing standard clinical results with quantitative PfMRI measurements, enabling early diagnosis, disease monitoring, and precise patient characterisation.

Conclusion

PfMRI provides a way to noninvasively quantify airway, parenchyma and pulmonary vascular abnormalities in chronic pulmonary disease. Rapid, robust and radiation-free measurements make PfMRI highly suitable for serial studies in both the research and clinical setting. In addition, the method is easily accomplished in patients including children, the elderly and patients with severe respiratory disease. PfMRI enables high temporal and spatial resolution measurements of abnormal ventilation, perfusion, airway and vessel dysfunction, and gas exchange. Such quantitative measurements offer novel ways to evaluate therapy efficacy. Despite challenges with the addition of PfMRI methods in clinical workflow, recent approvals and commercialisation have positioned PfMRI for future clinical integration, supplemented by standard pulmonary function tests with biomarkers relevant to chronic lung disease management, personalised medicine, and the targeting of treatable traits.

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Sex differences in respiratory function

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Sex differences in respiratory function are primarily due to smaller conducting airways and lungs in females. Differences in hormonal levels impact resting ventilation and may impact susceptibility and symptomatology of clinical conditions. <https://bit.ly/ERSM107>

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Sex differences in respiratory function are primarily manifest as smaller conducting airways and lung volumes in females. The smaller diameter of the large conducting airways in females relative to males is attenuated but persists when scaled for height or lung size. The smaller luminal area of the conducting airways results in a greater work of breathing and O₂ cost of respiration in females during exercise. However, much of the pulmonary response to exercise is similar between the sexes. Hormonal differences across the menstrual cycle impact ventilation at rest but not during exercise. Differences in hormones may also alter the presentation and symptoms of clinical conditions.

Introduction

This chapter describes sex differences in respiratory function. Our treatment of the topic will start with underlying anatomical differences that drive many of the sex differences in respiratory function during exercise (figure 1). We will then consider the effects of naturally occurring gonadal hormones and the impact of the menstrual cycle on respiratory function along with unresolved areas of interest. Prior to exploring the content, it is important to initially define what sex means, as it is often used interchangeably with gender. Here, we will focus on sex differences where sex is defined as “a set of biological attributes in humans and animals that is primarily associated with physical and physiological features” [1]. Gender rather is defined as “socially constructed roles, behaviours, expressions and identities of girls, women, boys, men, and gender diverse people” [1]. While we focus on sex, gender differences should not be disregarded and can be found in a series of articles elsewhere [2–4].

Taking synthetic hormones or having differences in hormonal expression at various points across the lifespan for a variety of reasons can potentially influence the sex differences we describe below. These influences can affect health, basic physiology and athletic performance. However, due to the nuances and ethical considerations, any discussion of the interaction

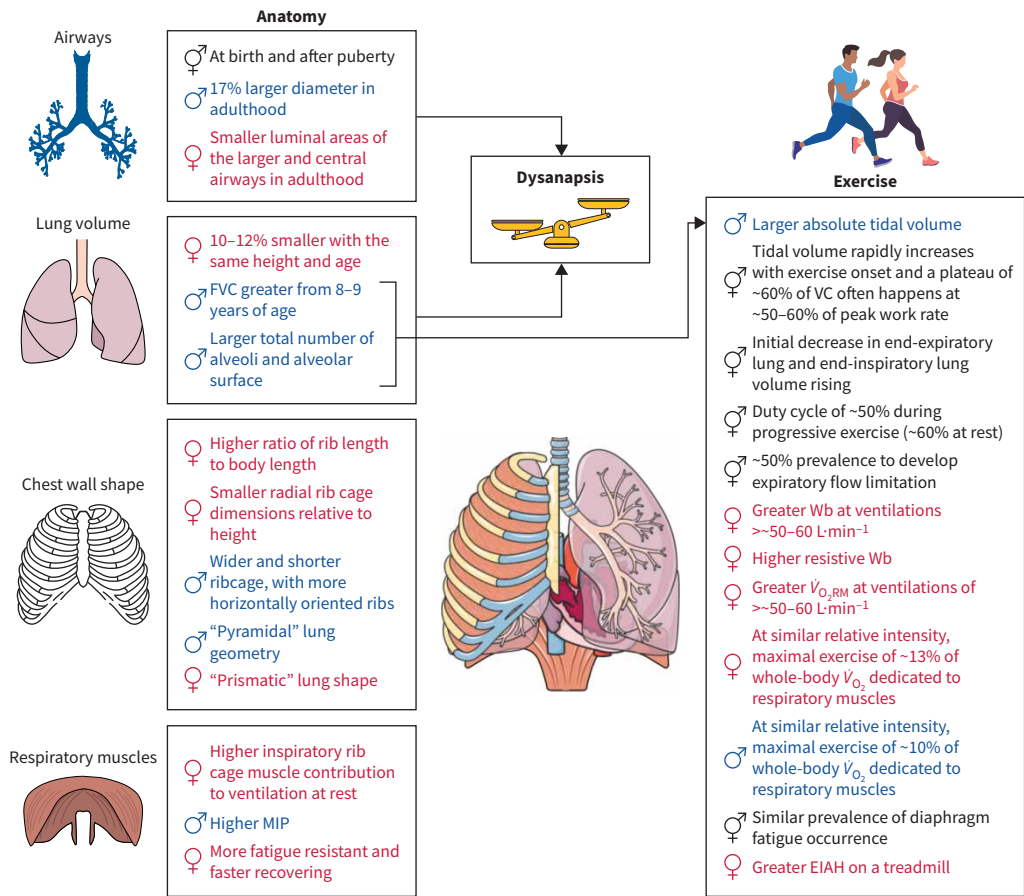


FIGURE 1 Schematic of differences and similarities in respiratory anatomy and exercise response between males and females. MIP: maximal inspiratory pressure; VC: vital capacity; Wb: work of breathing; $\dot{V}_{O_{2RM}}$: $\dot{V}_{O_2}$ of the respiratory muscles; EIAH: exercise-induced arterial hypoxaemia.

between synthetic hormones/differences in hormonal expression and sex differences in respiratory function is beyond the scope of this review, and the interested reader is directed to a recent article focusing on athletic performance [5].

Finally, when comparing males and females, one must consider if any observed differences are due to size or sex or some combination of the two. There are two common strategies often employed in an attempt to separate size *versus* sex effects. The first is to make comparisons at relative rather than absolute values, for example, a per cent of maximal $\dot{V}_{O_2}$ *versus* absolute $\dot{V}_{O_2}$ (L·min⁻¹). The second approach is to allometrically scale variables to account for body size differences, for example, $\dot{V}_{O_2}$ as mL·kg⁻¹·min⁻¹ *versus* L·min⁻¹. While both strategies have utility, their use and limitation should always be considered with the experimental question in mind.

Anatomical differences and dysanapsis

Lung volumes

The human pulmonary system can be thought of as anatomically comprising the lungs, airways, rib cage and respiratory muscles. While the gross anatomy of the pulmonary system does not

differ based on sex, there are several notable differences. Females tend to have smaller lung volumes than males – even when corrected for sitting height as a proxy for thoracic volume [6, 7]. The volume of adult female lungs is typically 10–12% smaller than that of the lungs of males with the same height and age. Others have made similar conclusions when correcting for “frame size” [8]. An analysis of spirometric data from approximately 2000 nonsmoking healthy participants found that FVC was greater in males than females >8–9 years of age and the difference persisted into adulthood [9]. There is also evidence that the number of alveoli differs by sex. For example, THURLBECK [10] studied the lungs of children (6–14 years) who had died from trauma or illness and found that the boys had larger lungs than the girls for the same stature, meaning that the boys had a larger total number of alveoli and a larger alveolar surface. Taken together, these observations emphasise the need to consider sex when considering lung volumes both for the purposes of evaluating lung function and the pulmonary response to exercise.

Airways

Most gross anatomical features of the airways tend to be similar between the sexes. The majority of airway resistance comes from the large conducting airways (*i.e.* trachea to the 5th or 6th generation) [11] and there is evidence for these generations to differ in size on the basis of sex. There appears to be a sex-based difference with respect to airway remodelling and hyperresponsiveness in asthma. Although this topic is beyond the scope of this chapter (see [12] for a review) a brief comment is warranted. The prevalence of asthma is lower in females than in males until the start of puberty, and higher in adult females than males. The disparity in asthma between the sexes and the change that occurs at the onset of puberty remains controversial and a current topic of study. In short, differences may relate to as yet unidentified anatomical factors, differences in inflammatory and immune responses, differences in sex hormones, and genetic or epigenetic influences. How the airways “behave” in response to conditions of high ventilation during exercise either acutely or in response to chronic training have yet to be clearly characterised.

Dysanapsis

MEAD [13] coined the term dysanapsis to explain the considerable variability of maximal expiratory flow rates between individuals with lungs of comparable volume [14]. Said differently, the term characterises the loose coupling between airway and lung size where there is a degree of disproportionate growth between the two constituent components of the respiratory system. The general framework is that the development of the airways is largely complete relatively early in gestation, whereas the air spaces continue to multiply into the postnatal period. Following this, lung volume increases can largely be attributed to the enlargement of air spaces [15]. Mead’s initial observations were made using a “functional” estimate of the lungs and airways, whereby a measure sensitive to airway size was divided by a measure sensitive to lung size. From these analyses, Mead calculated that the airways of adult males were approximately 17% larger in diameter than those of adult females [13]. These sex differences are in line with acoustic reflectance estimates of tracheal cross-sectional area [16]. More recent work, using high-resolution CT, has shown that the luminal areas of the larger and central airways are smaller in females than males even when matched for lung size or height [17–21]. These sex differences in airways do not appear at birth and begin at or after puberty [22]. Given that the airway luminal area is the primary determinant of airway resistance, the reported sex differences in airway size contribute to many of the male–female differences with respect to the pulmonary system’s response to whole-body exercise.

Shape of the chest wall

There is evidence to suggest that the shape of the lungs and rib cage differ based on sex. For example, at autopsy BELLEMARE *et al.* [23] found that the ratio of rib length to body length was

significantly greater in females and concluded that in females the ribs grow longer in relation to the axial skeleton than in males. Further work [24] showed that at all lung volumes, females tend to have smaller radial rib cage dimensions relative to height compared with males. In females, they also observed a greater inspiratory rib cage muscle contribution to ventilation at rest (greater rib cage contribution also occurs during exercise). More recent work, using CT reconstructions of rib cages, has reported a similar male–female difference with respect to the shape of the chest wall [25, 26]. Male rib cages were observed to be wider and shorter, with more horizontally oriented ribs relative to females. Moreover, males tended to have “pyramidal” lung geometry whereas females had a “prismatic” lung shape. The full implications of the observed differences in chest wall shape remain an open question.

Sex differences during exercise

Similarities in the response to exercise

The onset of dynamic whole-body exercise elicits an immediate ventilatory response through the coordination of the respiratory musculature and the neural output controlling exercise hyperpnoea. While our understanding of the control of exercise hyperpnoea is far from complete, the fundamental processes are not different between the sexes [27]. That is, for both sexes ventilation initially rises in proportion to metabolic demand until a threshold is reached, and the increase becomes exponential [28]. Moreover, once body size is accounted for, the sensitivity of the chemosensors is largely similar between the sexes during exercise [29–31]. Thereafter, much of the “normal” ventilatory response to exercise is similar between the sexes. While absolute values are typically larger in males, the relative change or patterns are not different. For a graphical illustration, the reader is directed elsewhere [3]. For example, tidal volume is typically larger in males, but both sexes have rapid increases with the onset of exercise and plateau at ~60% of vital capacity, which often happens at ~50–60% of peak work rate [32]. The absolute difference in tidal volume (and other volumes) is largely an effect of body size, with males typically being larger and thus having larger lungs [33]. Operating lung volumes also follow similar patterns between the sexes, with an initial decrease in end-expiratory lung volume to place the respiratory muscles in a mechanically advantageous position and end-inspiratory lung volume rising to allow for tidal volume expansion.

Expiratory flow limitation occurs when an individual reaches their mechanical ability to generate expiratory flow and can occur in healthy individuals during (near) maximal exercise [34]. Although females have proportionally smaller conducting airways and lungs, they do not appear to develop expiratory flow limitation more often than males; both have a prevalence of ~50% [35]. The exception may be endurance-trained individuals where females may develop expiratory flow limitation more often than males [36, 37]. As regular endurance training does not appear to substantially alter pulmonary function [38, 39], the highly trained females would have a substantial elevation in ventilatory demand with no change to capacity [40].

Respiratory energetics and the work of breathing

At ventilations above ~50–60 L·min⁻¹ females have a greater work of breathing (W_b) than males [36, 37, 41–44]. The W_b can broadly be divided into elastic, resistive, inertial and gravitational work [45], with the last two being minimal and typically disregarded. The differences in W_b between the sexes are due to higher resistive work in females [37, 42, 44, 46], which arises from smaller female conducting airways. The smaller airways result in a greater propensity towards turbulent flow, which exponentially increases the W_b as flow increases [47]. The link between airway size and W_b was demonstrated recently by pairing airway imaging data with CPET in the same participants [48, 49]. Although it is not possible to alter large conducting airway

calibre precisely, providing females with a physiologically analogous intervention as increasing airway size (heliox (~79% helium, 21% O₂) respiration, which decreases the propensity towards turbulent flow) eliminates the sex-difference in Wb [50].

Like other skeletal muscles, $\dot{V}_{O_2}$ of the respiratory muscles ($\dot{V}_{O_{2RM}}$) is linearly related to the work performed (i.e. the Wb). Since females have a greater Wb for a given ventilation, it is expected that their $\dot{V}_{O_{2RM}}$ will also be greater. Indeed, females have greater $\dot{V}_{O_{2RM}}$ at ventilations above which there are significant differences in Wb ($> \sim 50\text{--}60 \text{ L}\cdot\text{min}^{-1}$) [42, 51]. When comparing the $\dot{V}_{O_{2RM}}$ at a similar relative intensity, maximal exercise, the difference persists with females dedicating $\sim 13\%$ of whole-body $\dot{V}_{O_2}$ to the respiratory muscles compared with $\sim 10\%$ in males [42, 51]. Having a greater fraction of whole-body $\dot{V}_{O_2}$ dedicated to the respiratory muscles is not trivial as this would imply that a greater fraction of cardiac output is also directed towards the respiratory muscles [52, 53]. With the sympathetically mediated [54] bidirectional competition between respiratory and locomotor blood flow demonstrated [55, 56], females may have a greater redistribution of blood flow during exercise than males. The latter hypothesis follows the Fick equation but awaits precise experimental testing.

The higher Wb in females is achieved *via* relatively even activation of the respiratory muscles compared with that in males [57]. Specifically, some authors have shown that females have greater activation of the primary (diaphragm) and accessory respiratory muscles of the upper rib cage/neck (sternocleidomastoid, scalene) during progressive [57] and high-intensity constant load exercise [58, 59]. Other authors, however, do not find differences in respiratory muscle activation [60]. Potentially these differences in activation lead to or are the results of the diaphragm or other respiratory muscle fatigue. However, diaphragm fatigue occurrence during intense exercise had a similar prevalence and severity in both sexes [59]. Finally, it is important to consider that there are over 60 muscles known to play a role in pulmonary ventilation [61] and only a few have been experimentally compared between the sexes in terms of activation or fatigability [59, 62]. The distinction between classifying a muscle as nonrespiratory *versus* respiratory is somewhat artificial as many muscles involved in breathing also perform nonrespiratory functions such as postural support. There are known differences between female and male skeletal muscles, including difference in absolute muscular strength and power, energy metabolism, fibre type composition and contractile speed [63, 64]. It is unknown if these differences are equally present in respiratory musculature but there is reason to believe that sex differences in limb or locomotor muscle are equally expressed in the muscles of breathing.

Exercise-induced arterial hypoxaemia

During exercise, some ostensibly healthy individuals do not maintain their arterial O₂ levels near rest. The lowering of arterial O₂ tension/saturation is termed exercise-induced arterial hypoxaemia (EIAH) [65]. Some have demonstrated that EIAH occurs more often and to a more severe degree in healthy young females [66–69], while others show no differences between the sexes [70, 71]. One potential explanation for the discrepancy is the exercise modality, with running showing EIAH more often and to a more severe degree (regardless of sex) than cycling [72]. Indeed, the studies showing greater EIAH in females were conducted on a treadmill *versus* those showing no difference that were on a cycle ergometer. Clearly, more data using temperature corrected arterial blood gases to assess EIAH and potential sex differences are required.

Interaction of lifespan and sex

As we age there are unavoidable changes in pulmonary function [73]. Namely, the loss of elastic recoil and stiffening of the chest wall results in noticeable differences in the physiological response to exercise compared with that in younger individuals [74–76]. However, there does not

appear to be an interaction between sex and ageing in terms of ventilatory response. Like younger individuals, older females have a higher $\dot{V}_E$ for a given ventilation than older males [77], due to greater resistive $\dot{V}_E$ [44, 78], with the difference being of similar magnitude as that in younger individuals. Respiratory muscle recruitment patterns are similar to the $\dot{V}_E$, with both a sex and age effect that are independent of each other [57]. The exception is expiratory flow limitation for which older females appear to be especially prone to developing flow limitation to a more severe degree and at relatively lower exercise intensities than older males [77].

Exercise-induced dyspnoea

The sensation of unpleasant breathing or dyspnoea is often present to some degree in healthy individuals during intense exercise. However, ratings of dyspnoea are subjective and individually scaled, thus it should be viewed as a relative measure. Therefore, comparisons between the sexes should be carried out at relative exercise intensities (*e.g.* maximal exercise or 50% of peak exercise). At maximal exercise, there are no sex differences in dyspnoea between healthy individuals [20, 37, 58, 59, 77, 79–82]. While broad ratings of dyspnoea are not different, a select few descriptors of dyspnoea can show sex-based differences [81]. The origin of these differences in descriptors is likely psychological in origin as there has not been any experimental evidence demonstrating a manipulable causative mechanism. Rather, when the often-hypothesised causative mechanism ($\dot{V}_E$) is manipulated in the physiological realm, dyspnoea is unchanged [83], and cross-sectionally, airways size is unrelated to dyspnoea [20].

Sex hormones, the menstrual cycle and disease

Sex hormones and the control of breathing

The development of the respiratory control system experiences critical periods when an organism is susceptible to sensory input or environmental influences. Indeed, the respiratory control system is characterised by considerable plasticity during postnatal development [84–86]. The different exposure of males and females to gonadal hormones during late embryonic and early postnatal development may significantly affect the development of the respiratory control system [87, 88]. The sex steroid hormones are androgens, oestrogens and progesterone [89]. All the sex steroid receptors are present in the central nervous system in several respiratory-related brain regions (*i.e.* the nucleus of the solitary tract, the hypoglossal nucleus, the phrenic nucleus, and the carotid body) [90–93]. When the receptors of the sex steroid hormones are activated, they translocate to the nucleus to regulate target genes [94]. In addition, oestrogen and progesterone can be activated by a rapid, nongenomic mechanism at the plasma membrane [95, 96]. All three hormones are present in both males and females, although large changes in sex hormone levels occur from the perinatal period to later adulthood. In females, these changes are regulated by the menstrual cycle, and in males principally by testosterone. Males are exposed to high levels of testosterone in the perinatal period and at puberty, which gradually decline thereafter. After puberty, females experience important fluctuations in the levels of oestrogen and progesterone during the menstrual/oestrous cycle (figure 2) and pregnancy. These female fluctuations cease with increasing age, with a large decline in hormone levels at menopause.

The menstrual cycle, sex hormones and respiratory function

There is an effect of menstruation on respiratory function, although with contradictory findings (figure 2). The ventilatory response to hypoxia was reported to increase during the luteal phase (LP) at rest and during exercise [97–104] or unchanged [29, 105]. The response to hypercapnia increases during the follicular phase (FP) [98, 102, 106] or does not change [101, 105–109]. These responses point to a significant role of progesterone in the control of breathing. The menstrual cycle does not seem to affect ventilatory acclimatisation to high altitudes [110] and the ventilatory responses to mild voluntary exercise and passive movement [105, 111].

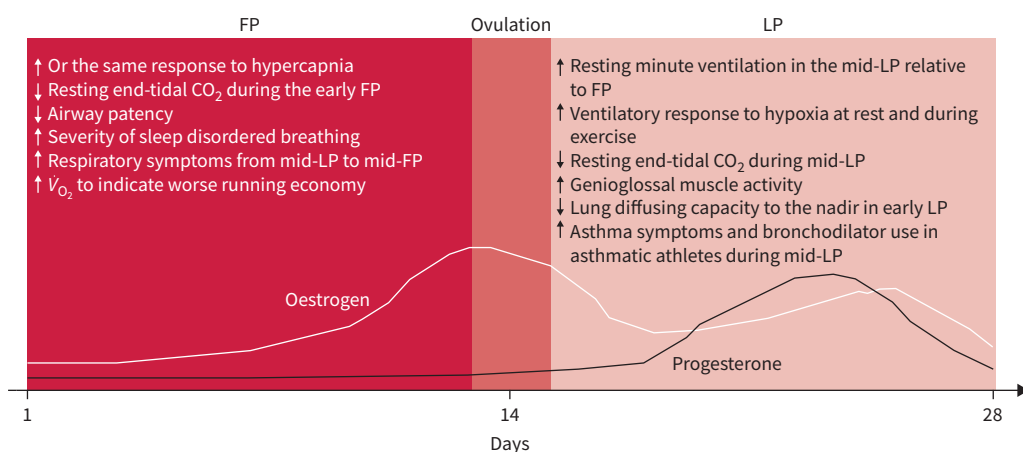


FIGURE 2 The effects on the respiratory system of the ~28-day human menstrual cycle. The follicular phase (FP) occurs in the first ~14 days with low levels of progesterone, while oestrogen levels gradually rise to a peak prior to ovulation (day 14). During the ~14 days of the luteal phase (LP), progesterone increases gradually to ~10-fold of the level during FP and then declines thereafter, while oestrogen undergoes a second and smaller increase and then declines.

The menstrual cycle phase affects neither exercise performance [112] nor exercise minute ventilation and physical performance at altitude [29, 105, 113]. The menstrual cycle phase affects resting minute ventilation, which increases in the mid-LP relative to FP. In addition, resting end-tidal CO_2 reduces during the early-FP and mid-LP (but not in the late-FP) compared with males [29]). The LP seems to be characterised by greater genioglossal muscle activity than the FP. In general, the activity of this muscle is greater in menstruating females than in postmenopausal females [114]. Airway patency seems to increase in the LP compared with the FP [115], reinforcing the idea that the severity of sleep-disordered breathing tends to be greatest in the FP [116, 117]. Any hormonal differences between the two phases of the menstrual cycle had no measurable effect on the adaptation of pulmonary $\dot{V}_{\text{O}_2}$ during the transition to moderate-intensity exercise [118]. During natural menstrual cycles, respiratory symptoms are most frequent from the mid-LP to mid-FP [119]. There is also an association between increased oestrogen levels and reduced levels of exhaled nitric oxide (a marker of airway inflammation). Increased progesterone levels are associated with increased exhaled nitric oxide. These variations are not observed among females using oral contraception [120]. These results explain the significant changes in asthma occurring throughout the menstrual cycle and they imply that sex hormones are important in airway physiology [121]. Running economy is better (lower $\dot{V}_{\text{O}_2}$) in the LP than in the FP [122].

The menstrual cycle, sex hormones and chronic lung disease

Female hormones also have an important role in lung function in chronic lung diseases. Epidemiologic data suggest that being female is a significant risk factor for morbidity and mortality from inflammatory lung diseases. Females appear to have worse prognoses than their male counterparts. There is, therefore, possible that sex-related hormones are important in mediating disease progression in asthma, COPD and CF with different mechanisms according to the disorder.

Sex hormones may modulate asthma because oestrogen promotes a T-helper type 2 response, while androgen promotes a T-helper type 1 response. Over the menstrual cycle, females with asthma experience cyclic changes not only in airflow but also in gas transfer and membrane

diffusing capacity. In these females, lung diffusing capacity reaches peak levels during menses to decline to the nadir in early LP. This further supports a hormonal effect on lung function [123]. The menstrual cycle phase is an important determinant of the severity of exercise-induced bronchoconstriction in female athletes with mild atopic asthma. Female asthmatic athletes may need to adjust their training and competition schedules to their menstrual cycle, as the mid-LP is accompanied by worsening asthma symptoms and increased bronchodilator use [124]. Sex hormones may modulate CF through oestradiol inhibiting chlorine anion secretion in the lung and upregulating mucus production in these patients [125]. In the pathophysiology of COPD, it may be relevant that oestrogens preferentially upregulate phase I enzymes, making the female lungs more susceptible to oxidant damage in response to cigarette smoke [126].

In addition, apnoeas, hypopnoeas and breathing oscillations that characterise sleep-disordered breathing are three times more common in males than females [127, 128]. The reason why this occurs more frequently in males is still an open question. Overall, males seem to have a higher upper airway resistance than females [115, 129], and they are more likely to develop pharyngeal resistance during sleep [130].

Unresolved questions

Humans can easily manipulate sex hormone levels through oral contraceptives and steroid derivatives. In the environment, there is also continuous exposure to endocrine-disrupting chemicals derived from industrial waste, agricultural products, leaching of polycarbonate plastics and products coated with epoxy resins [131–134]. These chemicals can mimic/disrupt steroid hormone signalling by acting on nuclear and membrane oestrogen (xenoestrogens) and androgen receptors (xenoandrogens). The respiratory-related consequences of this long-term exposure are still not well understood [135]. However, dedicated studies are needed. For example, there are inherent problems of hormonal residues associated with the consumption of meat and products derived from livestock treated with hormones [136]. Attempts have been made to correlate the levels of progesterone and oestrogen to ventilation. Some researchers show a correlation [101, 102, 114, 137], and others do not [110, 113]. These contradictory findings highlight the difficulties in conducting and interpreting this kind of study due to methodological differences, small numbers of subjects and natural variability of circulating hormones. Indeed, it is difficult to control for intersubject and intrasubject hormonal variations among cycles when considering the same cycle phases [29]. An additional caveat is body mass index, as subcutaneous abdominal fat is a nongonadal source of steroidal hormones in both sexes [138, 139]. Because of these problems in the interpretation of human data, animal models are implied to investigate sexual dimorphism in ventilation at rest and in response to hypoxia and hypercapnia.

The respiratory metaboreflex is defined as the reflex changes in cardiovascular function evoked by intense work of the respiratory muscles. This reflex is mediated by the sympathetic system [54]. It leads to a redistribution of blood flow in favour of the working muscles compared with the inactive muscles, with respiratory muscles “stealing” blood flow from active locomotor muscles. Cardiovascular control differs with sex, presumably because of the actions of oestrogen on autonomic control and/or to the peripheral effects of sex steroids on the vasculature [140]. Inspiratory muscle metaboreflex is attenuated in premenopausal females compared with young males [141–145]. However, female cardiovascular protection dramatically reduces after menopause. The arterial blood pressure response to the respiratory muscle metaboreflex is greater in older people than in sex-matched young individuals regardless of sex. The blood pressure response becomes similar between older males and postmenopause females. Taken together, these results indicate that the reduced circulating female sex hormones cause the abolition of the differences between males and females in the cardiovascular response to high levels of inspiratory muscle work [145, 146].

Conclusion

We have described a number of sex-based differences in the pulmonary system anatomy along with functional differences and similarities. However, we wish to end by emphasising that much work remains to be done to fully characterise any male–female differences. Furthermore, any observed differences may be influenced by ageing, environmental factors such as exposure to tobacco smoke or air pollution, active *versus* sedentary lifestyle, or interaction with other physiological systems. Appreciating and understanding male–female differences with respect to the pulmonary system may prove to be important when considering how to maximise the benefits of pharmacological or therapeutic interventions such as pulmonary rehabilitation.

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Environmental exposure and the ageing lung

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Ageing decreases lung function, increasing disease susceptibility. Structural and cellular changes, compounded by air pollution and oxidative stress, worsen chronic disease. Research is vital to understand mechanisms and threats to respiratory health. <https://bit.ly/ERSM107>

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Ageing leads to a decline in lung function, with vital parameters like FEV₁ and FVC decreasing from 35 years of age, and a sharper drop in FEV₁ after 65 years of age. Structural changes include reduced bronchiole density, enlarged alveoli and increased lung rigidity due to collagen build-up. Ageing also impairs the respiratory response to hypoxaemia and hypercapnia, and reduces mucociliary clearance. Cellular hallmarks such as telomere shortening, mitochondrial dysfunction and chronic inflammation contribute to lung ageing. Air pollution, a key risk factor for respiratory diseases like COPD, further exacerbates lung decline, particularly in older adults, due to oxidative stress. Pollutants cause oxidative damage and protein modifications, and may accelerate cellular senescence. While mechanistic insights remain limited, the impact of air pollutants on lung health is clear. Future research should address emerging threats like microplastics and the role of lifestyle and genetic factors, as political efforts to improve air quality continue.

Lung ageing

The process of ageing is associated with a gradual decline in organ function, which increases the susceptibility of elderly individuals to the onset of age-related diseases. Ageing is driven by a multifactorial process and can be observed at cellular, organ and organismal levels [1].

The role of lung health in ageing was acknowledged early on, as decreased pulmonary reserve was recognised as a risk factor for morbidity and mortality for pulmonary and extrapulmonary pathologies [2]. Chronic lung diseases (CLDs) result in age-related changes in lung function that are more evident in those whose initial lung capacity was low, and that can be accelerated by cellular stressors such as pollution, particulates or cigarette smoke [3]. Here, we discuss the structural and cellular changes that affect lung physiology and function in ageing.

Functional changes

During the natural ageing process, significant changes occur in the structure and function of the lungs [4]. Although the total lung volume remains unchanged [5, 6], physiological changes in the ageing lung lead to a decline in functional capacity.

Lung function peaks at 18–25 years of age and remains stable until ~35 years of age, after which it gradually declines [7, 8]. While total lung capacity remains largely constant, both FEV₁ and FVC begin to decrease after 35 years of age, resulting in reduced gas exchange capacity [9, 10]. The spirometric index FEV₁/FVC indicates that FEV₁ and FVC peak at 27 years of age in men and 20 years in women, subsequently declining, with a more rapid decrease in FEV₁ observed after 65 years of age [11]. This index is derived from spirometric measurements that reflect the degree of airflow obstruction, making it crucial for diagnosing and monitoring patients with lung disease. While both FEV₁ and FVC decrease with age, they do so at different rates. Notably, FVC alone has been identified as a strong predictor of mortality in the general population [12]. Genome-wide association studies have identified links between clinical measurements of FEV₁, FEV₁/FVC, and FVC and genetic variations at specific loci [13]. However, it remains uncertain whether these genetic variants contribute to accelerated lung ageing.

Vital capacity also diminishes with age. FRC, the volume of gas remaining in the lungs after a quiet expiration, increases with age, leading to alveolar distension and an increased load on the respiratory muscles [14]. This causes a 20% increase in the energy expenditure required for respiration in 60-year-olds compared with 20-year-olds. Age-related changes also affect the function of the chest wall and reduce the strength of the respiratory muscles [7, 15, 16]. Ageing is associated with both inspiratory and expiratory respiratory muscle strength reduction [15–17]. Alterations in pulmonary circulation, including a reduction in capillary blood volume and an increase in ventilation/perfusion mismatch, contribute to the decline in resting arterial O₂ levels [18].

Age-related variations in lung function parameters such as FEV₁/FVC ratios make interpreting lung function in older adults challenging, with factors such as height, gender, weight and ethnicity contributing to this variability. This complexity is further exacerbated in studies involving older adults (>65 years), as the relative influence of body composition, height and sex on lung capacity appears to differ from that in younger populations [19, 20]. In studies that include individuals >85 years of age, lung function values seem to increase again, which may be a result of the “survivor effect”, where only those in the best health live beyond 85 years [21]. Further research is necessary to establish reference standards for assessing lung function in the elderly.

Structural changes

The aged lung is marked by a reduction in bronchiole density and an increase in bronchiole diameter. Studies have shown that age-related changes occur in the microstructure of the acini, although the number of alveoli, alveolar ducts and capillary segments remains unchanged [21–24]. However, there is a significant increase in the size of the alveoli and the surface area of the alveolar-capillary membrane with age. The total alveolar area decreases from ~70 m² in individuals aged 30–39 years, to ~60 m² in those aged 70–79 years. Ageing might also lead to a local change in lung collagen content and thickening of the alveolar basement membrane. Changes in the extracellular matrix (ECM), including alterations in laminin, elastin and fibronectin, contribute to increased lung rigidity [25–27]. These changes result in a reduction in lung elastic recoil, an increase in lung volume at the end of expiration, and an uneven distribution of ventilation due to variations in lung compliance.

In older lungs, the response to hypoxaemia and hypercapnia is diminished, along with a reduced sensitivity to bronchoconstriction [28]. Measurements using oral occlusion pressure indicate a 50% decrease in the response to hypoxaemia and a 60% decrease in the response to hypercapnia in elderly compared with younger adults, suggesting a reduced capacity to process sensory information and generate appropriate neural responses [29].

Normal ageing reduces the mucociliary clearance of the lung [30–32]. With ageing there is a decreased ability to clear mucus from the lungs. Two mechanisms primarily contribute to this decline: 1) reduced cough strength; and 2) alterations in the body's ability to clear particles in the airways [32]. Reduced cough strength is mediated by a reduction in muscle strength [33]. There are alterations in both the clearance of large and small particles with ageing. Mucus transit times in both upper and lower airways slow down with ageing by roughly 50% [34, 35]. In older individuals, clearance of the small airways has been shown to reduce, as measured by retention of radiolabelled Teflon in healthy volunteers [36]. A myriad of changes in the airway epithelium can contribute to the slowing of mucociliary transport. There are structural changes to the airways at the cellular level and at the level of individual cilia, as well as changes in mucus composition that contribute to the alterations in mucociliary transit times. Decreases in ciliary motion (cilia beat frequency) do not seem to contribute to the observed defects, because unlike cigarette smoke, ageing does not change ciliary beat frequency [37].

Cellular changes

The degenerative mechanisms underlying the process of ageing are cellular mechanisms that LÓPEZ-OTÍN *et al.* [38] have described as the hallmarks of ageing. These hallmarks fulfil the following three conditions: 1) they manifest with ageing; 2) they accelerate ageing when they are experimentally induced; and 3) their therapeutic targeting decelerates, stops or reverses ageing. These hallmarks have recently been updated to include 12 traits of ageing, including telomere attrition, epigenetic alterations, loss of proteostasis, mitochondrial dysfunction, cellular senescence, chronic inflammation, and stem cell exhaustion [1]. Growing evidence suggests that many of these hallmarks are interconnected. Here, we focus on hallmarks predominantly affected by environmental exposures.

Altered proteostasis and mitochondrial dysfunction

The term proteostasis refers to processes that maintain a healthy proteome, including protein biosynthesis, folding, trafficking and degradation. Loss of proteostasis is one of the hallmarks of lung ageing [3] and is primarily triggered by oxidative stress, endoplasmic reticulum (ER) stress and epigenetic changes that collectively result in altered levels and activities of key players of the proteostasis network [39]. Ageing is associated with a decline in antioxidant capacity, with the accumulation of misfolded, aggregated and oxidatively modified proteins [3, 26, 40].

Numerous studies have established that protein carbonylation and nitration increase with ageing [41–44]. Protein carbonylation is an irreversible type of oxidative modification and involves various amino acids. Typically, alkaline side chains of, for example, proline, arginine and lysine are oxidatively deaminated, leading to the introduction of carbonyl groups [45, 46]. Importantly, protein carbonylation appears to be selective for specific proteins and alters their physicochemical properties [39, 43]. An example worth highlighting is carbonylation of SIRT1, a deacetylase whose activity is important for healthy ageing [47], where carbonylation of a cysteine residue impairs enzyme activity and leads to enhanced degradation [48, 49].

Protein nitration, the covalent addition of a nitro group to tyrosine residues, is a protein modification primarily mediated by reactive nitrogen species, which are formed from the

reaction between nitric oxide and reactive O₂ species (ROS), thus equally a result of oxidative stress [50]. Similar to carbonylation, nitration may lead to changes in enzymatic activity and degradation, resulting in aggregation [50–52]. Tyrosine nitration may also interfere with tyrosine phosphorylation and downstream signalling pathways [53, 54].

Finally, oxidative modification of proteins may lead to the generation or unmasking of neoepitopes, potentially triggering autoimmune disease [50].

Mitochondria are not only the cell's energy generators but are also one of the causes of inflammation and cell death [55]. Mitochondrial dysfunction is a central feature of ageing, contributing to a decline in cellular as well as tissue function [56]. As one of the factors leading to inflammatory responses, it is also responsible for maintaining a proinflammatory environment in the lungs of people suffering from CLDs [57]. There is an intimate link between mitochondrial function and proteostasis, as mitochondria are important for controlling energy metabolism and ROS formation, as well as in the response of mitochondrial proteostasis [58].

Senescence, stem cell exhaustion and chronic inflammation

Cellular senescence is a cell state characterised by the following four interdependent features: 1) prolonged and generally irreversible cell cycle arrest; 2) damage to DNA, protein and lipids; 3) the acquisition of a specific secretory phenotype; and 4) deregulated metabolism [59, 60]. Senescence is triggered under certain physiological conditions, including during embryonic development or during normal ageing, and principally serves a protective function, preventing uncontrolled cell proliferation to maintain tissue integrity. However, senescence is also induced by various exogenous stressors, intimately linked to oxidative stress, mitochondrial dysfunction and telomere shortening, and limits the regenerative potential of the lung [60–63]. Studies in mouse models of senescence and CLD as well as characterisation of clinical samples, suggest that cellular senescence primes the lung to disease and emphasise the potential of clearing senescent cells as a preventive or therapeutic approach in CLD [61, 64–72]. Senescent cells exhibit a senescence-associated secretory phenotype, consisting of inflammatory cytokines, interleukins and growth factors, which can affect cell and tissue repair as well as regeneration [73], and contributes to the chronic inflammation observed in ageing. This is also often referred to as “inflammageing” [1].

Telomere attrition

Telomeres are protective caps at the ends of chromosomes, which progressively shorten as cells divide during ageing, ultimately resulting in genomic instability, decreased stem cell potential, apoptosis and senescence [74]. Telomere length is therefore widely accepted as a surrogate marker for biological age. The shortening of telomeres can be induced by various factors, such as cell division, oxidative stress, DNA damage and telomerase activity. As telomere attrition induces senescence, it is not only a characteristic of ageing in general but also of cellular senescence, linking both hallmarks of ageing [1, 75].

Epigenetic alterations

Epigenetic reprogramming is defined as the alteration of epigenetic marks (DNA methylation, histone modifications and non-coding RNAs, for example) in an organism's genome. It is well established that the environment can influence epigenetic marks, and can affect gene activities and cell function in a persistent and even trans-generationally inheritable manner [76]. Changes in epigenetics that contribute to ageing are alterations in DNA methylation, post-translational histone modifications, chromatin remodelling, and changes in the function of non-coding RNAs [1].

Epigenetic alterations are closely associated with epigenetic clocks. The latter uses patterns of epigenetic changes, especially DNA methylation, to estimate biological age.

Environmental exposures affect and often exacerbate cellular ageing mechanisms as outlined below and visualised in figure 1.

Environmental exposure in the context of the ageing lung

Anthropogenic environmental exposures and adverse respiratory outcomes

Numerous epidemiological studies worldwide have demonstrated a significant correlation between elevated anthropogenic ambient air pollution and increased mortality in the general population [77, 78]. Improved air is associated with increased life expectancy [79, 80]. These findings have led to an update of the World Health Organization (WHO) global air quality guidelines [81] and to the recent adoption of a political agreement to improve air quality in the European Union by the European Parliament [82].

Increased mortality due to air pollution is closely linked to adverse respiratory health effects [62, 78, 83]. Air pollution is associated with subclinical worsening of lung function [84–86].

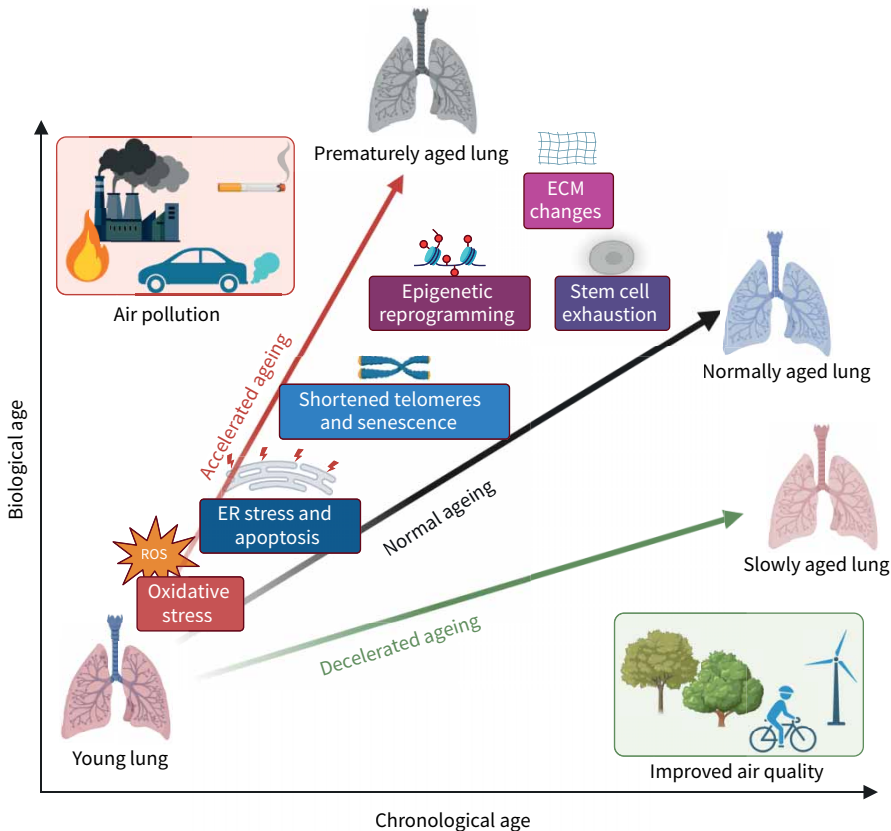


FIGURE 1 Accelerated lung ageing as a result of environmental exposure. Current evidence suggests that exposure to cigarette smoke and anthropogenic air pollutants accelerates lung ageing by exacerbating normal ageing mechanisms. Improved air quality may prevent this process and decelerate lung ageing. ROS: reactive O₂ species; ECM: extracellular matrix; ER: endoplasmic reticulum. Figure partially created using BioRender.com

It increases the risk of COPD [87–89], is the leading risk factor for COPD in never-smokers [90] and is associated with increased COPD exacerbations, morbidity and mortality [91–93]. Numerous air pollutants, including, most recently, particulate matter (PM), have been linked to increased lung cancer incidence and mortality [94, 95] and are classified as carcinogens by the International Agency for Research on Cancer (IARC) [96]. In recent decades, multiple studies have established a negative impact of ambient and indoor air pollution on paediatric and adult-onset asthma [97–99], on asthma exacerbations [100–103] and on near-fatal or fatal asthma attacks in children [104]. Newer studies have equally confirmed a long-suspected link between air pollutants and an increased risk of ILDs, particularly idiopathic pulmonary fibrosis [105–107]. A single-nucleotide polymorphism in the mucin-encoding gene *MUC5B*, which increases the risk for idiopathic pulmonary fibrosis, has also been shown to increase the risk of interstitial lung abnormalities in COPD, highlighting gene–environment interactions and genetic susceptibility in this context [108]. Hence, air pollution is unequivocally linked to worse respiratory health, as evidenced by consistent correlations with decreased lung function, as well as increased incidence and morbidity of a variety of CLDs.

In their guidelines on air quality, the WHO focuses on the following classical air pollutants, with convincing evidence of a negative impact on human health: PM_{2.5} and PM₁₀ (PM with an aerodynamic diameter of 2.5 and 10 µm, respectively), nitrogen dioxide (NO₂), sulfur dioxide (SO₂) and CO [81]. While strong associations between these pollutants and lung health have been demonstrated in the epidemiological studies outlined above, mechanistic evidence of the way in which these agents accelerate ageing or cause lung disease is lacking. This is partly a result of the inherent challenges in mimicking air pollution in experimental settings created by, for example, the heterogeneity of airborne PM, in terms of sources, components and physicochemical properties.

Most health-damaging air pollutants arise from the combustion of organic materials like fossil fuels and biomass. Tobacco smoke exhibits many compositional similarities to ambient air pollution, with several common constituents, including all the classical air pollutants mentioned above and volatile organic compounds. Given the well-known aetiological role of smoking in many CLDs, the effects of cigarette smoke on the lung have been intensively studied *in vitro*, *ex vivo* and *in vivo*, using a variety of exposure models [109–117]. We will therefore cover the cellular and molecular effects of cigarette smoke on lung ageing, in addition to those known for the major anthropogenic air pollutants (PM_{2.5}, PM₁₀, NO₂, SO₂ and CO).

Oxidative stress

It is well established that cigarette smoke contains ROS, including free radicals and other highly reactive species, like superoxide and hydrogen peroxide. These cause oxidative damage to critical cellular components, including mitochondria, DNA, proteins and lipids, generating secondary ROS that propagate oxidative stress even further. Systemic biomarkers exist that serve as measurable peripheral surrogates of these processes; examples include urinary 8-hydroxydeoxyguanosine (8-OHdG), which reflects oxidative DNA damage, and 8-isoprostane and malondialdehyde, which are markers of lipid peroxidation. Cigarette smoke also induces an inflammatory response, with immune cells generating additional ROS as part of their defence mechanism. Finally, cigarette smoke depletes critical antioxidants like glutathione, leaving cells more vulnerable to ROS [118, 119].

The ageing lung is more susceptible to oxidative stress, as ageing is associated with a decline in antioxidant capacity, with the accumulation of misfolded, aggregated and oxidatively modified proteins, and with mitochondrial dysfunction [3, 26, 40]. Exposure to cigarette smoke perpetuates and accelerates these processes and leads to premature lung ageing [62, 120]. Even though few

studies have addressed the impact of air pollutants in this context, there is evidence that these also increase oxidative stress *in vivo* [121–129]. This is supported by the fact that biomarkers of systemic oxidative stress like 8-OHdG, 8-isoprostane and malondialdehyde, are increased in the normal population and in patients upon exposure to ambient air pollution [130–134]. The major contributors to these changes appear to be PM_{2.5}, NO₂ and ozone [131, 133, 134].

Overall, cigarette smoke and anthropogenic air pollutants induce oxidative stress in the lung, with ageing lungs being more vulnerable to oxidative injury due to a decline in antioxidant capacity and the accumulation of damaged proteins.

Oxidative protein modifications

As a result of sustained oxidative stress, proteins are frequently oxidised, a process that involves various side chains and different oxidation chemistries [135]. In this context, protein carbonylation and nitration are the two most frequently assessed oxidative protein modifications.

Numerous studies have established that both modification types increase during ageing but also because of tobacco smoke exposure [41–44, 136–144]. Furthermore, protein carbonyl content is increased in patients suffering from ILD and COPD [145–149]. While it is plausible that classical air pollutants induce protein carbonylation or nitration in the lung (given that they induce oxidative stress), there is limited evidence to support this to date. Plasma protein carbonyl levels were shown to be modestly increased by PM_{2.5} in a study in the Czech Republic [150], and it has been reported that PM_{2.5} increases protein carbonyl content in a lung epithelial cell line [151]. Notably, the extent of carbonylation determines whether a carbonylated protein is directed towards the proteasome (moderately carbonylated), or whether proteasomal degradation is inhibited (excessively carbonylated) and irreversibly inactivated proteins accumulate and aggregate in the cell [48, 152].

NO₂ inhalation may cause protein nitration in the epithelial lining fluid and increase surfactant protein D crosslinking, causing defective microbial aggregation and impairing the host defence in mice [153]. Whether this occurs at ambient NO₂ concentrations is unclear, but it is known that air pollutants induce protein nitration in mouse retinæ and in pollen [154–156].

Hence, exposure to cigarette smoke and air pollution is likely to lower the threshold for damaged protein overload in the ageing lung, leading to cell death.

Altered proteostasis

Oxidative modification of proteins can result in increased proteasomal degradation in an adaptive response to moderate oxidative stress, or in aggregation of misfolded proteins upon inhibition of proteasome activity at higher ROS levels [48, 152, 157]. The latter triggers ER stress, where, at the expense of other gene expression programmes, biosynthesis of specific protein folding catalysts is induced to cope with the accumulation of misfolded proteins. ER stress is initially protective, but prolonged ER stress causes proapoptotic cell signalling, and the threshold for this response appears to decrease with ageing [158, 159]. Exposure to PM has been shown to induce ER stress in lung cells *in vitro* and *in vivo* [160, 161], and may thus predominantly induce the proapoptotic branch of the unfolded protein response in aged lungs. Interestingly, single-nucleotide polymorphisms in unfolded protein response genes have been linked to increased inflammatory biomarkers in induced sputum in elderly women, further strengthening the relationship between ER stress and adverse respiratory outcomes [162]. Notably, activation of ER stress is accepted as an aetiological factor in ILDs where genetic links as well as studies in patient samples, organoid systems and animal models strongly argue for

ER stress-linked apoptosis of the distal airway and alveolar epithelial cells as a disease-triggering event [163–167]. Studies performed *in vivo* also suggest a role for ER stress in emphysema and airway fibrosis [168, 169]. Activation of ER stress by air pollutants may thus contribute to the onset of CLD in aged individuals.

Autophagy is another major cellular proteolytic pathway that serves to degrade or recycle damaged or excess intracellular macromolecules. Upon oxidative and ER stress (amongst other stressors), an autophagosome forms that encloses the components designated for degradation, followed by fusion with lysosomes to enable degradation by lysosomal proteins in the autolysosome. Cigarette smoke induces autophagy in lung cells *in vivo* and *in vitro* [170–172]. While this helps clear dysfunctional proteins and mitochondria, a major source of ROS, it comes with an important pay-off: cilia consumption also increases, leading to shortened cilia upon cigarette-smoke exposure and impaired mucociliary clearance in the airways [173]. Sustained oxidative stress leads to an overload of the autophagy machinery, resulting in an accumulation of damaged proteins and dysfunctional mitochondria, which in turn perpetuate ROS production and trigger apoptosis, necroptosis and senescence [172, 174–177]. Importantly, upon ingestion by cells, PM accumulates in the lysosomes and causes lysosomal dysfunction upon release of its toxic constituents [178]. While PM has been shown to induce autophagy pathways *in vitro* and *in vivo* [179–182], lysosomal dysfunction presumably results in the accumulation of damaged macromolecules. The known age-related loss of autophagic activity thus exacerbates the accumulation of damaged proteins and dysfunctional mitochondria in the cell [183], potentially initiating a vicious circle that contributes to disease progression in the elderly.

ECM changes

With ageing comes a considerable change in ECM composition, crosslinking and dynamics [184–187]. Whether exposure to tobacco smoke or other air pollutants *per se* change ECM dynamics in the normal ageing lung is not currently known. However, ageing does come with altered levels of specific collagen types, chains and crosslinking enzymes, and possibly a shift from lysyl oxidase-mediated crosslinks to non-enzymatic advanced-glycation end product (AGE)-mediated crosslinks [184, 186]. As tobacco smoke is a source of reactive species inducing AGE formation [188] – in the skin, for example [189] – it is plausible that smoking and other environmental combustion processes induce AGE levels and thus also AGE-mediated crosslinking in the lung, compromising tissue elasticity.

Interestingly, a recent study has put forward another surprising mechanism for crosslinking changes in the lung ECM induced by inhaled fine PM: inhaled particles “hijack” the type IV collagen crosslinking enzyme peroxidase, leading to abnormally high crosslinking activity and a denser type IV collagen network. This, in turn, physically impairs immune cell recruitment to the tumour site and promotes lung carcinogenesis [190]. The study provides a compelling example of the functional protein corona, a collective term for proteins adsorbed to the surface of nano- and microparticles upon their interaction with biological environments [191]. The protein corona can profoundly change the properties of inhaled particles and may be responsible for many of the deleterious effects of PM [192–194]. Clearly, we need more studies to unravel the contribution of the protein corona of air pollution particles to lung disease.

Telomere shortening

The impact of tobacco smoking on telomere length is well documented: exposure to cigarette smoke induces telomere shortening *in vivo* and triggers telomere dysfunction *in vitro* [195–197]. Current and former smokers have shorter telomeres than age-matched never-smokers [184, 198] and the number of pack-years is dose-dependently associated with telomere length [199–201].

In contrast, the impact of ambient air pollution on telomere length remains controversial, but the following tentative conclusions can be made: prenatal exposure to air pollutants is associated with reduced telomere length [202]; and long-term adulthood exposure to air pollutants may shorten telomere length [203]. Interestingly, the most comprehensive study to date on the impact of PM₁₀, PM_{2.5}, black carbon and oxides of nitrogen (NO_x) on biological ageing revealed that none of these pollutants was significantly associated with telomere shortening in combined-sex models. However, exposure to black carbon was significantly associated with telomere shortening in males, indicating strong sex- and component-specific differences in biological ageing responses to environmental exposures [204].

It will be crucial to further investigate these associations given that shortened telomeres predispose to lung disease. Telomerase-deficient mice with short telomeres are more susceptible to cigarette smoke-induced emphysema [205]. Mutations in telomerase complex components, like the genes encoding telomerase reverse transcriptase (*TERT*), telomerase RNA (*TERC*) and dyskerin (*DKC1*), increase the risk of pulmonary fibrosis [206–208]. Hence, more high-quality studies on the impact of air pollution and biological ageing are necessary to confirm these relationships and investigate the underlying mechanisms.

Cellular senescence

Given the above-mentioned impact of tobacco smoke on telomere shortening, which causes replicative senescence on its own, it is not surprising that numerous studies have demonstrated induction of cellular senescence by exposure to tobacco smoke components in lung epithelial, endothelial, mesenchymal and immune cells [67, 186, 209–214]. Recent studies suggest a similar impact of PM, which can induce cellular senescence *in vitro* and *in vivo* [129, 215–217]. However, more work is needed, including epidemiological and mechanistical studies, to clarify the impact of various air pollutants on cellular senescence.

Epigenetic reprogramming

Cigarette smoking has a broad effect on epigenetic marks, including within genes that have been associated with smoking-related diseases like COPD and lung cancer, as well as lasting signatures that do not fully reverse upon smoking cessation [218–221]. Importantly, ageing comes with specific DNA methylation changes in blood cells and across multiple tissues that highly correlate with chronological and biological age [209, 210, 222]. Signatures representing this “epigenetic clock” are frequently used to assess premature ageing in disease or by environmental exposure, and their application has firmly established that cigarette smoking accelerates epigenetic ageing in the blood and lung cells of current and former smokers [211–213].

Exposure to air pollutants has been associated with epigenetic changes [214–217, 223–225], including adverse respiratory outcomes [105, 226, 227]. Fewer studies have monitored epigenetic ageing in association with contemporary levels of air pollutants, but they consistently show significant associations with epigenetic ageing [204, 228], including several sex-specific associations, with, for example, PM₁₀ inversely associated with DNA methylation age in men but not in women, and NO_x levels positively correlating with DNA methylation age in women but not in men [204]. More recent studies indicate that racial disparities also exist both in air pollutant exposure levels as well as in susceptibility to their effects, contributing to accelerated biological ageing in Black Americans [229, 230]. Interestingly, there appears to be a sensitive time window in early life where exposure to higher levels of PM_{2.5}, SO₂, and NO₂ associates with increased epigenetic age in later life [231].

In summary, these epidemiological studies strongly suggest that air pollution induces accelerated epigenetic ageing, which may predispose the lung to adverse respiratory outcomes.

Summary and future directions

Epidemiological research has unambiguously established a strong correlation between levels of classical air pollutants and adverse respiratory outcomes. This has resulted in a political commitment to improve air quality and recent legislation that sets stricter limits for PM_{2.5}, PM₁₀, NO₂ and SO₂ [82]. While this is encouraging, improvements in air quality will not happen overnight and the taken measures will vary in effectiveness across different countries and continents. Therefore, ongoing research remains crucial for the prevention and treatment of environmentally induced respiratory diseases. Specifically, we must deepen our understanding of the (epi)genetic, cellular and molecular mechanisms driving disease onset and progression due to air pollution using more advanced and relevant exposure systems and preclinical models.

The WHO advocates for further research into additional air pollutants, including ground-level ozone, black carbon, ultrafine particles, and sand and dust storms [81]. We are also exposed to numerous other compounds, such as microplastics and chemicals, as well as natural sources like pollen and other allergens. Many individuals continue to smoke or turn to alternative products, such as electronic cigarettes and heat-not-burn tobacco devices, which are often perceived as less harmful without sufficient independent evidence [232]. Nutrition has a major impact on respiratory outcomes too [233], and comorbidities in the elderly are very common, often resulting in the need for multiple medications. Importantly, elevated daily temperatures have been linked to telomere shortening [234], suggesting that climate change also accelerates aspects of ageing. However, for many of the above-mentioned exposures, their relationship with cellular and molecular changes in the lung and their contribution to accelerated lung ageing remains to be studied.

In addition to this, genetic predisposition determines susceptibility to disease and the temporal window of exposure has a strong impact on later outcomes [231, 235]. This complexity is reflected in a novel exciting approach termed “GETomics” which aims to understand the effects of dynamic interactions between genes (G) and the environment (E) by integrating information from basic omics (*e.g.* genomics, epigenomics, proteomics) and clinical omics (*e.g.* phenomics, physiomics, radiomics) with exposures (the exposome) over time (T) [236].

Assessing and integrating novel exposures and lifestyle factors as well as the development of adequate models that allow us to study molecular mechanisms will be of crucial importance to further use and improve this concept.

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Physiology, technology and the diagnosis of respiratory disease

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Respiratory physiology is in a period of rapid transition, thanks to advances in measurement technology and data management. Old measurements are being made more simply and accurately while new techniques are changing clinical practice. <https://bit.ly/ERSM107>

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Respiratory physiology forms a key part of the diagnosis and evaluation of patients with lung disease. Recent advances in engineering and data management have created new ways to explore and, if need be, modify established physiological concepts. Oscillometric measurement of lung mechanics during tidal breathing has been automated and applied to large populations. Wearable monitors supplement the information from exercise testing while the sensitivity of the respiratory system to disruption during sleep can be measured noninvasively. New oesophageal catheters have shed light onto the nature of breathlessness and the mechanisms underlying sleep apnoea. Simple tests of respiratory muscle function like sniff nasal pressure have proved to be good markers of respiratory deterioration and the need for ventilatory support in neuromuscular disease. Challenges remain about how to widen the uptake of physiological measurement but there is increasing use of domiciliary monitoring and early diagnosis in the community. The next few years will be exciting for physiologists and clinicians alike.

Introduction

As a discipline, respiratory physiology has always been intimately linked with the technologies available for making measurements. Examples of this include: the development of the pneumotachograph in the 1940s, which removed the need for water spirometers to measure ventilation [1]; the advent of accurate blood gas electrodes in the 1960s, which allowed for a proper understanding of respiratory failure [2]; and the creation of stable and accurate oximeters in the 1970s [3], which uncovered the unsuspected patterns of nocturnal desaturation in respiratory disorders [4]. In each case, the new technology was applied to one or more physiological problems and subsequently operationalised to become part of the routine diagnostic and, in some cases, therapeutic approaches to respiratory care.

This technological perspective has shaped the way we use physiological testing in diagnosis. In other medical disciplines, key measurements can be made on blood samples or bodily secretions, which are easily obtained with minimal patient cooperation. Even complex molecular

biological testing like reverse transcriptase PCR has been converted into a relatively simple self-administered test that has transformed the diagnosis of COVID-19 infection [5]. By contrast, the most widely used test of lung mechanics, spirometry, involves a significant amount of patient cooperation in making a maximal respiratory effort. One reason for the widespread use of pulse oximetry is its relative simplicity, although a failure to understand the underlying physiology and significance of O₂ saturation still limits its interpretation.

This *Monograph* has identified areas in which respiratory physiology is changing and how new methods of measurement and new understanding can be applied to improve diagnosis and evaluation in pulmonary medicine. Many of these advances build on the knowledge gained in the later 20th century, as seen in the reviews of lung mechanics [6], spirometry and plethysmography [7], and gas washout techniques [8]. Others reflect totally new approaches that apply classical physiological principles to new measurement approaches, as in the chapters by BAYAT [9] and MOZAFFARIPOUR *et al.* [10] on lung imaging. In this final chapter, I want to pick up on some of these themes and consider the barriers we need to break down to help physiological measurement achieve its true potential, as well as offer some pointers as to how this might happen. The collection of physiologically relevant data has been easier in the intensive care unit (ICU) where it is integral to improved patient outcomes. In this chapter, the focus is on physiological testing in spontaneously breathing people.

The scope of diagnostic physiology

Physiological testing does not of itself provide a specific aetiological diagnostic label. It describes the functional impact of a disease process on the respiratory system using objective measurements and has long been recognised to provide important prognostic information [11]. Interpreting the results of a test usually involves comparisons with “normal” subjects and this has led to an intense debate about the most appropriate way to define airflow obstruction, as discussed in chapter 5 [7]. However, empirical evidence supports the use of an FEV₁/FVC ratio of ≥ 0.7 as a way of distinguishing those at risk of a worse outcome, irrespective of age [12].

Some confusion has arisen due to the description in large cohort studies, predominantly of smokers, of individuals with preserved ratio but impaired spirometry (PRISm) [13, 14]. These people show an intermediate pattern of health risk during subsequent follow-up compared to nonsmokers with normal spirometry and those with airflow obstruction [14, 15]. This is an unsatisfactory concept from a physiological perspective and there are legitimate concerns about this diagnostic label [16, 17], as many PRISm subjects are obese and so may experience premature airway closure during a forced manoeuvre which may shorten their forced expiratory time. Longitudinal follow-up of PRISm subjects shows that there is migration between diagnostic groups, with 50% of PRISm cases developing obstruction or reverting to normal over 5 years of follow-up and a further 50% changing group at their 10-year visit [18]. The degree to which variable expiratory effort contributes to these findings remains unclear. Undoubtedly, some smokers do develop interstitial lung changes that are visible on CT scans but are not always reflected in their physiology [19]. Whatever the explanation for the PRISm phenomenon, it illustrates the need to set physiological findings in a wider context when they do not agree with other diagnostic features of known disease groups. It is always worthwhile repeating the physiological test when this is the case.

As described in chapter 6 [20], the development of airflow limitation during tidal breathing identifies obstructive lung disease patients with worse breathlessness who are more likely to deteriorate over time and experience exacerbations [21, 22]. The ability to measure tidal expiratory

flow limitation noninvasively and automatically [23], and the existence of objective standards for performing these tests [24], has opened up the prospect of better patient characterisation and even therapeutic intervention [25–28].

Relating these changes in airway mechanics to lung volume is essential, and the issues around lung volume measurement and interpretation are reviewed in chapter 5 [7]. One promising development is the advent of “cabinless” plethysmography, where the subject is connected to a sealed container of known volume during a brief respiratory interruption [29]. This method uses advanced mathematics and large patient populations to derive equations that predict total lung capacity with considerable accuracy. This “black box” approach to deriving physiological variables will likely become more widespread with the application of artificial intelligence (AI) to physiological measurement.

Finally, the use of the gas transfer factor has undergone something of a renaissance as it has been realised that this test offers information about prognosis, which complements that available from imaging [30, 31].

Two areas of physiology not previously considered merit some additional comment, as follows.

Control of ventilation during wakefulness and sleep

While the study of lung mechanics and gas exchange have led to the development of tools that are widely used in clinical practice, the same cannot be said for the control of ventilation. Yet this area of physiological enquiry plays a vital role in our understanding of breathlessness, on our ability to survive in adverse environments and during sleep.

Conventional descriptions of the ventilatory control system focus on the peripheral inputs that modulate respiration, especially changes in arterial blood gas tensions and mechano-receptor signalling, which monitor increased impedance to muscle shortening (figure 1) [32, 33]. The interaction of these signals with the respiratory pattern generating neurons identified in the brainstem of anaesthetised animals has been the focus of much research, but only modest enlightenment as a major influence on breathing in humans comes from the cerebral cortex. Studies using functional MRI have shown, somewhat frustratingly, that respiratory control is a “distributed” task within the cortex, with no single centre integrating the inputs and afferent responses [34]. For the clinician, simpler models based on the imbalance of the load on the respiratory system and the capacity to respond to this loading offer an easier way to understand the importance of ventilatory control in disease [35].

Until recently, the relationship of cortical function with respiration has been a “black box”. The use of loaded or restricted breathing to induce an uncomfortable sensation of unfulfilled inspiration has been central to the experimental study of breathlessness [34], although more detailed psychometric studies have found that the terms used to describe the sensation of breathlessness are more varied and more specific than first thought [36]. New approaches to this problem based on functional MRI data focus on a Bayesian or probabilistic modelling approach (discussed in more detail in [37, 38] and shown schematically in figure 2), where the observed inputs from peripheral sensors are related to anticipated sensations based on prior experience. This system involves the anterior insula cortex and helps explain how mood influences the sensation of breathlessness as well as being activated before the onset of exercise [39]. These technologies will allow for more rigorous testing of new concepts.

Physiology can explain why some interventions reduce breathlessness, such as the effect of bronchodilators on dynamic hyperinflation in COPD [40–43], and how pulmonary rehabilitation

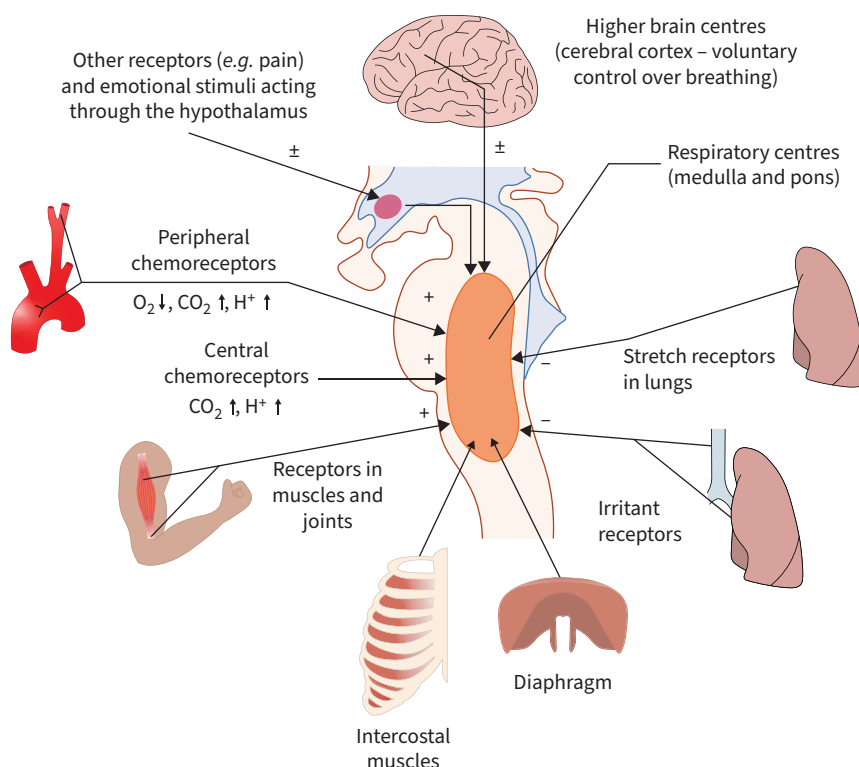


FIGURE 1 A classical model of the regulation of respiration as an interaction between the central respiratory pattern generator and peripheral inputs modulated by cortical influences. H^+ : hydrogen ion concentration. Reproduced and modified from [32] with permission.

programmes can lessen breathlessness by improving physical fitness, which reduces CO_2 production during exercise [44, 45]. Pharmacological attempts to modulate this essential physiological defence mechanism have generally been ineffective [46, 47], but recent physiologically robust studies have shown that hypnosis can modify the sensory response to respiratory load and the breathing pattern of healthy individuals [48]. More progress is being made by integrating electrophysiology of the respiratory muscles with the sensation of dyspnoea. Two relatively new approaches have been particularly valuable. The use of an oesophageal catheter that captures the diaphragmatic electromyogram (EMG) at multiple levels [49] has shown a good relationship between the EMG signal and breathlessness intensity in healthy subjects and in COPD patients [50], where it is a better guide to the severity of dyspnoea than the change in ventilation [51, 52]. Noninvasive monitoring of the parasternal EMG provides a respiratory signal that reflects central neural drive, and tracks the resolution of lung mechanics that follow a COPD exacerbation [53], as well as having prognostic significance for future relapse [54]. If this approach can be successfully operationalised as a routine measurement, it will have considerable clinical utility in a range of other settings [55, 56].

The role of the cortex in the generation of abnormal breathing patterns in individuals without other forms of respiratory disease remains uncertain. This is a complex field without a generally agreed upon objective definition [57], although the presence of inappropriately high levels of ventilation at rest and/or during exercise would be a good starting point to anchor the diverse

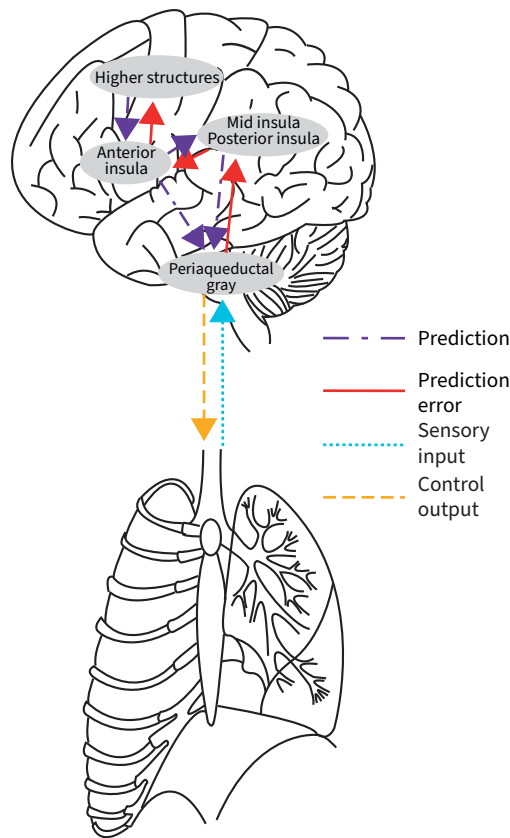


FIGURE 2 Potential neuroanatomical basis for breathlessness perception. Perception arises from the dynamic between bodily sensations and the brain's predictions. As such, anything that may affect predictions (affective state, learned expectations) can influence breathlessness independently of sensory input or lung pathology. The key brain areas include, but are not restricted to: the anterior insula, posterior insula and mid insula; higher brain structures, including the anterior cingulate cortex and the orbitofrontal cortex; and brain stem nuclei and midbrain structures, such as the periaqueductal gray. Reproduced and modified from [38] with permission.

symptoms of those suffering from these problems. In the absence of a simple way to evaluate the whole ventilatory control system, measurement of end-tidal CO_2 during CPET provides one way forward [58]. These issues are likely to become more important as the complex disorders that comprise long COVID syndromes are gradually disaggregated [59].

The most dramatic and frequent change in ventilatory control occurs daily, with the onset of sleep, when cortical inputs to the brainstem controller decrease and a series of stereotypical changes to the breathing pattern occur with each sleep stage [60], leading to a small overall fall in minute ventilation in non-rapid eye movement sleep. The response to chemoreceptor and mechanoreceptor input decreases [60–62] and tonic muscle tone falls in the voluntary muscles of the upper airways. This leads to increased upper airway resistance, hypoventilation and ultimately obstructive apnoea in people anatomically predisposed to a narrow airway, a problem only resolved by an arousal response from sleep with resulting sleep fragmentation [63, 64]. These processes were first described in the 1980s–1990s when it was also recognised that in some cases, they interacted with the ventilatory control system [65]. Here, the analysis of ventilatory control in terms of loop gain, a concept borrowed from engineering, has been very

helpful [66, 67]. This idea emphasises the dynamic nature of breathing pattern regulation when a small increase in arterial CO₂ tension (P_{aCO_2}) occurs at sleep onset and leads to a compensatory rise in minute ventilation. If the respiratory drive is relatively high, an overshoot in ventilation occurs that can lower the P_{aCO_2} below the apnoeic threshold during sleep. This so-called high controller gain represents ventilatory instability and explains why patients exhibit periodic breathing during sleep [68] or develop central sleep apnoea [69], a particular problem at altitude when persistent hypoxia increases the drive to breathing during wakefulness and P_{aCO_2} falls [67]. The presence of circulatory delay, as occurs in patients with reduced cardiac function, further enhances this unstable situation and leads to clinically significant sleep disruption [70]. The effects of high and low controller gain on breathing pattern are illustrated in figure 3, while table 1 highlights the factors that affect different aspects of the control system. Practical ways of assessing controller gain are now available, as set out in table 2.

The occurrence of central apnoeic events in patients with obstructive sleep apnoea (OSA) has been attributed to issues with loop gain. But not all “central” apnoea result from decreased respiratory drive, as is seen in neuromuscular diseases (NMDs) like Duchenne muscular dystrophy, where increased neural activation is observed when no airflow or respiratory effect is detectable [71]. More recently, the traditional paradigm in OSA of reduced muscle tone leading to obstruction in the face of increased respiratory drive, has been challenged by observations using new diaphragmatic EMG catheters, which show reduced neural drive to be a common finding in obstructive apnoeic events [72–74]. These findings will lead us to rethink how we address OSA, the most common sleep and breathing disorder.

Skeletal muscle physiology

The importance of the diaphragm, chest wall and accessory muscles for respiration is self-evident as they provide the motive force for ventilation; any failure, whether intrinsic or consequent on altered chest wall geometry, will impair the capacity of the respiratory system to sustain appropriate gas exchange. This has led to the development of numerous physiological

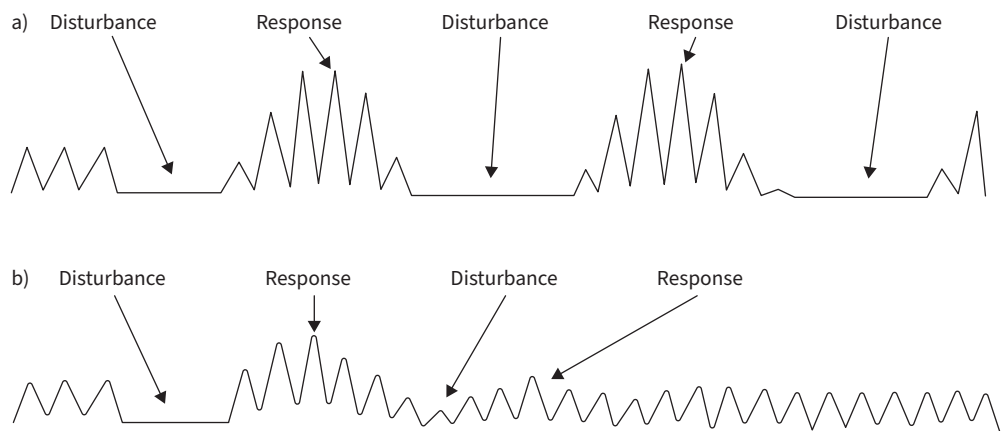


FIGURE 3 High and low loop gain. a) Loop gain ≥ 1 , breathing response exaggerated. b) Loop gain ≤ 1 , breathing response proportional to the disturbance. Loop gain is also defined as the ratio between the magnitude of the response and the magnitude of the disturbance that generated the response. If the response is greater than the disturbance, the loop gain is defined as ≥ 1 (a); if the response is less than or equal to the disturbance, the loop gain is defined as ≤ 1 (b). Reproduced and modified from [67] with permission.

TABLE 1 The measurement of loop gain

Definition of loop gain: the change in ventilatory drive to the ventilation it produces; this relationship is usually inferred from related changes in respiratory variables

Breath-holding during wakefulness

- The longer the breath-hold during wakefulness, the lower the loop gain when asleep
- The tidal volume of the largest breath measured at the end of the breath-hold divided by the duration of the breath-hold
- Usually measured with a 20-s breath-hold

CPAP manipulation during sleep

- When CPAP is turned off, ventilation falls; when CPAP is restored, there is a ventilatory overshoot reflecting the accumulation of CO₂
- Loop gain is derived by dividing the increase in post-CPAP ventilation from the previous baseline value by the decrease in ventilation from baseline when CPAP is withdrawn

Duty ratio measurement during periods of Cheyne–Stokes respiration

- The duration of periods of breathing is divided by the total duration of the hyperpnoeic–apnoeic cycle
- The longer the post-hyperventilation apnoea, the higher the loop gain

Use of specific software application during polysomnography

- The software applies a mathematical model to apnoea and post-apnoea hyperventilation to derive loop gain

CPAP: continuous positive airway pressure.

methods of assessing skeletal muscle function, which are well summarised in the most recent European Respiratory Society (ERS) statement [75]. Despite their centrality to breathing, specific physiological testing of the respiratory muscles has a limited place in current clinical practice. This is largely because other more “global” measures of respiratory function, like vital capacity and expiratory flow, reflect primary respiratory pathology more accurately. There was a flurry of interest in respiratory muscle function in the 1980s and 1990s, stimulated by the theory that chronic respiratory muscle fatigue might explain why some patients develop chronic respiratory failure, which proved not to be the case in most clinical settings [76]. As noted above, multilevel electrodes on oesophageal catheters have proven useful in understanding sleep and the relationship of respiratory loading to breathlessness. However, they remain an experimental tool rather than a clinical one, although parasternal EMG monitoring shows considerable promise for use in ICU [55] and even in domiciliary settings [54].

TABLE 2 Factors modifying different aspects of loop gain

Intrinsic factors

Respiratory controller gain

- Central chemoreceptor responsiveness
- Peripheral chemoreceptor responsiveness
- The neuromuscular effector system

Pulmonary controller gain

- Pulmonary CO₂ stores
- Lung volume, mainly FRC
- Pulmonary deadspace
- Ventilation/perfusion mismatching

Mixing gain

- Circulation time
- Pulmonary blood volume
- Diffusion delay within the CSF

Extrinsic factors

- Sleep stage-related change of arousal threshold
- Age
- Gender
- BMI
- Sleeping position
- Prior arterial CO₂ tension

BMI: body mass index; CSF: cerebrospinal fluid.

Respiratory muscle function testing is very important when the primary problem lies within the muscle rather than the lungs. In patients with intrinsic muscle weakness, gas exchange is relatively preserved until the disease becomes advanced, but there is a progressive fall in vital capacity which can be a useful marker of the need for ventilatory support [77]. Peak nasal sniff pressure reflects peak transdiaphragmatic pressure and is both simple and noninvasive to perform [78]. It appears to be a better reflector of prognosis in NMDs like amyotrophic lateral sclerosis than vital capacity [79, 80]. The ability to cough effectively is a key issue for patients with NMD and cough peak flow provides a relatively simple way of assessing this quantitatively [81]. A reduced cough peak flow is seen Parkinsonism [82]; in patients with stroke, reduced flow rates predict the risk of aspiration pneumonia [83]. Whether this simple technique is helpful in other respiratory conditions remains to be determined.

As elsewhere in respiratory physiology, the real advances come from improvements in imaging. The area of apposition of the diaphragm can be assessed noninvasively using ultrasound, which can indicate not only diaphragmatic thickness but also the degree to which the lungs are over- or under-inflated [84, 85]. This approach has been shown to be informative in the assessment of ventilated patients in the ICU [86]. In a different setting, loss of quadriceps muscle bulk measured by ultrasound has been shown to be an independent risk factor for future exacerbations in hospitalised COPD patients [87].

A better understanding of the integrated action of the respiratory system can be obtained by integrating imaging with other physiological approaches, as seen in the kinematic studies of chest wall volume change, which have been successfully applied in healthy subjects and COPD patients during exercise. This topic is reviewed in detail in chapters 4 and 9 of this *Monograph* [88, 89].

Challenges for respiratory physiology and new technologies

Many physiological concepts are now so well established that we accept their truth without question. Yet, unlike our colleagues in cardiology, pulmonologists have been reluctant to incorporate physiological measurement into their routine practice. Some of this reflects the failure of academic respiratory physiologists to make the case for the clinical value of objective measurement in disease. No physician would countenance the diagnosis of hypertension without measuring blood pressure, or of establishing a finding of a paroxysmal arrhythmia without extended electrophysiological monitoring. However, most physicians are still prepared to diagnose COPD without spirometry [90–92], the cardinal objective method to define this condition. Matters have improved substantially over the last three decades, especially in larger hospital centres, but an enormous number of people remain undiagnosed with conditions like COPD due to a reluctance to consider the diagnosis and conduct the key diagnostic test [93]. Addressing this problem is going to be just as important as new diagnostic aids as it has been with more established methods.

Several other factors have contributed to the image that respiratory physiological measurement is “difficult”, as follows.

The measurements and their interpretation

Unlike other disciplines, respiratory physiological testing often involves the active participation of the subject in the measurement process. This raises issues around patient comprehension, instruction clarity and inherent discomfort associated with the measurement process. Addressing these concerns has an important logistical impact on the time taken to perform the test, the need for someone to supervise what is done and the place in which testing is conducted. Physiological measurement technicians/respiratory technologists possess considerable skills in conducting

these complex procedures and are certainly essential to the effective conduct of many physiological tests. Standardised documents have been developed to ensure optimal spirometry and lung volume measurement [94, 95], with online certification of the operator's competence in spirometry [96, 97]. However, from the clinician's perspective, this focus on technical excellence has been a barrier to the wider implementation of physiological measurement in lung disease. As is often the case in pulmonary medicine, the ideal has become the enemy of the possible.

There is now a circular argument that because physiological tests are complex, time consuming and require additional personnel to conduct, they are expensive. In resource-limited healthcare systems (which include more traditionally affluent care environments) financial pressures make it hard to invest in resources like these, so access to measurement is hard. Clinicians adapt their practice in order to manage without these tests and in doing so, fail to make the case for more resources, which further restricts measurement to a limited number of centres. The consequences of doing this are hard to evaluate in many chronic respiratory conditions where diagnosis is delayed and monitoring is denied to patients. Hopefully, new developments in testing will change this perspective.

An under-appreciated issue is the way we interpret pulmonary function tests. Many clinicians accept a single measurement (that meets the specified performance standards) as representing the "true" physiological value, even though the measurement will necessarily incorporate an element of "noise" which contains its own biases. Some of this is down to the test itself (*e.g.* how much effort the patient made to cooperate on this occasion), but more variation arises from physiological factors. These are best described for spirometric variables but it is likely that they influence all physiological measurement. Airway smooth muscle tone varies throughout the day, contributing to the circadian variation in FEV₁, which can lead to clinically relevant differences in FEV₁ in more severe lung disease [98]. This also helps explain why the response to inhaled bronchodilators differs over time (figure 4) [99] and why routine testing is of limited diagnostic

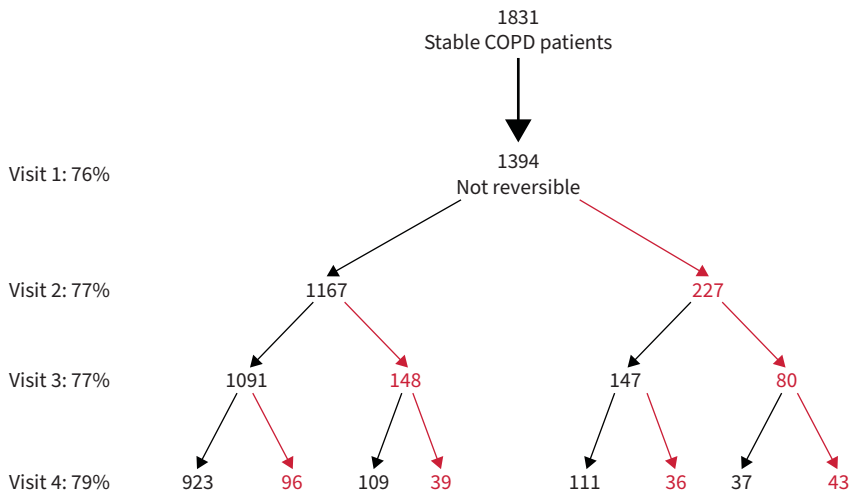


FIGURE 4 The reproducibility of the classification of reversibility in COPD subjects followed on four occasions over 1 year using the American Thoracic Society (ATS)/European Respiratory Society (ERS) reversibility criteria of a 12% and 200-mL change from baseline. Data are shown for people who did not meet the reversibility criteria at their first visit. A similar pattern of variation was seen in those who had an initial positive response. Note that although the mean percentage of responders across all patients is very similar at each visit, there was considerable variation in the individual responder status. Data from [99].

value [100]. Similar considerations apply to highly complex tests like respiratory sleep studies where a surprising amount of night-to-night variation exists, reflecting differences between night sleep structure and body position [101].

Ongoing and sometimes heated debates about the choice of appropriate normative values for spirometric indices adds another level of complexity. This is considered in more detail in chapter 5 of this *Monograph* [7]. The relatively radical revision in the standards for interpretation of spirometry [102] are based on logical principles and the best data in normal populations so far collected but require the non-expert to completely revise how they view this commonly used test and apply new criteria, as yet unvalidated, in the stratification of disease severity. In fact, the issues requiring change are relatively marginal, with most of the changes applying to a small section of the clinical population but not necessarily the general population [103, 104].

These issues around interpretation highlight the way in which physiological measurements in lung disease have become distanced from daily medical practice, by appearing to involve difficult and variable tests that specialists argue about interpreting. In reality, we need to accept that most testing is used to confirm a diagnosis and make a general estimate of disease impact. In the COPD context, the Global Initiative for Chronic Obstructive Lung Disease (GOLD) grading of severity encompasses a wide range of possible FEV₁ values in each grade that broadly relate to disease progression [105]. For those people who fall close to what are essentially arbitrary boundaries, more observation/investigation may be required to reach a final conclusion on their diagnosis [102].

All of these factors apply to the successful application of the new methodologies described in this issue of the *Monograph*, but by virtue of their novelty and parallel technical advances, they provide new opportunities to revive and extend the role of respiratory physiological testing in pulmonary diagnosis and management.

Opportunities offered by new approaches

Doing what we do now but better

Progress has already been made in the more widespread use of unsupervised spirometry in patients and the general population. In clinical trials, home spirometry provides “real world” evidence for the onset and persistence of bronchodilator action [106], the detection of clinical deterioration [107–112] and the identification of transplant rejection [113, 114]. During home monitoring, there is a consistent reduction in the absolute values for FEV₁ and FVC but not the ratio compared to laboratory testing [106, 109]. In the truly global Burden of Obstructive Lung Disease (BOLD) study, simple handheld spirometers yielded reproducible and clinically relevant data in a wide range of healthcare settings, demonstrating the possibility of making or excluding a diagnosis in tests outside of specialist centres [115]. Taking this a step further, coaching patients to perform spirometry using interactive online systems or smart phone apps that monitor test quality, has had some encouraging initial results [109, 116]; more systematic study and professional acceptance of the data is required before their use becomes more widespread [117]. Cost-effectiveness evaluation of the sensitivity and specificity of such an approach will also be welcome. However, given the rapid advances in technology, this looks like a “low hanging fruit”.

Digital assistance in the conduct and analysis of established physiological tests has led to significant improvements in the speed and reproducibility of many previously troublesome procedures, such as plethysmography. The resulting decrease in the time spent testing is a boon to patients and departmental finance officers alike. Likewise, simpler ways of measuring lung

volume should bring physiological measurements closer to the patient [29]. Further advances in this field are likely, especially given the rapid advances in AI, the availability and cost of which are set to fall dramatically.

New diagnostic approaches

This issue of the *Monograph* outlines some of the important new techniques that have become available to aid clinical diagnosis.

There are now robust standards and a growing list of clinical applications for oscillometry [24, 25], which is available in more portable forms suitable for use in the home. As is stressed in chapter 7 [118], this technique is complementary to data from spirometry but is a more sensitive way of identifying respiratory disease in the general population [119].

The pace of developments in diagnostic and functional imaging have been breathtaking, as illustrated in chapters 9, 13 and 14 [9, 10, 89], and this is an area in which physiological measurements can be made in new ways that provide a comprehensive picture of disease and how it impacts respiratory function. The growth in wearable technologies outlined in chapter 4 [88] will further increase this understanding.

Many of these approaches are suitable for telemonitoring, an issue discussed fully in the recent *ERS Monograph* on Digital Respiratory Healthcare [120]. The increasing use of “big data” and AI reviewed in that *Monograph* is likely to change how we make existing measurements, as illustrated by the development of “cabinless” plethysmography, which allows lung volume measurements to be made outside of the traditional laboratory setting [29].

New insights into disease

The availability of better physiology is already changing our understanding of how lung function is influenced by sex [94], ageing [121] and inhaled insults [122]. As previously noted, new technology like stable diaphragmatic EMG measurement can change our perception of the nature of breathlessness [34], the role of neural drive in respiratory muscle fatigue [123] and the nature of OSA [54]. A wealth of new diagnostic information and a growth in cohort studies of patients with and without established lung disease is generating enormous amounts of data. The application of so-called big data analysis coupled with AI algorithms has the potential to uncover important relationships that are not evident at the individual patient level. However, as always, care is needed when choosing the data used to train the AI system. At the single patient level, some of the enormous amount of information collected during an overnight polysomnography study or even modern CPET could be analysed using digitally assisted programmes.

Therapeutic end-points

Physiology already provides important outcome measures in trials of obstructive lung disease (the rate of decline of FEV₁) and ILD (the decline in FVC) but there is a need for more responsive measurements that relate better to the patient experience. Oscillometry shows promise here, at least in COPD, where an objective change in inspiratory reactance can be detected after a bronchodilator event in the presence of expiratory flow limitation [26]. More studies in a range of settings will be needed to convince regulators that new measurements are reliable and relevant enough to be accepted as an end-point in their own right [124–126]. Before this happens, physiology can fulfil other important functions, from permitting better stratification and election of patients for trials, to providing explanations as to how a therapy is effective, whether by changing lung mechanics during exercise or improving cardiac function in

patients with lung disease. In this regard, new imaging modalities have the potential to rapidly show improvements in pulmonary gas exchange [9] or modifications to airway and parenchymal structure [10].

Conclusion

Respiratory physiology will look very different in the next 10 years compared to the last 30. Routine tasks like spirometry and lung volume measurement, which have consumed the time of skilled personnel, will be more automated and easier for patients to access. Our capacity to understand the functional consequences of disease will also allow the dream of personalised medicine to be realised. However, for these goals to become reality, we will still need to understand the perspectives and hopes of our patients, which is why chapter 1 of this issue remains its most relevant [127].

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Respiratory physiology is a key clinical domain that directly affects patient care – a truth that is deftly demonstrated by the Guest Editors of this *Monograph*, the distinguished bioengineers Raffaele Dellacà and Andrea Aliverti. By integrating classical concepts with the latest advancements, the Guest Editors and authors provide a comprehensive overview of the field. This *Monograph* highlights cutting-edge diagnostic techniques and bridges the gap between basic physiology and clinical practice, offering insight into how physiological principles inform disease diagnosis and management. It also explores emerging challenges and research directions, including the impact of ageing, sex and the environment. Providing an accessible insight into the exciting world of physiology, this *Monograph* is a must for all clinicians, researchers and trainees in pulmonary medicine.

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