Environmental exposures, body composition and pulmonary function

How can we improve diagnostics?

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Abstract

Pulmonary diseases are influenced by numerous factors such as lifestyle, environment, genetics, and adipose tissue. A common factor for these diseases is that early and accurate diagnosis is beneficial for effective treatment. The improvement and development of diagnostic tools, including nanotechnology, offers the potential for more reliable diagnosis.

The main aim of this thesis was to improve our understanding of respiratory assessment through a comprehensive approach. This approach included studying the effectiveness of training and feedback in conducting spirometry tests to accepted diagnostic standard (paper I). Evaluating the association of body impedance analysis (BIA) and waist circumference in assessing the decline in lung function induced by excessive adipose tissue (paper II) and investigating the effects of weight change on lung function (paper III). In addition, the thesis investigated the potential of inhaled nano particles in individuals with impaired lung epithelium as a possible new diagnostic method (paper IV). Overall, these studies aimed to improve lung function diagnostics to make them more reliable, accessible and to advance the development of cutting-edge technological methods.

Our results show that the use of structured on-line feedback improve the quality of spirometry and that there is a potential gender differences in the effects of excess adipose tissue on lung function. Data also demonstrated a difference in clearance of inhaled nanoparticles between healthy and patients with impaired alveolar integrity, opening the possibility for new diagnostic approaches.

Keywords: Spirometry, Nanoparticles, Lungmedicine, Body Composition, Pulmonary Function Diagnostic, Bioelectrical Impedance Analysis (BIA) Spirometry, Particle Exposure, Healthcare, Lung Disease Management

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To my loved ones
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


II. Qvarfordt M, Lampa E, Cai G, Lind L, Elmståhl S, Svartengren M. **Bioelectrical impedance and lung function - associations with gender and central obesity: results of the EpiHealth study.** Submitted manuscript.

III. Qvarfordt M, Lampa E, Cai G, Lind L, Elmståhl S, Svartengren M. **The impact of weight gain on lung function - associations with gender, fat distribution and Interleukin 6: results from the EpiHealth study?** Manuscript


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Abbreviations

ADAM     Aerosol-Derived Airway Morphometry, method to assess airway dimensions by analyzing the deposition of monodisperse aerosol micro particles.
AiDA     Airspace Dimension Assessment, method to assess lung morphology using nano particles.
ATS      American thoracic society
BIA      Bioelectrical impedance Analysis, Method for Measuring Body Composition
BMI      Body mass index
COPD     Chronic obstructive lung disease
DLCO     Diffusing capacity for carbon monoxide, Measurement of gas exchange between air in the lung and the bloodstream
EpiHealth Cohort study in Sweden that focused on the interaction between lifestyle factors and genes in common disease
ERS      European respiratory society
FEV1     Forced expiratory volume after the first second, diagnostic measures in spirometry
FVC      Forced vital capacity, diagnostic measures in spirometry
GLI12    Global lung initiative 2012, reference values for lung function
LifeGene Cohort study in Sweden aimed at enhancing our understanding of how our genes, environment, and lifestyle influence our health.
IL6      Interleukin 6
IPF      Idiopathic lung fibrosis
UF particles Ultrafine particles
UFC particles Ultrafine carbon particles
$^{99m}$TcDTPA Technetium-99m-diethylenetriaminepentaacetic acid, method for testing the elimination of test particles from the lung.
1 Introduction

Pulmonary diseases are among the most common and serious health problems worldwide, influenced by lifestyle, environmental factors, and genetic predispositions (1-3). Accurate diagnosis at an early stage of the disease is often crucial for effective treatment and environmental adjustment (4-6). Spirometry, an important lung function test in the diagnosis of lung disease, is therefore also an indicator of general health (6-8). For spirometry to provide a reliable indication of lung health, the test must be performed in a manner that ensures its reliability (9-11). Currently, lung function tests are increasingly being performed outside of hospitals, particularly in primary- and occupational healthcare by several different professional groups with varying educational backgrounds which may complicate the reliability. Today, there are substantial technological advances that are already helping the healthcare system in providing more reliable and cost-effective diagnostics and treatment (12, 13). It is vital that we fully utilise these advances also in respiratory medicine. Furthermore, in clinical practice, lung function measurements are compared to reference values, which are expressed either as a percentage of the predicted value or as a z-score. These reference values are usually calculated using an equation that considers only three factors into account: Age, sex, and height (14-17). Weight is not usually included in these equations, as it does not greatly affect lung function, with the notable exception of cases with excess adipose tissue, which can considerably reduce lung volume (18-20). This is an important consideration in healthcare, especially because obesity has become an increasing global public health problem (21). There is a need to enable simpler spirometry procedures and ensure reliable assessment, while new methods are being developed. Therefore, rapid advances are being made in technologies such as nuclear medicine, magnetic resonance, and nanotechnology are being made for research and clinical applications. Nanotechnological methods and nano (or ultrafine) particles can be used both for diagnosis and treatment in healthcare (22-24) but the methods are still novel, and there is potential for further development.

This thesis emphasizes the need for advances both in the reliability of spirometry and in cutting-edge technological applications.
2 Background

2.1 Quality assurance of spirometry

To obtain reliable spirometry results the patient must cooperate actively and exert maximum effort. The procedure involves a full inspiration followed by a forced complete expiration until no more air can be expelled, as described in the guidelines of the European Respiratory Society (ERS) and the American Thoracic Society (ATS) (25, 26). The primary parameters measured by spirometry include forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1) as well as the ratio of FEV1 and FVC. The quality of the spirometry test quality is validated in two steps. Firstly, the patients each attempt is verified by mathematical algorithms that ensure that the manoeuvre starts forcefully and ends with minimal airflow indicating complete exhalation. Secondly, the attempt with the highest value of FVC and FEV1 is compared with the second-best attempt to check reproducibility. Thus, for a spirometry test to be of acceptable quality, it must consist of approved attempts that the patient can consistently reproduce (25, 26). Commonly spirometer devices present the two steps of feedback separately meaning that a patient may produce approved trials that are not reproducible, or vice versa. It is then up to the test leader to make a concluding quality assessment. These quality assurances can also be presented in an overall evaluation in a grading system created in accordance with professional guidelines (27). Thus, there are various ways to provide feedback, which differ depending on the spirometry software platform.

Since reliable results are the basis for accurate diagnostics especially when monitoring subtle changes in a patient's lung condition over time, there is extensive research on this topic (9-11, 26, 28-34). Efforts to improve and maintain the quality of spirometry include workshops, feedback from specialists, comprehensive training, and telemedicine collaborations with the common aim to provide patients with the most effective and accurate diagnosis possible. (9, 11, 28). Making use of artificial intelligence (AI) offers an interesting opportunity to utilise spirometer automation for easier, cost-effective for accessible quality improvement and training support (12, 13, 32).
2.2 Particles and particle deposition

The association between exposure to airborne particles and adverse health outcomes, including lung and cardiovascular disease, is well documented and affects individuals in both short and long term (35-42). This concern is even greater in occupational groups that regularly come into contact with airborne particles, such as industrial workers, firefighters, and welders, emphasising the importance of research in this area (42-44). Due to the nano (or ultrafine with aerodynamic diameter <100nm) particles large surface area in relation to their mass, nanoparticles are much more reactive. In many cases, this is an advantageous characteristic that can be utilised in for example catalyse chemical reactions. But it also raises safety concerns, especially if these particles are inhaled or ingested, which can lead to adverse health effects (45). For example, the use of silver nanoparticles for various industrial purposes is increasing, augmenting the risk of inflammatory, allergic, and other health effects from both acute and chronic inhalation exposure (46, 47). The exact mechanisms by which acute and/or chronic health effects are caused by inhalation of ultrafine (UF) particles is still unclear. Hypotheses suggest that inhaled particles either remain in the lungs or trigger an inflammatory reaction that subsequently affect the cardiovascular system, or they may translocate from the lungs to the vascular system, affecting areas prone to arteriosclerosis and leading to acute diseases such as stroke or ischaemic heart disease (48).

The impact of inhaled particles on the body depends not only on the concentration of particles, but also significantly on where the particles are deposited in the lung. Direct comparison to results from animal studies are difficult since the deposition pattern is different between animals and humans (49-51). Still, animal studies have documented varying degrees of particle translocation from the lung, as shown in the studies by Oberdörster et al. 2002, 2004, Kreyling et al. 2002, 2009, 2020, Semmler et al. and Elder et al. 2006 (52-57). The deposition of inhaled airborne particles in the respiratory system is influenced by the aerodynamic properties of the particles, respiratory patterns and the structure of the airways and occurs by impaction, sedimentation or diffusion (58). Compared to larger particles, UF particles are deposited to a large extent by diffusion. This opens new possibilities for use in diagnostics, as the deposition is related to residence time and airway diameter but not as sensitive to a higher respiratory flow rate. In addition, studies have shown that nanoparticles are better able to penetrate poorly ventilated and diseased lung regions compared to micro particles and reduce particle loss in the oropharyngeal and upper airways (59, 60). The interest in nanotechnology has increased dramatically and has expanded to a wide range of applications (61-63). New diagnostic methods have been developed, such as Airspace Dimension Assessment (AiDA), a technique for assessing lung morphology by measuring the deposition of inhaled nanoparticles in the lungs (64). Petersson-Sjögren et al. 2022 showed a significant correlation between the AiDA method and the
spirometry test. Moreover, the AiDA method provides information about the lung with the effective airspace radius variable which cannot be measured by standard methods. Another method that uses particles, Aerosol-Derived Airway Morphometry (ADAM), assesses airway dimensions by analysing the deposition of monodisperse aerosol particles, which are typically between 0.8–1.0 µm in size (65). The method uses larger particles (0.8–1 µm), that are then deposited by sedimentation. This requires a low-flow breathing method that complicates measurements in individuals with respiratory disease and often requires determination of static lung volume (60) which demonstrates the advantages of nanoparticles in this area.

2.2.1 Indium-labelled ultrafine particles

In previous research, our team has developed a novel method for tracking the translocation of UF carbon particles using a modified Technegas® generator to label the particles with Indium-111 (111In-UFC), which offers important improvements over the conventional Technegas aerosol labelled with Technetium-99m (Tc99m) (66-68). The 111Indium-UFC aerosols have a longer radioactive half-life of 2.8 days (versus 6 hours for Tc99m) which enable to study the particle a longer period.

The method in short, the aerosol was developed to study the deposition, retention, and translocation of particles from the lungs. Translocation meaning the inhaled particles enter the bloodstream from the alveoli, for example. The physical properties of the generated aerosol have shown a stable particle size and a high labelling efficiency, which ensures reproducibility and chemical stability. (66-68). The study protocol includes continuous monitoring and follow-up for one week using gamma camera images to assess deposition and clearance. This method demonstrates that 111Indium-labelled UF carbon particles can be an effective tool for long-term studies on the health impact of inhaled nanoparticles and may provide important insights into their interaction with biological tissues and potential pathways for systemic distribution (68).

2.3 DTPA clearance

Diethylenetriamine pentaacetic acid (DTPA) clearance from the lungs is a diagnostic method to assess the permeability of the alveolo-capillary barrier to measure the air-blood barrier permeability of water-soluble substances (69-71). This method involves inhaling an aerosol containing Technetium-99m-labeled DTPA and monitoring its transfer from the alveoli to the bloodstream using a gamma camera. The method can be used for analysing ventilation distribution, assessing the permeability of the lung epithelium, chronic interstitial diseases, and acute inflammation. It is also elevated in smokers and in
conditions of increased blood flow in the lung or be influenced by rapid breathing patterns (69, 72-74).

2.4 Body constitution

Obesity is associated with various health issues and represents a major public health challenge (75, 76) including a number of respiratory complications such as reduced lung volume, asthma and obstructive sleep apnoea (18, 77-80). Excess adipose tissue can affect the respiratory system in various ways. Central obesity (elevated abdominal fat) increases intrathoracic pressure and impedes lung expansion, resulting in decreased tidal volume, increased respiratory rate, and overall decreased lung capacity. At the cellular level, adipose tissue can exacerbate systemic inflammation, although the mechanisms are not yet fully understood (78, 81-84).

Previous studies have shown that central obesity is a significant factor affecting lung function, particularly in relation to the distribution of body fat (85). The distribution of body fat can be described to be either central or peripheral: central (or android) obesity, more common in males, involves fat accumulation around the abdomen and visceral organs, while peripheral (or gynoid) obesity, more common in females, involves fat accumulation around the hips, thighs, and upper extremities (86). Given these differences, it is plausible that lung function could be affected differently in men and women, although this has not always been consistently demonstrated in past studies.

In the clinical setting, lung function measurements are compared to calculated reference values and represented as either a percentage of the predicted value or a z-score (25, 26). Reference values for lung function are usually determined using an equation that incorporates the variables: age, sex, and height. Body fat is not taken into account, although previous studies have consistently shown that obesity impairs lung function (81). Furthermore, lung function is also impaired in normal-weight individuals with excess adipose tissue and in people with mild obesity (18, 77, 83, 87). What is currently lacking is an established consensus on how adipose tissue should be measured in the context of healthcare and lung assessment.

Body Mass Index (BMI) is widely used to assess overweight and obesity, despite its limitations in accurately assessing body composition (88, 89). High BMI can imply both increased and decreased lung volume depending on your body constitution. For example high BMI associated muscle mass may be associated with large lung volumes (90) and an increased waist circumference, used in the definition of the metabolic syndrome indicate central obesity, has shown in previous studies have a stronger association with reduced lung volume than BMI (85). Although the obesity is associated with decreased lung volume, the optimal method to evaluate obesity's impact on lung function remains unresolved. For clinical relevance, any assessment method should be
simple, standardized, and informative. Bioelectrical Impedance Analysis (BIA) is an alternative method for assessing body composition (91-94). The relationship between BIA and lung volume, however, has not been fully explored. BIA offers a digital method easy to use and standardized method for assessing body composition in a clinical context. Thus, it would be valuable to investigate BIA's effectiveness in providing a more accurate understanding of obesity's effect on lung function and to examine if and how it correlates to lung function.

2.4.1 Evaluating body composition and Bioelectrical Impedance Analysis

Body weight and body composition is linked to lung function and an important component in assessing health status (77). Currently, there's no standard method for obesity measurements when assessing lung function impairment. While BMI is frequently used, it falls short in assessing body fat distribution and differentiating between fat mass and fat-free mass. Research, including a study by Byberg et al. (2018), shows that early body measurements related to weight can predict better lung function in adolescence due to larger lung sizes associated with greater stature (90). However, BMI alone does not reliably predict lung function.

Waist circumference is included as part of the definition of metabolic syndrome, which makes it central in the assessment of overweight and obesity (27). However, research shows that waist circumference, a better indicator of central obesity and part of metabolic syndrome definitions, is more closely associated with lung function decline than BMI (85, 95). Although it is a simple and inexpensive method, it also has the disadvantage that it requires training and practise and that it is easy to make measurement errors, which, as studies have shown, are relatively common. The method is described in guidelines, but it is unclear how familiar those who use the method clinically are with these guidelines (96-98).

Advanced methods like Bioelectrical Impedance Analysis (BIA) and Dual-Energy X-ray Absorptiometry (DXA) provide more accurate distinctions between body tissues. DXA is the standard for body composition assessment, yet BIA is more practical for routine use in healthcare due to its non-invasive nature, cost-effectiveness, and ease of standardization (93, 99-101). Although not commonly associated with lung function impairment BIA could provide valuable information and should be investigated.
2.5 LifeGene and EpiHealth

2.5.1. LifeGene

LifeGene is a population-based research initiative a collaboration between seven universities in Sweden hosted by the Karolinska Institute in Stockholm (www.lifegene.se) with the aim gaining a better our understanding of the distribution and causes of chronic and infectious diseases through epidemiological research. Together with a questioner, body measurements such as blood pressure, height, weight, and spirometry were included in the physiological assessment of subjects. The LifeGene study was preceded by an extensive pilot study. In this study, we refer to the pilot study as LG1 which included 5043 participants and the main LifeGene study as LG2 which included the first 4379 participants. LG1 and LG2 different models of office spirometers were used, each equipped with its own software. The quality of spirometry was rated using a standardised system with five specific quality grades, where a higher grade indicating approved quality according to ERS and ATS standards (25, 27).

Two models of office spirometers, each with their own software, were used during this study, one in LG1 and another for LG2. Both provided immediate feedback on individual effort (exhalations) via on-screen displays. However, they differed in the way the overall session quality grade was presented: LG1 provided immediate, real-time overall quality feedback during the test, while LG2 provided this feedback after the session was completed. This discrepancy between LG1 and LG2 allowed a comparative analysis of the effectiveness of automated real-time feedback on test quality, depending on when the feedback was given.

2.5.2 EpiHealth

EpiHealth is a population study that aims to deepen our understanding of the distribution and ethology behind chronic and infectious diseases through epidemiological studies. This collaborative project involves 15 partners from universities and the healthcare system. The aim is to facilitate the creation of screening systems that improve prediction, treatment pathways and cost-effectiveness in healthcare, as well as the establishment of advanced epidemiological infrastructures, including registries, biobanks and technological platforms. EpiHealth is a population study that aims to deepen our understanding of the distribution and ethology behind chronic and infectious diseases through epidemiological studies. The aim is to facilitate the creation of screening systems that improve prediction, treatment pathways and cost-effectiveness in healthcare, as well as the establishment of state-of-the-art epidemiological infrastructures, including registries, biobanks and technological platforms (102).
3 Objectives

The main objective of this thesis was to improve our understanding of respiratory diagnostics through a multifaceted approach that includes the effectiveness of training and feedback in performing high-quality spirometry testing (paper I), the comparative predictive value of body impedance analysis (BIA) versus traditional measures in the assessment of obesity-induced lung function deterioration (paper II), and the long-term effects of weight gain on lung function, including the dose-response relationship and the mediating role of inflammation, taking into account factors such as gender (paper III). Lastly studying the translocalisation of inhaled UF particle in individuals with damaged lung epithelium as a possible new diagnostic method (paper IV). Overall, these studies aimed to develop lung function diagnostics that are both broader and more accessible and reliable, as well as a step towards the development of advanced technological methods.

3.1 Paper I
To investigate whether, after a short training period, test leaders are able to perform spirometry tests that meet ERS and ATS quality standards (25) and whether they can consistently achieve high quality results using automated on-

3.2 Paper II
The study aimed to investigate the connections between obesity, inflammation, and weight changes from early adulthood on respiratory health. Both the long-term effects of weight gain on lung function and the study the association of inflammation, considering variables such as gender, age, activity, and smoking.

3.3 Paper III
The aim of this study was to assess the impact of adipose tissue measured by Bioimpedance Analysis (BIA) in relation to waist circumference as a predictor decline of decline in lung function thus improving the assessment of the impact obesity on pulmonary health and investigating BIA's utility in clinical settings, using data from the EpiHealth study (102).
3.4 Paper IV
The aim of this study was to determine whether individuals suffering from a disease-causing damage in the alveoli i.e. COPD, and Idiopathic pulmonary fibrosis (IPF), have a higher translocation rate of UF particle from the lung into the blood than previously documented in healthy individuals. The method could then potentially be used in diagnostics to guide interventions, such as determining the initiation of treatment for IPF.
4 Material and method

4.1 Data collection

**Paper I** is a method evaluation study that investigated how quickly test leaders can be trained to perform spirometry tests that meet internationally recognised quality standards.

**Paper II** is a cross sectional study investigating how Body Impedance Analysis is related to lung function in the context of obesity and central obesity.

**Paper III** is a cohort study investigating the relationship between, weight gain from young adulthood to middle age, fat mass, inflammation, and their association with lung function.

**Paper IV** This is an experiment trial investigating whether inhaled nanoparticles can translocate the air-blood barrier in people suffering from IPF or COPD.

4.1.1 Paper I

The quality of spirometry assessments was analysed using data from the LifeGene project, a collaborative research initiative hosted by Karolinska Institute in Stockholm involving six universities in Sweden (www.lifegene.se). The project recruited people aged 18 to 45, who either received a postal invitation or registered on the LifeGene website. The pilot study, LG1, included 5043 participants, followed by an 18-month evaluation phase before the actual study, LG2 was conducted. The first 4379 participants were enrolled in our study. Participation involved both a comprehensive questionnaire and a visit to the test centre for health assessments, including blood and urine tests, measurements of height, weight, and girth circumferences and with tests for bioimpedance, audiometry, and spirometry, following the European Respiratory Society (ERS) and American Thoracic Society (ATS) guidelines 2005 (25). The spirometry tests, for which three to eight trials scheduled, measured lung function parameters such as FVC, FEV1, and FEV1/FVC ratio. The test leaders, mainly nurses and biomedical technicians had little to no prior experience in spirometry, underwent training that included a day of lectures and practical exercises, focussing on the use of the computerized quality parameters spirometry software's to ensure test
quality. The programs evaluated the tests using 'office grade' reproducibility criteria that met ATS/ERS standards and achieved a Grade 1 or 2 / A or B quality, with specific grading criteria for the spirometers outlined in Table 1 (25, 27). Both study phases, LG1 and LG2, utilized different spirometers. In LG1 the software provided an immediate overall quality feedback during the test, whereas in LG2 the quality feedback was only provided after the test in the summary report.

This different set-up allowed variation allowed the effect of real-time quality feedback on test accuracy to be analysed. The study was approved by the Swedish regional ethics committee.

<table>
<thead>
<tr>
<th>Grade</th>
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<tbody>
<tr>
<td>1</td>
<td>At least two approved tests. Difference in both FEV1 and FVC of &lt;100mL</td>
<td>A</td>
<td>At least two acceptable manoeuvres, with the highest two FEV1 values matching to within 100 mL and the largest two FEV6 values within 100 mL</td>
</tr>
<tr>
<td>2</td>
<td>At least two approved tests. Difference in both FEV1 and FVC of 101-150mL</td>
<td>B</td>
<td>At least two acceptable manoeuvres, with the FEV1 values matching to within 101 to 150 mL</td>
</tr>
<tr>
<td>3</td>
<td>At least two approved tests. Difference in both FEV1 and FVC of 151-200mL</td>
<td>C</td>
<td>At least two acceptable manoeuvres, with FEV1 values matching to within 151 to 200 mL</td>
</tr>
<tr>
<td>4</td>
<td>At least two approved tests. Difference in both FEV1 and FVC of &gt;201mL</td>
<td>D</td>
<td>Only one acceptable manoeuvre, or more than one, but the FEV1 values not matching to within 200 mL (with no interpretation)</td>
</tr>
<tr>
<td>5</td>
<td>No approved tests</td>
<td>F</td>
<td>No acceptable manoeuvres (with no interpretation)</td>
</tr>
</tbody>
</table>

4.1.2 Paper II

Our study utilised data from the EpiHealth study data (102), to study the effects of weight gain on lung function, considering the lifestyle that influences respiratory health.

Participants completed a questionnaire and attended a visit at the test centre for health evaluations including blood and urine analysis, height, weight, and girth measurements, as well as bioimpedance, and spirometry tests, performed according to the guidelines of the European Respiratory Society (ERS) and the American Thoracic Society (ATS) 2005 (25). Bioimpedance was measured using Tanita BC-418MA analyser and lung function with the MiniSpir spirometer. Lifestyle factors such as smoking habits, physical activity, and education level were analysed, to assess their impact on lung health. The EpiHealth study initially included
25,444 participants aged 45–75 years. Subjects missing data on weight, height, bioimpedance, or those with highly unlikely lung function measurements (FEV1 < 0.8 L or > 7 L and FVC < 1 L or > 9 L) were excluded, leaving 22,706 individuals for analysis in this study. See figure 1 for exclusion chart.

Statistical analysis of Weighted Quantile Sum Regression (WQS) was used to assess the impact of body composition on lung function taking lifestyle factors variables into account. WQS is a statistical method. In contrast to linear regression mode using classic least squares method, which predicts the average outcome based on the predictor variables, quantile regression estimating specific percentiles, such as the median, of the response variables under varying conditions of the predictors. This method proves to be particularly useful when variables correlate closely. The study received approval from the Swedish regional ethics committee.

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**Figure 1**: Flow chart for exclusion in paper II, III

- **EpiHealth participants** = 25,444
- **Subjects** = 23,308
- **Subjects** = 23,306
- **Included subjects’ paper II** = 22,706
- **Missing data weight at age 20 or weight change < -21 kg or > 66 kg**, **n = 3,958**
- **Included subjects’ paper III** = 19,348
- **Subgroup paper III** = 2,052
4.1.3 Paper III

For the third study, data from the EpiHealth study (102) were used, to which participants aged 45 – 75 years were invited, resulting in a cohort of 25,444 individuals. Weight change was calculated as current weight minus weight at 20 years of age, excluding extreme changes (weight changes less than -21 and more than 66 kilogrammes). Missing data or implausible lung function metrics (FEV1 < 0.8 L or > 7 L and FVC < 1 L or > 9 L). Missing smoking history data were addressed by generating 29 imputed datasets using multiple imputation, enhancing the analysis's accuracy and power. The final sample comprised 19,348 participants. See figure 1 for exclusion chart.

As described in paper II, measurements of body circumference, blood pressure, height, waist and bioimpedance (BIA), were performed in addition to spirometry. Lifestyle factors such as smoking habits, educational level and physical activity were categorised into three statuses into three levels according to intensity and duration. Self-reported weight at age 20 minus current weight was used to calculate weight change. A subgroup of 2,052 participants underwent interleukin-6 (IL-6) analysis using OLINK Multiplex panels, which analysed IL-6 levels along with other proteins.

Lung function was assessed according to international guidelines (25) and calculating z-scores using the Global Lung Function Initiative (GLI) equations (26).

Statistical analysis focussed on the relationship between weight change and lung function, using linear regression models and adjusting for confounders like education level, smoking status, and physical activity. The study was approved by the Swedish regional ethics committee.

4.1.4 Paper IV

Participants with COPD or IPF were invited to participate in the study. They underwent 99mTc-DTPA aerosol clearance tests, spirometry, and diffusion capacity (DLCO) measurements, and the results were compared with reference values for a Scandinavian population by Hedenström et al (16, 17). The 99mTc-DTPA aerosol (droplet size was 1.3 µm) prepared using a commercial kit, was inhaled via a SmartVent™ system (Diagnostic Imaging Limited, Welford, UK). Each subject received approximately 100 MBq of 99mTc-DTPA and breathed normally during supine gamma camera imaging. Imaging lasted 45 minutes with a two-headed gamma camera (Symbia T16, Siemens Healthcare, Erlangen, Germany), tracking the pulmonary clearance and calculating the clearance half-life of 99mTc-DTPA from the decay-corrected time activity curve.

In brief, the study procedure consisted of using a commercial first-generation Technegas generator (Cyclomedica, Kingsgrove, Australia) to produce particles. Particle size and concentration were measured before and after
exposure using a Scanning Mobility Particle Sizer system (SMPS; TSI, Incorporated Particle Instruments, Shoreview, MN, USA). The efficiency of labelling, or the extent to which particles were labelled with the radioactive marker upon exposure, was assessed immediately after production. Participants inhaled through a mouthpiece, with a nasal clip, until approximately 5 MBq of activity was detected by a radiation-protection proportional counter (Berthold Technologies GmbH & Co, Bad Wildbad, Germany). See figure 2 for the experimental set-up. Post-exposure, the distribution of UF aerosol in the thorax and abdomen was imaged using a gamma camera (Symbia T16, Siemens Healthcare, Erlangen, Germany) with medium energy collimators. Imaging was performed after exposure and continued at intervals of 2, 24, 72, 168, and 240 hours. Blood samples were collected at each imaging session, and participants provided daily urine samples throughout the follow-up period. Indium activity in blood and urine was analysed using a sodium-iodine detector (Wizard Gamma Counter, PerkinElmer Inc., Waltham, MA, USA), and free activity levels were determined using a dialysis membrane diffusion technique. See figure 3 for the study protocol.

Corrections were made activity leaching and mucociliary clearance were applied to adjust total lung activity clearance measurements, as detected by the gamma camera, to reflect actual lung particle clearance. The study received approval from the Swedish regional ethics and radiation protection authorities.

Figure 2: Setup for particle generation and exposure. Indium was manually loaded into the modified Technegas generator. Following particle generation, the vacuum pump activated, drawing the aerosol into the Mylar balloon. Particle sizes were then verified through the sampling line before starting inhalations, which were measured by the pneumotachograph upon opening the manual valve.
Figure 3: The follow-up after inhaling ultrafine graphite particles tagged with 111In-dium included measuring activity across the thorax and abdomen using a gamma camera with a wide field of view. Leaching tests on a filtered sample of the inhaled aerosol were conducted in vitro using dialysis membrane diffusion and a sodium-iodine well chamber. Blood samples, collected during each hospital visit, and 24-hour urine outputs, stored in 10-liter bottles from day 2, were analysed for activity concentration using a sodium-iodine counter. The levels of free activity in both blood and urine samples were determined using the dialysis membrane technique.
5 Results

5.1 Summary of main results

This thesis covered several aspects of lung function diagnostics, from the impact of automated diagnostic tools to the effect of body fat on lung function and new nanotechnology – all four papers focusing on pulmonary diagnostics. In the first study, we assessed the quality of spirometry in two groups, LG1 and LG2. The quality was notably higher in LG1, demonstrating the importance of real-time on-screen feedback. The second paper investigated the relationship between body fat percentage and lung function and showed that increased body fat, particularly in the waist and trunk areas, negatively affects FEV1 and FVC values, with gender-specific impacts on how body fat distribution affects lung function.

The third paper extended on these findings by examining the effects of body fat and weight gain on lung function in 19,348 participants, again showing that obesity rates were similar between genders, but women had higher fat mass on the legs and hips. This study also noted gender differences in the effects of weight gain on lung function. Further analysis of IL6 levels showed that an increase in IL6 had a more negative effect on lung volume in females than in males.

In the last paper, we compared individuals with COPD and IPF, finding no significant aerosol deposition differences but notable differences in particle translocation. These nuanced differences emphasize the complex relationship between lung function, body composition, and respiratory disease and highlight the need for considering personal factors approaches to managing and understanding pulmonary health.

5.2.1 Paper I

In this study, we analysed lung function using spirometry data from two groups: LG1, with 5043 participants aged 6-74, and LG2, with 4379 participants aged 10-80 (see table 1, paper I).

There was a significant difference in the quality between LG1 and LG2. In LG1, 92% of spirometry tests were of approved quality according to ERS and ATS 2005 standard (25). Opposed to LG2 which 73% of the spirometry’s achieved approved quality (see figure 1, paper I). After 40 subjects 94% reached approved level in LG1, 73% in LG2 (see figure 2, 3 paper I).
Notably, the failure rate (grade, 5 or F) for completing an approved spirometry attempt was significantly lower in LG1 at 1%, compared to 8% in LG2. The test leader’s performance also varied, with a notable improvement from the initial tests to after 40 subjects in LG1 both with consistently high quality. Thus, the learning curve for test leaders plateaued, suggesting early adaptation to spirometry testing procedures.

5.2.2 Paper II
This study used data from the EpiHealth study assessing the relationship between body fat and lung function. Participant demographics were analysed by body fat percentage (BF%). Of the initial 25,444 participants aged 45 – 75 years, we included 22,706 individuals by excluding participants with incomplete data or implausible lung function measurements. The average age of 60.3 years, with 44% of participants being male (see table 1, paper II). Both sexes had similar rates of obesity by according to BMI, however females exhibited higher body fat mass, particularly on the legs and hips.

Lung function, which was analysed in relation to BF%, showed that higher body fat was associated with lower z-score FEV1 and FVC values, while the FEV1/FVC ratio remained stable (see table 3, paper II). Strong correlations were observed between BIA variables, waist circumference, and lung function, suggesting that higher body fat and waist circumference were associated with decreased lung function, even after accounting for factors including smoking, education level and physical activity.

The influence of body fat distribution on lung function differed between the sexes. In men, waist circumference and trunk fat had a similar impact on FVC, whereas, in female, trunk fat had a greater impact. This pattern was consistent with FEV1, where waist circumference was a stronger predictor for men and trunk fat was more significant for women (see figure 1a, 1b paper II).

5.2.3 Paper III
This study investigated the effects of body fat and weight gain on lung function in 19,348 participants, with an average age of 60.3 years, 44% of whom were male also using data from the EpiHealth study (102) (see table 1 paper III). Body fat percentage was determined using BIA and categorised into quartiles. Notably, obesity rates were similar between sexes, with 17% of men and 15% of women having a BMI $\geq 30$, although females had higher fat mass, particularly on the legs and hips, which correlated with weight gain.

Lung function, as assessed by z-score FEV1 and FVC measurements, decreased with increasing weight change, while the FEV1/FVC ratio remained stable (see table 3a, 3b, paper III). Regression analysis showed weight gain affected lung function differently by gender, based on initial weight at age 20
and subsequent gain, male showing a more pronounced negative effect (see table 4, paper III).

An analysis of a subgroup of 2,052 participants in whom IL6 levels were measured showed no significant gender difference in IL6 concentration. Nevertheless, an increase in IL6 levels in female was associated with a greater decline in lung function for females than males, even after adjusting for trunk fat mass and weight gain, as well as lifestyle factors (see table 4, figure 6a, 6b table III).

5.2.4 Paper IV

In this study, as expected, there were no significant demographic differences between the groups of participants with COPD or IPF. However, smoking history was significantly higher COPD individuals had compared to those with IPF. While lung diffusing capacity for DLCO showed no significant difference between the groups, the IPF group had a significantly faster DTPA clearance rate than the COPD group (see table 1 corrected, paper IV).

Particle deposition in the lungs differed markedly, with IPF patients showing a lower deposition fraction (25.8%) than COPD patients (42.0%), although the deposition patterns between central and peripheral lung regions did not differ significantly in either group (see table 3, paper IV). The clearance of particles varied widely between participants, demonstrating a broad range from approximately 20% to nearly 70% after ten days in both groups. COPD and IPF patients showed a rate of UF particle translocated from the lungs, from 21.0% and 24.9% respectively at 7 days post-exposure, to 30.6% and 34.1% respectively after 10 days (see figure 3, paper IV). Ten days after exposure, the combined level of unbound activity in saline was remarkably low at median 1.6% (IQR 1.2–3.5%) and 1.7% (IQR 1.2–1.8%) in the subject groups. This indicates a high degree of chemical stability (see table 4, paper IV).
Table 1, paper IV (corrected). Patient clinical characteristics and pulmonary function data. Values are median and interquartile range (IQR).

<table>
<thead>
<tr>
<th></th>
<th>COPD (4 Female, 7 Male)</th>
<th>IPF (n=4 Female, 5 Male)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>75.0 (71.0 – 80.5)</td>
<td>76.0 (66.7 – 81.2)</td>
<td>ns</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.0 (170.0 – 178.5)</td>
<td>169.0 (158.2 – 180.7)</td>
<td>ns</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79 (72.5 – 87.5)</td>
<td>75.0 (68.5 – 82.0)</td>
<td>ns</td>
</tr>
<tr>
<td>Smoking (PY)</td>
<td>39.1 (20 - 69) ± 13.5</td>
<td>12.8 (0.3 - 33) ± 11.2</td>
<td>0.0002</td>
</tr>
<tr>
<td>FEV1 (% of predicted)</td>
<td>47.4 (40.3 – 66.5)</td>
<td>91.0 (78.0 – 94.0)</td>
<td>0.0001</td>
</tr>
<tr>
<td>FVC (% of predicted)</td>
<td>69.0 (66.4 – 98.8)</td>
<td>82.0 (77.0 – 99.0)</td>
<td>ns</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>47.2 (42.3 – 52.3)</td>
<td>72.0 (70.0 – 74.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DLco (% of predicted)</td>
<td>54.0 (53.0 –63.7)</td>
<td>53.0 (47.0 – 54.0)</td>
<td>ns</td>
</tr>
<tr>
<td>DTPA clearance half-life (min)</td>
<td>59.1 (41.4 – 84.8)</td>
<td>33.2 (23.9 – 48.7)</td>
<td>0.015</td>
</tr>
</tbody>
</table>

FEV1 = force expiratory volume; FVC = force vital capacity; DLco = Diffusion capacity for carbon monoxide; PY = cigarette exposure expressed as packs per year; ns = not significant; DTPA = labelled diethylene triamine penta-acetate. Lung function reference parameters from Hedenstrom et al. 1985 and 1986.
6 Discussion

6.1 Paper I

In the LifeGene study, forced spirometry tests were conducted in both an initial pilot (LG1) and the first six months (LG2) on a general population at a single testing centre—a rarity in this type of research. This study aimed to evaluate the impact of automated real-time quality grading during tests (LG1) compared to post-session reporting (LG2) on test quality, while monitoring quality progression.

Both LG1 and LG2 demonstrated high compliance with ATS and ERS quality and reproducibility standards, though LG1 showing significantly better quality compared to LG2. This difference was already evident in the first ten subjects tested by each test leader and persisted throughout the study, emphasizing the potential of direct feedback to improve test quality. Previous studies have confirmed the positive impact of feedback on spirometry test using other more costly and time-consuming methods such as workshops or consulting experts (30, 31, 103, 104). Our findings suggest that automated real-time AI, feedback during testing sessions can also significantly improve quality. Comparatively, the BOLD study (29) and the World Trade Centre Responder program (103) showed almost as high test reproducibility, yet direct comparisons are complicated due to differing quality measures and study designs. Furthermore, in contrast to LifeGene’s single-centre approach, these studies involved multiple clinics and had more extensive education and training for the test leaders. Rapid quality improvement was observed in the first 40 participants in LG1 and LG2, which is contrary to established guidelines that recommend for more extensive training (25). This can be compared with educational studies which have demonstrated that providing direct feedback to students is a successful method and facilitates learning (105-107). This fast-learning curve may also be due to the centralised test centre environment which encouraged an informal knowledge sharing among test leaders. The study suggests that even with minimal initial training in large population studies, high quality spirometry can be achieved rapidly in up to 90% of cases, emphasising the important role of frequent testing and collective learning. Finally, the identical conditions between LG1 and LG2, apart from the spirometry equipment, emphasise the effect of real-time on-screen quality feedback on improving spirometry test quality.
6.2 Paper II, III

In this large-scale cohort study, we studied the impact of weight gain, fat mass, body composition waist circumference and inflammation on lung function, using data from the EpiHealth project in two separate papers (II, III). The results showed that fat mass and weight gain are associated with reduced lung function. There was also a gender difference in body fat distribution, males having more trunk fat and female having more fat distributed on the hips and legs. In addition, data showed that BIA can be used as a predictor of reduced lung function in both sexes, with waist circumference providing further information regarding lung volume, especially in males.

We also examined the role of inflammatory marker IL6 on lung function, in relation to adipose tissue and weight gain. Our results identified sex differences in how fat mass, weight gain, and IL6 levels associate to affect lung capacity. Elevated IL6 levels were associated with reduced lung function for both sexes. Moreover, adjusting for weight gain and trunk fat, data showed that IL6 will have a significantly stronger negative association to lung function for female than for male.

As expected, our results show gender-specific differences in body fat which is consistent with previous studies (19, 82, 85). Females exhibited higher total fat mass and more hip and leg fat, while males showed greater abdominal fat. These trends are consistent with documented gender differences in subcutaneous and visceral fat distribution (86). This emphasises the importance of body composition and fat distribution for lung function.

IL6, an indicator of inflammation (108, 109) and associated with impaired lung function and mortality (109, 110). Previous studies have also shown that females have a higher expression of IL6 in males (111). In this study, both males and females had equally level of IL6. However, the results showed a significant difference in the association between of IL6 and lung function after adjusting for fat mass and weight gain, which is interesting.

The use of BIA and waist circumference as tools for obesity measurement relative to lung function presents both advantages and disadvantages. While waist circumference is a straightforward measure associated with obesity-related health issues as part of the definition of metabolic syndrome the procedure requires the practitioner to perform manual journal entries, which introduces a potential source of error. There are established guidelines for correctly measuring waist circumference (96); however, research indicates that these are not always adhered to in clinical practice (96, 98). The BIA method, being digital and capable of direct integration with medical records, requires no special training and is straightforward to standardize. Therefore, it should be considered a viable alternative for use in health screenings (99, 112).

In conclusion, the studies show the complex relationship between obesity, inflammation, and lung function, and the need to account for sex-differences and adipose tissue affect lung function. Our results support the development
of new public health approaches and medical evaluations to reduce the effects of obesity on lung health.

6.3 Paper IV

The fourth study investigated the clearance of UF carbon particles from the lungs of patients with damaged alveolar membranes, including COPD and IPF subjects, and compared these results with data from healthy individuals from a previous study (66). The results showed that there was no significant difference in particle translocation between the IPF and COPD groups, both of which had higher levels of particle translocation compared to healthy subjects (see figure 3 in paper VI). These results contrast with the differences of DTPA clearance which was more rapid in the IPF patient. Notably, clearance of UFC particles did not correlate with spirometry parameters, indicating a distinction between the effects of airway and alveolar damage. DTPA clearance is used to measure lung epithelial permeability to evaluate for example alveolar inflammation. Previous studies have shown a rapid clearance in IPF patient but weak correlations High Resolution Computed Tomography (HRCT) (113). Clearance may be influenced by respiratory patterns such as shallow, rapid breathing and can also be affected by increased perfusion (72). This may explain why the IPF group exhibits faster clearance rates compared to the COPD group.

There was no significant difference between the groups in diffusing capacity for carbon monoxide (DLCO). These findings suggest that these methods reflect different mechanisms. However, this could also be a consequence of the small number of participants. The severity and extent of the disease can vary among COPD and IPF patients. In addition, we observed that approximately 2% of the isotope was no longer associated with UFC particles in saline 10 days post-generated, indicating a minimal particle leaching.

Our research group has developed this method and has already demonstrated that healthy subjects show no or very little translocation of nanoparticles after 7 days (66). The complexity of this type of study and that permeability, is influenced by various factors, including particle size, concentration, and solubility limits the number of comparable human studies. Mills et al. 2006 showed that carbon nanoparticles remain in the lungs for up to 6 hours post-inhalation (114). The results from Möller et al. 2007 indicate that most inhaled UF carbon particles remain in the lung's outer regions and airways and only minimally systemic spread or accumulate in the liver within 48 hours (115). Unique to our study are the groups of disease that participated and the relatively long duration over which we monitored the subjects.

However, our study did not investigate the specific mechanisms underlying translocation of particles into the lung. For both patient groups, bound activity
in the bloodstream peaks at 24 hours post-inhalation and then declines exponentially, suggesting a dual-phase particle translocation pattern. The presence of bound activity in urine indicates renal clearance of UFC particles, though only a small fraction of these particles is cleared via the kidneys, possibly limited by their size (see figure 4 in paper VI). This observation is consistent with previous study Cho et al (2011) where only particles under 10 nm are likely to be cleared through glomerular filtration.

Clearence of particles from the lung in this study could include passive diffusion through damaged alveolar barriers or mucociliary transport into the gastrointestinal tract. Interestingly, mucociliary transport did not appear to play a significant role in UFC particle clearance in this study, suggesting passive transport through damaged alveolar barriers is more plausible and to some extent the smaller airways. Given that this is a human experiment, we are limited to an amount of particles that falls below the limit of detection threshold when dispersed in the thorax, which would otherwise have been interesting to investigate. Nonetheless, animal studies have shown pulmonary particle translocation and accumulation at vascular sites with inflammation (116).

The study also observed an imbalance between the total activity excreted from the lungs and the cumulative activity found in the blood and excreted through the urinary system, suggesting a possible accumulation of UFC particles in organs like the liver. Given the different pathological causes of alveolar damage in COPD and IPF the mechanisms of particle clearance need to be further research to clarify these processes more clearly, taking into account the limitations imposed by the degradation of the isotope-particle complex and the need for quality control of the labelling to ensure accurate measurement of bound activity.

6.4 Ethical considerations

The ethical considerations concerning this thesis primarily focus on participant welfare, data integrity, and the implications of the findings on broader public health policies.

In the LifeGene study, we utilized two different spirometers to investigate whether one method generated more reliable results. Had this been a study designed solely for this purpose, we would have had additional ethical considerations, especially since part of the participants might not receive the same level of reliability in their spirometry measurements. However, this study was conducted retrospectively using existing datasets, meaning the spirometers were not switched intentionally for the study; rather, we took the opportunity to examine the topic within this unique setup.

LifeGene and EpiHealth have highly similar study designs. Such studies provide participants with extensive information about their health. A
challenge may arise in post-study management. Are individuals with health issues provided with adequate assistance or information on how to seek help?

Furthermore, this thesis highlights gender differences in how our most common public health issues are addressed, emphasizing the problem of research areas that fail to adequately consider and base their findings on both genders.

Moreover, the study involving pulmonary clearance of UF carbon particles in patients with compromised lung function touches upon the ethical issue of exposing vulnerable populations to potential risks without a clear understanding of the mechanisms behind particle translocation and accumulation.

This research underscores the ethical imperative to conduct thorough risk assessments and maintain a balance between advancing scientific knowledge and protecting participant health.

6.5 Strength and limitations

The studies discussed offer significant insights into pulmonary health, each with its strengths and limitations.

In paper I, the LifeGene study showed the efficacy of real-time automated quality feedback in forced spirometry tests on improving test outcomes, surpassing those with post-session reporting. This finding emphasizes the potential of instant feedback to elevate the quality of respiratory testing without extensive technician training is paramount for high-quality results. The strength of the project lies in that except for the spirometer's software LG1 and LG2 offer a large number of subjects and nearly identical study conditions for example, single-centre and training of the test leaders. This provides an excellent opportunity to investigate the impact of feedback systems on quality and learning. However, the study's limitation lies in its approach and the absence of data on trial numbers, which could have further confirmed the influence of real-time feedback on testing efforts.

Papers II and III focused on the impact of fat mass, weight gain, waist circumference on lung function using the EpiHealth project data. These papers show the significance of abdominal fat (waist circumference) in lung volume impairment, particularly in males, and introduce trunk fat mass and inflammation as influential factor for females. A major strength of the course lies in the large number of participants and the fact that all examinations were conducted using the same equipment and consistent study protocols, thereby ensuring clear standardization. However, limitations of the study that we don’t have measured weight gain patterns from age 20 to the study period. Also, weight at 20 years was self-reported, making it less reliable which could limit our analysis. The measurement of IL6 was performed with the OLINK system, which varies between different batches. Within a batch, the measurements are
comparable; however, some systematic errors may occur in direct comparisons with other materials.

Paper IV explored the pulmonary clearance of UF carbon particles in individuals with compromised lung function. The study found no significant clearance differences between COPD and IPF patients, suggesting different mechanisms for particle clearance that do not correlate with traditional spirometry parameters. The primary strength of the study lies in the method being used. We utilize a technique previously developed by our group, characterized by a robust bond between particle and activity, allowing us to track the particle over a relatively extended period. An additional strength that should be emphasised is that these experiments were conducted on humans. We know that deposition patterns differ between humans and animals, which makes this study interesting. The primary limiting factor in this study is the restriction on the types of particles permitted for use, which also constrains detection outside the lung and also the absence of a healthy control group in the same study.
7 Concluding remarks & future aspects

In this thesis, my objective was to address pulmonary diagnostics from the perspective of my profession, “Biomedicinsk analytiker” with the focus to improve diagnostics - both the challenges encountered in clinical daily practice and to explore the potential in future diagnostic methods.

These studies collectively highlight several opportunities for future research in pulmonary diagnostic methods - both in clinical practise and large-scale epidemiological studies.

7.1 Paper 1

The inspiration for the first study appeared from a long-standing clinical demand for large volume of spirometry tests and, consequently, an understanding of how to perform accurate spirometry’s that provide a reliable basis for interpretation. There are internationally acknowledged guidelines to achieve high-quality examinations (25). These guidelines are embedded in the form of algorithms in our spirometry devices, intended to offer quality feedback. We demonstrated that automated direct feedback enhances quality. This feedback procedure is not always utilized in clinical practice, leading to more subjective quality assessments. Beyond the uncertainty this leads to in diagnostics, it complicates the ability to monitor patients over time. It's important to note that a spirometry test not approved according to guidelines can still provide important understanding of a patient's condition and should not go to waste. Also, achieving the quality standards can be challenging even for healthy patients; for example, young individuals may struggle to maintain the breathing manoeuvre for the duration required for approval. This has likely contributed to more subjective interpretation of the guidelines, contributing to diagnostic uncertainty. Fortunately, the new guidelines published in 2019 Graham et al. have addressed this issue, incorporating a grading system similar to the one used in the LifeGene pilot study (LG1), and introducing a quality category of "clinically useful" aside "approved quality" which permits the interpretation of spirometry results that do not meet quality standards be useful in a clinical context (26). These improvements have been shown to enhance the quality of testing, leading to more reliable diagnoses and healthcare (26, 31, 117). As AI is rapidly emerging as a tool in other diagnostic areas, and it could have a large
potential application in spirometry. Hopefully in the future, research in using AI as a more incorporated tool both in training test leaders and in patient interactions enhancing diagnostic accuracy, would be beneficial. We believe that greater measurement accuracy is of great benefit, especially when it comes to defining what is not a result within standard limits. The actual inability to obtain reproducible lung function results within an individual may be due to illness that should be differentiated from poor measurement quality (118).

Besides, how should we manage participant data that do not achieve acceptable quality levels? In most epidemiological studies, spirometry is encouraged to be performed according to ERS and ATS guidelines, yet spirometry’s failing to meet the required quality are rarely excluded. The approach to this issue needs further clarification. Data variability increases with poorer quality (see figure 4), and in larger studies, this variability likely has no statistical significance. However, the question remains regarding where the threshold lies. When do we need to adjust our data for substandard quality, and when does it not affect the results? Complicating matters is the potential risk of excluding spirometry data not meeting quality standards, which might also eliminate an important group of subjects. There are studies suggesting that the sickest patients, who are at the greatest health risk, are among those unable to perform spirometry adequately. Through the LifeGene study, we demonstrated the significant advantage of real-time, automated feedback in improving spirometry test quality, challenging the notion that extensive training is mandatory for high compliance with ATS and ERS standards (2). This suggests that even with short initial training, a real-time feedback mechanism can quickly elevate test quality in large-scale studies, which initiates an interesting area for future research within pulmonary function diagnostics.
7.2 Paper II, III

After ensuring diagnostic accuracy the next step involves comparing an individual's spirometry measurement to a reference value to distinguish between health and disease. The development of reference values is both crucial and complex, and there are numerous studies dedicated to this. In the development of a reference material one of the challenges lies in the balance of which body measurement parameters should be considered. The body measurements must be strongly correlated with lung function while also being practical and feasible for implementation in the clinical setting. Height, age, and gender are standard, but has been shown in various studies, that fat mass and body constitution effect lung function (18, 83, 87, 89, 119). But how to use this knowledge in a clinical setting remains still unclear.

In these two studies we demonstrated that waist circumference has a more significant impact on lung function in men, while trunk fat has a larger effect on women. This is puzzling but could reflect the pronounced mechanical impact of abdominal obesity best measured with waist circumference, which then dominates other mechanism. Furthermore, weight gain from age 20 to middle age is negatively associated with lung function for both sexes. But, despite similar levels of IL6, female lung function is more adversely affected by IL6 than men's when adjusting for abdominal obesity. Inflammation, or chronic inflammation, is associated with a multitude of diseases including
pulmonary impairment, although the association is not fully elucidated (120-124). It is known that chronic inflammation can lead to impaired lung function, but the reverse relationship may also hold true. Studies have demonstrated a negative relationship between inflammation and the lung function measures FEV1 and FVC, although results from longitudinal studies differ (121, 124-128). Kalhan et al, 2010 showed that systemic inflammation in young adults is linked to impaired lung function in middle-aged individuals (126). Fogarty et al 2007 and colleagues did not find evidence supporting the idea that systemic inflammation directly diminishes lung function (125). However, they noted an association between decreased lung volume and elevated levels of inflammatory markers, suggesting that reduced lung volume could lead to systemic inflammation (125). Gan et al 2004 showed that decreased lung capacity was linked to higher systemic inflammatory markers, which could hold significant implications for the pathophysiology and treatment of individuals with stable COPD (121). Fat mass impacts lung function through various mechanisms, including mechanical effects from abdominal obesity altering thoracic pressure and cellular-level influences and evidently the specific mechanism underlying this association has yet to be clarified. Numerous studies have highlighted the mechanical impact of abdominal fat (81, 82, 85, 95, 129). The cellular impact is also significant but challenging to delineate. Jenkins et al 1991 and Chen et al 2007 have demonstrated that even mild obesity has discernible effects, underscoring the difficulty in distinguishing the contributions of different mechanisms (83, 95). This is a challenge due to body measurements that are strongly correlated, and the effect of one mechanism may be more pronounced, potentially overshadowing the results. In these studies, we employed statistical methods to differentiate the effects of body measurements that are strongly correlated with each other, enabling the distinction between central obesity (increased waist circumference) and trunk fat.

Thus, the mechanisms related to fat mass, body constitution and inflammation differ between the sexes. Given the gender differences in body fat distribution and the cellular distinction of fat tissues along with varying characteristics and properties, it is likely that there is a gender-specific difference in the impact of adipose tissue on lung function. These observations challenge the adequacy of waist circumference and BMI as a sole measure of obesity’s impact on pulmonary function and advocate for more targeted measures that consider gender differences and specific fat distribution patterns. Today, technically progressed methods like bioimpedance analysis offer a means to measure fat quantity, enhancing reliability limiting test leader bias an error during manual data transfer. The test potentially assumes a more significant role in diagnostics in the future. In these two studies we emphasized the gender differences in how fat distribution impacts lung function and identified additional mechanisms, such as inflammation, where gender differences are noticeable. Obesity is a growing global health, so identifying methods to quantify its
impact on the lungs to distinguish fat mass’s effect from disease is imperative for enhancing diagnostics.

7.3 Paper VI

The final part of the thesis addresses potential future diagnostic methods and risk assessment. Lung diagnostics must be comprehensive and accessible, yet there's also a need to develop more advanced techniques. Nanotechnology has had rapid advancements, and our ability to utilize this technology in medicine has significantly expanded. Currently, there are new techniques to produce nanoparticles for, diagnostics, and treatment within pulmonary function diagnostics like described in Jakobsson et al 2016 that created a device to assess the deposition of nanoparticles in the respiratory tract through a single inhalation AiDA (64). In our study, we explored the clearance of UF carbon particles in individuals with compromised lung function, focusing on patients with COPD and IPF. Our findings indicate a significant clearance of UFC particles in both COPD and IPF patients, in contrast to healthy controls, with no notable difference between the two patient groups. This contrasts with the distinct differences observed in the washout rate of DTPA aerosol and diffusing capacity for carbon monoxide (DLCO), suggesting that UFC particle translocation may provide insights into the integrity of the alveolar epithelial barrier rather than just airway or alveolar damage. A factor that might be used to as one indicator on when to initiate treatment in subjects with IPF.

Typically, insoluble particles are phagocytosed by macrophages in the alveoli. Particles passing through the lungs are largely trapped in the liver where Kupffer cells (equivalent to macrophages) process them. Completely insoluble particles are retained for extended periods in lung tissue, as noted by Philipson K, Camner and others in studies involving gold-195 labelled particles. When the particle number is high relative to the mass, the signals to macrophages can become unclear. Soluble materials are excreted via the kidneys. Particles phagocytosed in the lungs can, to some extent, be expelled via the airways and swallowed. They may also pass deeper into the lung.

The impact of nanoparticles on health is an evolving area of research with limited human studies, particularly involving patients with impaired lung function. A crucial distinction in our studies, which are conducted on humans rather than animals, is the significant difference in how the lungs function regarding elimination and passage. Studying individuals with diseases and demonstrating how they differ from healthy subjects offers insights not as readily achievable with animal studies. Thus, the strength of studying the species of interest, particularly when aware of such differences, is substantial. Furthermore, studying humans provides more directly applicable results compared to animal experiments. Animal studies have shown nanoparticle translocation from the lungs to other organs, aligning with our observations.
However, our study's observational nature and methodological differences from other studies highlight the need for further research to elucidate the mechanisms of pulmonary clearance and extrapulmonary accumulation. Our results suggest that mucociliary transport plays a minor role, if any, in UFC particle clearance in patients with lung damage, pointing towards passive diffusion through a damaged alveolar barrier as a probable major mechanism. This is supported by the presence of different pathologies in COPD and IPF affecting alveolar integrity, which may facilitate UFC particle leakage into the bloodstream.

Although UF particles contribute only slightly to the total mass of particles, but significantly to their number, and high doses can cause oxidative stress, inflammation and other toxic effects. To understand their effects, it is necessary to understand the deposition, retention, clearance and excretion processes. Studies on human exposure to UF particles are inconsistent. Some show systemic translocation to organs such as the liver, while others find that UPs remain in the lung (24, 25). Chou et al 2022 study showed that is that silver nanoparticles are mainly cleared from the lungs through the digestive system, rather than the urinary tract (22). Kreyling et al 2020 followed over 28 days, healthy rats after inhaled 20 nm silver nanoparticle aerosols, which were processed and excreted via the gastrointestinal tract and feces.

Furthermore, particles cleared from the lungs may accumulate in vascular sites or organs such as the liver. Specifically for Indium, it is also excreted from the body via the liver to the gastrointestinal tract.

Given the complex interplay between lung function, particle clearance, and health outcomes, future research should focus on:

- Understanding the specific pathways and mechanisms of nanoparticle clearance and translocation in individuals with different lung pathologies.
- Investigating the systemic effects of nanoparticles, including their potential accumulation in organs other than the lungs, to better understand their health implications.
- Exploring the role of inflammation and macrophage activation in the clearance and systemic distribution of nanoparticles.
- Enhancing detection methods for nanoparticles in the body to facilitate more accurate and comprehensive studies on their distribution and health effects.
- Expand the investigative methods in a future study using the same approach, such as employing more advanced gamma imaging techniques or correlating gamma images with CT scans to demonstrate that translocation occurs in the damaged areas of the lung.
8 Populärvetenskaplig sammanfattning


Utvecklingen av automatiserad kvalitetsbedömning, BIA, tillsammans med diagnostik med ultrafina partiklar, belyser behovet av fortsatta teknologiska utveckling inom både diagnostiska verktyg och nya medicinska tillämpningar, vilket är avgörande för att hantera lungsjukdomar mer effektivt och att ge både en bredare och mer specifik vård.
9 Acknowledgments

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A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)