Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Public Health and Nursing

Kirsti Sørås

The associations between exposure to two different road-paving materials and lung function and club cell secretory protein in plasma and urine

Master's thesis in Public Health
Supervisor: Sindre Rabben Svedahl
Co-supervisor: Rikke Bramming Jørgensen
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Trondheim, May 2021,

Kirsti Sørås

Acronyms and abbreviations

FVC = Forced vital capacity (1)

FEV1 = Forced expiratory volume in one second (1)

MMEF = Maximum mid-expiratory flow (1)

PEF = Peak expiratory flow (1)

FEF25-75% = Mean forced expiratory flow between 25% and 75% of FVC (1)

FEFX% = Instantaneous forced expiratory flow when X% of the FVC has been expired (1)

FET = Forced expiratory time (2)

FEV_1% = The percentage ratio between FEV_1 and FVC (2)

CC16 = Clara cell protein (3)

PM = Particulate matter (4)

$PM_{0.1}$ = Particles up to 0.1 micrometers in aerodynamic equivalent diameter, ultrafine particles (4)

$PM_{2.5}$ = Particles up to 2,5 micrometers in aerodynamic equivalent diameter, fine particles (4)

PM_{10} = Particles up to 2,5 micrometers in aerodynamic equivalent diameter, coarse particles (4)

ELISA = Enzyme Linked Immunosorbent Assay (5)

Central Definitions

Particulate matter: the sum of all solid and liquid particles suspended in the air which came from different sources and are different in chemical composition and size (6)

Clara cell protein: a 16-kDa protein secreted by Clara cells and other non-ciliated cells of both the bronchiolar and bronchial epithelium (7)

In vitro: For biological processes and reactions, responses created outside the body of an organism, in an artificial environment. Experiments performed on cells grown in the laboratory (5)

In vivo: Directly translated "in life". For biological processes and reactions, responses that take place in living organisms (5)

Abstract

Background: This master thesis was part of work package 2 in the NIPH project «Preventive measures to reduce the adverse health impact of traffic-related air pollution (PrevenTAP)». The project was a collaboration between the Norwegian Institute of Public Health (NIPH), the Norwegian Public Roads Administration, St. Olav's Hospital, the Norwegian University of Science and Technology (NTNU), the Norwegian Geological Institute (NGU), and Trondheim Municipality. The experiments in work package 2, «Exposure to different types of road dust and reaction from the airways and cardiovascular system in humans» were carried out by the Department of Occupational Medicine, St. Olav's hospital in collaboration with the Department of Industrial Economics and Technology Management at NTNU and the Clinical Research Ward, St. Olav's hospital.

Epidemiological studies have shown that air pollution must still be considered a severe threat to public health globally. Particulate matter generated from road wear is a significant contributor to this pollution. Findings in previous experiments with human cell cultures have shown a difference in inflammatory response when exposed to dust from different rock types.

Aim: The purpose of the master's thesis was to investigate whether there were differences in the influence on lung function after exposure to dust from two types of stone commonly used in Norwegian road surfaces.

Methods: The study was designed as a randomized, double-blind, cross-over study. 23 healthy volunteers were exposed to dust in 2 rounds with exposure from two different rock types (Ottersbo and Bjønndalen), as well as a round of dust from lactose powder that was used as a placebo. Spirometry and blood tests were performed before and after the exposures.

Results: A few statistically significant changes were shown after the exposures. FVC was statistically significant ($p = 0.016$) reduced by 1.12% 4 hours after exposure to Ottersbo, compared to Lactose. FVC was also statistically significant ($p = 0.045$) declined by 1.75% 24 hours after exposure to Bjønndalen, compared to Ottersbo. A reduction in FEV1 of 1.48% ($p = 0.061$) and a decline in FEF25%% of 4.55% was observed after exposure to Bjønndalen. Uteroglobin levels in plasma were reduced after all three exposures, statistically significant after 4 hours for both Ottersbo ($p = 0.011$) by 14.52% and Bjønndalen ($p = 0.003$) by 11.29%, compared with Lactose. After 24 hours, Bjønndalen was still significantly reduced by 22.38% ($p = 0.012$) compared with Lactose. No significant changes were shown for uteroglobin in urine.

Conclusion: In this study, only small changes in lung function were revealed after exposure to rock dust from the two different rock types we examined, the changes are expected to have little clinical significance at the individual level. The results suggest a tendency for Bjønndalen to be more potent than Ottersbo, however, due to a small sample and great individual differences the results should be interpreted with caution. Large individual differences in the response could indicate that some individuals are more vulnerable, also among young, presumably healthy people, which could be interesting to study further before one can conclude.

Relevance: Population growth and increased urbanization can contribute to more pollution in cities and will mean that a larger proportion of the world's population lives in areas with harmful levels of air pollution. More individuals are thus exposed to potential negative health effects. More research to understand how particles affect health seems relevant.

Sammendrag

Bakgrunn: Denne masteroppgaven var en del av arbeidspakke 2 i FHI-prosjektet «Preventive measures to reduce the adverse health impact of traffic related air pollution (PrevenTAP)». Prosjektet var et samarbeid mellom Folkehelseinstituttet (FHI), Statens vegvesen, St. Olavs hospital, Norges teknisk- naturvitenskapelige universitet (NTNU), Norges Geologiske institutt (NGU) og Trondheim Kommune. Forsøkene i arbeidspakke 2, «Eksponering for ulike veimaterialer og reaksjoner i luftveiene og hjerte- og karsystemet», ble gjennomført av Arbeidsmedisinsk avdeling ved St. Olavs hospital, Institutt for industriell økonomi og teknologiledelse ved NTNU og Forskningsposten ved St. Olavs hospital.

Epidemiologiske studier har vist at luftforurensning fortsatt må betraktes som en alvorlig trussel mot folkehelse globalt. Svevestøv generert fra veislitasje er en vesentlig bidragsyter til denne forurensningen. Funn i tidligere forsøk med humane cellekulturer har vist en forskjell i inflammatorisk respons når de utsettes for støv fra ulike bergarter.

Hensikt: Formålet med masteroppgaven var å undersøke om det var forskjeller i påvirkning av lungefunksjonen etter eksponering for støv fra to steintyper som er mye brukt i norske veidekker.

Metode: Studien var designet som en randomisert, dobbelt-blindet, cross-over studie. 23 friske frivillige ble eksponert for støv i 2 runder med eksponering fra to ulike steintyper (Ottersbo og Bjønndalen), samt en runde med støv fra laktosepulver som ble brukt som placebo. Spirometri og blodprøver ble utført før og etter eksponeringene.

Resultat: Få statistisk signifikante endringer ble målt etter eksponeringene. FVC var statistisk signifikant (p=0,016) redusert med 1,12 % 4 timer etter eksponering for Ottersbo, sammenlignet med Laktose. FVC var også statistisk signifikant (p= 0,045) redusert med 1,75 % 24 timer etter eksponering for Bjønndalen, sammenlignet med Ottersbo. En reduksjon i FEV1 på 1,48 % (p=0,061) og en reduksjon i FEF25%% på 4,55 %, etter eksponering for Bjønndalen ble observert. Uteroglobinnivå i plasma ble redusert etter alle tre eksponeringene, statistisk signifikant etter 4 timer for både Ottersbo (p=0,011) med 14,52 % og Bjønndalen (p= 0,003) med 11,29 %, sammenlignet med Laktose. Etter 24 timer var Bjønndalen fortsatt signifikant redusert med 22,38 % (p=0,012), sammenlignet med Laktose. Ingen signifikante endringer ble vist for uteroglobin i urin.

Konklusjon: I denne studien ble det kun påvist små endringer i lungefunksjonen etter eksponering for steinstøv fra de to ulike steinstypene vi undersøkte, endringene er forventet å ha liten klinisk betydning på individnivå. Resultatene antyder en tendens til at Bjønndalen er mer potent enn Ottersbo, men det var et lite utvalg og store individuelle forskjeller som gjør at resultatene må tolkes med forsiktighet. Store individuelle forskjeller i responsen kunne tilsi at noen individer er mer sårbare, også blant unge, antatt friske mennesker, som kunne være interessant å studere videre før man kan konkludere.

Relevans: Befolkningsvekst og økt urbanisering kan bidra til mer forurensning i byene og vil medføre at en større andel av verdens befolkning bor i områder med skadelige nivåer av luftforurensning. Flere individer er dermed utsettes for potensielle negative helseeffekter. Mer forskning for å forstå hvordan partikler påvirker helsen synes relevant.

1. INTRODUCTION

1.1 Air pollution and health

The association between air pollution and death has been assumed since the 19th century (8). Measures to limit air pollution have resulted in a gradual improvement in air quality in Europe from the 1960s (8). Still recent studies show a connection between the current concentration of particles in city air and health damage (8, 9). Despite reductions in emissions and ambient concentrations, air quality remains poor in many areas of Europe (10). The 2019-report from the European Environment Agency states air pollution as the most important environmental risk to human health in Europe, a significant proportion of Europe`s population is still exposed to air pollution levels higher than the levels recommended by WHO guidelines (10).

In a period of pollution control in association with the Beijing Olympics 2008 a study showed significant improvements in several biomarkers, supporting the idea of adverse effects of air pollution on human health (11). Several recent studies regarding air quality during the ongoing covid-19 pandemic reveals a significant reduction in particulate matter levels from emission from traffic and industrial sources, as a consequence of social distancing and lockdown (12-15). It has been hypothesized that the results of these measurements give positive effects on both public health and the economy (12, 15).

The EU project ESCAPE (European Study of Cohorts for Air Pollution Effects) investigated the association between various air components and long-term health outcomes in Europe. Exposure to airborne dust with a diameter of less than 2,5 micrometers was associated to reductions in lung function in school children (16), stroke (17) and lung cancer (18). These associations are relatively small. However, considering the high number of people that are exposed, this is a serious health issue (19).

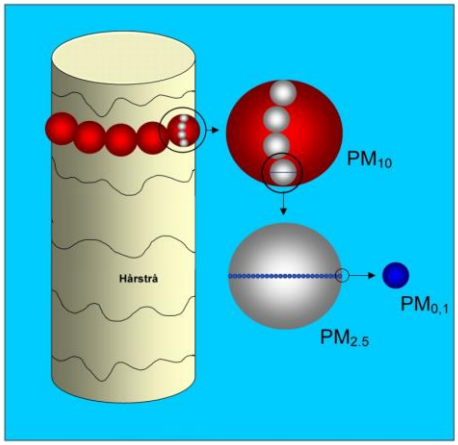
The health effects of air pollution may be an overall effect of several types of components working together (20). This may explain why health effects are observed at lower levels in population studies than in experimental studies (20). However, there is little knowledge about possible interactions between different types of particles and the mechanisms for such effects, especially at low concentrations (20).

Air pollution is considered globally as the environmental factor leading to the greatest loss of health in high-income countries (21). A recent Norwegian report on public health estimates

that air pollution in the form of airborne dust led to between 1100 and 1500 premature deaths in Norway in 2016 (22). Epidemiological and experimental studies have suggested that children and elderly (23-26), as well as individuals with preexisting cardiovascular (27) or respiratory diseases (28, 29) are more susceptible to air pollution.

1.2 Particle fractions

Particulate matter are airborne particles and includes several different types of pollutants from various sources. The adverse health effects of particulate matter have been well documented in both clinical studies and population studies (20, 30, 31). Particulate matter (particles, PM) varies in size and composition. In the matter of public health, $PM_{2.5}$ is defined as fine fraction and, PM_{10} as the coarse fraction and $PM_{0.1}$ as the ultrafine fraction (4, 32). Figure 1 illustrates the sizes of particulate matter.



Figur 1 Illustration of the size $PM_{2.5}$ and PM_{10} compared to a human hair strand. Source: FHI (32)

Exposure to particulate matter stimulates inflammatory reactions that may cause several diseases and adverse health effects (32). Experimental studies with humans and animals have shown that airborne dust reaches different parts of the airways and lungs depending on the properties of the particles, such as size, shape, and surface properties.

The airways consist mainly of three regions, as illustrated in figure 2. The extrathoracic region (including the nose, throat, and the larynx), the tracheobronchial airways, and the gas exchange region (the pulmonary or alveolar region) (33).

In occupational medicine particle size fractions may be referred to as inhalable, thoracic, and respirable (International Standards Organisation, sitet by Davies & Henderson,2009) (34). Inhalable dust is the fraction of airborne particles which is inhaled thorough the nose or

mouth. In general terms the inhaled fraction includes all particles <100 micrometers (34). Thoracic dust fraction is the airborne fraction of inhaled particles that penetrate beyond the larynx, like PM_{10}. In general, the thoracic fraction includes all particles <50 micrometers and compromises about 50% of total airborne particles (34). Respirable fraction defines particles that penetrate to the alveoli region. In general terms the respirable fraction includes all particles <16 micrometers (majority 10 micrometers) and having a 50 % cut of about 4 micrometers (34). $PM_{2.5}$ are slightly less of size than defined respirable dust and will be deposited in the same region.

When breathing through the nose at rest, particles smaller than 100 micrometers, and most of the particles larger than 10 micrometers and will be deposited in the upper respiratory tract (as shown in figure 2). A significant proportion of particles of 4 micrometers or less will be deposited in the lower airways, where the gas exchange occurs, and where it is assumed that they will be able to do the most damage (5).

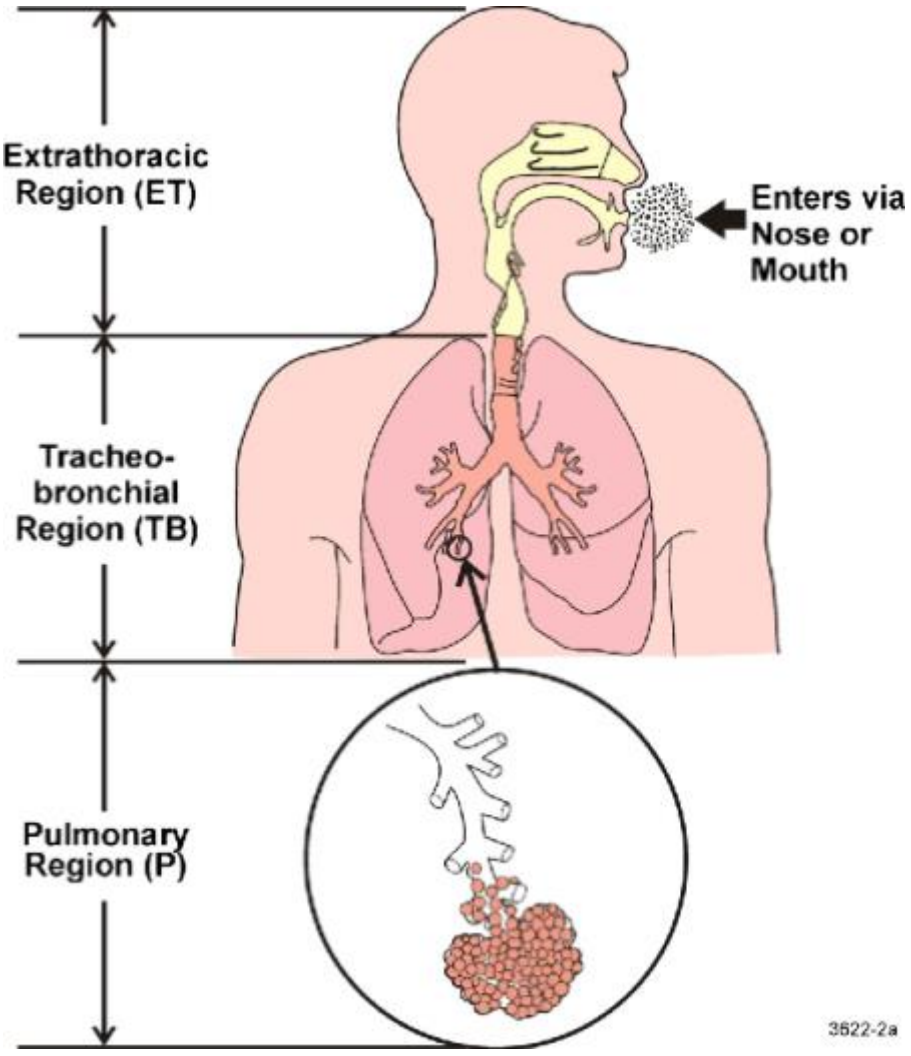


Figure 1 Display of the human respiratory system. Source: Cheng, 2014 (35).

Airborne dust can come from various sources, e.g., road dust caused by traffic. During periods of high particulate matter pollution, mineral particles from road surface wear can dominate. Hence for road surface, it may be important to consider rock types which produces as little dust as possible and also gives dust with little potential to trigger health effects, in addition to having the desired mechanical properties (5). Studies has suggested that associations between $PM_{2.5}$ and mortality depend more on components and sources than mass solely (36, 37). As legal limits so far have been referring to concentrations of PM, more understanding of the significance of composition is needed (10, 38). From a public health focus, it may be cost-effective to identify specific sources of particulate matter which gives adverse health effects (37).

Regarding air pollution from road traffic, it is common to distinguish between exhaust and non-exhaust emissions, the latter referring to brake, tire and road dust resuspension (10). Exhaust emissions are declining as a result of strict controls on EU emission limits (10, 39, 40), as a consequence non-exhaust emission is relatively increasing (41). In recent years, there has been an increasing interest in investigating the relevance of non-exhaust emissions (39-42). Road wear is one of the most important sources of airborne dust (22). In Norway and other northern countries, studded tires in the winter season are a major source of road dust (43-45).

A study examining road wear particles and their effects on health, exposed human cells to road wear particles caused by studded tires. The authors concluded that the inflammatory response to such road wear particles is almost similar to that of exposure to particles from urban streets, more so than to exposure to particles from the subway and at least as inflammatory as when exposed to particles from diesel exhaust (45).

Although there is uncertainty regarding the association between exposure to particulate matter and adverse health effects, and the complete understanding of the effect on health remains unclear (46), there are several hypotheses in research literature. Oxidative stress or inflammation can occur after inhaling particles (47). That may contribute to a systemic inflammatory condition, which further activates hemostatic pathways, impairs vascular function, and accelerate atherosclerosis and cause cardiovascular effects (48). It is established that inhaled ultrafine particles can diffuse rapidly into the systemic circulation, which may be relevant to particulate pollution-related cardiovascular disease and mortality (49).

Epidemiological studies have indicated associations between increased $PM_{2.5}$ exposure and hospitalization with lung infections (50-52), it has been proposed that exposure to fine particles alters the inflammatory response to infectious agents (50).

Seaton et al. suggested that ultrafine particles can cause alveolar inflammation which further stimulates both acute affection of blood coagulability as well as the release of mediators that can induce acute respiratory disease in susceptible individuals (53). According to a recent review children, females, and males with respiratory diseases appears to be more susceptible to air pollution (54).

Solid epidemiological data suggest that exposure to particulate matter is associated with adverse lung effects (55-59). However, the choice of study populations represents a limitation to these studies. These are studies on vulnerable groups, e.g., children, elderly, people with lung diseases, or populations with high occupational exposure. Furthermore, most studies base their assessments of exposure levels on observations from outdoor measuring stations, which can lead to measurement errors (60). Personal exposure to a given pollutant will vary from one individual to another, e.g., based on differences in activities and residential environments (61). When breathing, one does not inhale a single pollutant, but rather a complex mixture of several pollutants. Studies have indicated that personal measurements do not correlate with measurements on fixed monitors (61-63).

1.3 Lung function

To quantify effect of exposure in experimental or observational studies, spirometry may be used. Spirometry is an important screening test of general respiratory health, often used in combination with clinical assessment. In a standardized matter, it measures the volume and flows in how an individual inhales or exhales (1). In performing spirometry test it is common to follow guidelines for standardization of spirometry (1), based on recommendations of the American Thoracic Society and the European Respiratory Society.

The spirometric test should be starting with a rapid, full inspiration, followed by a blasting and complete expiration, before ending with full inflation (1, 2).

FVC (forced vital capacity) and FEV_1 (forced expiratory volume in 1 second) are often considered the primary variables of spirometry (64), and together with PEF (peak expiratory flow) they reflect flow and volume of the large airways.

FVC may be reduced in case of reduced lung compliance, chest deformity, muscle weakness or airways obstruction (65). The maximum acceptable variability for a measurement to be considered reliable is <5% on repeated measurements in one subject, and FVC is considered a reliable test (66).

FEV_1 is the amount of air that comes out of the lungs the first second of a forced expiration, after filling your lungs completely (1). FEV_1 reflects the resistance or obstruction in the airways and may also be reduced in the same conditions that reduces FVC (65). The FEV_1% is the percentage ratio between FEV_1 and FVC, what proportion of FVC that is emptied in one second. FEV_1% is normally reduced with age (67). PEF is the maximum expiratory flow. After maximal inspiration a maximum forced expiration maneuver starts without hesitation (1). PEF reflects the resistance in the airways.

Both long-term and short-term exposure to particulate matter (both $PM_{2.5}$ and PM_{10}) has been associated with reductions in FVC, FEV_1 and, PEF in healthy adults (60, 68, 69). Diurnal variability is shown for PEF, FEV_1, FEF25, FEF 50, $FEF_{25–75\%}$, and FEF75% in a population of healthy students, with generally lower values in the morning, increasing during the day till it peaks in the afternoon, then gradually falls against morning (70).

Other variables measured by spirometry may be $FEF_{25\%}$ and $FEF_{75\%}$, instantaneous forced expiratory flow when 25% and 75 % of the FVC, respectively, has been expired (71). $FEF_{25–75\%}$, or maximal mid-expiratory flow rate (MMEF), is the forced expiratory flow between the 25^{th} and the 75^{th} percentile of FVC, i.e. the middle half of the FVC (72). To some degree it reflects the presence of airway obstruction in the small peripheral airways when the flow through large airways is not obstructed (65). $FEF_{25–75\%}$ addresses the measuring of small airways (71, 73). There are arguments that FEF-rates should not be considered in clinical practice because of vide variability (74), and further that FEF has a substantial individual variation and rarely indicates respiratory failure in spirometry with normal FVC and FEV_1 (75). The validity of FEF relies a great deal on FVC measurement and effort in expiration (1).

FET, the forced expiratory time, is the time from the beginning to the end of a forceful expiration after a full inspiration (76), in 1966 Lal et.al. published findings from a study presenting FET less than 5 seconds in subjects without airway obstruction, and FET more

than 6 seconds in subjects with airway obstructions. A more recent study showed a mean FET of 10 seconds in a healthy non-smoking population, increasing values with age and higher BMI, and further supports that FET is prolonged in healthy subjects with airflow limitations (77). FET relies heavily on effort.

Several studies on occupational exposure to different types of dust showed a decline in lung function. A population with tunnel construction workers had a lower mean of FVC, FEV_1 and $FEF_{25–75\%}$ compared to a control population which was less exposed (78). Cement factory workers precented with significantly lower FVC, FEV_1, and FEV_1/FVC in a study comparing them to healthy volunteers (79). Asphalt workers had significantly lower FEV_1 and $FEF_{50\%}$ compared to control group in a Norwegian study from 2007 (80).

In a chamber study, small decrements in FEV1 and FVC were shown in a small sample of current- and ex-smokers after two hours of exposure to concentrated ambient fine particles, mostly from nearby traffic (81). A small, but statistically significant decrease in PEF following exposure to a 3-hour exposure to diesel exhaust was revealed in another chamber study with healthy volunteers (82).

There are few studies conducted on healthy, voluntary, young adults who represent most of the workforce. In 2014-2015, Wang et.al. conducted a study with 36 healthy, non-smoking students. They tested the short-term effect of exposure to $PM_{2.5}$ on lung function and serum Club cell secretory protein (CC16). They also investigated whether the permeability of the blood-air barrier, here measured by serum CC16, plays a role in reduced lung function caused by $PM_{2.5}$. Wang et al. found that acute exposure to $PM_{2.5}$ was associated with a decrease in lung function parameters and an increase in serum CC16. The effect on circulating CC16 occurred earlier than the effect on lung function, both lasting up to 24 hours after exposure (60).

1.4 Uteroglobin

Clara cell protein 16 (CC16), is a 16-kD protein secreted by the Clara cell (3). Clara cell secretory protein 16 (CC16) is also known as “Club cell secretory protein” and “uteroglobin”, and the latter will be used in this thesis. The Clara cell is located at the terminal bronchioles and belongs to the non-ciliated secretory epithelial cell (83), the cell contributes significantly in maintaining pulmonary homeostasis (84). In addition to playing an important role in the immune system, it has anti-inflammatory and anti-fibrous properties. Uteroglobin has been investigated as a potential biomarker related to pulmonary epithelial damage in a number of

diseases (83). Further, uteroglobin is assumed to have a protective role against inhaled toxic agents on the respiratory tract (3). In conditions with increased permeability of the lung epithelial barrier, such as exposure to toxins, serum levels of lung secretory proteins are higher than usual (85-88). Serum uteroglobin is considered a good, non-invasive marker to assess the integrity of the respiratory epithelium (89), and average levels in healthy non-smokers is between 10 and 15 μg/l. Uteroglobin is eliminated by renal excretion and can be detected in urine samples (90). Uteroglobin is also secreted postrenal, from the prostate, thus higher concentration has been detected in urine samples from males (90, 91).

A diurnal variability in uteroglobin has been observed in studies, with lower levels in serum at daytime, and opposite higher in daytime than night in urine (85).

In patients with chronic obstructive pulmonary disease (COPD) and smokers lower levels of serum uteroglobin has been observed (7, 92, 93) this might be an effect of damage to the Clara cells in individuals with chronic airways disease, and thereby reduced secretion of uteroglobin (85, 93). A population study in Spain observed decreased levels of serum uteroglobin in subject with airflow limitation measured by spirometry (94).

A study of controlled exposure to wood smoke showed an increase at 17 percent in serum level of uteroglobin 24 hours post-exposure, urine levels were not statistically changed (85). Serum levels were also found to increase in firefighters immediately after exposure to fire smoke (95, 96). A chamber study showed increased serum levels of uteroglobin at 2- and 4 hours after exposure to ozone compared to filtered air (97). In a longitudinal study performed in China, measuring four rounds of 72 hours personal exposure to $PM_{2.5}$, Wang et.al. found that serum levels increased within 2 hours after exposure, with a lasting increase up to 24 hours (60). A Norwegian study showed increased levels of uteroglobin in serum in asphalt pavers after the asphalt season, compared to the control group of engineers and plant operators (93). Conversely, a chamber study exposing young healthy, non-smoking adults to PM at ambient air levels, compared to filtered air, showed no detectable changes in either uteroglobin levels in urine or serum (98). Also, no changes were revealed in spirometric parameters (98).

1.5 Previous studies of mineral particles

Previous experiments with human cell cultures and animal studies have shown differences in the pro-inflammatory potential of different mineral particles. It has been suggested that high

levels of plagioclase (a mineral of the feldspar group) have lower potential to trigger pro-inflammatory response (99, 100) . However, in vitro experiments do not provide information on where in the lungs the particles are deposited or how quickly they are removed from the lungs (100, 101).

Quartz has consistently given a higher inflammatory response in cell experiments (bronchial epithelial cells and macrophage-like immune cells) than potassium feldspar (102). However, these responses were measured on other parameters than what was assessed in this current study.

The two materials compared in this study are not very different overall, both consist a great deal of plagioclase. Quartz diorite has a higher level of quartz, and rhomb porphyry contains more potassium feldspar than quartz diorite (102). Other materials showed greater inflammatory potential, but the two were chosen as they are both common in asphalt on Norwegian road surfaces.

1.6 Study aims

To examine whether the type of road surface has an impact on health, the current project aimed to investigate whether similar differences can be shown in in vivo experiments, i.e., when humans are exposed to dust from different stone materials commonly used in road surfaces.

To investigate this, the project aimed to examine the effect on levels of inflammatory markers from exposure to pure stone dust of different types of stone used in road surfaces to see if there is a difference, and whether possibly a particular type of stone or stone mineral will have more effect on health than other types.

This project compared dust from stone produced at Ottersbo Pukkverk Fosen (quartz diorite)- referred to as “stone A” and Bjønndalen Pukkverk Nittedal (rhomb porphyry) - referred to as “stone B”.

In this master thesis the focus will be on possible impacts on lung function measured by spirometry and levels of uteroglobin in both plasma and urine, from exposure to different type of mineral dust.

1.7 Research questions

In healthy volunteers, does exposure to particulate matter from stone from Ottersbo and/or stone from Bjønndalen affect lung function and levels of uteroglobin in plasma and urine? Is there a difference between the two materials? Further, what are the covariation between spirometry scores and uteroglobin levels?

2. METHODS

2.1 Ethics

This master thesis is part of work pack 2 in the project PrevenTAP "Preventive measures to reduce the adverse health impact of traffic-related air pollution". PrevenTAP is a collaboration between the National Institute of Public Health (FHI), the Norwegian Public Roads Administration, St. Olavs Hospital, Norwegian University of Science and Technology (NTNU), Geological survey of Norway (NGU) and Trondheim Municipality.

The study was approved by the Regional Ethics Committee, Central Norway (ref. nr. 2018/924). All participants were informed about the study and gave a written consent. Potential disadvantages in participating were carefully described in the written information letter, and it was ensured that the levels of exposure did not exceed the Norwegian Labor Inspection Authority`s limit values.

For confidentiality, the participants were assigned individual study IDs. The project was funded by the Research Council of Norway, the Better Health program.

All study procedures were conducted in compliance of the principles of Good Clinical Practice (103). The participants were covered by the Patient Injury Act. Any unexpected medical findings that indicated the need for follow-up of health personnel were handled by a medical doctor at the Department of Occupational Medicine, St. Olavs Hospital. Participants received modest financial compensation for each attendance in the project and a bonus for completing all attendances.

2.2 Study participants

The study participants were 24 presumed healthy, non-smoking university students, aged 20-28 years (mean age 23,6). Fourteen of these were female, ten were male. Previous or present lung diseases or chronic diseases such as rheumatism, ulcerative colitis, and Crohn`s disease were exclusion criteria. The participants were instructed to abstain from alcohol and hard work-out sessions the 36 hours prior to testing, and to try to have "normal" sleep behavior the days before testing.

During the study, these 24 were divided into 6 groups of 4 participants. One participant withdrew from the study after the first day of testing. This participant was not replaced.

Table 1: Baseline characteristics of participants

Characteristic	All (N= 23)	Women (n= 13)	Men (n= 10)
Age, y	23.6 ± 2.1	23.9± 1.3	23.1 ± 2.9
Height, cm	171.8 ± 9.8	166.2 ± 7.9	179.0 ± 7.3
Weight, kg	71.2 ± 15.0	63.6 ± 10.5	81.0 ± 14.4
BMI, kg/m^2	24.0 ± 4.3	23.0 ± 3.2	25.4 ± 5.4

Values are expressed as mean ± SD

2.3 Study design and procedure

The study had a randomized, double-blind, crossover design. The order of the exposures (material A, B or C) was randomized for each group. Except for the occupational hygienist who controlled the randomization, the rest of the study team and the participants were blinded for which dust was used every time.

The study was conducted from September to December 2019. Due to availability, one visit for two of the participants had to be postponed until the beginning of January 2020. During this period, the 6 groups of participants each met at three occasions for exposure testing, one of the tests involved inert placebo dust (lactose), the other two tests with material A and material B. Between each dust they were exposed to, there was a wash-out period for approximately a month.

Plan for exposure is shown in figure 4.

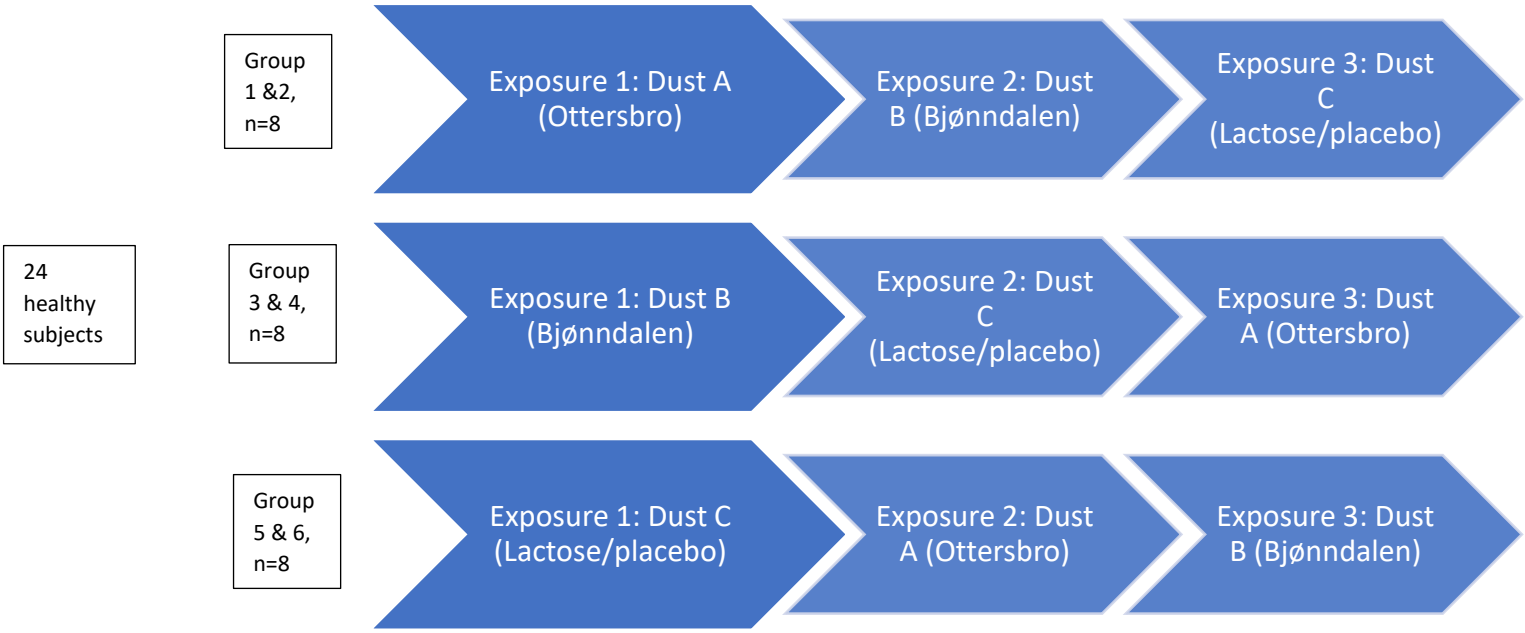


Figure 2 Schedule of exposure

The participants were exposed for four hours for each dust. On the days of exposure, the four scheduled participants met 30 minutes apart, as several procedures/tests were performed before exposure.

During one exposure day there were 4 time points of tests. See table 2 for a display of the procedures done.

Before entering the exposure chamber the participants were asked about symptoms such as cough, dyspnea, burning eye sensation, nasal congestion, or other symptoms. This was followed by three measurements of blood pressure and heart rate. Participants rested for five minutes before the blood pressure measurement and for one minute between each measurement.

In addition to blood and urine samples and spirometry, the testing procedure involved measuring the fraction of exhaled nitric oxide (FeNo), exhaled breath condensate (EBC), and a bronchial provocation test with Methacholine. After these tests, the participants entered the exposure chamber for 4 hours. Once every hour they were to use a step board for 15 minutes to increase respiratory rate and thus volume of inhaled dust. After each time a participant

finished on the step board, he/she switched seats with the next participant to use the step board. The rest of the time they could sit and relax.

Table 2: An overview of procedures.

Procedure	T1~ (before exposure)	T2~ (immediately after exposure)	T3¨ (4 hours after exposure)	T4¨ (24 hours after exposure)
Blood pressure and heart rate	X	X	X	
FeNo	X	X	X	X
Spirometry	X	X	X	X
Bronchial provocation test (Methacholine)			X	
Exhaled breath condensate (EBC)	X	X	X	
Blood samples*	X		X	X
Urine samples		X		X

*Analyses: High sensitivity crp, fibrinogen, leucocytes, thrombocytes, d-dimer, coagulation factor VIII, Von Willebrandts factor, Club cell secretory protein (CC16), TNF-alpha, Interleukins
~= performed at NTNU
¨= performed at St. Olavs Hospital

Testing procedures were performed on two sites: at Department of Industrial Economics Technology Management, at the division of Health, Safety and Environmental Management, at NTNU and at the Clinical Research Ward, St. Olavs hospital, both in Trondheim. The rationale for using two sites was that the exposure chamber was located at NTNU, so procedures at time T1 and T2 was done there (Table 2). Four hours after T2 the participants met at St. Olavs hospital for further tests (T3), as the device for Methacholine test was located there. Tests on T4, 24 hours after exposure was also performed at St. Olavs hospital (Table 2).

Only spirometry and blood- and urine samples were further addressed in this thesis, as stated in the introduction.

2.4 Exposure

Dust exposures were conducted in a custom-designed, closed chamber with controlled supply and exhaust of air at NTNU (Gløshaugen campus). Before entering this chamber, the

participants were asked to wear a protective coverall to avoid contamination of their clothes. The participants were supervised during the whole exposure time via a large window to the to the adjacent room. The room was equipped with one large table and 4 chairs, all easily clean materials, such as plastic furniture and linoleum floor.

The chamber was sufficiently ventilated to ensure that the CO_2 concentration was not too high. The air to the chamber was taken from a ventilation system with an extra hepa filter to ensure that the air was completely clean. There were difficulties in obtaining high enough concentrations of the placebo dust. To compensate for this, air changes in the chamber were made with a somewhat lower frequency for placebo dust (2.06 times per hour) than for dust A and B (3 times per hour).

Dimensions of the chamber: 3,56 meters deep, 3,31 meters wide and 2,97 meters high. Floor area 11,78 m^2 and volume 35 m^3. The mean temperature in the chamber was 20.8°C, ranging from 18.1 to 23.1°C. The mean room humidity was 36%, ranging from 22-49%.

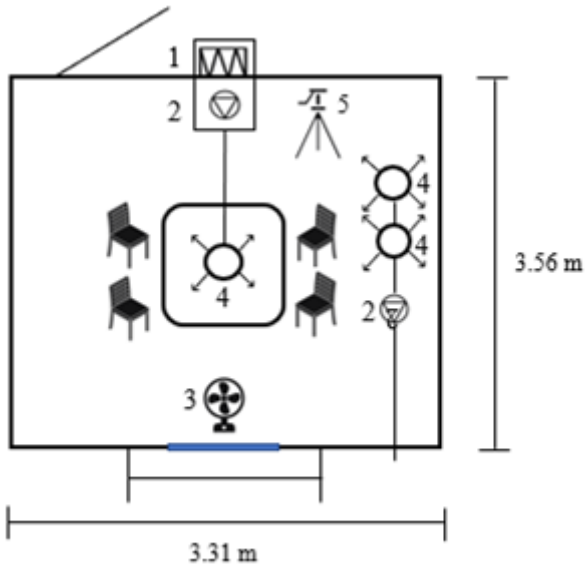


Figure 3 Exposure chamber. Figure by T.B. Nitter, 2021 (104)

Symbols: 1= air filter, 2= pump, 3= table fan, 4= circular diffusor, 5= test stand

Stone from the two quarries (Bjønndalen and Ottersbo) was crushed into fine-grained dust using the Los Angeles (LA) method. A dust aerosol generator distributed the dust equally out in the chamber via a supply hose. For both dust A and B, a TSI 3400 generator was used, and for dust C a TSI 3410 generator.

To ensure full agitation of the dust, a table fan was placed near the window in the chamber.

Limits for average exposure for 8 hours are defined to a maximum of 5 mg/ m^3 respirable dust fraction and 10 mg/ m^3 "total dust" by the Norwegian Labor Inspection Authority. Levels of exposure in this project were monitored by dust meters to correspond with these limits.

The exposure chamber was equipped with measuring instruments for PM_1, $PM_{2.5}$, PM_{10}, respirable dust fraction, thoracic dust fraction, "total dust" and ultrafine (nano) particles.

2.5 Personal exposure monitoring

Each participant carried a personal monitor in the breathing zone to measure average personal exposure to respirable dust fraction during the 4 hours of exposure. The measurement was gravimetrically determined.

Total dust and $PM_{2.5}$ were also determined gravimetrically, measurement was made stationary in the chamber, and then corrected from the direct measurement of how the concentration in the chamber varied over the day and what was the variation during the period the participant was in the chamber.

All equipment was calibrated before and after measurement.

2.6 Biological markers

Several markers were analyzed from blood samples, urine samples and exhaled breath condensate.

The blood and urine samples were centrifuged and frozen directly on the sites. Several sample tubes were drawn at the same time. For every tube to be centrifuged in time we used two centrifuges on both sites, as the tubes were to be centrifuged on various centrifuge spin settings immediately after they were collected. At NTNU: Hettich, Universal 320 and Hettich Rotina 35 1705-01 Bench-model. At St. Olavs: Hettich Rotina 420R and Hettich Rotina Universal 32R. Periodic maintenance is performed regularly on the centrifuges.

Blood was collected in BD vacutainer tubes. The blood samples were centrifuged on 2000G for 15 minutes at 4 degrees of Celsius, and separated plasma was then divided into 10 aliquots. The urine samples were centrifuged at 2500G for 15 minutes at 4 degrees, then divided into 5 aliquots.

Both plasma and urine samples were put in a -20 degrees freezer at NTNU. At the end of each test week all samples were transported on ice to St. Olavs Hospital, (clinical research facility) and put in a -80 degrees freezer with continuous temperature monitoring. Later the samples were shipped on dry ice to FHI (Oslo) for analysis.

Directly after the participants exited the exposure chamber, they delivered a urine sample. This was considered the baseline test. The second urine sample was collected on site the day after exposure, 24 hours post exposure. This also ensured that urine was collected at the same time of day, to avoid any diurnal variation.

Analyzes on uteroglobin in plasma and urine was measured with ELISA-kit, according to manufacturer's instructions.

2.7 Lung function

Spirometry was measured with a computerized spirometer (Spirare, version 3, Oslo, Norway). As testing was performed at two sites, two devices, the same software model, were used. Spirare sensors (SPS330) were used at both sites, with different Serial numbers (SN: 217665 at NTNU site, and SN: 217665 at St. Olavs Hospital site). The devices were calibrated before each test, as specified by the manufacturer. On each exposure day spirometry was done before entering the chamber, after they came out, 4 hours later the same afternoon and 24 hours after exposure. Per guidelines of European Respiratory Society, a minimum of three measures were performed each time to ensure reliability (1). The best of three acceptable readings was accepted. Spirometry was performed by 5 different study nurses, each with considerable experience with the procedure. To reduce measurement error, one strived to ensure that the same study nurse performed all tests on all timepoints the same day. Spirometry was performed in sitting, upright position, and participants wore nose clips during the procedure.

The lung function parameters examined in this study were FVC (forced vital capacity), FEV_1 (forced expiratory volume in 1 second), FEV_1% (percentage ratio between FEV_1 and FVC), PEF (peak expiratory flow), $FEF_{25\%}$ (forced expiratory flow when 25 % of FVC has been exhaled), $FEF_{75\%}$ (forced expiratory flow when 75 % of FVC has been exhaled), $FEF_{25-75\%}$/MMEF (maximal mid expiratory flow) and FET (forced expiratory time).

2.8 Statistical analysis

Data from the participant who withdrew after the first day of exposure are not included in any analyzes, to avoid the mean being affected.

For the choice of further statistical analyses, normal distribution was first tested. Data were tested for normality by Shapiro-Wilk`s test. The Shapiro-Wilk gives exact significance values, and it is considered more accurate than the Kolmogorov-Smirnov test, which gives an approximation of significance values. For small samples Shapiro-Wilk is appropriate. A significant value indicates that data is not normally distributed (105). All analyses were performed with SPSS version 25.0.

Before testing for normality, the file was split for time (immediately after exposure, 4 hours after exposure, and 24 hours after exposure) and material (A, B, and C). Normal distribution of data could not be assumed for all variables by the Shapiro -Wilk test. Generally, normality tests have low power in small sample sizes. Thus, plots and graphs were also visually inspected.

Percent change was calculated in SPSS (post-exposure level/baseline * 100)-100.

As all participants were exposed for each dust, and measurements were made on all subjects before and after exposure, they all serve as their own controls. This has the advantage of reducing the impact of inter-individual variation between the participants.

When data is not normally distributed, Wilcoxon matched-pairs test is the appropriate statistical test for comparing paired samples (106). Wilcoxon signed-rank test is a non-parametric alternative to paired samples T-test (105).

Nonparametric statistical tests calculate the differences between the observations and ranking them in increasing order. Zero-values are excluded from the rank numbers so *n* is the number of non-zero differences, so *n* might be less than the original sample (105).

For any significant findings revealed by the Wilcoxon signed-rank test, the effect size was calculated using the equation for effect size for Wilcoxon signed-rank test, $r = Z/\sqrt{N}$. Value for Z is given from SPSS, N is the total number of observations which z is based on. Effect size 0.1 is considered a small effect, 0.3 medium effect, and 0.5 large effect (105).

Finally, to test for correlation/covariation between spirometry data and uteroglobin in urine and plasma, Spearman rank correlation coefficient was performed, as assumption of normality is not required for this test (106). Spearman rank correlation test was also used to check for any correlation between gender and uteroglobin levels.

3. RESULTS

Use of descriptive statistics, such as mean, is sensitive to non-normal distribution. Before analysis, all variables were checked for normality, as judged by the Shapiro-Wilk test and histograms. Some of the variables were skewed and the dataset contained some significant outliers, and normal distribution could not be assumed for all variables. For descriptive purposes, the data are presented using median values, along with a 95 % confidence interval for the median.

3.1 Spirometry measurements

In table 3, the median percent change, with corresponding 95 % confidence intervals, in spirometric parameters are shown.

Table 3: Percent change in spirometric parameters: (from baseline)

Immediately after exposure	**Ottersbo (Quartz diorite) -A**	**Bjønndalen (Rhomb porphyry) - B**	**Lactose (Placebo) -C**
FVC	-0.67 [-1.32 – 1.20]	-2.67 [-2.83 - -0.53]	-0.52 [-1.10 – 0.74]
FEV_1	-0.43 [-1.63 – 2.02]	-1.48 [-2.12 - -0.39]	0.38 [-0.82 – 2.22]
FEV_1%	0.00 [0.00 – 2.53]	0.00 [0.00 – 1.35]	1.32 [0.00 – 3.66]
PEF	-0.76 [-2.22 – 1.26]	-1.65 [-2.92 – 4.57]	0.00 [-3.49 – 4.68]
$FEF_{25\%}$	-2.72 [-3.89 – 1.08]	-1.22 [-2.63 – 1.17]	3.12 [-1.24 – 7.77]
$FEF_{75\%}$	1.04 [0.00 – 6.03]	3.32 [-1.74 – 8.33]	8.86 [0.49 – 12.59]
$FEF_{25-75\%}$	1.16 [-2.70 – 3.60]	-0.20 [-2.28 – 3.60]	4.38 [-0.30 – 8.67]
FET	0.00 [-6.85 – 9.43]	-2.08 [-8.22 – 13.79]	-1.35 [-8.62 – 11.76]
4 hours after exposure	**Ottersbo (Quartz diorite) -A**	**Bjønndalen (Rhomb porphyry) - B**	**Lactose (Placebo) - C**
FVC	-1.12 [-2.63 - -0.36]	-1.80 [-2.07 - -1.07]	-0.44 [-1.36 – 1.57]
FEV_1	-1.42 [-2.32 – 0.00]	-0.60 [-1.93 – 0.00]	0.28 [-0.57 – 1.59]
FEV_1%	0.00 [0.00 – 2.30]	0.00 [-1.02 – 1.33]	0.00 [0.00 – 1.25]
PEF	-3.24 [-4.15 – 0.00]	-3.13 [-4.56 – 0.23]	-2.74 [-5.07 – 0.75]
$FEF_{25\%}$	-2.67 [-3.33 – 0.39]	-1.41 [-4.08 – 4.90]	0.52 [-4.68 – 5.67]

$FEF_{75\%}$	2.08 [-5.52 – 4.65]	-0.37 [-3.17 – 3.70]	0.71 [-6.16 – 3.81]
$FEF_{25-75\%}$	1.62 [-4.83 – 3.02]	0.91 [-0.71 – 1.88]	1.67 [-1.02 – 5.73]
FET	8.57 [0.00 – 20.37]	-4.44 [-6.98 – 18.52]	8.57 [-1.96 – 22.22]
24 hours after exposure	**Ottersbo (Quartz diorite) - A**	**Bjønndalen (Rhomb porphyry) - B**	**Lactose (Placebo) - C**
FVC	-1.10 [-2.40 – 0.00]	-1.75 [-3.39 - -0.59]	-0.38 [-1.98 – 0.74]
FEV_1	-0.68 [-2.79 – 1.71]	-1.72 [-3.67 - -0.34]	0.79 [-1.32 – 1.49]
$FEV_1\%$	0.00 [0.00 – 1.32]	0.00 [0.00 – 2.38]	0.00 [-1.01 – 3.66]
PEF	0.63 [-1.43 – 2.24]	-0.46 [-2.37 – 2.34]	-2.64 [-5.04 – 2.87]
$FEF_{25\%}$	-2.61 [-5.51 – 2.84]	-4.55 [-6.73 – 0.73]	2.18 [-3.06 – 6.22]
$FEF_{75\%}$	0.58 [-2.96 – 6.77]	-0.80 [-3.32 – 6.25]	-4.76 [-7.45 – 9.22]
$FEF_{25-75\%}$	-1.20 [-3.41 – 2.58]	-0.57 [-2.82 – 3.49]	0.44 [-3.31 – 6.71]
FET	0.00 [-3.70 – 19.05]	0.00 [-3.70 – 13.16]	13.64 [0.00 – 25.49]

Presented with median and 95% confidence interval of the median

3.1.1 FVC

According to the Wilcoxon signed-rank test (presented in Table 4) a statistically significant decrease from baseline to 4 hours post-exposure was observed in FVC, comparing material Ottersbo (A) and Lactose (C), (z=-2.419, p=0.016), with a medium effect size (r= .36). From baseline and 24 hours following exposure, FVC was statistically significantly lower following exposure to Bjønndalen (B) than Ottersbo (A), (z= -2,008, p=0.045), with a medium effect size (r=.3).

3.1.2 FEV_1

Using the Wilcoxon signed-rank test, a borderline statistically significant decrease was observed comparing Bjønndalen (B) to Lactose (C) from baseline and immediately after exposure, (z=-1.872, p= 0.061), with a medium effect size (r=.28).

3.1.3 $FEF_{25\%}$

Comparing Bjønndalen (B) to Lactose (C), a borderline significant decrease was observed from baseline and 24 hours after exposure, (z=-1.916, p=0 .055), with a medium effect size (r=.28).

3.1.4 Remaining spirometric parameters

No statistically significant changes were observed in any other markers measured, i.e., FEV_1/FVC, PEF, $FEF_{75\%}$, $FEF_{25–75\%}$, and FET.

Table 4: Wilcoxon signed ranks test, changes in spirometric parameters

	Comparing A to B (B-A)		**Comparing A to C (C-A)**		**Comparing B to C (C-B)**	
	Z	Asymp. Sig. (2-tailed)	Z	Asymp. Sig. (2-tailed)	Z	Asymp. Sig. (2-tailed)
T1-T2	**From baseline to immediately after exposure**					
FVC	-1.721[b]	0.085	-0.532[b]	0.594	-1.737[c]	0.082
FEV_1	-1.445[b]	0.148	-0.472[c]	0.637	-1.872[c]	0.061
FEV_1/FVC	-0.411[c]	0.681	-1.426[c]	0.154	-0.811[c]	0.417
PEF	-0.502[c]	0.616	-0.396[b]	0.692	-0.076[b]	0.939
$FEF_{25\%}$	-0.167[c]	0.867	-1.065[c]	0.287	-1.035[c]	0.301
$FEF_{75\%}$	-0.015[b]	0.988	-0.821[c]	0.411	-0.548[c]	0.584
$FEF_{25-75\%}$	-0.289[b]	0.773	-1.232[c]	0.218	-1.491[c]	0.136
FET	-0.081[b]	0.935	-0.228[c]	0.819	-0.685[c]	0.493
T1-T3	**From baseline to 4 hours post-exposure**					
FVC	-0.146[c]	0.884	-2.419[c]	**0.016***	-1.810[c]	0.070
FEV_1	0.000[d]	1.000	-1.445[c]	0.148	-1.121[c]	0.262
FEV_1/FVC	-0.254[b]	0.800	-0.029[b]	0.977	-0.373[b]	0.709
PEF	-0.471[b]	0.638	-0.213[c]	0.831	-0.730[c]	0.465
$FEF_{25\%}$	-0.365[c]	0.715	-0.928[c]	0.354	-0.076[b]	0.939
$FEF_{75\%}$	-0.114[c]	0.909	-0.049[c]	0.961	-0.380[b]	0.704
$FEF_{25-75\%}$	-0.209[c]	0.835	-0.684[c]	0.494	-0.309[c]	0.758

FET	-0.715[b]	0.475	-0.167[c]	0.867	-0.974[c]	0.330
T1-T4	**From baseline to 24 hours post-exposure**					
FVC	-2.008[b]	**0.045***	-0.091[b]	0.927	-1.308[c]	0.191
FEV_1	-1.506[b]	0.132	-0.396[b]	0.692	-1.492[c]	0.136
FEV_1/FVC	-0.263[c]	0.793	-0.164[b]	0.870	-0.510[b]	0.610
PEF	-0.380[b]	0.704	-1.354[b]	0.176	-0.913[b]	0.362
$FEF_{25\%}$	-1.430[b]	0.153	-0.274[c]	0.784	-1.916[c]	0.055
$FEF_{75\%}$	-0.137[b]	0.891	-0.441[b]	0.659	-0.183[b]	0.855
$FEF_{25-75\%}$	-0.152[c]	0.879	-0.715[c]	0.475	-0.714[c]	0.475
FET	-0.167[b]	0.867	-1.628[c]	0.104	-1.719[c]	0.086

a. Wilcoxon Signed Ranks Test **b.** Based on positive ranks. **c.** Based on negative ranks. **d.** The sum of negative ranks equals the sum of positive ranks.

*=statistically significant level p≤0.05

3.1.5 High-low-close plots, median percent change with 95 % confidence intervals, spirometry

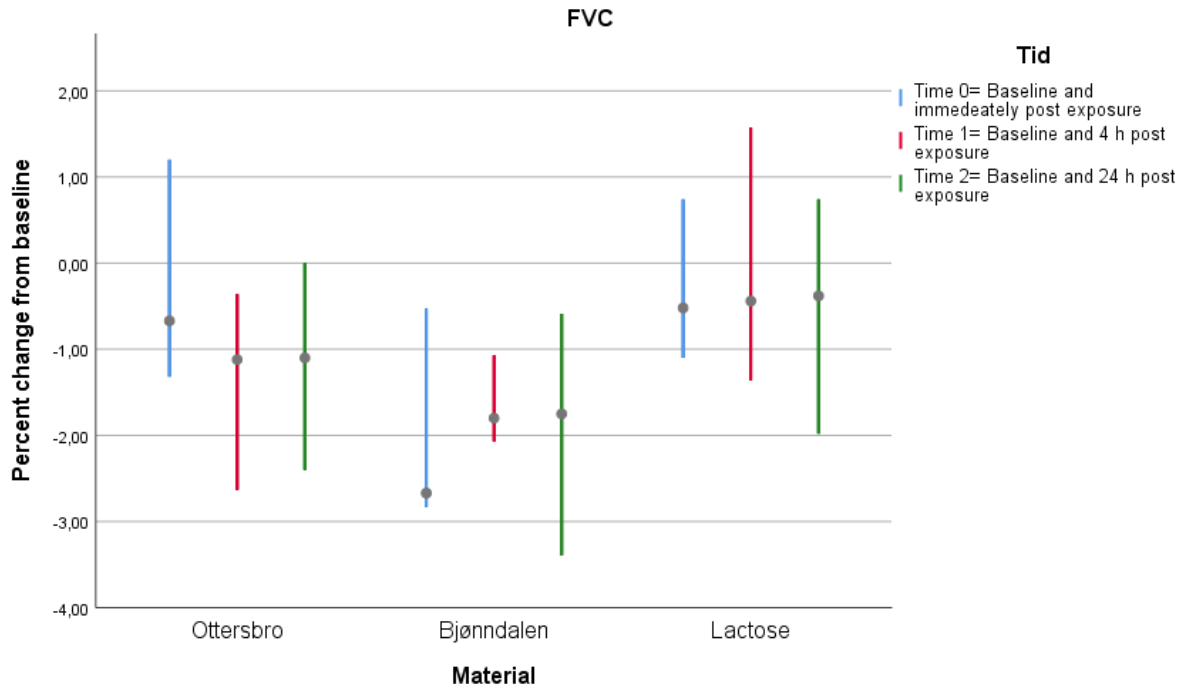


Figure 4 High-low-close plots, median percent change with 95 % confidence intervals, FVC

As shown in figure 6, the largest decrease in FVC from baseline to immediately after exposure was observed for material Bjønndalen (B), reversing somewhat to 4- and 24-hours post-exposure. The decrease at 24 hours post-exposure to Bjønndalen (B) at -1.75 percent was statistically significant compared to Ottersbo (A). For material Ottersbo (A) and Lactose (C), there was a similar minor decrease from baseline to immediately after exposure. For Ottersbo (A), the decrease increased slightly for the measurements after 4 - and 24 hours post-exposure, the decrease of 1.12 percent at 4 hours reaching statistical significance compared to Lactose (C).

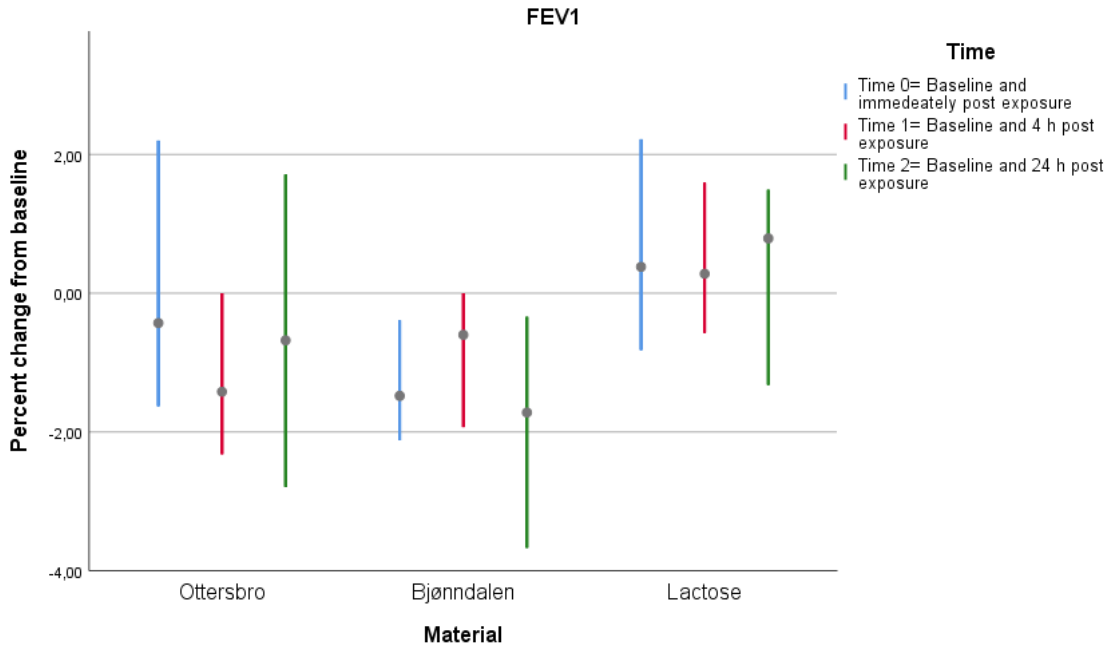


Figure 5 High-low-close plots, median percent change with 95 % confidence intervals, FEV1

The plot in figure 7 showed a decrease in FEV_1 for both material Ottersbo (A) and Bjønndalen (B), while for Lactose (C), a slight increase in measurements at all time points. As for FVC, the decrease in FEV_1 also increases at 4 hours post-exposure from immediately after exposure for Ottersbo (A). The decrease of 1.48 percent immediately after exposure to Bjønndalen (B) reaching a borderline statistical significance compared to Lactose (C).

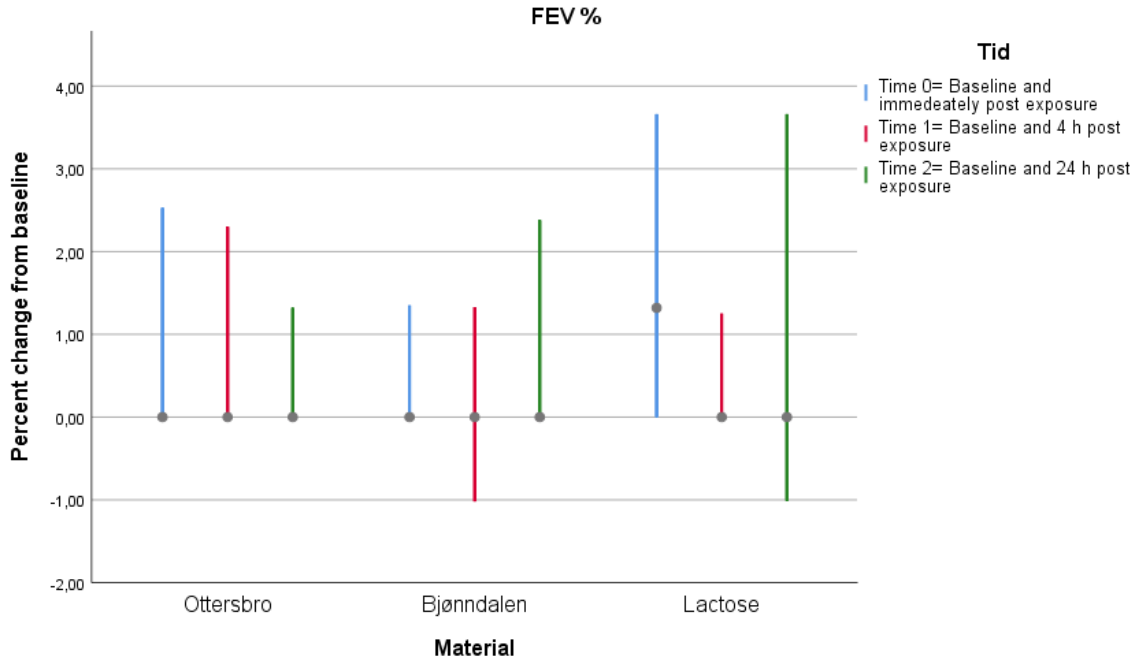


Figure 6 High-low-close plots, median percent change with 95 % confidence intervals, FEV1%

FEV_1 % seems to be unaltered at all time points after exposure for both material Ottersbo (A) and Bjønndalen (B) on any time points, only for material Lactose (C), there was an increase for FEV_1% immediately after exposure (figure 8).

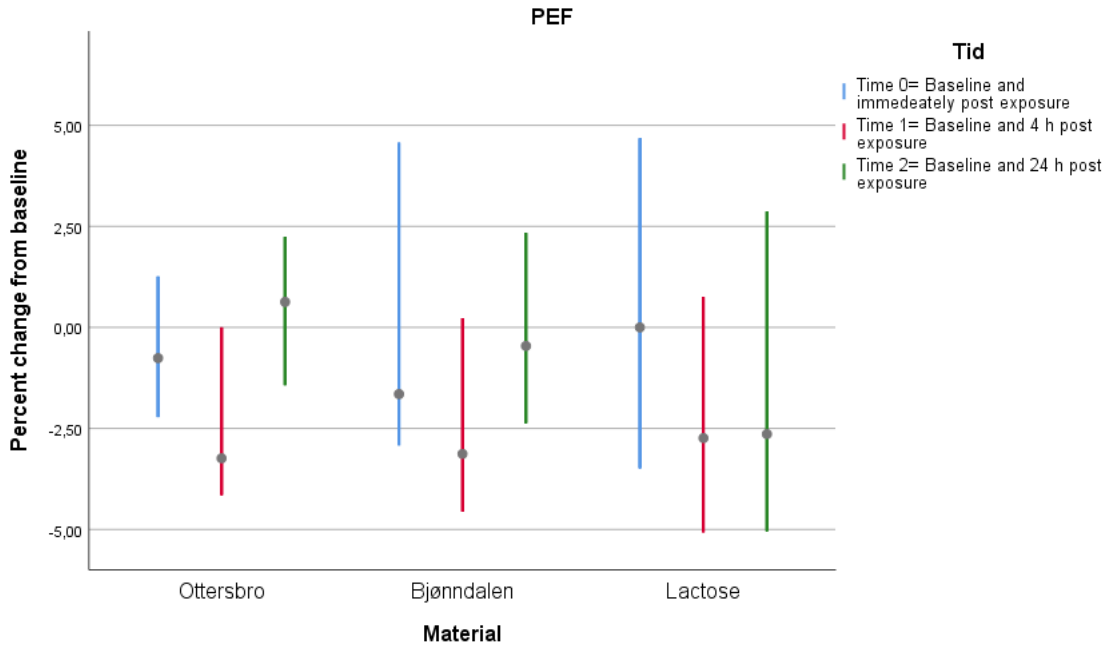


Figure 7 High-low-close plots, median percent change with 95 % confidence intervals, PEF

For material Ottersbo (A) and Bjønndalen (B), a similar increasing decline for PEF from immediately after exposure to 4 hours post-exposure (figure 9), back to nearly baseline levels 24 hours post-exposure for Bjønndalen (B), and above baseline for Otterbro (A). No change

in PEF for Lactose (C) immediately after exposure, decrease on 4- and 24-hours post-exposure.

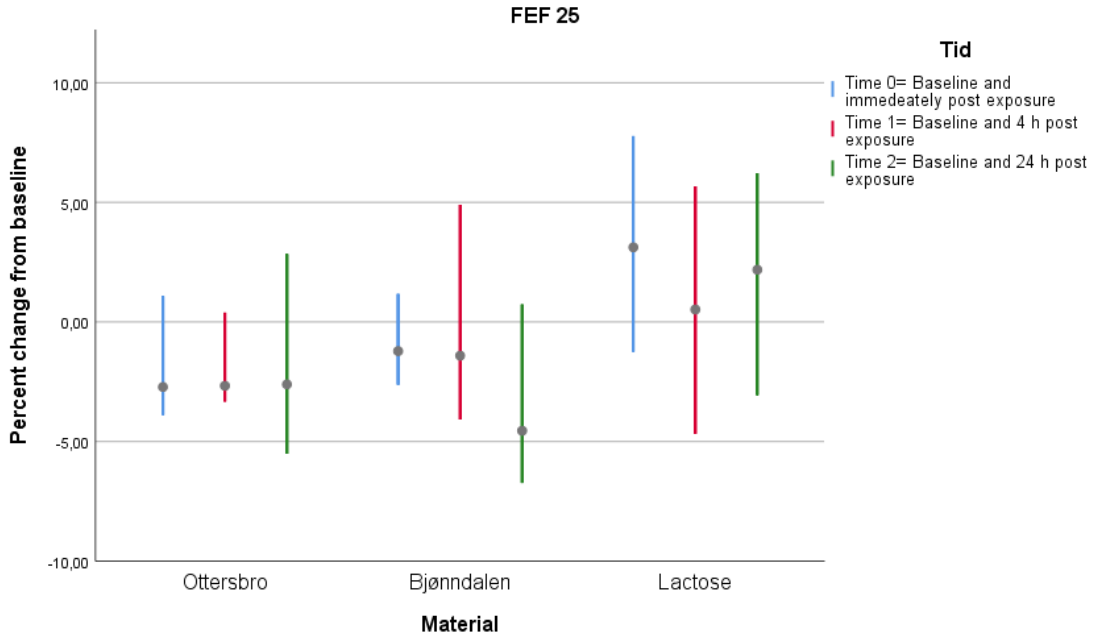


Figure 8 High-low-close plots, median percent change with 95 % confidence intervals, FEF25%

As shown in figure 10, FEF25% decreased immediately after exposure, 4- and 24 hours post-exposure remained unaltered from immediately after exposure for Ottersbo (A). For Bjønndalen (B), an increasing decline on the three measurements post-exposure, after 24 hours the decrease of 4.55 percent which is statistically significant compared to Lactose (C). FEF25% increased immediately after exposure to Lactose (C).

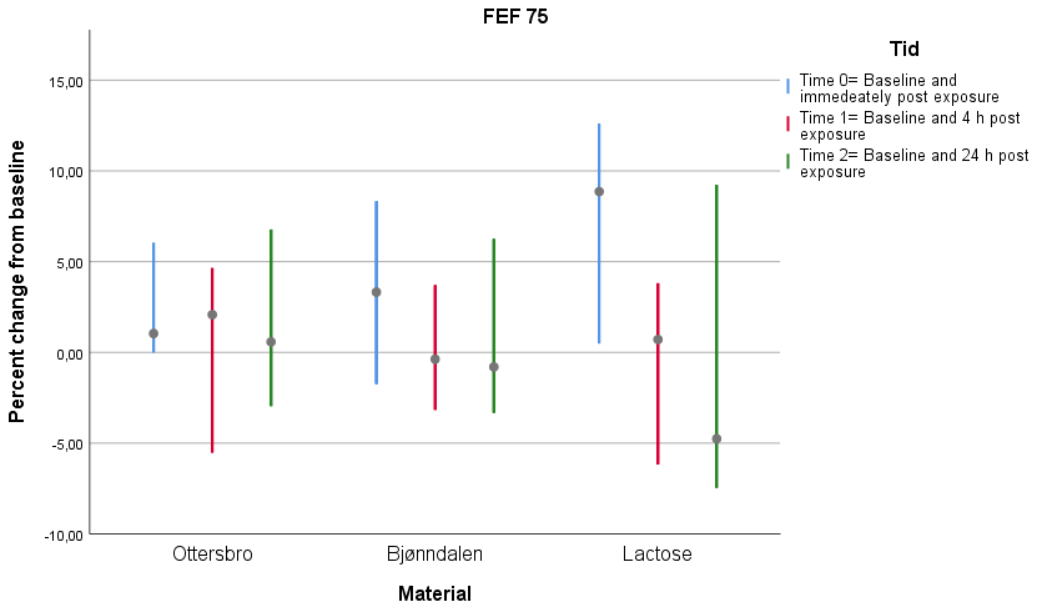


Figure 9 High-low-close plots, median percent change with 95 % confidence intervals, FEF75%

There was a slight increase in $FEF_{75\%}$ after exposure to Ottersbo (A), the largest increase 4 hours post-exposure (figure 11). For Bjønndalen (B), the largest increase occurred immediately after exposure, $FEF_{75\%}$ was back to baseline levels on 4- and 24-hours post-exposure. A larger increase in $FEF_{75\%}$ immediately after exposure to Lactose (C), baseline levels 4 hours post-exposure, and a greater decrease on 24 hours post-exposure.

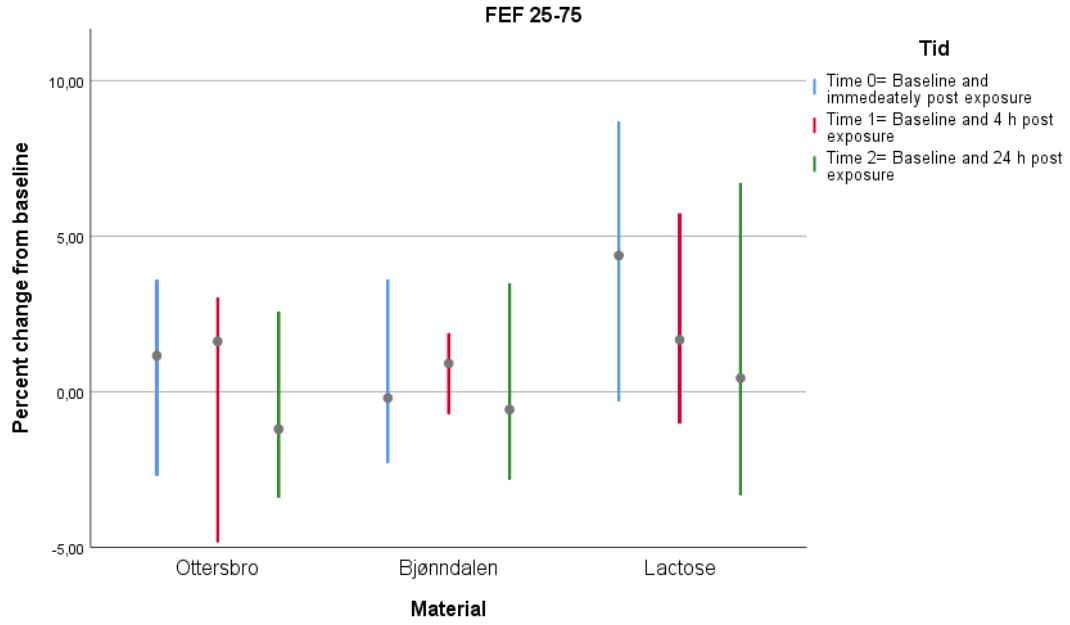


Figure 10 High-low-close plots, median percent change with 95 % confidence intervals, $FEF_{25-75\%}$

Figure 12 showed that $FEF_{25–75\%}$ increased slightly immediately and 4 hours post-exposure to Ottersbo (A), a minor decrease from baseline level to 24 hours post-exposure. For Bjønndalen (B), a slight increase at 4 hours post-exposure, other measurements were corresponding to baseline levels. Immediately after exposure to Lactose (C), a greater increase in $FEF_{25–75\%}$ occurred, decreasing against baseline levels 24 hours post-exposure.

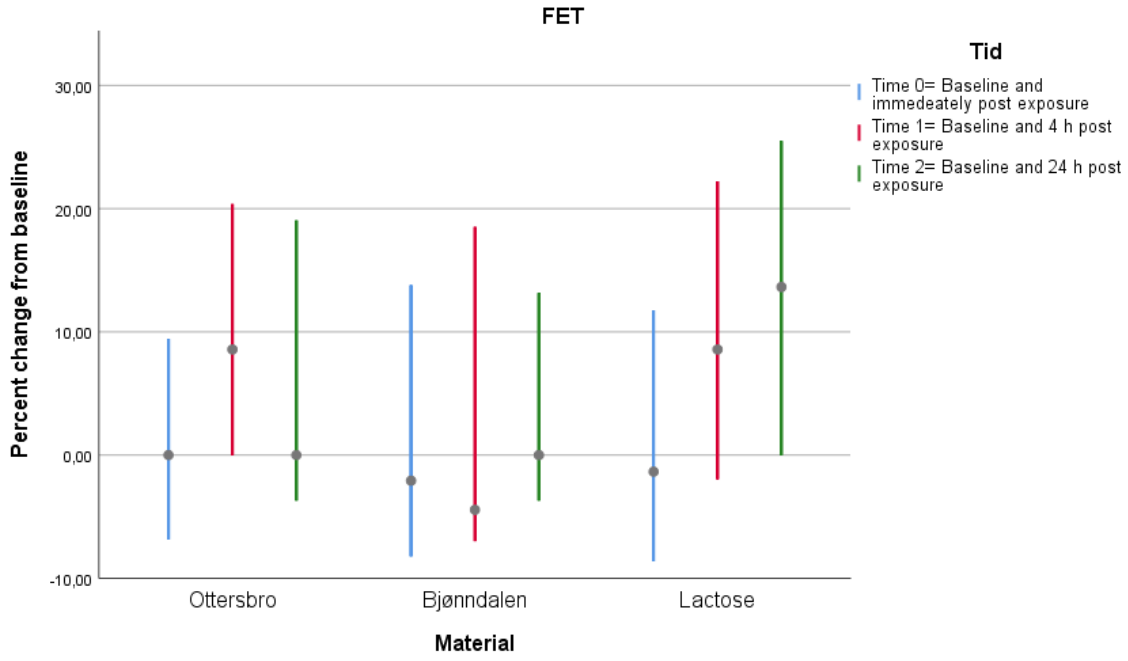


Figure 11 High-low-close plots, median percent change with 95 % confidence intervals, FET

FET increased 4 hours post-exposure for material Ottersbo (A), baseline levels immediately after- and 24 hours post-exposure, as displayed in figure 13. After exposure for material Bjønndalen (B), small decreases immediately after and 4 hours post-exposure, back to baseline levels 24 hours post-exposure. No change immediately after exposure to Lactose (C), increasingly for 4 hours post-exposure, to above 10 percent increase on 24 hours exposure.

3.2 Uteroglobin levels

Table 5 displays the median percent change in uteroglobin levels, with the corresponding 95% confidence intervals.

Table 5: Percent change in uteroglobin levels (from baseline):

4 hours after exposure	**Ottersbo (Quartz diorite) - A**	**Bjønndalen (Rhomb porphyry) - B**	**Lactose (Placebo) - C**
Uteroglobin plasma	-14.52 [-22.98 – 7.23]	-11.29 [-19.57 - -1.83]	-25.20 [-41.08 - -21.78]
24 hours after exposure	**Ottersbo (Quartz diorite) - A**	**Bjønndalen (Rhomb porphyry) - B**	**Lactose (Placebo) - C**
Uteroglobin plasma	-28.30 [-34.78 - -19.71]	-22.38 [-28.57 – 12.68]	-28.20 [-40.06- -18.33]
Uteroglobin urine *	-7.53 [-20.62 – 42.68]	3.64 [-15.28 – 55.85]	0.00 [-26.51 – 71.05]

*Baseline immediately after exposure.

Table 6: Wilcoxon signed ranks test, changes in plasma and urine uteroglobin values:

	Comparing A to B (B-A)		Comparing A to C (C-A)		Comparing B to C (C-B)	
	Z	Asymp. Sig. (2-tailed)	Z	Asymp. Sig. (2-tailed)	Z	Asymp. Sig. (2-tailed)
T1-T3	**From baseline to 4 hours post-exposure**					
Uteroglobin plasma	-0.669[c]	0.503	-2.549[b]	0.011*	-3.003[b]	0.003*
T1-T4	**From baseline to 24 hours post-exposure**					
Uteroglobin plasma	-1.490[c]	0.136	-0.730[b]	0.465	-2.524[b]	0.012*
T2-T4	**From immediately after exposure to 24 hours after exposure**					
Uteroglobin in urine	-0.517[c]	0.605	-0.669[c]	0.503	-1.034[b]	0.301

3.2.1 Uteroglobin levels in plasma

The levels of uteroglobin in plasma decreased from baseline to both 4 hours and 24 hours post-exposure for all the three different exposure settings (presented in Table 6).

Comparing Ottersbo (A) and Lactose (C) the difference in decrease from baseline to 4 hours after exposure reached statistical significance (z= -2.549, p=0.011), to a medium effect size (r=.38). A significant decrease was also observed from baseline and 4 hours after exposure comparing Bjønndalen (B) and Lactose (C) (z= -3.003, p=0.003), with a medium effect size (r=.45).

Further, comparing Bjønndalen (B) to Lactose (C) the difference in decreases from baseline to 24 hours post-exposure reached statistical significance (z= -2.524, p=0.012), with a medium effect size (r=.37).

3.2.2 Uteroglobin in urine

Baseline values for uteroglobin in urine showed great individual variance.

For uteroglobin in urine, no significant difference in change from baseline to 24 hours after exposure was observed between the three exposure-materials.

Spearman`s correlation test indicated a significant positive association between gender and baseline uteroglobin in urine ($r_s(138)=.74$, p=<0.001) and further between gender and plasma levels of uteroglobin ($r_s(138)=.34$, p=<0.001), with higher baseline levels for males compared to females.

3.2.3 High-low-close plots, median percent change with 95 % confidence intervals, uteroglobin

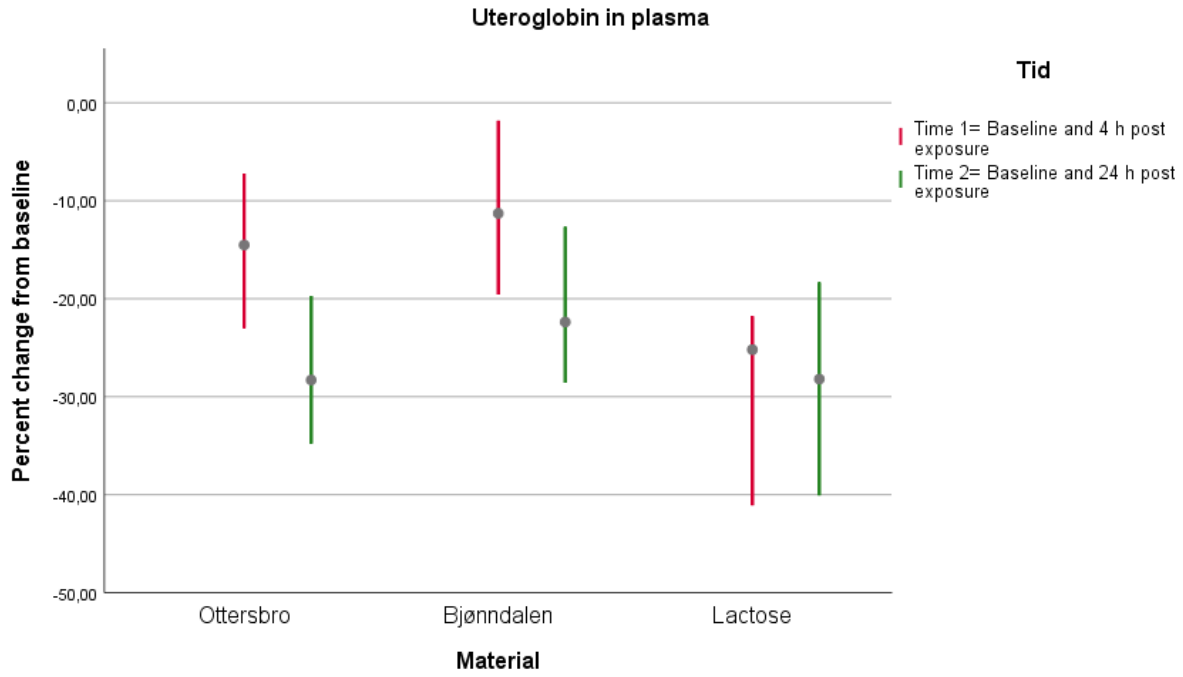


Figure 12 High-low-close plots, median percent change with 95 % confidence intervals, Uteroglobin in plasma

Uteroglobin levels in plasma had the greatest decrease after exposure to Lactose (C), on both 4- and 24-hours post-exposure. The decrease in uteroglobin at 24-hour post-exposure to Ottersbo (A) reached the same level as for Lactose (C) at this time point.

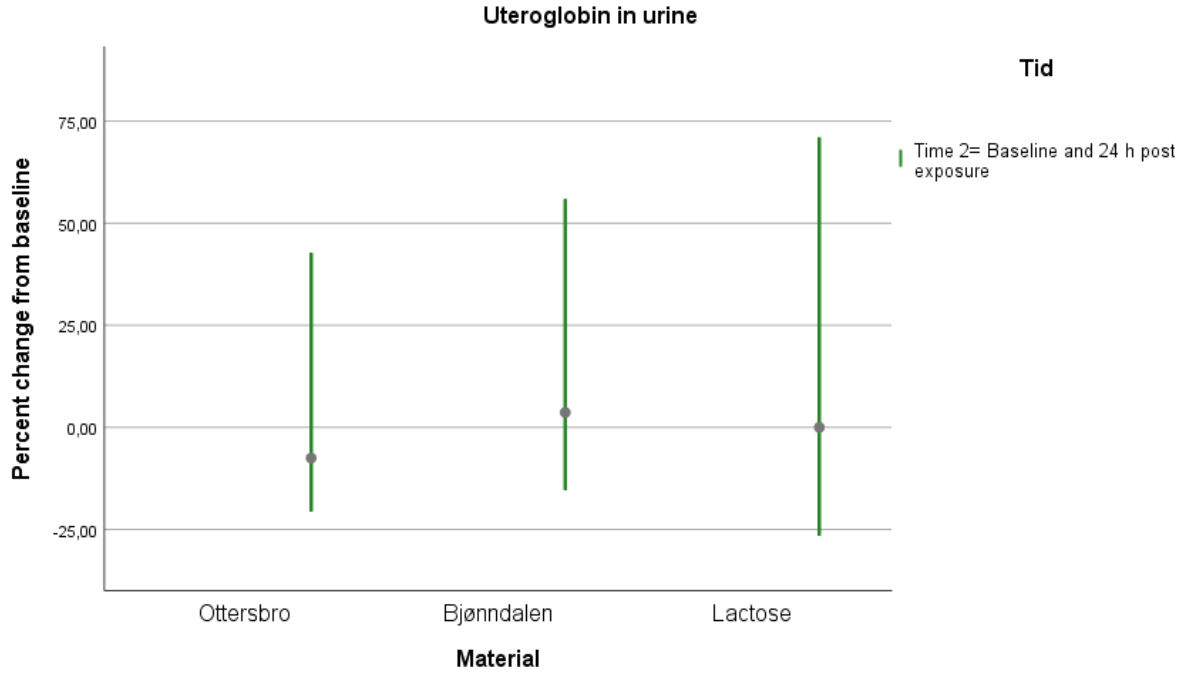


Figure 13 High-low-close plots, median percent change with 95 % confidence intervals, Uteroglobin in urine

For material Ottersbo (A), there was a minimal decrease in uteroglobin levels in urine from baseline to 24 hours post-exposure. After exposure to Bjønndalen (B), uteroglobin levels in urine increased slightly, with no changes after exposure to Lactose (C). For uteroglobin in urine, the baseline test was immediately after exposure.

3.3 Covariation between spirometric values and uteroglobin levels in urine and plasma

A Spearman`s correlation test was done to investigate possible correlations between some spirometry indexes and uteroglobin-levels.

The result showed a significant positive correlation ($r_s(44)=.43$, $p=0.003$) between PEF and uteroglobin level in urine after exposure to Ottersbo (A).

For Bjønndalen (B), there was a significant positive correlation ($r_s(44)=.48$, $p=0.001$) between FVC and uteroglobin in urine. A positive correlation was also observed between FVC and uteroglobin level in plasma ($r_s(44)=.41$, $p=0.005$). A positive correlation was also obtained between PEF and uteroglobin in urine ($r_s(44)=.62$, $p<0.001$).

4. DISCUSSION

The aim of this study was to investigate whether exposure to particulate matter from two different types of stone compared to a placebo dust would affect lung function and levels of uteroglobin in plasma and urine, if there would there be a difference between the two, and to determine whether there was a covariance between spirometry and uteroglobin.

4.1 Discussion of the results

The results indicate few changes of statistical significance for spirometric parameters following exposure.

For Ottersbo (A) it seems several parameters are most affected 4 hours post-exposure. FVC, FEV_1, and PEF decreases with 1.12, 1.42 and 3.24 percent, respectively. A simultaneous increase at 8.67 percent in FET appears. Prolonged FET, and decreased FEV_1 and PEF, may be an expression of airflow limitation (65, 77). However, only the change for FVC reaches the level of statistical significance($p=0.016$), compared to placebo.

FVC decreased 1.80 percent 4 hours following exposure to Bjønndalen, borderline significant ($p=0.070$) compared to lactose. 24 hours post-exposure Bjønndalen (B) FVC remains declined by 1.75 percent from baseline, which compared to Ottersbo is statistically significant ($p=0.045$). Immediately following exposure to Bjønndalen (B) FEV_1 was reduced with 1.48 percent, a borderline statistically significant change from baseline ($p=0.061$) compared to placebo dust. 24 hours post-exposure Bjønndalen (B) $FEF_{25\%}$ decreased 4,55 percent from baseline, borderline statistically significant ($p=0.055$) compared to placebo dust. The value of FEF-rates in clinical use has been assumed to be low (74, 75).

No other measurements of spirometric parameters at any time points showed statistically significant changes.

The high-low plots suggest some trend for Bjønndalen (B) having the largest decrease for both FVC and FEV_1, followed by Ottersbo (A). All changes, however, are considered modest without clinical significance on the individual level. None of the decreases in FVC exceeds the range of variability between two measurements which may be accepted at <5% (66).

Decreases in plasma levels of uteroglobin were shown post-exposure to all three materials. Significant changes at -11.29 and -22.38 percent, at 4- and 24 hours post-exposure to

Bjønndalen compared to placebo dust, respectively. Compared to placebo dust, exposure to Ottersbo was followed by a statistically significant change of -14.52 after 4 hours. Baseline levels showed some individual variations, this may be explained by different numbers of Clara cells (107).

A slight increase of 3.64 percent in urine levels following exposure to Bjønndalen (B) and a slightly larger decrease of 7.53 percent after exposure to Ottersbo (A), were observed. Levels of uteroglobin in urine was unaltered after exposure to placebo dust. Exposure to toxins has been followed by an observed increase in serum levels of uteroglobin (85, 95-97). Our results were inconsistent with these observations.

Bräuner et.al., showed no detectable changes in uteroglobin levels, or spirometric parameters, following exposure to ambient levels of urban air (98). The authors suggest variations in time and dose of exposure as a possible explanation for discrepancies from results in previous studies (98). Heterogeneity in results may be due to differences in study design and small sample sizes. Particulate matter studied may have different compositions and be obtained from different areas. Several studies are conducted over a long period, and over time the composition of particulate matter may have changed (98).

Some correlations of statistical significance were shown between uteroglobin levels and spirometric parameters. After exposure to dust from Bjønndalen (B), there was a significant positive correlation between the changes in FVC and uteroglobin in both plasma and urine. Uteroglobin in plasma and FVC was simultaneously reduced following exposure to dust from Bjønndalen. From immediately post-exposure to 24 hours after exposure, there was a slight increase in FVC and urine levels of uteroglobin. Further, a positive correlation was also obtained between PEF and uteroglobin in urine for both Ottersbo (A) and Bjønndalen (B). However, the measured changes in spirometric parameters and levels of uteroglobin in urine was very small. It remains uncertain whether the correlations found might be due to simultaneous biological responses caused by the exposure to dust from Bjønndalen and Ottersbro.

4.2 Discussion of method and design

Most of the participants had no experience in performing a spirometry test before. The measurement relies a great deal on technique, and possibly it may have been appropriate to train participants in this procedure before the first test day. Not all started on the same

exposure dust. That may reduce the risk of systematic error from lack of training, which may be an issue as spirometry technics might approve the more times participants performed the test.

It was aspired that the same technician performed all tests on the same participant. Due to availability, this was not possible at all measurements.

Any possible diurnal variation in spirometry measures (70) was accounted for in this study, as participants entered the chamber at same time of day on all three exposure sessions. Similarly, spirometry test was also measured at same time of day on all occasions.

The baseline urine test was collected immediately after exposure, contrary to before exposure. The second urine test was collected 24 hours post-exposure, as it corresponded with attendance at the site for scheduled tests the day post-exposure. The original plan was to take a baseline urine sample in the morning at the day of exposure. The participants were supposed to bring a sample of the morning urine to the site. Due to insecurity about whether all the participants would remember this every time it was decided to collect urine samples on-site to ensure it was done on the same time points each time. This also assured control over any possible diurnal variability and preanalytical factors as equal time to centrifuge and freeze of urine samples.

In addition to circadian rhythms, biomarkers may vary for several factors, e.g., genetic characteristics, gender, age, and diet. The statistical methods that were chosen, where every participant serves as their own control, minimize these factors as possible confounders. Furthermore, all study participants were young adults, healthy, non-smokers, without chronic diseases. They were also asked to keep their diet regular on exposure days and the days before.

This study was conducted on healthy volunteers, with exposure for four hours within acceptable limits. Such acute, short-term exposure to young, healthy individuals is considered mild and reversible (108). Some of the measurements showed considerably individual differences with great outliers. Children, elderly, and individuals with preexisting conditions has been assumed to be more susceptible to air pollution (23-29). Still, it is possible some individuals are more susceptible, also in a sample of presumed healthy young people.

The study participants received modest financial compensation for their participation in this study. It could be argued that this would compromise their voluntarily consent. However,

such compensation may be appropriate if amount is considered reasonable for time spent and inconveniences (109).

4.3 Strengths and limitations of the study

This study and its design have several strengths. Chamber exposure allows controlled exposure, unlike studies based on fixed outdoor monitors or personal monitors on participants exposed to air pollution as a mixture of components. Personal monitoring by monitors in the breathing zone could give quite an accurate estimation of exposure level. Changes in the weather, temperature, humidity, geographical/area differences, or other pollutants could not confound the exposure.

The experimental setup was identical in all sessions; hence it was a possibility to compare the effects to the two types of dust, against placebo. As all participants could not be tested at the exact same time there is a hypothetic chance that exposure might differ, even so, measurements showed quite similar exposure rates for the two active dusts. Between the exposure sessions, there was a period of 4 weeks, which reduced the risk of carry-over effects between the exposures.

The study also had some limitations. It was a relatively small sample size, data was victim of great individual variances, and it was not normally distributed. It is likely that in a bigger sample size normal distribution could have been assumed. Small sample sizes could be more susceptible for uncontrolled confounding and it could be difficult to argue that the sample is representative for a population. Nevertheless, studies with smaller sample sizes also contribute to existing and future knowledge (106). The number of participants was decided on an empirical basis, and what would be possible to carry out with regards to resources. It was hypothesized that one would not be able to prove statistically significant changes for all parameters, but some. Wide confidence intervals for some measurements at some time points should thus be expected and demonstrates some of the uncertainty of these results.

There was a problem achieving a high enough concentration of placebo dust, this was considered not an issue as the purpose of the placebo was mainly to make participants believe and feel like they were exposed to dust on the same levels also on this test days, due to the blinding design of the study.

Chamber studies make it possible to study the acute effects of individual air pollutants. However, one fails to reproduce the natural exposure with the mixture of different

contaminants or time variations that are the case in epidemiological studies (110). Meanwhile, causality may be problematic to establish in epidemiological studies (111). It is difficult to determine the exact contribution to human disease and mortality of each specific source of air pollution, as all components have a variable and a relatively minor role (112).

4.4 Comparison with previous studies

There is a discrepancy between the results in this study, indicating Bjønndalen (Rhomb porphyry) might be more potent than Ottersbo (quartz diorite), conversely to studies on cell cultures where quartz diorite showed a higher inflammatory response (100, 102).

There are controversies about whether the instillation as a route of administration (in animal studies) is equivalent to inhalation. Generally, in procedures with instillation considerable higher concentrations than would be inhaled by individuals in a real-world setting are used (112). There are standard models for comparing doses in in vitro and in vivo studies. Even so, several factors to make this conversion for this study are missing, e.g., factors calculating the proportion of the amount of dust inhaled that is deposited (33). Thus, it is difficult to compare doses between studies on cell culture and this chamber study.

Furthermore, there is a complex interaction between a toxin and the biological system, obviously missing in in vitro studies where single cells are removed from the body before exposed to a toxin (33). The actual dose of exposure from a toxin deposited in the lung also depends on the extent of clearance of the substance. Furthermore, whether it stays in the lungs or is translocated to another organ. This will depend on where in the lungs the substance is deposited, and the physical and chemical properties of the substance (33).

The immune system is complicated, with many factors working together. Part of this interaction is missing in in vitro studies. Accordingly, arguments that in vivo studies should be the gold standard for understanding the effect of exposure to particulate matter are made (113). There are also significant individual differences in the immune system, which should also be considered (113).

Previous experimental chamber studies on humans with similar exposure settings were not found in my literature search. A few chamber studies, somewhat similar regarding exposure time and controlling by placebo as the current study, showed divergent results. A chamber study comparing a 2-hour exposure to ambient particles to filtered air showed a small, but statistically significant decrease in both FVC and FEV_1, the time points for measuring

corresponded partly with this current study (before, immediately after, 3 hours- and 22 hours post-exposure) (81). In Sweden, a small sample sized chamber study compared diesel exhaust to filtered air, in 3-hour exposure, FEV1 and FVC were unaltered, as were serum uteroglobin, However, a small but statistically decrease in PEF were shown 75 minutes post exposure to diesel exhaust (82). Uteroglobin levels in both serum and urine, and spirometric parameters remained unaltered after 24 hours of exposure to particulate matter of urban air, in a Danish chamber study (98). These statistically significant changes revealed in these studies, are of low clinical significance. These studies also had relatively small sample-sizes, ranging from 11-29 participants. Indeed, they are only somewhat comparable due to differences in exposure time, components of particles, and one sample consisting of smokers and ex-smokers.

Possibly, exposure at higher levels in an experimental setting would produce more results, which could have been of interest, but certainly unethical. In controlled experiments, it is challenging to choose the right level of exposure to demonstrate possible health effects when clearly, one does not want to exposed participants to unacceptable risk. Further, the inclusion of subjects presumably more susceptible in experimental studies might produce more results. However, it would undoubtedly be ethically problematic.

5. CONCLUSIONS

In our experimental setting, we were able to unveil only minor changes in lung function after short-term exposure to moderate concentrations of mineral dust from the two different types of stone materials used in road pavement that we studied. Some of these changes reached statistical significance. Although the measured post-exposure declines in lung function are expected to be reversible and of miniscule impact for the health on individual level, they might indicate differences in health effects from exposure to different types of dusts, that might be of importance in cases of long-term environmental exposure. Great individual variability in some of the markers of lung function and uteroglobin may indicate that some of the participants are more susceptible to exposure. Our results suggest a possible tendency towards Bjønndalen being more potent compared to Ottersbo and placebo, however due cautions is needed in the interpretations of these results, due to the many limitations, including few subjects, small effects, and high variability.

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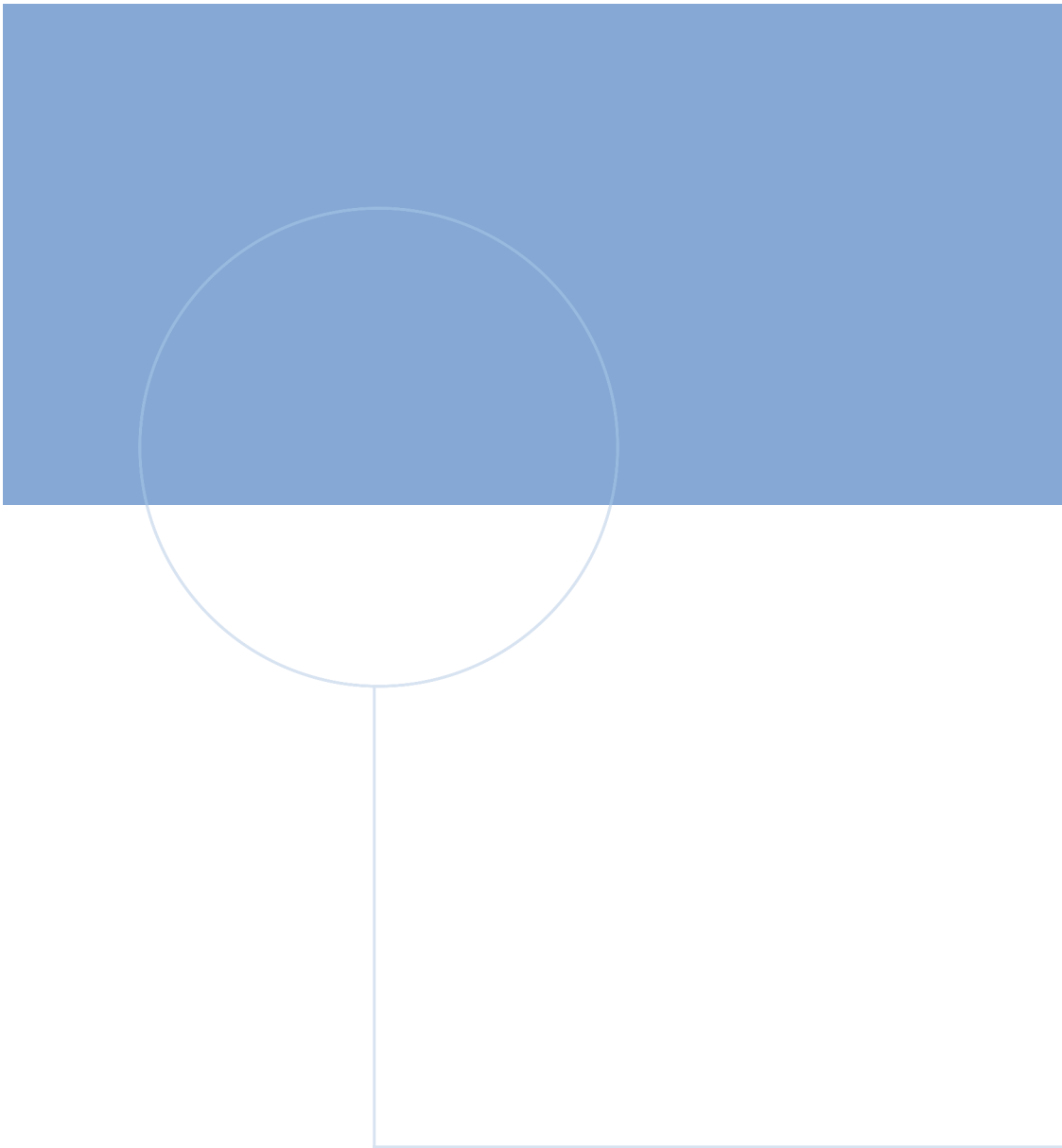

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