Inflammatory effects of exposure to different stone types used in Norwegian asphalt

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ABSTRACT

During the winter in Scandinavian countries, up to 90% of traffic-related particulate matter (PM) is from non-exhaust emissions sources such as asphalt wear. Measures to reduce urban PM have focused mainly on exhaust emissions, while the contribution from asphalt has received less attention. *In vitro* studies suggest that the composition of asphalt can affect the inflammatory potential of road dust. Using a whole-body human exposure chamber, we have explored whether different stone materials used in Norwegian asphalt impose different inflammatory reactions in plasma of healthy volunteers. Our results show no acute increases in the inflammatory markers SP-D, P- selectin, or CC16. However, quartz diorite induced an apparent increase in ICAM-1, not seen for rhomb porphyry or placebo dust (lactose). Although this did not reach statistical significance, it resembles previously observed fibrinogen-effects, and may suggest that different types of stone minerals provoke different inflammatory reactions in humans compared to placebo dust.

INTRODUCTION

An adult human at rest inhales approximately 14,000 L of air every day. If that air contains hazardous chemical pollutants, it can adversely affect human health. Each year, exposure to air pollution, such as particulate matter (PM), causes hundreds of thousands of premature deaths in Europe (European Environment Agency, 2020), and many previous epidemiological studies have shown statistical associations between exposure to PM and an elevated risk of hospital admission and premature mortality
(Chen & Hoek, 2020; Wei et al., 2019).

All PM of 10 µm or less in aerodynamic diameter (PM_{10}) can be inhaled and deposited in humans' respiratory tracts. The location in which the particles are deposited in the airway, along with the chemical composition of particles inhaled, determines the health effects of the exposure (Elvidge, Matthews, Gregory, & Hoogendoorn, 2013; Pöschl, 2005).

Emissions from exhaust and non-exhaust sources, such as tire wear, brake wear, and road surface abrasion, contribute approximately equally to traffic related PM_{10} emissions. Implementation of numerous regulations and vehicle-control mechanisms, such as parking fees and low emission zones, have significantly reduced traffic exhaust emissions (Sousa Santos et al., 2020). The non-exhaust emissions, which are affected by the number of breaking events, road surface characteristics, and characteristics of the vehicle, such as weight, type of tires, and engine power (European Commission, 2014), remain a challenge. Due to the use of studded tires during the winter in Scandinavian countries, up to 90% of PM_{10} measured in urban areas may come from stone particles found in asphalt (Johansson, Norman, & Gidhagen, 2007; Kupiainen et al., 2005). Incentives for reduced carbon emissions have increased the number of electric vehicles used in Norway significantly in the last few years (Bjerkan, Nørbech, & Nordtømme, 2016). However, an average electrical vehicle is 24% heavier than an average diesel or gasoline vehicle, leading to increased wear of asphalt, tires, and brake pads, consequently increasing concentration of PM from non-exhaust emission sources (Timmers & Achten, 2016).

Inflammation is a complex immunological reaction following various pathways (Prasad, Tyagi, & Aggarwal, 2016). It can be measured using various biological markers found in urine, blood, exhaled breath and bronchoalveolar lavage (BAL) fluid.

The lung's alveolar surface is covered with a pulmonary surfactant consisting of specific proteins, all with different functions (Hermans & Bernard, 1999). One essential specific protein found in the pulmonary surfactant is Clara Cell protein (CC16). CC16 is an anti-inflammatory protein secreted by Clara cells, which are mainly located in the lung's respiratory bronchioles (Lakind et al., 2007; Singh et al., 1988). Another protein secreted by the airways is Surfactant Protein D (SP-D). SP-D has an essential role in the

immune system. During inflammation, the SP-D concentration decreases in the lungs and increases in the circulatory system (Tajima et al., 2020). Platelet-Selectin (P-selectin), a lectin-like molecule expressed by endothelial cells, plays an important role in mediating inflammation by promoting adherence of leukocytes to activated platelets and endothelium (Hayashi et al., 2000). Elevated P-Selectin concentrations have been detected in the plasma of animals and patients with inflammatory disease (Gearing & Newman, 1993; Krieglstein & Granger, 2001). Another inflammation-related protein is Intercellular adhesion molecule-1 (ICAM-1), which plays a critical role in the local accumulation of inflammatory cells (Hua, 2013).

The mineralogy of asphalt depends on which stone aggregate is being used. In some previous *in vitro* studies, different inflammatory potentials have been documented during exposure to stone aggregates of various mineral compositions. One of the main findings was that feldspar-rich stone materials showed little pro-inflammatory potential (Becher et al., 2001; Hetland et al., 2000; Øvrevik et al., 2005), and the choice of stone types may significantly affect human health. In a recent study from Norway, the interactions of six different stone types, namely, quartzite, anorthosite, rhomb porphyry, dacite, quartz diorite, and hornfels, with HBEC3-KT cells and THP-1 macrophages were investigated. Quartzite, rich in quartz mineral and with smaller amounts of muscovite and anorthite, was the most cytotoxic of the particle samples in both cell types and was also the most potent in HBEC3-KT cells with regards to the pro-inflammatory response. Anorthosite and hornfels, which consist mainly of the minerals K-feldspar, plagioclase, and quartz, were most potent in THP-1 cells (Grytting et al., 2021). *In vitro* studies have provided information on mechanisms of response, but we cannot be confident that the magnitude of responses and exposure concentrations applied are representative of, or relevant to, human health effects (Bernstein et al., 2004).

The objective of this chamber study was to investigate the inflammatory potentials in humans following short-term controlled exposure to dust from the stone types quartz diorite and rhomb porphyry with different mineral composition. The present study focuses on cardiovascular, systemic effects.

METHODS

In this study, healthy volunteers were exposed in a double-blind manner to three different types of dust: quartz diorite, rhomb porphyry, and lactose, with the latter serving as a negative control.

Study subjects

Twenty-four healthy volunteers were recruited to participate in this study: however, one person withdrew from the study after the first exposure session. In groups of four, the remaining 23 subjects (10 males, and 13 females) were exposed to the two stone types and lactose in three separate exposure sessions, each lasting four hours. Only healthy people without a known chronic inflammatory illness or any other respiratory disease were accepted as participants in this study. Additionally, prior to each exposure sessions, the subjects were asked about their general health using a standardized questionnaire.

Exposure occurred two days a week. Each exposure session started at 9 AM, with the subjects entering the exposure chamber at 30-minute intervals. Thus, the last person left the chamber at 2:30 PM. To avoid hang-over effects, at least three weeks separated the exposure sessions. To maintain the anonymity of the subjects, they were given unique identification numbers (IDs) that were used for data storage purposes.

Blood and urine samples

Blood samples were collected from the subjects approximately 30 minutes prior to exposure (baseline readings) and 4 h and 24 h after exposure. Urine samples were collected immediately following exposure (baseline readings) and 24 h after exposure. The blood samples were collected in two different clinics: one was located in a separate room next to the exposure chamber and was where baseline blood and urine samples were collected, and one was situated at St. Olavs Hospital, approximately a 20-min walking distance from the exposure chamber, and used for the collection of the post-exposure samples (4 h and 24 h after exposure).

The blood samples were collected in BD vacutainer K2EDTA tubes. After sampling, the samples were immediately centrifugated at 2000 x g for 15 min at 4 °C, then pipetted in 0.5-1 ml aliquots into ten Eppendorf tubes. The urine samples were centrifugated at 2500 x g for 15 min at 4 °C and pipetted in 1 ml aliquots into five Eppendorf tubes. Following centrifugation, plasma and urine samples were stored at - 20 °C. At the end of each exposure day, all samples were transported to the Clinical Research Facility at St. Olav's Hospital at Trondheim University Hospital and stored at -80 °C until transporting to Norwegian Institute of Public Health for analysis. CC16 in plasma and urine was analyzed using DuoSet® ELISA (RnD Systems, Inc., Biotechne, USA), Nunc Maxisorb plates (Thermo Scientific, USA) and Sunrise absorbance microplate reader with Magellan software ver. 4.00. (Tecan Trading AG, Switzerland). SP-D, P-Selectin, and ICAM-1 were analyzed using Luminex® Assays (RnD Systems, Inc., Biotechne, USA) and Bio-plex 200 instrument with Bio-Plex Manager software ver. 6.2. (Bio-Rad, USA).

Exposure chamber and dust generation

The exposure chamber was 11.8 m^2 in size, with a total air volume of 35 m^3. The air change rate was adjusted for each of the exposure materials so that the PM

concentrations were approximately the same across exposure days and as close to the 8-h occupational exposure limits for total dust (10 mg/m^3) and respirable dust (5 mg/m^3) as possible.
The exposure chamber had mixed ventilation, i.e., the air was supplied to the room through one air terminal located on the ceiling and extracted through two extraction diffusers, in the ceiling. The air supply was filtered through a HEPA filter before being supplied to the exposure chamber. Using TSI DustTrak 8533, the concentrations of PM_1, $PM_{2.5}$, respirable dust, PM_{10}, and total dust were monitored for two hours before each exposure session started. The PM concentrations measured were less than 0.01 mg/m^3, which confirmed that the air was adequately filtered and that the exposure chamber was cleaned sufficiently between exposure sessions.
During exposure, the subjects were seated at a table located in the middle of the exposure chamber. For moderate exercise, the subjects were required to use a step-board for 15 minutes each hour. Each hour they also had to rotate one seat around the table to reduce the variation in exposure concentration due to location within the room.
The dust was dispersed to the room through a flexible silicone tube connected to an aerosol dust generator (TSI 3410 L during exposure to the two stone aggregates, and TSI 3400 during exposure to lactose). The silicone tube was connected through a hole, located approximately 1.3 m above floor level, on one of the four walls of the chamber.
During exposure, the concentrations of various PM fractions were measured every minute using a TSI DustTrak 8533. In addition, both stationary and personal (respirable fraction) gravimetric samples were taken. Lactose powder easily agglomerates, as it contains more moisture than quartz diorite and rhomb porphyry. Therefore, the exposure chamber looked equally dusty during the lactose exposure days as during exposure to rhomb porphyry and quartz diorite. However, the achieved concentrations were low during lactose exposure, with median concentrations of total dust and respirable dust of 5.7 mg/m^3 and 0.3 mg/m^3, respectively. During exposure to quartz diorite and rhomb porphyry, however, higher concentrations were achieved, with median concentrations of total and respirable dust of 20.8 mg/m^3 and 5.4 mg/m^3, respectively, for quartz diorite and 22.4 mg/m^3 and 5.7 mg/m^3, respectively, for rhomb porphyry.

Quartz diorite and rhomb porphyry were chosen because these stone types are used in the asphalt of the largest, most trafficked cities in Norway. These stone types also have similar size distributions and induced pro-inflammatory responses in human bronchial epithelial cells (BEAS-2B) and THP-1 macrophages, with significantly higher responses (CXCL8 and TNF-α) for quartz diorite in THP-1 macrophages (Grytting et al., 2021). Lactose is an inert powder and should not trigger any inflammatory reactions. Thus, it was used for the control exposure in this study. Although filtered air has been used in most previous controlled exposure studies, lactose powder was used in our study so that the exposed subjects and the researchers would not know what type of exposure was being used.
The mineral compositions of quartz diorite and rhomb porphyry were measured using X-ray diffraction (XRD) analyses, and the results are shown in Figure 1. The quartz diorite mainly consisted of plagioclase (32%), and quartz (27.5%), while the rhomb porphyry consisted mostly of plagioclase (47%) and K-feldspar (31%).

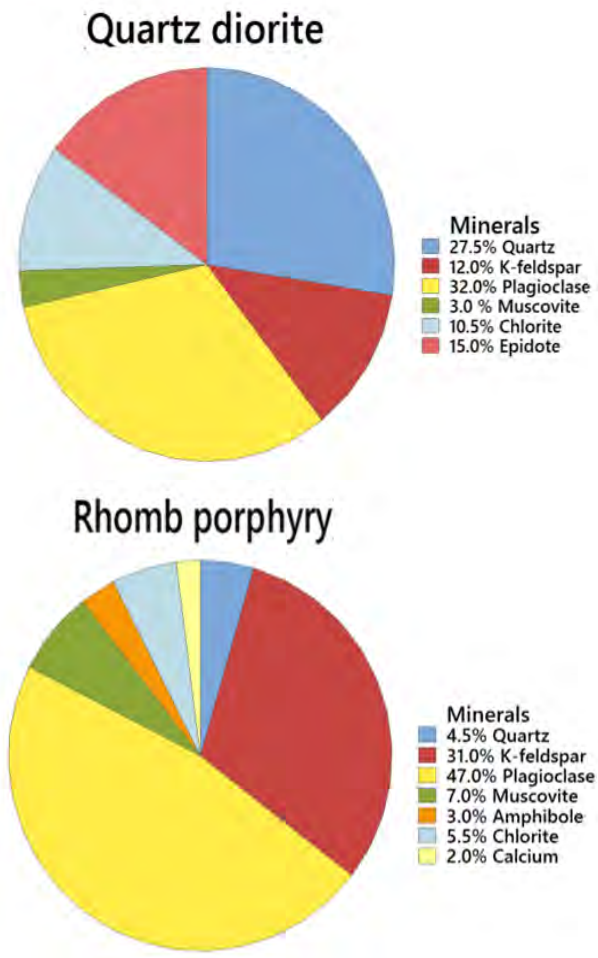

Figure 1: The mineral compositions of quartz diorite and rhomb porphyry

Statistical analysis

In this study, each individual was used as his/her own control, and the differences in reactions over time were the outcomes of interest. With the exception of CC16 in urine, which had normalized residuals after its log transformation, the remaining variables were positively skewed, and analyses were performed using the non-parametric Wilcoxon signed-rank test. In this test, the absolute change from baseline was calculated for each subject, and the absolute changes observed for each material were paired two and two. Statistical significance refers to $p<0.05$ (two-tailed). For the log transformed CC16, linear mixed effect models were used. For descriptive statistics, the percent change from baseline was calculated. Due to the non-normality of the remaining variables, the median values and median 95% confidence intervals (CIs) were used for graphical purposes. Spearman's correlation was used to analyze non-parametric bivariate correlations.

Before the study started, a power calculation was performed, with a two-sided 5% significance level, based on different mean values for 24 subjects. A mean change of at least 1.25, with a standard deviation of 1/3 of this value, was assumed to be sufficient for achieving statistically significant differences for at least some of the parameters included.

The study was approved by the Regional Ethics Committee (REK) with approval no.: 260381. All participants provided written informed consent before inclusion and were also given detailed oral information concerning the study.

RESULTS

The median percent change from the baseline score and 4 h after exposure and between the baseline score and 24 h after exposure for the inflammatory markers measured in plasma (CC16, ICAM-1, SP-D, and P- Selectin) and urine (CC16) are shown in Figures 2 and 3.

As shown in Figure 2, the median percent change in ICAM-1, i.e., the change from baseline and 4 h after exposure, was greatest post-exposure to quartz diorite (+3.75%). According to the Wilcoxon signed-rank test, however, the change observed in ICAM-1 at 4 h did not reach statistical significance for either material. However, from baseline and 24 h post exposure, quartz diorite and lactose caused a statistically significant reduction in plasma ICAM-1 levels when compared to the absolute change observed following exposure to rhomb porphyry.

For SP-D, a median decrease from baseline was observed for all three exposure materials (Figure 2). According to the Wilcoxon signed-rank test, no statistically significant differences among the three materials were observed.

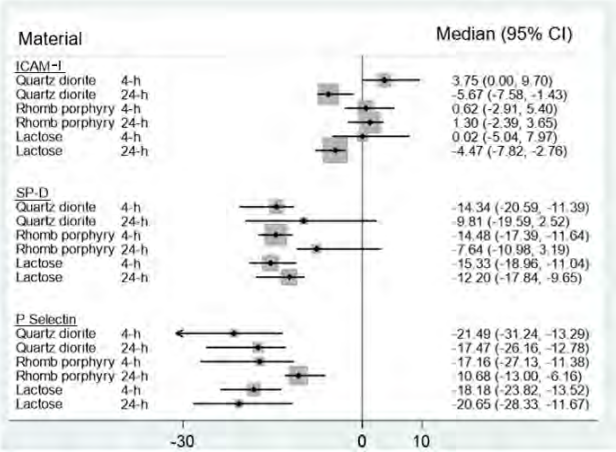

Figure 2: Median percent changes from baseline for plasma concentrations of ICAM-1, SP-D, and P-Selectin. 4 h is the percent change from 4 h post-exposure and baseline. 24 h is the percent change from 24 h post-exposure and baseline.

Although the median concentration observed for P- Selectin decreased post-exposure to all exposure materials (Figure 2), the Wilcoxon signed-rank test

indicated that the absolute change observed between 24 h post-exposure and baseline was significantly different comparing rhomb porphyry and quartz diorite ($z=1.95$, $p=0.05$), and rhomb porphyry and lactose ($z=2.59$, $p=0.01$).

The median plasma concentration of CC16 decreased from baseline after exposure to all three materials (Figure 3). According to the Wilcoxon signed-rank test for the plasma concentration of CC16, the absolute differences observed between 4 h post-exposure and baseline when comparing quartz diorite and lactose reached statistical significance ($z=2.55$, $p=0.01$). A statistically significant difference was also observed when comparing rhomb porphyry and lactose ($z=3.00$, $p=0.00$). No statistically significant differences from baseline to post-exposure were observed for quartz diorite and rhomb porphyry. At 24 h post exposure no statistical differences were observed between the materials. Furthermore, no apparent effects were observed in urine levels of CC16 at 24 h (Figure 3).

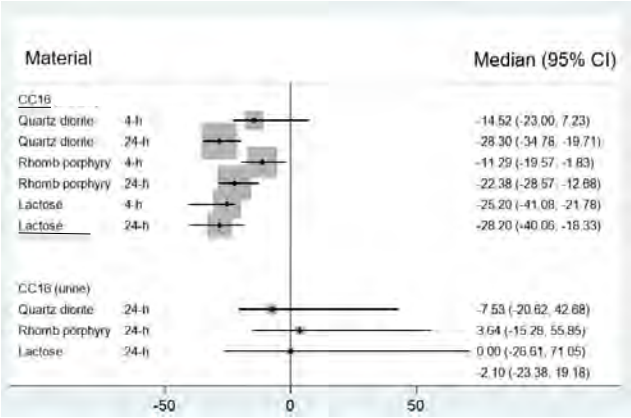

Figure 3: Median percent changes from baseline for plasma and urine concentrations of CC16. 4 h is the percent change from 4 h post-exposure and baseline. 24 h is the percent change from 24 h post-exposure and baseline.

Notably, in eight subjects, quartz diorite caused an increase in CC16 measured in plasma 4 h post-exposure. Similarly, rhomb porphyry exposure increased the plasma levels of CC16 in six subjects 4 h post-exposure. By contrast, an increase in plasma CC16 was only observed for one subject post-exposure to lactose (Figure 4).

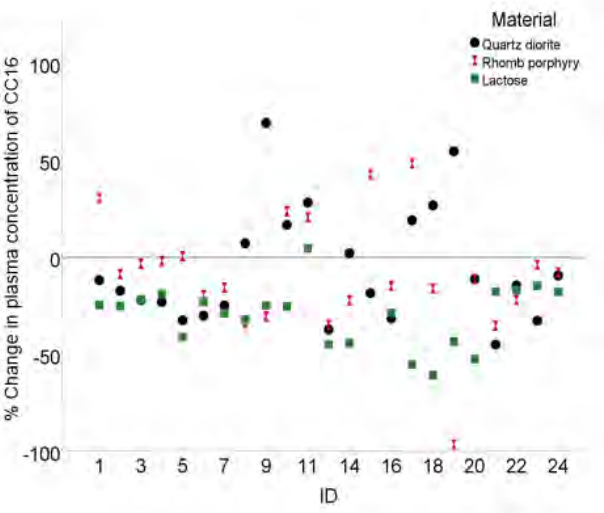

Figure 4: The individual % changes (between 4 h after exposure and baseline) in plasma concentrations of CC16

DISCUSSION

Outdoor air pollution, such as PM, can enter the indoor environment by infiltrating the building envelope and mechanical or natural ventilation system (Leung, 2015). The ratio between the indoor and outdoor environment is challenging to determine, considering it depends on many factors (Chen & Zhao, 2011). A previous study found that 75% of the indoor air pollution variation could be explained by the concentrations measured outdoors (Cyrys, Pitz, Bischof, Wichmann, & Heinrich, 2004). When discussing health effects caused by indoor air pollution sources, outdoor air pollution is also essential for assessing the total risk associated with exposure.

The adverse health effects following short-term and long-term exposure to air pollution is well established; however, the underlying mechanisms are not yet fully understood, making it difficult to determine exactly which biomarkers should be measured to characterize the health effects caused by exposure to PM.

In this study, the plasma concentrations of CC16, SP-D, ICAM-1, and P-Selectin, plus the urine concentration of CC16, were measured. To the best of our knowledge, this is the first study in which the inflammation potential has been investigated in humans after controlled exposure to such different types of stone aggregates used in asphalt. The PM concentrations used in this study were set in such a manner as to not exceed the 8-h occupational exposure limit values for total dust (10 mg/m^3) and respirable dust (5 mg/m^3). However, the exposures used in this study largely exceed the PM concentrations in Norwegian cities, in which the ambient air quality criteria is 30 µg/m^3 of PM_{10} a day.

Several underlying factors, such as gender, age, body mass index (BMI), ethnicity, and exercise, affect the levels of inflammatory markers found in blood and urine, and many of these factors are not yet fully understood. In our study, however, these factors were controlled for, as each subject was used as their own control.

For the CC16 concentration measured in plasma, a median decrease from baseline was observed following exposure to all exposure materials. However, 4 h after exposure to quartz diorite and rhomb porphyry, the CC16 concentration measured in plasma increased for eight and six subjects, respectively, while an increase was observed for just one subject following exposure to lactose. The differences observed in absolute change from baseline reached statistical significance when comparing quartz diorite and lactose and rhomb porphyry and lactose. However, no significant difference was observed between quartz diorite and rhomb porphyry.

The increase observed in plasma concentration of CC16 suggests that for some of the healthy subjects exposed, the inflammatory response was more pronounced following exposure to the two stone minerals compared to what was observed following exposure to lactose.

As a biomarker for acute and chronic pulmonary damage, CC16 has received some criticism, especially when used as a single biomarker of effect and when used in uncontrolled epidemiology or survey studies. This criticism is due to its lack of specificity in specific exposures (Lakind et al., 2007). In several previous controlled exposure studies, when used as a marker of inflammation in healthy subjects, CC16 has provided ambivalent results. In some studies, no significant or

consistent associations were found between controlled exposure to particle-rich air or wood smoke and CC16 measured in plasma and urine (Bräuner et al., 2009; Muala et al., 2015; Zuurbier et al., 2011). In one study, however, in which ICAM-1, SP-D, and CC16 were among the measured biomarkers, after 18 healthy individuals had been exposed to diesel exhaust and filtered air, the concentration of CC16 increased slightly, but not statistically significantly, immediately post-exposure to diesel exhaust (Xu et al., 2013).

In our study, reductions were observed for SP-D and P-Selectin, post-exposure to all exposure materials. In a previous Norwegian study, where exposure to PM was studied for professional ski waxers, the plasma concentrations of CC16 and SP-D were significantly lower following both exposure and non-exposure; however, the concentrations where more significantly reduced following exposure. It was speculated that the permeability of the alveolar-endothelial barrier was not compromised by exposure to ski wax aerosols and that the PM had an effect on pulmonary cells secreting these pneumoproteins (Freberg et al., 2016). In our study, no statistical difference was observed in SP-D concentration from baseline to post-exposure to the three exposure materials. Hence, the physiological relevance of this response remains unclear.

In previous studies, ICAM-1 has been found to both increase and decrease following exposure to diesel exhaust particles and filtered air (Takizawa et al., 2000; Xu et al., 2013). In our study, a median percent increase was observed 4 h post exposure to quartz diorite; however, this increase was not statistically significant compared to the other two materials. Notably, we have recently reported that exposure to quartz diorite, but not rhomb porphyry or lactose, increased plasma fibrinogen levels in the same experiments (Nitter, Hilt, Svendsen, Buhagen, & Jørgensen, 2021). Fibrinogen is known to induce ICAM-1 expression in the vascular endothelium (Harley, Sturge, & Powell, 2000). Thus, it is tempting to speculate that these quartz diorite-associated effects could be connected. However, while the ICAM- 1 increase was observed 4 h post-exposure, the effect on fibrinogen was observed after 24 h.

In the study conducted by (Grytting et al., 2021), where the same stone aggregates were used to study acute pro-inflammatory responses in human bronchial epithelial cells (BEAS-2B) and THP-1 macrophages, quartz diorite was found to be significantly more potent than rhomb porphyry in THP-macrophages, but not in BEAS-2B cells, suggesting a potential for airway effects due to mineral exposure. The results of the present study suggest that stone aggregates also may increase the levels of selected pro-inflammatory markers in plasma. Furthermore, the responses to quartz diorite in particular, seemed greater compared to those following exposure to lactose powder.

CONCLUSIONS

No acute increases were observed for SP-D and P-selectin concentrations in the blood of healthy individuals. Following exposure to the two stone aggregates, the CC16 concentrations measured in the plasma of some subjects increased, which could indicate that some subjects reacted more strongly to the stone minerals than the lactose powder. The non-significant increase observed in the ICAM-1 concentration 4 h post-exposure to quartz diorite, is resembling earlier observations on plasma fibrinogen levels, and is further suggesting that the stone aggregates may be more potent than the lactose powder. Although the results indicate that the mineral composition of inhaled stone dusts could affect blood levels of induced inflammatory markers in humans, the observed responses were relatively low and deviated from the effects of placebo dusts only for selected endpoints. Thus, the mechanisms and potential relevance of these systemic effects induced by stone PM remains to be clarified.

LIMITATIONS AND FURTHER STUDIES

Considering that some healthy subjects might be more vulnerable to certain chemicals or chemical compositions, one might question whether 23 subjects is sufficient when the responses caused by short-term exposure are as low as those observed in the present study. Another limitation is the number of exposure sessions, as each subject was only exposed to each material once. Furthermore, the lactose concentration should be more similar to the concentration of the two stone types. Regardless, we still believe that our results provide valuable information concerning the potential health effects of non-exhaust emissions. Hopefully, exposure to non-exhaust emission sources will be given more attention in future research.

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