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Effects of low-intensity and high-intensity cycling with diesel exhaust exposure on soluble P-selectin, E-selectin, I-CAM-1, VCAM-1 and complete blood count

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ABSTRACT

Background Exposure to particulate matter 2.5 µm or less ($PM_{2.5}$) that contains transition metals may play a role in systemic oxidative stress and inflammation. Exposure to diesel exhaust (DE) can increase adhesion molecules, which are important in the inflammatory response; however, it is unclear how exercising in DE affects adhesion molecules and how exercise intensity modulates this response.

Aim To determine how DE exposure during exercise of varying intensities affects adhesion molecules and markers of systemic inflammation.

Methods Eighteen males performed 30 min cycling bouts at low intensity and high intensity (30% and 60% of power at VO_{2peak} (peak oxygen consumption) and a control condition (rest)). Each trial was performed once breathing filtered air (FA) and once breathing DE (300 µg/m^3 of $PM_{2.5}$, six trials in total). Prior to, immediately post, 1 and 2 hours post exposure, blood was drawn to measure parameters of a complete blood count and soluble (s) platelet-Selectin, endothelin-Selectin, intracellular cell adhesion molecule (sICAM)-1 and vascular cell adhesion molecule (sVCAM)-1. Data were analysed using repeated-measures analysis of variance.

Results Two hours following high-intensity exercise, sICAM-1 was significantly less in DE compared with FA (p=0.008). Immediately following rest (p=0.013) and high-intensity exercise (p=0.042) in DE, sICAM-1 was significantly greater than immediately following low-intensity exercise in DE. There were no significant differences in other markers between DE and FA.

Conclusions Based on this study, healthy individuals may not experience an acute increase in adhesion molecules and systemic inflammatory markers from exercising in DE compared with FA, and higher exercise intensities do not appear to increase the likelihood that DE will affect adhesion molecules and systemic inflammatory markers.

What are the new findings?

- Exercising in diesel exhaust (DE) did not increase adhesion molecules.
- High-intensity exercise in DE lowered soluble intracellular cell adhesion molecule-1 and did not affect soluble vascular cell adhesion molecule-1, soluble platelet-Selectin, soluble endothelial-Selectin, lymphocytes, neutrophils, monocytes and eosinophils.
- Higher intensity exercise did potentiate any effects of DE.
- Healthy individuals do not appear to experience an acute increase in adhesion molecules and systemic inflammatory markers from exercising in DE.

INTRODUCTION

Exposure to particulate matter (PM) leads to cardiovascular events such as myocardial infarction[1] and increases the risk of cardiovascular mortality.[2] One way in which air pollution may perturb the cardiovascular system is through an increase in pulmonary and systemic oxidative stress and inflammation, causing vascular endothelial dysfunction.[3] Adhesion molecules such as intracellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) play a role in the inflammatory response through the binding and margination of leucocytes and then sequestration to the site of inflammation.[4] Increased soluble levels of ICAM-1 (sICAM-1) are associated with increased risk of myocardial infarction, angina and cardiovascular mortality.[5] Ambient $PM_{2.5}$ (PM of 2.5 µm or less) exposure[6] and in vitro exposure to diesel exhaust (DE)[7 8] have been shown to increase concentrations of sICAM-1 and soluble VCAM-1 (sVCAM-1). Furthermore, DE exposure increases expression of ICAM-1 and VCAM-1 in the bronchial tissue.[9] Thus, pollution-mediated increases in these molecules could contribute to the adverse pulmonary and cardiovascular effects of air pollution exposure.

Exercise reduces the risk of cardiovascular disease and cardiovascular mortality by improving endothelial function, assisting in

the maintenance of blood lipids and lowering blood pressure and inflammation.[10 11] However, during exercise the increase in minute ventilation and oronasal breathing increases the dose of air pollution and the proportion of PM that deposits in the respiratory tree,[12 13] and thus could exacerbate any air pollution-related effects on adhesion molecules and inflammation. Therefore, the purpose of the present study was to determine how DE exposure during exercise of varying intensities affects adhesion molecules and markers of systemic inflammation. We hypothesised that exposure to DE would increase adhesion molecule and inflammatory marker concentration and that any physiological effects of DE would be magnified as exercise intensity increases.

MATERIALS AND METHODS

Eighteen recreationally active males volunteered for the study. Participants were considered sufficiently active for inclusion in the study if they met Canada's physical activity guidelines.[14] Each participant was a non-smoker and had no history of respiratory or cardiovascular disease. The Clinical Research Ethics Board of the University of British Columbia approved this study. Prior to all visits, participants were asked to refrain from exhaustive exercise and alcohol for 24 hours, caffeine for 6 hours and food or non-water beverages for 2 hours. Each participant performed all trials at the same time of day. Participants were also asked to maintain the same pretest routine including the same mode of travel to the laboratory and pretest meal, and were asked to restrict vitamin supplementation for the duration of the study. The sample size was calculated based on a minimal detectable difference in soluble platelet-Selectin (sP-Selectin) of 2.8 ng/mL using an effect size of 0.87 (*f*), a power of 0.8 and an alpha of 0.05[15] and a minimal detectable difference in neutrophils of 49.2% increase using an effect size of 0.65 (*f*), a power of 0.8 and an alpha of 0.05.[16]

Experimental design

Data collection for this study occurred as part of a larger study, and overall methods are explained in detail elsewhere.[17–19] Briefly, each participant attended the laboratory on seven occasions. The initial visit served as a familiarisation with all study procedures and performance of a maximal exercise test on a cycle ergometer. Details of the maximal exercise test, as well as the exercise testing equipment can be found in detail elsewhere.[17–19]

On testing days 2–7, participants performed 30 min trials of low-intensity cycling, high-intensity cycling or rest. Each intensity was performed once in filtered air (FA) and once in DE with a target concentration of 300 µg/m^3 of $PM_{2.5}$, for a total of six trials, each of which was separated by a 7-day period. Exercise intensity and the exposure (FA and DE) were randomised. To avoid experimental bias, both the participant and the research assistant collecting the data were blinded to the exposure of FA or DE. Work rates on cycling days were based on the peak power achieved during the maximal exercise test. Low-intensity cycling was set at 30% of the power at peak oxygen consumption (VO_{2peak}) (mean (SD): 96.1 (17.7) W) and high-intensity cycling was set at 60% of power at VO_{2peak} (192.2 (35.3) W). Control exposures involved sitting for the same period of time (30 min), but without performing exercise. Information regarding the exercise set-up can be found in detail elsewhere.[17 19] Prior to, immediately post, 1 hour and 2 hours post exposure, blood was drawn to measure parameters of a complete blood count and adhesion molecules that are detailed below.

Outcome measures

Blood samples were taken from the right antecubital fossa with a 21-gauge needle. White blood cell (WBC), neutrophil, monocyte, lymphocyte, eosinophil, platelet, red blood cell (RBC), haemoglobin concentrations and haematocrit (Hct) were measured in a commercial laboratory. Blood samples were also taken to determine concentrations of sICAM-1, sVCAM-1, sP-Selectin and soluble endothelial-Selectin (sE-Selectin). These samples were immediately centrifuged at 1500*g* for 20 min to separate plasma from formed elements. Plasma was extracted, frozen and stored at −80°C until assayed. Plasma concentrations of sICAM-1, sVCAM-1, sP-Selectin and sE-Selectin were determined in duplicate using commercially available Luminex assay kits (Human Adhesion Molecule Luminex Performance Assay; R&D Systems, Minnesota, USA), according to the procedures outlined by the manufacturer and using a Luminex 200 System (Luminex, Ontario, Canada). The intra-assay coefficient of variation for sICAM-1 was 4.4%, sVCAM-1 was 3.8%, sP-Selectin was 4.4% and sE-Selectin was 3.6%. Of the 432 planned blood samples, two were unable to be collected for technical reasons. To prevent complete exclusion of those subjects with missing measurements and based on the recommendations of a statistician, the missing values were imputed using regression.[20]

As plasma volume may change during exercise, levels of sICAM-1, sVCAM-1, sP-Selectin and sE-Selectin were adjusted for changes in plasma volume from baseline, which is explained in detail elsewhere.[17 18]

Exposure set-up

All exposures were performed using an environmental exposure booth and that is explained in detail elsewhere,[21] but was modified only in that the generator load was kept constant at 2.5 kW and not cycled. For DE exposures, participants were exposed to calibrated, aged and diluted DE with a target concentration of 300 µg/m^3 of $PM_{2.5}$. For FA exposures, participants were exposed to room air after it was concentrated and then passed through a high-efficiency particulate air filter. All equipments used to determine pollutant concentrations are also explained in detail elsewhere.[17–19] Briefly, in-booth PM mass concentration measurements were made using a Tapered Element Oscillating Microbalance (Model 1400a; Rupprecht & Pattashnick, Albany, New York,

USA). A TSI Scanning Mobility Particle Scanner (Model 3936; TSI, Shoreview, Minnesota, USA) classified the particle size distribution between 2.5 nm and 1000 nm. DE was chosen to represent a mixture similar to that in an urban street canyon. For example, in a street canyon in close proximity to a major highway peak particle number concentration (PNC) was similar to experimental exposures conducted in our laboratory, where PNC exceeds 300 000 particles/cm^3.[21 22] Furthermore, 30 min peak carbon monoxide concentrations in downtown street canyons exceed carbon monoxide concentrations within our laboratory (17.5–35 parts per million (ppm) vs 11.2 ppm in the current study).[21 23] This dose of DE is occupationally relevant and has been experienced by miners, construction workers, mechanics and dockside workers.[24–26] The concentration of $PM_{2.5}$ is approximately 1 order of magnitude greater than 24 hours ambient standard in Canada.

Statistical analysis

Statistical analyses were completed using SPSS V.20 software (SPSS, Chicago, Illinois, USA), and analyses were chosen through consultation with a PhD statistician. For each parameter, data were analysed using a 2 (exposure: FA vs DE) × 3 (intensity: rest, low intensity, high intensity) × 4 (time: pre, post, 1 hour post, 2 hours post) repeated measures analysis of variance (ANOVA). Significance was set at $p<0.05$. For all repeated measures ANOVA, the Huynh-Feldt adjustment was used to correct for violations of sphericity. Main or interaction effects were further analysed using pairwise comparisons, and significance was adjusted to account for multiple comparisons using the Sidak adjustment, which is explained in detail elsewhere.[17–19] Briefly, the p-values represented in this manuscript have been inflated to incorporate the Sidak adjustment, meaning that remains at 0.05. Baseline data were analysed using a 2 (exposure: FA vs DE) × 3 (intensity: rest, low intensity, high intensity) repeated measures ANOVA. All means are reported with SD in parentheses.

Patient and public involvement

We did not involve patients or the public in the design of the research project or research questions. The public were recruited as participants, which has been previously explained in detail.

RESULTS

$PM_{2.5}$ levels were 9.3 (6.20) and 302.1 (6.50) µg/m^3 for FA and DE, respectively. Mean PNCs during FA and DE exposures were 0.14×10^4 and 61.60×10^4 (n/cm^3). Mean nitrogen dioxide concentrations during FA and DE exposures were 0.04 (0.04) and 0.58 (0.15) ppm. Mean nitrogen oxide concentrations during FA and DE exposures were 0.02 (0.02) and 7.00 (0.09) ppm. Mean carbon monoxide levels during FA and DE exposures were 3.00 (0.40) and 13.9 (2.10) ppm. Mean baseline values for all parameters for the participants across the 6 testing days were not significantly different ($p>0.05$; table 1). All participants performed all six trials, although three participants were unable to finish the high-intensity trial in DE due to volitional exhaustion. In individuals who were unable to finish the first high-intensity trial, the second high-intensity exercise trial was designed to mimic the first; therefore, the duration in second trial was matched to that of the first trial.

Soluble ICAM-1

There was a significant three-way interaction (exposure-by-intensity-by-time) for sICAM-1 (figure 1, $p=0.037$). Post hoc analysis showed that 2 hours following high-intensity exercise sICAM-1 was significantly less in DE compared with FA (figure 1c; $p=0.008$, 12.37×10^4 (5.49×10^4) vs 13.60×10^4 (4.92×10^4) pg/mL). Immediately following rest ($p=0.013$; 13.11×10^4 (4.82×10^4) pg/mL) and high-intensity exercise ($p=0.042$; 12.97×10^4 (5.17×10^4) pg/mL) in DE, sICAM-1 was significantly greater than immediately following low-intensity exercise in DE (11.61×10^4 (5.11×10^4) pg/mL). Immediately following rest in DE, sICAM-1 was significantly greater than 2 hours following rest (figure 1A; $p=0.017$; 13.11×10^4 (4.82×10^4)

Table 1 Mean pre-exposure complete blood count and adhesion molecules averaged over 6 experimental test days in 18 recreationally active males; mean (SD)

Complete blood count							
WBC (10^9/L)	RBC ((10^{12}/L)	Haemoglobin (g/L)	Hct	RDW (%)	Platelet count (10^9/L)	Neutrophils (10^9/L)	Monocytes (10^9/L)
5.16 (1.24)	0.0049 (00000.28)	145 (7.72)	0.43 (0.02)	12.57 (0.49)	208.19 (44.97)	2.95 (0.93)	2.95 (0.93)

Adhesion molecules			
sICAM-1 (pg/mL)	sVCAM-1 (pg/mL)	sP-Selectin (pg/mL)	sE-Selectin (pg/mL)
12.78×10^4 (4.91×10^4)	89.64×10^4 (19.55×10^4)	5.21×10^4 (1.72×10^4)	2.92×10^4 (1.05×10^4)

Hct, haematocrit; RBC, red blood cell; RDW, red cell distribution width;sE-Selectin, soluble endothelial-Selectin; sICAM-1, soluble intracellular cell adhesion molecule-1; sP-Selectin, soluble platelet-Selectin; sVCAM-1, soluble vascular cell adhesion molecule-1; WBC, white blood cell.

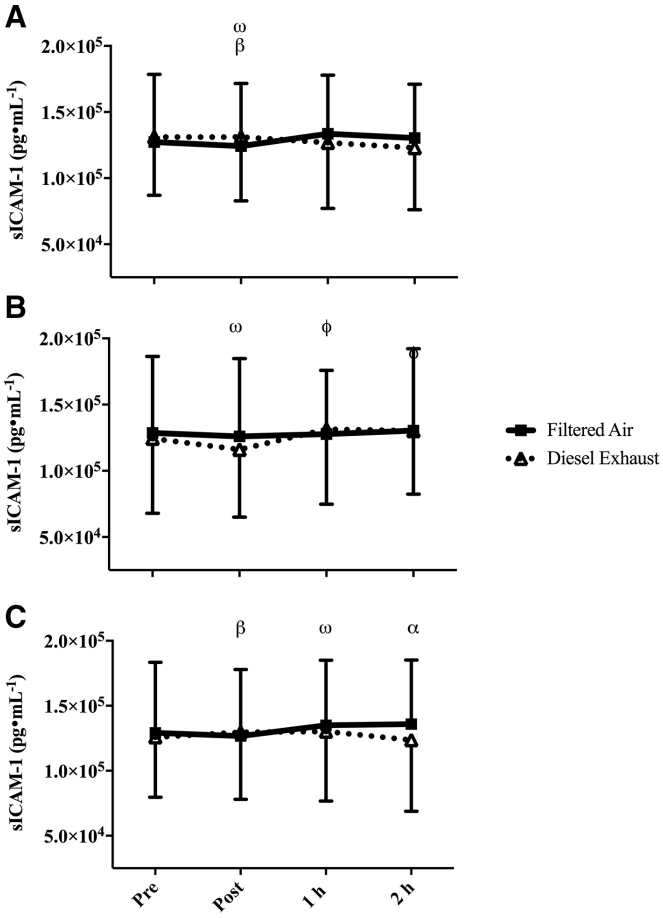

Figure 1 sICAM-1 in 18 recreationally active males prior to and following 30 min of (A) rest, (B) low-intensity cycling or (C) high-intensity cycling in DE and FA. α=significantly less than FA at the same time point; β=significantly greater than immediately post low-intensity exercise (DE only); Φ=significantly greater than post (DE only). DE, diesel exhaust; FA, filtered air; sICAM-1, soluble intracellular cell adhesion molecule-1. ω= in DE only significantly greater than 2 hour post (rest and high-intensity), significantly less than 1 and 2 hour post (low intensity)

vs 12.29×10^4 (4.68×10^4) pg/mL). One hour (p=0.013; 13.14×10^4 (5.65×10^4) pg/mL) and 2 hours (p=0.02; 13.03×10^4 (4.78×10^4) pg/mL) following low-intensity exercise in DE, sICAM-1 was significantly greater than immediately following low-intensity exercise in DE (figure 1B; 11.61×10^4 (5.11×10^4) pg/mL). One-hour post high-intensity exercise in DE sICAM-1 was significantly greater than 2 hours post high-intensity exercise in DE (p=0.027; 13.02×10^4 (5.36×10^4) vs 12.37×10^4 (1.30×10^4) pg/mL). There were no significant changes in sICAM-1 in FA.

Soluble VCAM-1

There was a significant intensity-by-time interaction (p<0.001) for sVCAM-1 (figure 2). Post hoc analysis revealed that 1 hour post (p=0.001; 98.13×10^4 (23.13×10^4) pg/mL) and 2 hours post (p=0.016; 96.76×10^4 (24.36×10^4) pg/mL) low-intensity exercise sVCAM-1 was significantly greater than prior to low-intensity exercise (88.36×10^4

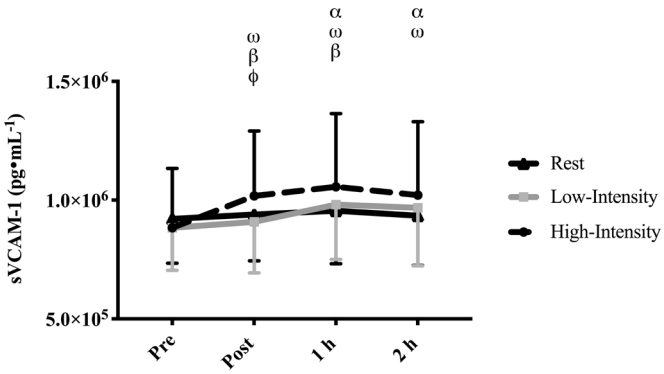

Figure 2 Vascular cell adhesion molecule-1 (sVCAM-1) in 18 recreationally active males prior to and following 30 min of rest, low-intensity cycling or high-intensity cycling. α=significantly greater than pre in the corresponding intensity (low-intensity exercise); ω=significantly greater than pre in the corresponding intensity (high-intensity exercise); β=significantly less than high intensity at the corresponding time point (immediately post high vs immediately post low intensity, 1 hour post high intensity vs 1 hour post rest); Φ=significantly less than 1 hour post in the corresponding intensity (low-intensity exercise).

(17.89×10^4) pg/mL). sVCAM-1 was also greater at 1 hour post low-intensity exercise (p=0.001; 90.89×10^4 (21.48×10^4) vs 98.13×10^4 (23.13×10^4) than immediately post-exercise. Similarly, immediately following (p<0.001; 101.81×10^4 (27.35×10^4) pg/mL), 1 hour following (p<0.001; 105.60×10^4 (30.82×10^4) pg/mL) and 2 hours following (p=0.003; 102.15×10^4 (30.92×10^4) pg/mL), high-intensity exercise sVCAM-1 was significantly greater than prior to high-intensity exercise (88.40×10^4 (24.98×10^4) pg/mL). sVCAM-1 was significantly lower immediately post low-intensity exercise compared with immediately post high-intensity exercise (p=0.015; 90.89×10^4 (21.48×10^4) vs 101.81×10^4 (27.35×10^4) pg/mL). sVCAM-1 was also significantly lower immediately 1 hour following rest compared with 1 hour following high-intensity exercise (p=0.047; 95.52×10^4 (22.32×10^4) vs 105.60×10^4 (30.82×10^4) pg/mL). There were no significant differences in sVCAM-1 between DE and FA.

sP-Selectin and sE-Selectin

There was a main effect of intensity (p=0.022) and time (p=0.01) for sP-Selectin. Prior to exercise/exposure, sP-Selectin was significantly less than 1 hour post-exercise/exposure (p=0.032; 5.21×10^4 (1.78×10^4) vs 5.90×10^4 (2.04×10^4) pg/mL). Post hoc analysis did not reveal any significant differences when comparing exercise intensity. There were no significant differences in sP-Selectin between DE and FA. There were no main or interaction effects for sE-Selectin.

Complete blood count

There was a significant intensity-by-time interaction for WBC (p<0.001; figure 3A), neutrophils (p<0.001; figure 3B), monocytes (p<0.001; figure 4A), lymphocytes (p<0.001; figure 4B), haemoglobin (p=0.039) and

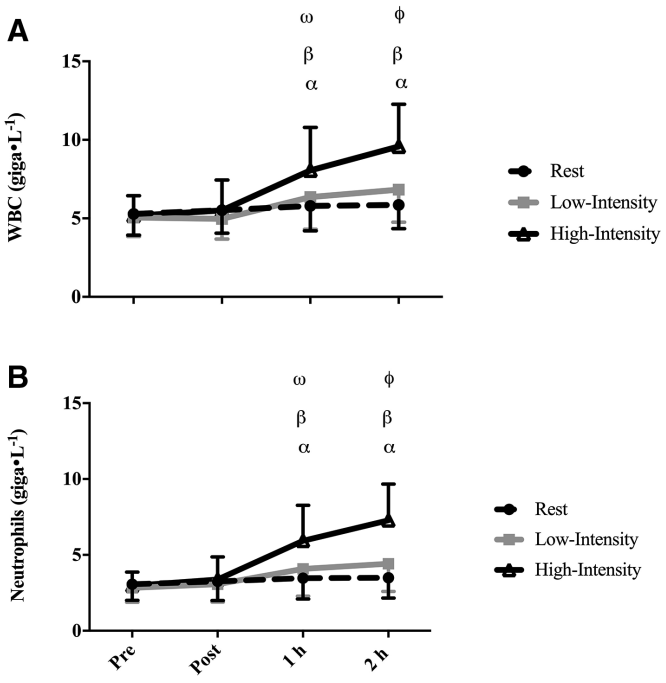

Figure 3 (A) White blood cell (WBC) count or (B) neutrophils in 18 recreationally active males prior to and following 30 min of rest, low-intensity cycling or high-intensity cycling. β=significantly greater than pre-exercise and post-exercise (for all intensities aside from neutrophils where during rest 1 hour is only significantly different than post); Φ=significantly difference between rest and low-intensity exercise; α=significantly less than high intensity at the corresponding time point for both low intensity and rest; ω=significantly less than 2 hours (low and high intensity only).

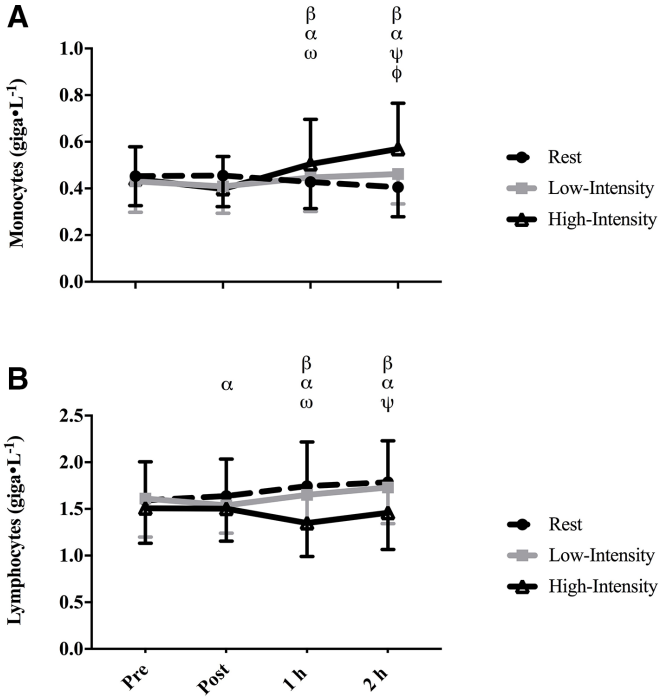

Figure 4 (A) Monocytes or (B) lymphocytes in 18 recreationally active males prior to and following 30 min of rest, low-intensity cycling or high-intensity cycling. β=significantly different from post-exercise for all intensities (monocytes: rest is only significant at 2 hours, lymphocytes: high intensity is only significant at 1 hour); Φ=significantly different between rest and low-intensity exercise; α=significantly less than high intensity at the corresponding time point for both low intensity and rest (lymphocytes: at the post time point significance only occurred for rest vs high intensity); ω=significantly less than 2 hours in the corresponding intensity (high intensity only); Ψ=significantly different from the pre-exercise (monocytes: rest and high intensity only, lymphocytes: rest only).

platelets (p=0.016). Following exercise, WBC, neutrophils and monocytes increased over time and the increase was intensity dependent with greater increases over time seen with high-intensity exercise. In contrast, lymphocytes decreased with exercise intensity. For detailed comparisons of WBC, neutrophils, lymphocytes, monocytes, haemoglobin concentration and platelets, see online supplementary files 1 and 2. There were no other main or any other interaction effects for WBC, neutrophils, monocytes, lymphocytes, eosinophils, platelet count, RBC, haemoglobin concentration and Hct.

DISCUSSION

This is the first study to determine the effects of DE exposure with exercise of varying intensities on adhesion molecules and markers of inflammation. We found that sICAM-1 was significantly lower immediately following low-intensity exercise compared with immediately following high-intensity exercise or rest in DE but not FA. We also found significantly lower levels of sICAM-1 2 hours following high-intensity exercise in DE compared with FA. Following 30 min of low-intensity and high-intensity exercise, sVCAM-1, sP-Selectin, sE-Selectin, WBC, neutrophils, monocytes, lymphocytes, eosinophils, platelet count, RBC, haemoglobin concentration and Hct were not significantly different between DE and FA conditions.

Soluble ICAM-1

The finding that sICAM-1 was significantly lower in DE compared with FA, 2 hours following high-intensity exercise, could be related to circulating levels of nitric oxide or endothelin-1. In patients with hypertension, circulating endothelin-1 is related to ICAM-1[27] and in cerebrovascular endothelial cell lines, endothelin-1 upregulates ICAM-1.[28] As part of a larger project with the same study design, we found that 2 hours post exposure, endothelin-1 was significantly lower in DE versus FA.[17] Additionally, we found that endothelin-1 was higher 2 hours post high-intensity exercise when compared with 2 hours post low-intensity exercise and 2 hours post rest.[17] While the described study did not find a significant difference in endothelin-1 between DE and FA 2 hours post high-intensity exercise, the DE-FA difference 2 hours post exposure appeared to be driven by the high-intensity condition.[17] As endothelin-1 appears to be associated with ICAM-1,[27 28] the lower endothelin-1 may result in a

lower sICAM-1 2 hours post high-intensity exercise in DE in the current study.

The lower sICAM-1 in DE compared with FA is similar to the findings of Frampton *et al*,[29] who found that ICAM-1 expression on leucocytes decreased in response to ultrafine particulate matter (UFP) in a dose-dependent manner. While the current study and Frampton *et al*,[29] measured ICAM-1 differently, sICAM-1 may derive from the proteolytic cleavage of the membrane-bound form[30] and thus sICAM-1 may serve as a surrogate marker of cellular expression. The rationale proposed by Frampton *et al*,[29] for the lower ICAM-1 following UFP exposure includes a greater sequestration of leucocytes into the lung parenchyma due to minor lung inflammation and the potential for UFP to cause leucocyte cellular apoptosis. However, we did not find support for this rationale, as we found no significant differences in WBC or surrogate markers of lung inflammation between FA and DE.[18] These authors[29] also suggested that UFP may adsorb soluble cytokines and thus reduce its inflammatory potential,[29] which is a potential explanation for the lower sICAM-1 hours post high-intensity exercise in DE in the current study.

We also found that sICAM-1 was significantly greater following high-intensity exercise compared with low-intensity exercise in DE. Exercise causes a shift of granulocytes from the marginated pool to the circulatory pool.[31] Leucocytes can marginate in the lung, and there is an inverse relationship between transit time and leucocyte margination.[31 32] For leucocyte margination to occur, they require a sufficient transit time through the pulmonary capillaries.[31 32] During high-intensity exercise, the higher cardiac output would decrease transit time through the pulmonary capillaries, which could decrease leucocyte sequestration and margination. The decreased sequestration and margination coupled with exercise-induced shear stress that causes shedding of ICAM-1[33 34] could have resulted in greater levels of sICAM-1 following high-intensity exercise compared with low-intensity exercise in DE.

Complete blood count

In the current study, white blood cells, neutrophils and monocytes increased following exercise; the magnitude of the increase was exercise intensity dependent, but there were no effects of DE on the response. The finding that WBC and neutrophil counts increased with exercise intensity, without a further increase with DE exposure, suggests that acutely, DE may not initiate a systemic inflammatory response through WBC. The increase in WBC and neutrophils with exercise intensity is not surprising as acute exercise results in a biphasic response of blood neutrophils. Initially there is a rapid blood neutrophilia, followed by a second delayed increase a few hours later, and the response increases as exercise duration and intensity increase.[35 36] The initial increase in neutrophils may be related to the release of neutrophils from the vascular wall caused by shear stress and catecholamines.[37] The secondary increase in neutrophils, which would occur at a similar time to 1 hour and 2 hours post exposure in the current study, is due to a release of more WBCs from the bone marrow in response to increased cortisol levels.[37]

The current study did not find that exposure to DE modified the exercise response of WBC or neutrophils. The lack of effect of DE on the neutrophilic response to exercise contrasts with the unblinded study of Jacobs *et al*[38] who found that cycling in traffic for 20 min significantly increased neutrophil count more than cycling in a laboratory without exposure to air pollution. Acute psychological stress increases inflammatory cytokines[39 40]; therefore, the difference in findings between the current study and Jacobs *et al*[38] could be related to the stress response to noise or cycling in traffic. It is also possible that in the study by Jacobs *et al*,[38] PM exposure from automotive tire and brake wear, which has a high oxidative potential, could have played a role in the observed effects.[41] Furthermore, one cannot rule out the role of other pollutants such as ozone or re-entrained dust that are not otherwise present in a laboratory setting.

Limitations

This study employed a double-blind, cross-over and counterbalanced design to minimise variability caused by between-subject differences. Despite the strength of study design, there were several limitations that warrant consideration. The study was powered based on detectable differences in sP-Selectin and neutrophils, and we cannot discount that other endpoints were inadequately powered. DE exposure contains a mixture of PM and gaseous pollutants. Gaseous components include carbon monoxide, carbon dioxide, oxygen, water vapour, nitrogen oxides, sulfur compounds and VOC.[42] Additionally, DE contains PM in the fine (<2.5 µm: $PM_{2.5}$) and ultrafine (<0.1 µm) range. The PM within DE is composed of elemental carbon, adsorbed organic compounds and small amounts of sulfate, nitrate, metals and trace elements.[42 43] However, the chemical composition of DE and particle size vary significantly with engine type, operating conditions and fuel formations.[42] Therefore, the mixture within the current study likely differs from ambient conditions and other laboratories using DE, which may explain why we did not observe significant differences in WBC, sVCAM-1, sP-selectin and sE-Selectin in DE compared with FA. Despite this consideration, DE was chosen as a model air pollution mixture as it contains both gaseous and particulate pollution and represents a mixture similar to that in an urban street canyon with significant heavy goods truck traffic. We cannot discount that physiological responses will vary when exercise duration, the time course of post-exercise measures and the fitness level or health status of our participants is different and this may have led to some non-significant findings. We chose a 30 min exercise bout to represent a cycle commute[44]; however, we cannot predict how our results would have been different following longer

duration exercise. Since the changes in sICAM-1 were small and the concentration of PM in DE was high, the clinical significance is unclear.

CONCLUSIONS

This is the first study to assess the acute effects of 30 min of rest, low-intensity and high-intensity cycling with DE exposure in healthy males on adhesion molecules and markers of systemic inflammation, such as WBC. We hypothesised that exposure to DE would increase concentrations of adhesion molecules and markers of inflammation and that any physiological effects of DE would be magnified as exercise intensity increases. Despite these hypotheses, we found that following low-intensity exercise in DE, sICAM-1 was significantly lower compared with immediately following rest and high-intensity exercise in DE. Additionally, sICAM-1 was significantly lower 2 hours following high-intensity exercise in DE compared with FA. All other measured adhesion markers and markers of systemic inflammation were not different between DE and FA. Based on the results of this study, healthy individuals do not appear to experience an acute increase in adhesion molecules and systemic inflammatory markers from exercising in DE. However, to substantiate this claim, more research is needed to determine the longer-term effects of DE exposure during exercise as well as different compositions of air pollution during exercise.

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