Acute Responses of Inflammatory Markers of Cardiovascular Disease Risk to a Single Walking Session

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Background: Markers of inflammation are emerging as novel indices of cardiovascular risk. These markers have been shown to alter acutely after intense exercise; however, the effects of more moderate intensity exercise in healthy individuals is not known. Walking forms a cornerstone of physical activity promotion, so the inflammatory response to this exercise merits investigation. This study evaluated the effects of a 45-min walk on C-reactive protein (CRP) and interleukin 6 (IL-6), in sedentary, overweight men. *Methods*: Fifteen men (49.7 ± 5.9 y) walked for 45 min at 60 to 70% of predicted maximum heart rate. Fasted blood samples were taken prior to and immediately 1 hr and 24 h post-walk. *Results*: IL-6 decreased from 1 h post-walk to 24 h post-walk ($P < 0.01$). No significant changes were observed in CRP. *Conclusions*: These findings suggest that 45 min walking at 60 to 70% $HR_{max\text{-}p}$ causes a decrease in IL-6 24 h post-exercise, but does not evoke a significant response in CRP levels.

Key Words: exercise, health, interleukin 6, C-reactive protein

There is increasing recognition that traditional risk factors do not fully account for the occurrence of cardiovascular disease (CVD). Almost one-half of the 1.3 million individuals who develop myocardial infarction in the US each year have either normal or only moderately increased cholesterol.[1] Although atherogenesis was formerly considered a lipid storage disease, recent advances have highlighted the role of inflammation and underlying cellular and molecular mechanisms.[2] Two markers of inflammation, interleukin 6 (IL-6) and C-reactive protein (CRP), have been linked with tissue damage and infection[3] and the progression of atherosclerosis.[4] Recent work has illustrated that IL-6 can predict future myocardial infarction and mortality risk in healthy individuals and those with coronary syndromes.[5,6] CRP has a strong association with cardiovascular risk, which is consistent, dose-related, and independent.[7]

The health-related changes associated with exercise may be due in part to acute responses that persist following each exercise session.[8] CRP and IL-6 have

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been shown to alter acutely with exercise. IL-6 may increase following prolonged aerobic,[9] maximal,[10] eccentric,[11] and anaerobic[12] exercise while CRP has been shown to increase following prolonged aerobic[13] and anaerobic exercise.[12] These studies, however, have examined the response of trained individuals to high-intensity/volume exercise, where the activated phagocytic process is maximized. Exercise of a lower intensity where phagocytosis is minimized, might enhance the vascular clearance of some pro-inflammatory molecules, and provide a protective effect from further endothelial damage. Few studies have considered how sedentary individuals would respond to moderate-intensity activity as recommended by public health guidelines.

Walking is a popular[14] moderate-intensity exercise[15] and forms the cornerstone of many activity promotion strategies. The inflammatory response to walking therefore merits investigation. Two studies have utilized walking when examining the acute phase response.[16, 17] The treadmill protocol of Fiotti and colleagues,[16] however, included a fixed stop of 1 min every 5 min, with peripheral artery disease (PAD) patients. Furthermore, CRP values were not reported. Similarly in the study of Signorelli and co-workers[17] the subjects also had PAD, and walked to the onset of calf muscle cramps while the controls walked for a maximum of 5 min. No study has examined the effects of walking for durations ≥ 30 min on IL-6 and CRP in healthy but sedentary subjects. The purpose of the study, therefore, was to evaluate the acute effects of a single walking session on CRP and IL-6, in sedentary, overweight, but otherwise healthy men.

Methods

Study Design

The experimental protocol consisted of an initial screening assessment followed, at least 1 wk later, by a single exercise trial conducted over 2 d. The Research Ethics Committee at Queen's University, Belfast, approved the study. Subjects were recruited from a register of outpatients at the Ulster Hospital and from staff at the University of Ulster, by means of telephone contact, email, and post. All subjects were sedentary,[18] overweight males. Subjects gave their written informed consent and completed a health history questionnaire. Exclusion criteria were: age > 60 years, resting blood pressure ≥ 159/99 mm Hg, body-mass index (BMI) > 45 kg/m^2, current smokers, individuals with known CVD, pain or discomfort in the chest, dizziness, or shortness of breath with usual activities. Also, those taking medications known to interfere with the cardiovascular disease risk markers being measured were excluded from the study. Physiological characteristics of the subjects who took part in the study (n = 15) are shown in Table 1.

A prospective power of the test calculation was performed[19] and 15 subjects were identified as the required number to observe a statistical difference in IL-6 between time intervals at 95% probability.

Preliminary Measures

During the screening visit, height and body mass were recorded using a stadiometer (Seca model 770, Vogel & Halke, Hamburg, Germany) and scales (Seca model 707, Vogel & Halke) respectively. Waist circumference was measured at the level of the trunk where the girth is minimal. If there was no noticeable indent the tape

Table 1 Physiological Characteristics of Subjects (n = 15)

	Mean	SD	Range
Age (y)	49.7	5.9	39 – 57
Body mass (kg)	100.1	19.5	80.6 – 163.1
Height (m)	1.8	0.1	1.7 – 1.9
BMI (kg/m^2)	30.9	4.9	24.9 – 45.7
Body fat (%)	27.6	5.2	21.9 – 43.4
Waist girth (cm)	105.3	5.8	96.3 – 117.0
Hip girth (cm)	110.8	4.7	103.0 – 122.0
Waist:hip ratio	0.95	0.03	0.90 – 1.01
Systolic BP (mm Hg)	123.7	9.1	109.5 – 137.0
Diastolic BP (mm Hg)	76.4	5.8	70.0 – 90.5
Resting HR (beats/min)	64.7	7.3	52.5 – 80.5

was located at the umbilicus. Hip circumference was measured according to established procedures.[20] Body-fat percentage was assessed by bioelectrical impedance analysis (Bodystat 1500, Bodystat Ltd., Isle of Man, UK) using recommended procedures.[21] Resting blood pressure was recorded as the mean of 2 measurements using a validated automated device (Omron 705CP, Omron Matsusaka Co. Ltd., Japan), taken a minimum of 2 min apart, after the subject had rested in a supine position for at least 5 min. Subjects were then familiarized with walking on the treadmill at various speeds and slopes.

Experimental Trial

Subjects were instructed to fast for 10 h and refrain from strenuous physical activity for 24 h prior to testing. On day 1 of the exercise trial, subjects arrived at the Human Performance Laboratory between 7:30 AM and 9:00 AM. Subjects were asked to report any acute illnesses or infections, as these are associated with increased CRP levels.[22] None were reported. Following 5 min rest in a supine position, a blood sample was obtained by venipuncture. Subjects then completed a 45-min treadmill walk at 60 to 70% of age-predicted maximum heart rate ($HR_{max\text{-}p}$). The walk was preceded by a 3-min warm-up period. Heart rate was measured throughout the test by short-range telemetry (Accurex Plus; Polar Electro, Kempele, Finland). Ratings of perceived exertion (RPE) using the Borg 15-grade scale[23] were recorded every 5 min using recommended instructions.[21] Energy expenditure was estimated using the subject body mass and walking speed.[24] Venous blood samples were then taken immediately and 1 h post-exercise. On day 2 of the experimental trial, subjects returned to the laboratory after a 10 h fast. A final venous blood sample was taken 24 h after completion of the 45-min treadmill walk.

Blood Analysis

Full blood count was measured on fresh EDTA samples on a Sysmex SE 9500 (Sysmex, Milton Keynes, UK). Serum samples were separated, frozen at –22 °C, and

analyzed within 5 months for IL-6 and CRP. IL-6 was measured using a Quantikine high sensitivity kit (R & D Systems, Abingdon, UK) on a microplate autoreader (model EL 311, Bio-tek, Winooski, VT). Between-assay imprecision for IL-6 at concentrations of 0.49, 2.78, and 5.65 pg/L were 9.6, 7.2, and 6.5% CV. CRP was measured using a full-range, high sensitive immunoturbidimetry method (Randox Laboratories, Crumlin, UK) on a Hitachi 717 analyzer (Roche Products Ltd., Herts, UK). Between-assay imprecision for CRP was 1.82%, 1.85%, (n = 95) at mean concentrations of 2.18, 4.93 mg/L, and 2.38% at a mean of 21.50 mg/L (n = 70). Hematocrit was corrected by 1.5% for plasma trapped between erythrocytes.[25] Hemoglobin and hematocrit were then used to correct all variables for changes in plasma volume.[26]

Statistical Analysis

The data were analyzed using a one-way ANOVA with repeated measures. Where differences emerged, Bonferroni comparisons were used to identify which time points were significantly different. Statistical significance was established at $P < 0.05$. All descriptive data is presented as mean (± standard deviation) and all outcome measures are presented as mean (± standard error of the mean) unless otherwise stated.

Results

Interleukin 6

Mean pre-exercise IL-6 was 1.92 ± 0.29 pg/mL. Corresponding values at immediately post-, 1 h post-, and 24 h post-exercise were 1.73 ± 0.18, 1.76 ± 0.16, and 1.37 ± 0.14 pg/mL, respectively. There was a significant decrease from 1 h post-exercise to 24 h post-exercise. The IL-6 response to the 45-min treadmill walk is illustrated in Figure 1.

C-Reactive Protein

Mean CRP at pre-exercise was 1.95 ± 0.63 mg/L. Corresponding values were 1.91 ± 0.61, 1.92 ± 0.63, and 2.36 ± 0.76 mg/L at immediately post-, 1 h post-, and 24 h post-exercise, respectively. No significant effects were observed.

Full Blood Count

White blood cells significantly increased from baseline (5.66 ± 0.27 × 10^9/L) to immediately post-exercise (6.29 ± 0.30 × 10^9/L) and remained increased at 1 h post-exercise (6.29 ± 0.30 × 10^9/L). Neutrophils significantly increased from baseline (3.24 ± 0.18 × 10^9/L) to immediately post-exercise (3.76 ± 0.23 × 10^9/L) and remained significantly elevated at 1 h post-exercise (3.84 ± 0.24 × 10^9/L). Baseline levels of lymphocytes, monocytes, eosinophils, and basophils were 1.7 ± 0.14, 0.47 ± 0.04, 0.2 ± 0.02, and 0.05 ± 0.01 × 10^9/L, respectively. There were no significant effects observed.

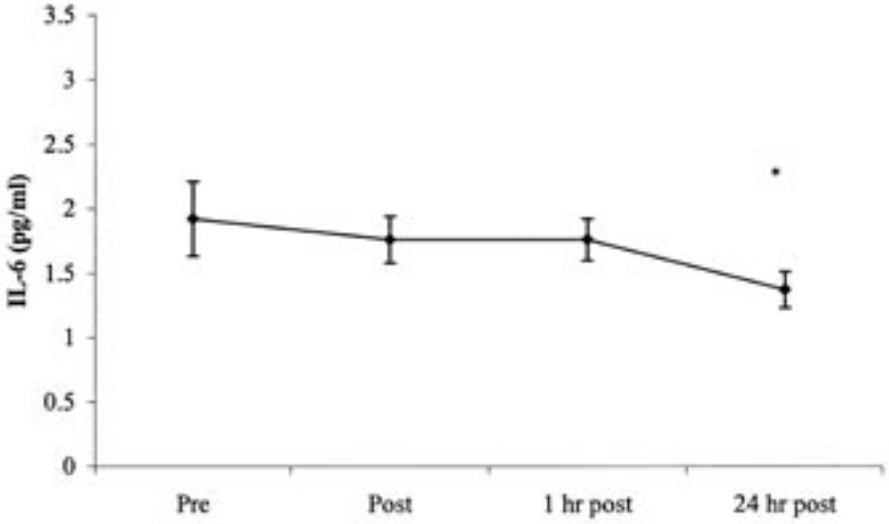

Figure 1 — Interleukin 6 (IL-6) before and after 45 minutes walking. Values are means ± standard error of the mean. *Significant difference from 1 h post-exercise ($P < 0.01$).

Exercise Intensity

The mean HR recorded during the 45-min walk was 112.2 ± 5.7 beats/min. This corresponded to an exercise intensity of 65.8 ± 1.7% $HR_{max\text{-}p}$. The mean RPE during the treadmill test was 11.4 ± 1.0. The mean estimated energy expenditure during the 45-min walk was 315 (± 61.45) kcal.

Discussion

The unique aspect of the current study was that the acute response of novel inflammatory markers of CVD risk was examined following exercise of a type prescribed in current physical activity promotion strategies. The main findings are that walking for 45 min at a moderate intensity (~ 66% $HR_{max\text{-}p}$) causes a significant decrease in IL-6 levels at 24 h post-exercise, and does not alter the acute-phase protein CRP.

The mean BMI of subjects in the present investigation was 30.9, which classifies them as "Class 1 obese" according to the Expert Panel on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults.[27] Overweight males were deliberately chosen for the present investigation due to their increased CVD risk. Several studies have noted a positive relationship between adiposity and CRP,[28] and its primary stimulus, IL-6.[29] The fact that up to 30% of total circulating IL-6 originates from adipose tissue[7] could mean that obesity resembles a low-grade inflammatory state.[4] This might be a considerable public health burden given the rising prevalence of overweight and obesity.[30]

IL-6 is a potent stimulator of the acute phase response and has recently emerged as a novel risk factor for CVD risk. Risk stratification levels for IL-6 in CVD risk have yet to be established, although comparison can be drawn to a recent prospective study of 14,916 apparently healthy men followed-up over 6 y

for future myocardial infarction.[5] The mean baseline IL-6 level of subjects in the present investigation (1.92 ± 0.29 pg/mL) were in the third quartile of Ridker and colleagues' cohort, which is associated with a relative risk of 2.8 compared to those in the lowest quartile. There was no significant change in IL-6 levels immediately after, or 1 h following the 45 min walk. There was a significant decrease, however, from 1 h post-exercise to 24 h post-exercise. These findings are in contrast to the preponderance of research in the area which show elevated IL-6 levels immediately post-exercise.[31,32] The intensity/volume of exercise performed by the subjects in the present investigation was considerably less than the aforementioned studies, which could account for the conflicting findings. This is consistent with previous suggestions that the magnitude of IL-6 response is dependent on the intensity of effort.[33]

Only one other study has been identified which demonstrated attenuated IL-6 levels post-exercise. The effects of 30 min of treadmill walking (3 km/h) was examined in patients with peripheral arterial disease and control subjects.[16] The treadmill walk included a fixed stop of 1 min every 5 min, as these conditions were the heaviest strain tolerable by patients. IL-6 significantly decreased immediately post-exercise in both patients and controls, and this reduction was also present 4 h later. The authors, however, could not hypothesize as to the reason for the observed reduction in IL-6 nor the implication of this finding. The present study shows the unique finding of a significant reduction in IL-6 levels at 24 h post-exercise. As Fiotti and colleagues did not obtain a blood sample after 4 h, it is not known whether IL-6 levels would have been significantly reduced at 24 h in their subjects. Similarly, whether IL-6 concentration returned to baseline levels in the hours after the last sampling time in the present investigation is unknown.

Although systemic changes similar to an acute phase response after a single exercise session tend to be small and transient, local tissue responses might be of sufficient magnitude and duration to have biological relevance.[34] A fall in IL-6 at 48 h after admission to hospital with unstable angina was associated with an uncomplicated in-hospital course, while an increase was associated with an elevated risk of coronary events.[35] Further work is required to examine whether a transient exercise-induced decrease in IL-6 levels, of the order found in the present study, translates into a meaningful attenuation in CVD risk. It is worth considering that given the attenuation of IL-6 levels at 24 h post-exercise, IL-6 might be kept at suppressed levels if an individual were to walk on a daily basis. The mechanism underlying the decrease in IL-6 found in the current study is at present unclear and further work is required to elucidate the issue.

Baseline CRP values in the present study were 1.95 ± 0.63 mg/L. CRP levels of < 1, 1 to 3, and > 3 mg/L correspond to low-, moderate-, and high-risk groups, respectively, for future cardiovascular events.[22] The mean baseline CRP level classifies subjects as being at moderate risk of future cardiovascular events. There were, however, no significant effects observed for the acute effect of 45 min walking on CRP levels.

In keeping with the results of the present investigation, five other studies did not find a significant elevation in CRP levels following exercise. Sixty minutes of level and 40 min of downhill treadmill running did not evoke significant changes in CRP.[36] Similarly, elevated CRP was not seen as an acute consequence of typical training of elite female netball and soccer teams.[37] Twenty-four maximal eccentric arm curls did not evoke significant alterations in CRP levels in 14 untrained

males.[38] Five minutes of treadmill walking did not evoke a significant change in CRP values immediately post-exercise, in healthy elderly control subjects.[17] Finally, CRP level did not alter following 30 min of treadmill walking in patients with peripheral artery disease and controls.[16] It has been suggested that a dose–response relationship might exist for the acute phase responses following exercise, where the dose of exercise is the product of exercise intensity multiplied by duration.[34] The reasons for the lack of change in CRP following exercise observed in the present study are not clear; however, one cannot exclude the possibility of a change in the production-clearance ratio of this metabolite. It could also be that the sample size was insufficient to detect a change in CRP over time.

Cytokines operate both as a cascade and as a network in stimulating the production of acute-phase proteins.[39] As IL-6 is the primary stimulus for CRP, their effects should not be discussed in isolation. Cytokine-induced gene expression and subsequent de novo protein synthesis takes time; thus significant elevation of acute phase plasma proteins require 12 to 24 h to occur.[34] A significant reduction in IL-6 levels was noted at 24 h post-exercise in the present investigation. It is therefore plausible that had there been a subsequent sampling time, allowing another 12 to 24 h, an effect on CRP levels might have been detected. This snapshot approach with 3 post-exercise blood samples does not allow a complete picture of the IL-6 changes to be described. In the study of Fiotti and co-workers[16] reduced IL-6 levels were found immediately post-, and 4 h post-exercise, while no changes in CRP was reported. Again, it is likely that had another sampling time been included, possibly 12 to 24 h later, an effect on CRP concentrations could have been detected. These observations highlight important areas for future research.

In summary, the findings of the present investigation suggest that 45 min moderate-intensity walking at ~ 66% $HR_{max\text{-}p}$ causes a decrease in IL-6 at 24 h post-exercise, and does not evoke a significant acute-phase response as measured by CRP concentrations. Whether the acute reduction in IL-6 translates into attenuated CVD risk is an important avenue for future work, given the potential benefits for public health.

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