

The Flow-mediated Dilation Response to Acute Exercise in Overweight Active and Inactive Men

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Objective: Inflammation has been found to play a role in the etiology of cardiovascular disease as well as provoke endothelial dysfunction. Inflammatory cytokines associated with endothelial function are interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α). IL-6 is exercise intensity dependent and has been shown to inhibit TNF- α expression directly. The aim of this study was to investigate the interaction of IL-6 and TNF- α on endothelial function in response to acute exercise in overweight men exhibiting different physical activity profiles.

Methods and Procedures: Using a randomized mixed factorial design, 16 overweight men (8 active, maximal exercise capacity ($\text{VO}_{2\text{peak}}$) = 34.2 ± 1.7 , BMI = 27.4 ± 0.7 and 8 inactive, $\text{VO}_{2\text{peak}}$ = 30.9 ± 1.2 , BMI = 29.3 ± 1.0) performed three different intensity acute exercise treatments. Brachial artery flow-mediated dilation (FMD) and subsequent blood samples were taken pre-exercise and 1 h following the cessation of exercise.

Results: Independent of exercise intensity, the active group displayed a 24% increase ($P = 0.034$) in FMD following acute exercise compared to a 32% decrease ($P = 0.010$) in the inactive group. Elevated ($P < 0.001$) concentrations of IL-6 following moderate (50% $\text{VO}_{2\text{peak}}$) and high (75% $\text{VO}_{2\text{peak}}$) intensity acute exercise were observed in both groups; however, concentrations of TNF- α were unchanged in response to acute exercise ($P = 0.584$).

Discussion: The FMD response to acute exercise is enhanced in active men who are overweight, whereas inactive men who are overweight exhibit an attenuated response. The interaction of IL-6 and TNF- α did not provide insight into the physiological mechanisms associated with the disparity of FMD observed between groups.

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INTRODUCTION

Obesity has emerged as a problematic epidemic in the United States and has recently been considered an independent risk factor for atherosclerotic cardiovascular disease (1). Atherosclerosis is a progressive inflammatory disease characterized by the accumulation of lipids and fibrous elements in medium to large arteries (2) and is a manifestation of endothelial dysfunction (3,4). Brachial artery flow-mediated dilation (FMD) is a measure of nitric oxide dependent vasodilation and is a non-invasive measurement of endothelial function. Unsurprisingly, individuals who are overweight or obese exhibit an increase in systemic inflammation (5) and are more likely to develop risk factors and comorbidities of cardiovascular disease. There appears to be a positive relationship between overweight and all-cause mortality in men (6); however, overweight men who are active *may* have a lesser risk for heart disease than their sedentary counterparts (7).

Inflammation has been found to play a role in the pathogenesis of coronary heart disease and provoke endothelial dysfunction (8,9). High sensitive C-reactive protein (CRP) (10), Tumor necrosis factor- α (TNF- α) (11), and

Interleukin-6 (IL-6) (12) are all bio-markers of systemic inflammation which have been associated with future atherosclerotic events. Recently, IL-6 has been proposed to provide anti-inflammatory effects (13) and accepts the role as a myokine (14), a cytokine secreted from active skeletal muscle. The production of IL-6 is exercise intensity dependent (13) and has been shown to inhibit TNF- α expression directly in cardiac muscle (15) and skeletal muscle (13). In a longitudinal study, Drenth and colleagues (16) found that exercise (training) increased IL-6, attenuated TNF- α , and reduced the acute inflammatory response. Consequently, the augmentation of FMD associated with acute exercise (17,18) may be linked to the exercise-induced IL-6-mediated TNF- α suppression, which has not been investigated. The physiological mechanisms underlying the anti-atherogenic effects of the exercise associated improvements in endothelial function remain ambiguous (19). The specific counteracting effects of IL-6 observed on TNF- α (13,15) have led us to believe that the exercise-induced increase in IL-6 may play a role in regulating the pro-inflammatory response and subsequent enhancement in FMD.

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The aim of this study was to investigate the interaction of IL-6 and TNF- α on the FMD response to acute exercise of different intensities in overweight men exhibiting different physical activity profiles. It was hypothesized that (i) the FMD response following acute exercise would be enhanced in overweight active men when compared to their inactive counterparts, (ii) baseline CRP and TNF- α would be elevated in the inactive men who are overweight compared to their active counterparts, (iii) IL-6 would exhibit a positive exercise intensity dependent association, which would decrease the concentrations of TNF- α and provide a possible mechanism associated with the improvement in FMD.

METHODS AND PROCEDURES

Experimental design

In a randomized mixed factorial experimental design, subjects performed three different acute exercise treatments, separated by at least 2 days apart. Before each exercise investigation day, subjects were instructed to report to the Indiana University Clinical Exercise Physiology Laboratory at 7:30 AM having (i) fasted from the night before, (ii) abstained from exercise for 24 h, and (iii) abstained from caffeine and tobacco for 12 h. Following an initial left upper extremity venous catheter placement, brachial artery FMD and subsequent blood samples were taken pre-exercise and 1 h following the cessation of exercise. Subjects were asked to remain in the laboratory and perform sedentary activities between measurements.

Subjects

Sixteen overweight men ages 46–68 participated in this investigation. Subjects were classified into either an active ($n = 8$) or inactive ($n = 8$) group. Overweight was defined as having a BMI ≥ 25 kg/m². Physically active was defined following the Surgeon General's guidelines (20); 30 min of moderate physical activity most days of the week. Subjects were excluded if they (i) had before diagnosis of cardiovascular disease, pulmonary disease, or diabetes, (ii) could not exercise at 75% VO_{2peak} for 45 min, (iii) had orthopedic problems that would limit their exercise, or (iv) were on any medications that influence vascular compliance. Subjects were asked to abstain from prophylactic aspirin and vitamin supplementation 3 days before the start of the study and throughout the investigation time period. All procedures were approved by Indiana University's Committee for the Protection of Human Subjects and the Institutional Biosafety Committee. Written informed consent was obtained from each subject before participation in the study.

Risk stratification and screening

Eligibility to participate in this investigation was determined by risk stratification. A risk stratification form detailing information of medical/family history of disease, height and weight, and current medications was completed by all subjects following initial informed consent. In addition, a fasting venous blood draw was performed to obtain CRP, total serum cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol. All subjects were reported to be non-smokers.

Maximal graded exercise test

The maximal graded exercise test was performed to measure the subjects' maximal exercise capacity (VO_{2peak}) and obtain each subject's individual exercise treatment intensity. Briefly, subjects walked at a pre-determined speed on a motor-driven treadmill with the grade increasing 2.5% every 2 min until volitional fatigue. Expired gases were collected into a mixing chamber through a unidirectional flow mouthpiece and analyzed using a Sensor Medics 2900 Metabolic Cart (Sensor Medics, Yorba Linda, CA). A maximal test was confirmed using the American College of Sports Medicine maximal exercise test criteria.

FMD acquisition/analysis

Endothelial function was measured via brachial artery FMD. FMD was measured pre-exercise and 1 h following the cessation of exercise. For each FMD measurement, subjects were instructed to lie supine in a climate-controlled room (22–24 °C) for 20 min to establish a hemodynamic steady state. The brachial artery was imaged longitudinally by a 2D high resolution Sonoace Pico ultrasound system (Universal Medical Systems, Bedford Hills, NY) using a 7.0 MHz linear transducer, placed 2–10 cm above the antecubital fossa. Once a clear image was obtained, the transducer was stabilized in a holder and the position was marked to ensure the same placement for each FMD measurement. The average diameter and blood velocity for 10 cardiac cycles were recorded and analyzed for baseline values. Following baseline measurements, A 5 × 84 cm forearm occlusion cuff (D.E. Hokanson, Bellevue, WA) was placed around the subjects' right forearm and rapidly inflated (E-20 rapid cuff inflator, D.E. Hokanson, Bellevue, WA) to induce occlusion for 5 min at 250 mm Hg and subsequent reactive hyperemia. Doppler measurements of peak hyperemic blood velocity were made during the first 10 s following cuff release at an isonation angle of 70° before switching back to continuous 2D ultrasound imaging for the remainder of the 2-min collection period. Electrocardiogram gating was used to capture end-diastolic arterial diameters, triggered by each QRS complex, which were analyzed using the Vascular Analysis Integrative System (Medical Imaging Applications, Coralville, IA). The highest 10-s averaged interval throughout the 2-min post occlusion collection period represented the peak hyperemic diameter. Hyperemic velocity and baseline diameter were converted to hyperemic local shear stress using the following equation (21): hyperemic local shear stress (arbitrary units) = $8 \times \mu \times V_H / D_{BL}$, where μ is blood viscosity, assumed to be 0.035 dyne × s/cm², V_H is peak hyperemic velocity, and D_{BL} is baseline diameter. FMD is expressed as a percent increase in diameter from baseline and calculated as: FMD = (peak hyperemic diameter – baseline diameter)/baseline diameter. All image acquisitions were performed by the same investigator. In addition, all measurement analyses were performed by the same investigator who was blinded to the exercise treatment.

Acute exercise treatments

Immediately following pre-exercise measurements, subjects performed either a low (25% VO_{2peak}), moderate (50% VO_{2peak}), or high (75% VO_{2peak}) intensity treadmill walking session for 45 min. Each exercise treatment was separated by at least 2 days to eliminate any training effect. Oxygen uptake (VO₂) was measured through a Sensor Medics 2900 metabolic cart between the 5th and 10th min of each exercise treatment to confirm the appropriate exercise intensity. The work rate was adjusted if the VO₂ was not within $\pm 5\%$ of the target exercise intensity. Expired gases were then measured again between the 10th and 15th min to confirm the new exercise intensity. Heart rate (electrocardiogram), blood pressure (BP) (auscultation), and rating of perceived exertion (Borg Scale 6–20) were measured every 5 min throughout each exercise session.

Laboratory procedures

All blood samples were collected from each subject and transferred into EDTA tubes after an overnight fast (pre-exercise) and 1 h following the cessation of exercise. All blood was centrifuged immediately at 1,000 × g for 15 min. The plasma supernatant was immediately transferred into microtubes and stored at –80 °C until analyzed. Concentrations of TNF- α and IL-6 were determined through enzyme immunoassay according to manufacturer's specifications (R & D Systems, Minneapolis, MN). The mean detection limits of the kits are 0.106 pg/ml and 0.039 pg/ml for TNF- α (4th generation) and IL-6 (3rd generation), respectively. All samples were run in triplicate. Sample concentrations that fell above a coefficient of variation of 20% were re-run. The mean concentration of each sample was used in the statistical analyses. Concentrations of CRP, triglycerides, cholesterol,

and lipid sub-fractions (high-density lipoprotein and low-density lipoprotein) were obtained via standard laboratory procedures and collected only for pre-investigation characteristic values.

Statistical analysis

Descriptive statistics and independent *t*-tests were used to compare subject demographics and exercise-induced physiological variables between groups. To determine the FMD, IL-6 and TNF- α response to acute exercise, comparisons were made using three-way (group \times intensity \times time) mixed factorial ANOVA's (SPSS, Chicago, IL: V 14.0). For any significant interactions, simple main effects were employed. When indicated by a significant *F*-ratio, Dunnett's post-hoc analysis was performed to identify differences. All data are expressed as mean \pm s.e.m. Statistical significance was set at $P < 0.05$.

RESULTS

Subject characteristics

Sixteen subjects were recruited for this investigation and placed into either an active ($n = 8$) or inactive ($n = 8$) group based on their physical activity profile. **Table 1** summarizes the subject's characteristics. The active group reported more physically active days per week when compared to the inactive

Table 1 Subject characteristics

Variables	Inactive ($n = 8$)	Active ($n = 8$)	<i>P</i>
Age (years)	56.9 \pm 2.6	59.9 \pm 2.8	ns
BMI (kg/m ²)	29.3 \pm 1.0	27.4 \pm 0.7	ns
Systolic blood pressure (mm Hg)	118.1 \pm 2.8	115 \pm 4.7	ns
Diastolic blood pressure (mm Hg)	79.4 \pm 2.7	73.3 \pm 2.3	ns
Physical activity (days/week)	0.7 \pm 0.3	4.4 \pm 0.5	<0.001
VO ₂ peak (ml/kg/min)	30.9 \pm 1.2	34.2 \pm 1.7	ns
Total cholesterol (mg/dl)	181.9 \pm 12.2	205.1 \pm 15.1	ns
HDL cholesterol (mg/dl)	48.0 \pm 5.5	54.3 \pm 5.3	ns
LDL cholesterol (mg/dl)	100.6 \pm 9.4	129.1 \pm 13.0	ns
Triglycerides (mg/dl)	166.8 \pm 27.0	108.4 \pm 15.8	ns
Fasting glucose (mg/dl)	95.0 \pm 1.6	92.4 \pm 3.2	ns
C-reactive protein (mg/l)	4.0 \pm 2.2	1.5 \pm 0.3	ns

Values are mean \pm s.e.m.

HDL, high-density lipoprotein; LDL, low-density lipoprotein; ns, not significant, VO₂peak, maximal exercise capacity.

Table 2 Physiological variables in response to acute exercise

Variables	Low (25% VO ₂ peak)		Moderate (50% VO ₂ peak)		High (75% VO ₂ peak)	
	Inactive	Active	Inactive	Active	Inactive	Active
Heart rate (bpm)	87.8 \pm 3.6	75.5 \pm 2.9	113.9 \pm 5.3	105.4 \pm 3.3	143.1 \pm 4.3	139.4 \pm 4.1
Systolic blood pressure (mm Hg)	122.8 \pm 4.7	126 \pm 6.3	142.4 \pm 5.8	132.1 \pm 5.6	164.6 \pm 6.7	157.6 \pm 88.2
Diastolic blood pressure (mm Hg)	78.6 \pm 4.1	74.1 \pm 3.8	74.5 \pm 3.3	70.5 \pm 2.0	75.1 \pm 4.0	69.9 \pm 3.0
Rating of perceived exertion	8.0 \pm 0.4	7.7 \pm 0.2	11.3 \pm 0.6	11.4 \pm 0.6	14.2 \pm 0.5	13.7 \pm 0.4
VO ₂ (ml/kg/min)	8.0 \pm 0.2	8.7 \pm 0.4	15.6 \pm 0.7	17.3 \pm 0.8	23.3 \pm 1.1	25.8 \pm 1.3
VO ₂ (%)	26.1 \pm 0.5	25.6 \pm 0.6	50.4 \pm 0.5	50.5 \pm 0.4	75.2 \pm 1.0	75.4 \pm 0.6

Values are mean \pm s.e.m.

VO₂peak, maximal exercise capacity.

group (4.4 \pm 0.5 vs. 0.7 \pm 0.3; $P < 0.001$), whereas all other physical characteristics were similar ($P > 0.05$) between these two groups.

Heart rate, BP, rating of perceived exertion, and VO₂ in response to acute exercise

Table 2 illustrates the heart rate, BP, rating of perceived exertion, and oxygen consumption (VO₂) in response to acute exercise of different intensities. Oxygen consumption is presented as the absolute VO₂ obtained in response to acute exercise, as well as the VO₂ obtained during exercise expressed as a percentage of VO₂peak. No differences ($P > 0.05$) in exercise-induced physiological variables were identified among acute exercise intensities between groups.

FMD in response to acute exercise

Figure 1 illustrates the effect of exercise intensity on the FMD response 1 h following the cessation of exercise in overweight men who are active and inactive. Although exercise intensity did not influence the FMD response, a significant ($F_{1,14} = 14.22$; $P = 0.002$) group \times time interaction indicated the two groups responded differently. Pre-exercise FMD was similar ($F_{1,14} = 0.21$; $P = 0.654$) between groups; therefore, the FMD values were normalized to reflect the absolute response to exercise. **Figure 2** illustrates the group \times time interaction for post-exercise FMD normalized to pre-exercise values. Collapsing for exercise intensity, the active group displayed a 24% increase ($F_{1,14} = 5.49$; $P = 0.034$) in FMD following acute exercise compared to a 32% decrease ($F_{1,14} = 8.95$; $P = 0.010$) in the inactive group. Baseline arterial diameters, baseline and hyperemic blood velocities, and hyperemic local shear stress are presented in **Table 3**. For both groups, baseline diameters decreased ($P < 0.05$) following the low intensity acute exercise, no change ($P > 0.05$) was observed for the moderate intensity exercise and an increase ($P < 0.05$) following high intensity acute exercise was observed. In addition, baseline blood velocity was decreased ($P < 0.05$) 1 h following exercise in both groups collapsing for exercise intensity. The active group exhibited a decrease ($P < 0.05$) in hyperemic velocity following acute exercise which was independent of exercise intensity, whereas no change was observed in the inactive group. It is important to note that no differences ($P > 0.05$) in hyperemic local shear stress upon cuff release, a stimulus

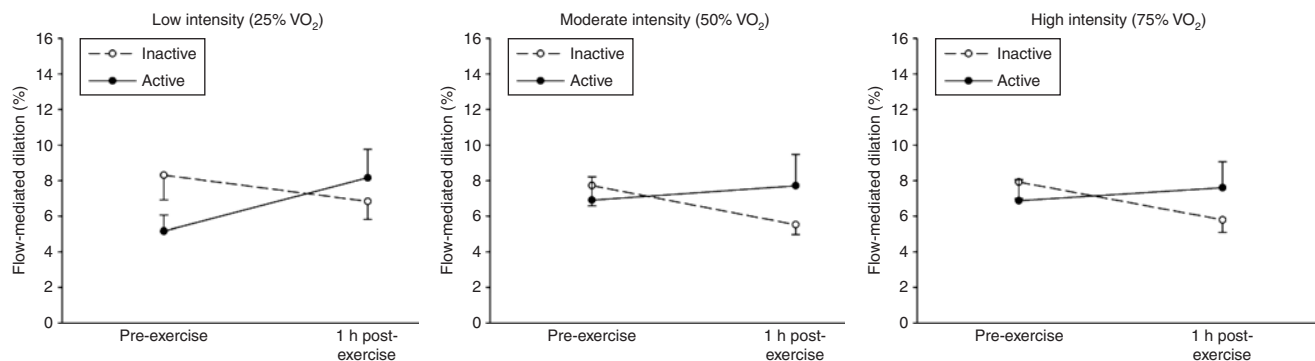


Figure 1 The effect of exercise intensity on the flow-mediated dilation (FMD) response 1 h following the cessation of exercise between groups. Note: A significant group \times time interaction exists independent of exercise intensity.

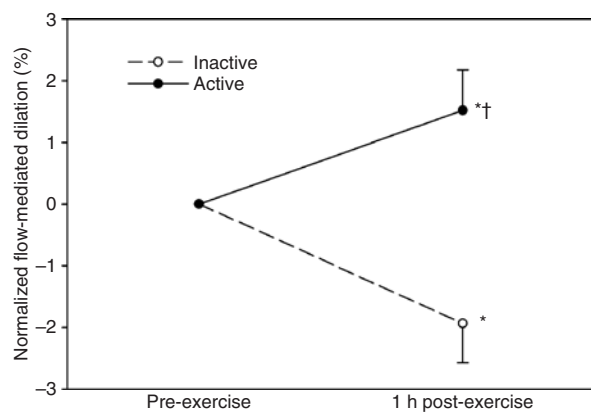


Figure 2 Illustration of the group \times time interaction, collapsing for exercise intensity, of flow-mediated dilation (FMD) normalized to pre-exercise values. Asterisk denotes significant from pre-exercise values. Dagger denotes significantly different from the inactive group.

for vasodilation, were observed for either group throughout the analysis.

IL-6 in response to acute exercise

The mean coefficient of variation for all IL-6 samples run was $8.7 \pm 1.5\%$. Pre-exercise concentrations of IL-6 were similar ($F_{1,14} = 1.38$; $P = 0.259$) between the active and inactive groups (1.3 ± 0.3 pg/ml vs. 1.8 ± 0.3 pg/ml, respectively). An ANOVA indicated a significant ($F_{2,28} = 27.17$; $P < 0.001$) intensity \times time interaction for the IL-6 response to exercise. **Figure 3** illustrates, for both groups, a significant increase in plasma concentrations of IL-6 following moderate and high intensity acute exercise ($F_{1,28} = 27.32$; $P < 0.001$ and $F_{1,28} = 99.61$; $P < 0.001$, respectively), whereas no change ($F_{1,28} = 0.19$; $P = 0.669$) in IL-6 was observed following low intensity exercise.

TNF- α in response to acute exercise

The mean coefficient of variation for all TNF- α samples run was $10.8 \pm 1.6\%$. No differences ($F_{1,14} = 0.31$; $P = 0.584$) in pre-exercise concentrations between groups were observed. In addition, no change ($F_{1,14} = 0.00$; $P = 0.983$) in plasma TNF- α in response to acute exercise was observed in either group. **Figure 4** illustrates plasma concentrations of TNF- α in response to different intensities of acute exercise between groups.

DISCUSSION

The aim of this study was to investigate the interaction of IL-6 and TNF- α on the FMD response to acute exercise of different intensities in overweight men exhibiting different physical activity profiles. Our findings support the main hypothesis that FMD in response to acute exercise would be enhanced in overweight active men when compared to their inactive counterparts; however, the pro- and anti-inflammatory state pre- as well as post-exercise may not explain the disparity in FMD observed between groups.

It is important to note the significant changes in brachial artery characteristics following acute exercise. A change in baseline diameter following low and high intensity exercise was observed in both groups. We are unable to explain the unexpected slight but significant vasoconstriction observed following low-intensity exercise. Perhaps this physiological response may be a consequence of the waiting room climate. In addition, we feel that there are no physiological implications associated with this slight change in diameter. Furthermore, the change in baseline blood velocity among exercise intensities was similar between groups. One would expect an enhanced FMD following an increase in hyperemic velocity; however, a decrease in hyperemic velocity, collapsing for exercise intensity, was observed only in the active group. The significant changes in brachial artery characteristics following acute exercise do not explain the disparity of FMD observed in this study and should not discount the robustness of our results.

Subjects were classified as either active or inactive based on the Surgeon General's guidelines (20). In addition, evidence supports the use of a single self-reported physical activity question as a method to classify subjects as either active or inactive (22). Although VO_2 peak was similar between these two groups, a reduced likelihood for developing cardiovascular disease has been observed following moderate habitual physical activity with no modification in VO_2 peak (23,24).

It was hypothesized that the state of global systemic inflammation would differ between overweight men who exhibited different physical activity profiles. Although the baseline characteristic of CRP does not exhibit a statistical difference, there appears to be a trend toward separation between the two groups. The disparity (non-significant) in CRP, BP, and

Table 3 Pre- and post-exercise artery characteristics by group

Variables	Low (25% VO ₂ peak)		Moderate (50% VO ₂ peak)		High (75%VO ₂ peak)	
	Pre-exercise	1 h post	Pre-exercise	1 h post	Pre-exercise	1 h post
Inactive group (n = 8)						
Baseline diameters (mm)	4.21 ± 0.12	4.15 ± 0.18 ¹	4.08 ± 0.16	4.12 ± 0.18	4.04 ± 0.15	4.30 ± 0.19 ¹
Baseline velocity (cm/s)	21.0 ± 3.4	16.5 ± 3.2 ¹	20.5 ± 2.4	18.5 ± 2.4 ¹	22.2 ± 2.6	21.5 ± 3.0 ¹
Peak hyperemic velocity (cm/s)	107.0 ± 3.8	99.1 ± 8.0	96.2 ± 8.1	104.4 ± 5.6	98.9 ± 4.0	103.2 ± 5.4
Hyperemic local shear stress (AU)	7.2 ± 0.4	6.8 ± 0.7	6.7 ± 0.7	7.3 ± 0.7	6.9 ± 0.4	6.8 ± 0.4
Active group (n = 8)						
Baseline diameters (mm)	4.16 ± 0.16	4.00 ± 0.14 ¹	4.04 ± 0.15	4.04 ± 0.13	4.02 ± 0.15	4.09 ± 0.17 ¹
Baseline velocity (cm/s)	18.5 ± 2.2	14.2 ± 2.1 ¹	18.5 ± 3.6	13.4 ± 1.2 ¹	15.1 ± 1.9	13.6 ± 1.1 ¹
Peak hyperemic velocity (cm/s)	83.7 ± 7.7	77.2 ± 6.3 ¹	89.3 ± 7.9	82.5 ± 9.0 ¹	99.0 ± 8.0	78.5 ± 6.5 ¹
Hyperemic local shear stress (AU)	5.7 ± 0.6	5.5 ± 0.5	6.3 ± 0.7	5.7 ± 0.6	7.0 ± 0.6	5.5 ± 0.6

Values are mean ± s.e.m.

AU, arbitrary unit; VO₂peak, maximal exercise capacity.

¹Significant from pre-exercise.

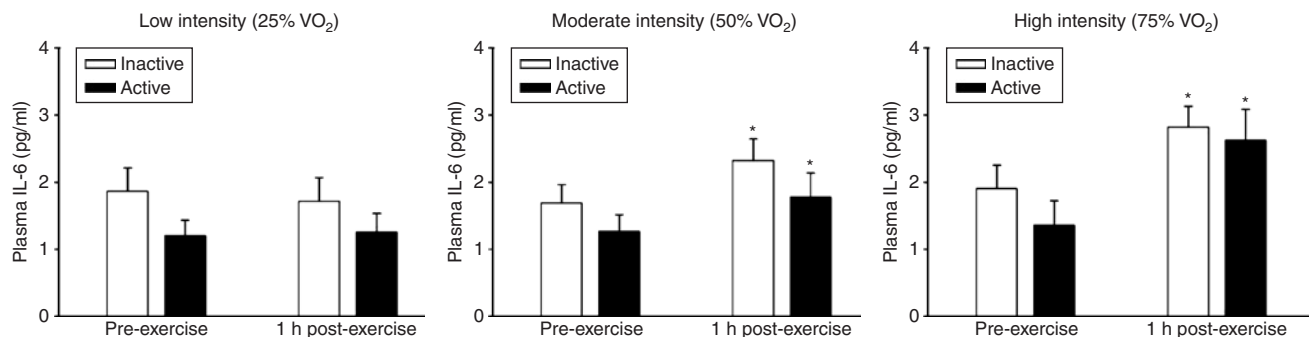


Figure 3 The effect of acute exercise intensity on plasma concentrations of interleukin-6 (IL-6) between groups. Asterisk denotes significant from pre-exercise values.

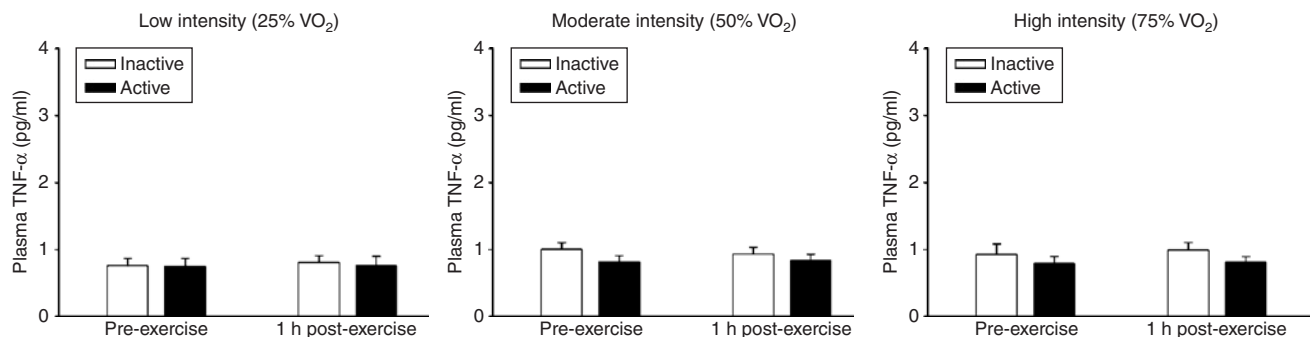


Figure 4 The effect of acute exercise intensity on plasma concentrations of tumor necrosis factor-α (TNF-α) between groups.

triglycerides observed between groups may in fact be reflected of subject's physical activity profile, body fat distribution, or a combination thereof. Elevated BMI, the only measure of body composition performed in this study, is associated with higher concentrations of CRP (5). In addition, contrary to our original hypothesis, there is no difference in the pre-exercise concentrations of TNF-α between groups. This likeness in TNF-α may be explained by a recent study by Van Guilder and colleagues (25) who found similar concentrations of TNF-α between normal weight and obese subjects; however, the obese who presented

with metabolic syndrome exhibited significantly elevated concentrations of TNF-α when compared to the normal weight subjects and the obese without metabolic syndrome. Moreover, the similar TNF-α response to acute exercise leads us to believe that the change in FMD observed in overweight men is independent of this plasma marker of inflammation.

TNF-α is postulated to be the first responder of the pro-inflammatory cytokine family, whereas IL-6 is typically the last responder (16). The production of IL-6 during exercise is related to the intensity and duration of the exercise (13). The

findings of this study strongly support this phenomenon. It is possible that the increase in IL-6 in response to moderate and high intensity exercise inhibited the TNF- α response; however, the response of IL-6 and TNF- α to acute exercise was similar in both groups, which does not explain the different response in endothelial function. Although no changes in plasma TNF- α were observed, evidence supports an increase in myocardial TNF- α expression immediately following an acute bout of moderate intensity exercise of similar duration (26). It is important to note that a possible increase in TNF- α may have appeared before the 1 h post-exercise time period evaluated in this study; again, the similar cytokine concentrations pre- and post-exercise observed between the two groups would suggest the timing of the post-exercise measurement did not influence the difference in FMD observed. There are many benefits from habitual exercise that may elucidate cardioprotective effects and explain the different FMD response observed between the two groups.

There is substantial evidence suggesting that habitual exercise enhances FMD in healthy as well as clinical populations (27,28). Exercise training has been shown to augment blood flow and shear stress, which in turn increases nitric oxide production, leads to an increase in nitric oxide bioavailability (29), and enhances endothelial function (30). A strong association exists between inflammation and training intensity (31,32) which may have an impact on the FMD response to exercise. In this study, both groups received the same relative exercise intensity (stimuli) which is evident by the similar response of physiological variables. Goto and colleagues (33) have found an improvement in endothelial function following moderate intensity exercise training, whereas no change following low or high intensity training was discovered. Although this study investigated the FMD in response to *acute* exercise; low, moderate, and high intensity elicited similar FMD. Evidence suggests that an acute bout of exercise can reduce triglycerides, reduce BP, increase high-density lipoprotein cholesterol, improve insulin sensitivity, and improve glucose homeostasis (34); therefore, utilizing an acute exercise model to investigate the anti-atherogenic effects in endothelial function is supported. In general, the findings of this investigation support the school of thought that being fit in overweight may reduce the hazards of obesity (6,35,36). More specifically, habitual exercise may reduce vascular expression of nicotinamide adenine dinucleotide phosphate oxidase which results in decreased local reactive oxygen species generation (37), increase antioxidant status (38), and/or improve insulin sensitivity (39).

The increase in oxygen uptake during acute exercise produces an increase in oxidative stress (OS) (40), which has been shown to impair endothelial function (41). The acute exercise-induced OS may conceptually appear to contradict the enhancement in FMD observed following exercise (17,18). Evidence supports an exacerbated OS response to a single bout of exercise in obese subjects (40); however, active individuals have a greater resistance to acute exercise-induced OS (38). The extent of oxidative damage is not only a factor of reactive oxygen species generation, but also the capacity

of antioxidant defense (38). The resistance to acute exercise-induced reactive oxygen species may be through the mechanism of (exercise) training-induced enhanced antioxidant capacity (38). Although, subjects were instructed to abstain from vitamin supplementation throughout this investigation, the dietary influence of antioxidants between the active and inactive overweight men is unknown and may have influenced the FMD response. The balance of pro- and antioxidant status may provide explanation to the endothelial function observed in response to acute exercise in this study. In addition, the improvement in insulin sensitivity associated with habitual exercise in obesity may be related to changes in skeletal muscle fatty acid metabolism and the enhancement of post absorptive fat oxidation (42). We can only speculate to the possible mechanism(s) involved in the different FMD responses observed in this study. The physiological adaptations proposed may support the potential mechanism(s) associated with our findings; however, to our knowledge they have not been investigated in overweight or obesity following an oxidative challenge such as acute exercise.

In conclusion, this study is the first of its kind to evaluate the FMD response to acute exercise of different intensities in overweight men. Our findings support the hypothesis that the FMD response to acute exercise is enhanced in overweight active men when compared to the attenuation observed in their inactive counterparts; however, the interaction of IL-6 and TNF- α does not support the disparity in FMD observed in response to acute exercise. In general, it appears that physical activity profile is an independent determinant of the FMD response to acute exercise observed between the two groups. More specifically, habitual physical activity may result in an improvement in OS, antioxidant capacity, insulin resistance, and/or a combination of all three which may explain our results; however, future investigations of this kind in overweight and obesity are warranted.

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DISCLOSURE

The authors declared no conflict of interest.

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