Original Article

Inflammatory biomarkers are unrelated to endothelial-mediated vasodilation in physically active young men

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ABSTRACT

Aron A, Hargens TA, Guill SG, Herbert WG. Inflammatory biomarkers are unrelated to endothelial-mediated vasodilation in physically active young men. *J. Hum. Sport Exerc.* Vol. 7, No. 2, pp. 581-588, 2012. Endothelial dysfunction has an important role in genesis of atherosclerosis and is depicted by a series of inflammatory and endothelial biomarkers. Shear stress arising from repeated episodes of increased blood flow with physical activity (PA) is a possible mechanism that improves vascular endothelial function. Our purpose was to examine whether inflammatory markers mediate the association between PA and endothelial function. Subjects were young, healthy men recruited according to recreational PA habits: high-active (n=21) vs. sedentary (n=17). Active subjects reported >45 min/day of moderate-vigorous physical activities, >4 days/week over 6 months, while sedentary subjects reported no recreational physical activity. Fasting serum samples were analyzed for C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). Endothelial function was determined using flow-mediated dilation of the brachial artery induced by post ischemic reactive hyperemia. Hyperemia response was greater in the high-active than in sedentary men (30.2±8.2 vs. 24.3±5.2 mL/min/100mL; P<0.001). There were no differences between the groups with respect to CRP, TNF-α, or IL-6. Concentrations of these inflammatory biomarkers were unrelated to reactive hyperemia differences attributable to PA. Improved hyperemic response seen in young physically active subjects may be influenced by factors beyond the inflammatory factors, e.g., enhanced nitric oxide production. Physical activity was associated with an increased vascular function in young adults, although a diminished inflammatory state was no revealed. Additional research is needed to clarify the role of PA on cytokine indicators of inflammation and how this relates to endothelial function. **Key words**: ENDOTHELIAL DYSFUNCTION, CRP, TNF-A, IL-6, PHYSICAL ACTIVITY

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INTRODUCTION

Coronary artery disease (CAD) is considered one of the main causes of death and disability leading to high health care expenses in developed countries. CAD is characterized by a long asymptomatic phase of development, which starts as early as the first decade of life and progresses eventually to the formation of atherosclerotic plaques. Evidence suggests that endothelial dysfunction occurs early in the process of atherogenesis and precedes the development of morphological atherosclerotic changes that can contribute to lesion development and later clinical complications (Ross, 1993).

Subjects with known cardiovascular disease (CVD) (Schroeder et al., 1999), or even cardiovascular risk factors (Benjamin et al., 2004), exhibit impaired endothelium-dependent vasomotor responses. Pharmacological interventions are associated with reduction in cardiovascular mortality and morbidity by improving endothelial function (Jarvisalo et al., 1999). Animal (Niebauer et al., 1999) and human (DeSouza et al., 2000) studies suggest that exercise training can enhance endothelium-dependent vasodilation in the forearm in healthy subjects and patients with chronic heart failure (Hornig et al., 1996). Specifically, animal studies report that the increase in arterial wall shear stress during exercise results in increased nitric oxide-synthase expression and enhanced vasodilator capacity (Sun et al., 1994). Human studies suggest that exercise training leads to sustained whole-body improvement of vascular function, not only within vascular beds of the trained muscle (Green et al., 2002). This relationship is independent of adiposity and the underlying mechanism may be modulated by suppression in systemic inflammation (Steiner et al., 2005). It is speculated that physical inactivity causes a proinflammatory profile as regular muscle contractions mediate signals using messengers to suppress proinflammatory activities at distant sites as well as within the active skeletal muscle (Bruunsgaard, 2005). A high self-reported degree of physical activity is associated with attenuated circulating levels of TNF-α, IL-6 and CRP compared with those devoted to a sedentary lifestyle, independently of gender, age, smoking habits, BMI, total cholesterol, blood glucose, and blood pressure (Panagiotakos et al., 2005).

Venous occlusion plethysmography, a simple non-invasive method for testing endothelial function, provides the ability to detect subtle vascular abnormalities in asymptomatic patients with classic cardiovascular risk factors (Dakak et al., 1998). This method has been widely used to study the effect of several cardiovascular risk factors on endothelial function such as hypercholesterolemia, diabetes mellitus, cigarette smoking and aging (Taddei et al., 1995; Makimattila et al., 1999).

To determine if physical activity influences endothelial function in young healthy men, we measured responses of forearm blood flow to reactive hyperemia, an index of endothelium-dependent vasodilation. Furthermore, we examined whether inflammation could impact this association in a young adult population.

METHODS

Subjects

Thirty-eight young (age = 20.8 ± 2.2 yr), normal weight (BMI = 22.4 ± 1.6 kg/m^2) men were recruited to participate in this study. Participants were free of any known cardiovascular or metabolic diseases. Diabetics, asthmatics and individuals taking any medication that may affect cardiovascular function were excluded from participation. Subjects with smoking history and individuals with acute medical conditions (e.g., orthopedic injury), active infection and/or on pharmacotherapy with known vascular effects (e.g., anti-inflammatory therapy, cardiovascular medications) were disqualified. According to recreational physical activity habits, subjects were divided in high-active (n = 21) vs. sedentary (n = 17) groups. Active subjects

reported more than 45 min/day of moderate-vigorous physical activities, for more than 4 days/week, over the last 6 months, while sedentary subjects reported no moderate intense physical activity for the same period. The study was approved by the Institutional Review Board of Virginia Polytechnic Institute and State University.

Anthropometrics

Body mass (kg) and height (cm) were used to calculate Body Mass Index (BMI, kg/cm^2). Measures were obtained while subjects were in lightweight clothing and socks. Total body fat was quantified using dual energy x-ray absorptiometry (DXA) (Hologic QDR 4500A. Bedford, MA). All measurements from DXA were obtained and analyzed by one investigator with weekly scans of an external soft tissue bar being used to calibrate the DXA measurements.

Venous occlusion plethysmography

Subjects were instructed to refrain from food, alcohol or caffeine for 12 hours, and vigorous physical activity for 24 hours before the procedures. Upon arrival, each rested in a supine position in a quiet, dark, air-conditioned room (constant temperature, 22°C to 24°C) and blood pressure was measured. Prior to evaluation of their basal forearm blood flow, subjects maintained the supine resting position for 10 minutes. During this time, blood pressure cuffs were positioned around the left upper arm and wrist, and a mercury-in-silastic strain gauge was placed at the widest part of the forearm. The strain gauge was connected to a plethysmograph (EC-5R system, Hokanson, Inc; Bellevue, WA). The forearm was extended, slightly supinated and supported by a foam block ensuring the arm was above heart level. Immediately before the blood flow measurements, hand circulation was occluded by inflating the wrist cuff to a pressure 50 mm Hg greater than the subject's measured systolic blood pressure. The upper arm cuff was inflated to 50 mm Hg for 7 seconds in each 15-second cycle to occlude venous outflow from the arm, using a rapid cuff inflator (EC-20, Hokanson, Inc; Bellevue, WA). Resting blood flow was determined by calculation of the mean of middle three measurements taken within 2 min. The forearm blood flow response during reactive hyperemia was evaluated. Arterial occlusion was achieved by inflating the cuff on the upper arm to 50 mm Hg above systolic blood pressure for 5 minutes. After release of the upper arm cuff, forearm blood flow was measured every 15 seconds for 3 minutes. Following the test all readings were manually analyzed to eliminate any cuff artifacts using proprietary software (NIVP3 version 2.9, Hokanson, Bellevue, WA). Blood flow was expressed as milliliters per minute per 100 ml of forearm tissue volume.

Biochemical analyses

Blood samples were obtained by venipuncture following an overnight fast. After the blood collection, plasma and serum were separated via centrifugation and frozen at -80°C for batch analysis upon completion of human testing. All biochemical analyses were performed with commercially available kits, according to the manufacturers' protocols. Serum TNF-α was measured by high sensitive enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN). Plasma CRP and Il-6 concentration levels were quantified via a custom, multiplex sandwich ELISA (SearchLight, Pierce Biotechnology, Rockford, IL.). Interassay coefficients of variation were 10.4% for CRP, 9.2% for IL-6, and 8.6% for TNF- α.

Exercise testing

Each subject performed a maximal cycle exercise test on an electronically-braked ergometer (Ergometrics 800, SensorMedics, Yorba Linda, CA). Subjects began 1-minute warm-up by pedaling at a self-selected rate between 50–80 rpm with a constant load of 25 W/min. Load was increased automatically by 15 W/min until volitional fatigue occurred. The treadmill tests were terminated according to the published guidelines (American College of Sports Medicine, 2006).

Statistical analysis

Statistical analyses were performed using SPSS for Windows, version 17.0 (SPSS Statistical Software, Chicago, IL). Data for descriptive characteristics and peak aerobic power are presented as mean ± SD and independent t-tests were used to evaluate group differences in these measures. Forearm reactive hyperemia was compared between the two groups using repeated measures analysis of variance (ANOVA). Further ANOVA was used to compare the inflammatory markers between groups. The difference in CRP, IL-6 and TNF-α between active and sedentary groups adjusted for total body fat percentage was evaluated using analysis of covariance. Pearson correlation was used to examine the relationships between reactive hyperemia and inflammatory cytokines. Significance level was set a priori at 0.05.

RESULTS

The subject characteristics are shown in Table 1. Both groups were similar with respect to age, height, weight and BMI. Sedentary subjects had a lower VO_2peak compared with the active group (34.4 ± 7.0 vs. 44.1 ± 9.5 ml/kg/min; P = 0.001). The sedentary group maintained higher body fat than the physically active group (18.9 ± 4.9 % vs. 14.0 ± 3.0 %; P = 0.001), but the groups did not differ on BMI. Early hyperemic response, immediately after release of the pressurized cuff (0 sec), was ~33% higher (P = 0.01) in the active group but not different from the sedentary group at any time point thereafter (Figure 1). Serum TNF-α levels did not differ between groups, 0.86 ± 0.2 for sedentary vs. 0.84 ± 0.2 ng/mL for active (P = 0.7). The other two proinflammatory cytokines showed similar values in both groups, 0.54 ± 0.6 mg/L for CRP and 3.99 ± 2.8 ng/mL for IL-6 and 0.9 ± 1.3 mg/L for CRP and 3.5 ± 2.9 ng/mL for IL-6 in sedentary and active groups, respectively (Figure 2). Post-occlusion reactive hyperemia did not correlate with CRP (R = 0.001, P = 0.99), IL6 (R = 0.06, P = 0.72) or TNF-α (R = 0.02, P = 0.89). Our analysis adjusted for fat percentage did not reveal any differences in plasma levels of inflammatory markers between the two groups (P = 0.97).

Table 1. *Participant characteristics.*

	Active (N=21)	**Sedentary (N=17)**	***P***
Age (years)	20.7 ± 2.5	21.0 ± 2.1	0.65
Weight (kg)	71.1 ± 8.1	70.4 ± 7.4	0.78
Height (cm)	177.5 ± 6.7	176.7 ± 6.1	0.71
BMI (kg/m^2)	22.5 ± 1.8	22.4 ± 1.6	0.76
Total body fat (%)	14.0 ± 3.0	18.9 ± 4.9	0.001
VO_{2peak} (ml · kg^{-1} · min^{-1})	44.1 ± 9.5	34.4 ± 7.0	0.001

DISCUSSION

In this study, we examined how physical activity could have an impact on forearm vasodilatory response to reactive hyperemia and inflammatory process. Active subjects had significantly higher mean hyperemic response compared with sedentary individuals.

Endothelial dysfunction has been linked to many forms of vascular disease development, including advanced atherosclerosis, hypertension, and a diminished angiogenic response (Widlansky et al., 2003). Inflammation has been demonstrated to be an important contributor to endothelial dysfunction. A recent study (Fichtlscherer et al., 2000) reported that high CRP serum levels were associated with blunted systemic endothelial vasodilator function in patients with coronary artery disease and that this relationship was transitory as the inflammation reduces. There is evidence suggesting a correlation between levels of CRP and lower brachial artery flow-mediated dilation (Brevetti et al., 2003). Even in healthy volunteers, induced inflammation causes endothelial dysfunction (Bhagat et al., 1996). These authors showed that a very brief exposure to endotoxin impaired endothelium-dependent relaxation for many days, and the degree of the impairment was much greater than that produced by chronic risk factors.

Our positive correlation between high levels of habitual physical activity and post ischemic reactive hyperemia has been confirmed in previous studies involving old (Rinder et al., 2000) and young (Palmieri et al., 2005) healthy individuals. The mechanisms responsible for these exercise benefits can derive from the enhanced nitric oxide production through augmented shear stress (Tinken et al., 2010) or lower pro-inflammatory states (Ribeiro et al., 2010). This second mechanism did not seem to work in our study of young apparently healthy men, as we did not observe any relation between the level of habitual leisure time PA and any markers of vascular inflammation. Serum and plasma levels of CRP, IL6 and TNF-α were not different between the active and sedentary groups. These findings are in conflict with previous studies that have reported an independent association between PA and lower levels of fibrinogen, CRP and other blood markers of inflammation (Pitsavos et al., 2003). These discrepancies are possibly due to the younger age of our subjects compared to previous studies. Several other studies have reported a lack of association between inflammatory factors and physical activity in a population even younger than ours (Kelly et al., 2007; Thomas et al., 2008). One possible explanation was the body weight or body composition confounding influence as certain adipokines and inflammatory markers are stimulated by adipose tissue. Although our sedentary subjects had a higher body fat percentage than the active group, there were still no differences in plasma levels of inflammatory markers between the groups after adjusting for total body fat percentage. Thus, we believe, a more considerable change in adiposity is required before inflammatory cytokines can be altered in young adults. Furthermore, two previous studies found that inflammation, as assessed by multiple inflammatory markers, was not related to measures of endothelial function once results were adjusted for effects of traditional cardiovascular risk factors (Kathiresan et al., 2006). This hypothesis cannot be applied to present study as our subjects were free of any CVD and they were closely matched in age and weight. In young men, the impact of physical activity on endothelial function may not be prominently influenced by low inflammation status. Physical activity modulates systemic inflammation by multiple mechanisms, including decreases in cytokine production by adipose tissue, reduction in skeletal muscle cytokines expression or attenuation in mononuclear cell production of atherogenic cytokines (Kasapis & Thompson, 2005). These mechanisms may require exposure for a longer time to high levels of physical exercise in order to initiate changes in inflammatory cytokines.

There are several limitations to this study. The cross-sectional design of this study cannot establish causality but only generate hypotheses. Another limitation of the present study concerns the technique used for assessing endothelial function. Measurements of endothelial function using intra-arterial agonist infusion are now regarded as more definitive. However, the noninvasive technique of reactive hyperemia we employed is a widely used method for assessing the resistance vessels' endothelial function, as is safe and reproducible (Alomari et al., 2004). In our study, as well as in most of the epidemiological studies, physical activity was measured by self-reported leisure time physical activity. These questionnaires only

provided course estimates of intensity and duration of physical activity, with no information as to specific types of activity.

CONCLUSIONS

As a response to high volume of physical activity, young adult men displayed an improved hyperemic response. This response was unrelated to subject's status for inflammatory cytokines. Further work is needed to identify how inflammatory pathways are linked to physical activity in young adults and to determine the pathophysiological mechanisms underlying such an association.

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