Associations of monocytes, neutrophil count, and C-reactive protein with maximal oxygen uptake in overweight women☆

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Received 4 April 2008; received in revised form 11 June 2008; accepted 8 July 2008
Available online 10 September 2008

Keywords
Monocyte count;
Neutrophil count;
High-sensitivity C-reactive protein;
Maximal oxygen uptake

Summary
Background: Increased aerobic capacity can reduce the incidence of cardiovascular disease and mortality. Inflammation plays a pivotal role in the pathogenesis of atherosclerosis.
Aims: This study was designed to elucidate whether aerobic capacity is associated with inflammatory status.
Methods: The subjects included 90 overweight women [age, 51.5 ± 10.7 yrs; body mass index (BMI), 28.1 ± 2.5] with coronary risk factors who were outpatients at our institution. A multistage graded submaximal exercise stress test was performed on an electric bicycle ergometer to determine the estimated maximal oxygen uptake (˙VO2 max).
Results: Univariate regression analyses showed that monocyte, neutrophil counts, and high sensitivity C-reactive protein (hs-CRP) levels were correlated with fasting triglycerides, fasting insulin, BMI, and waist circumference, while ˙VO2 max was negatively associated with monocyte and neutrophil counts, but not with hs-CRP. Stepwise multivariate regression analysis demonstrated a strong association of monocyte count with the fasting triglyceride and ˙VO2 max (r² = 0.260, p < 0.0001). Neutrophil count was also found to be associated with fasting triglyceride and fasting insulin (r² = 0.114, p < 0.0001), while hs-CRP was associated with fasting triglyceride and waist circumference (r² = 0.151, p < 0.0001).

☆ Notice of grant support: This study was supported in part by a grant from the Japanese Ministry of Education, Culture, Sports, Science and Technology (No. 16500445).
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0914-5087/$ — see front matter © 2008 Published by Elsevier Ireland Ltd on behalf of Japanese College of Cardiology.
doi:10.1016/j.jjcc.2008.07.010
Conclusions: These results suggest that $\dot{V}O_2$ max is a sensitive factor that reflects the inflammatory status and might support cardiovascular protective effects of aerobic exercise in overweight women.

Introduction

It is well known that physical inactivity is associated with a significantly higher incidence of coronary artery disease (CAD) [1], and that increases in aerobic capacity can reduce the incidence of CAD and mortality [1,2]. On the other hand, there is accumulating evidence that inflammation plays a pivotal role in the pathogenesis of various stages of atherosclerosis, including the initiation and progression of atheroma formation, plaque instability and rupture, and post-angioplasty restenosis [3–8]. Adhesion of leukocytes, neutrophils, and monocytes to the endothelium, induced by cell adhesion molecules, and subsequent migration of leukocytes across the endothelium into the vessel wall are characteristic steps in the process of inflammation [6–9]. In addition, white blood cell count has recently been shown to be a predictor of cardiovascular events [10] and there is also evidence that a reduction in body weight decreases the levels of inflammatory markers [11,12]. Although several studies have reported that lower physical activity levels are associated with higher levels of inflammatory markers such as high-sensitivity CRP (hs-CRP) and white blood cell counts [13–15], Rawson et al. [16] reported that hs-CRP levels correlated with body mass index (BMI), but not with physical activity levels. Therefore, at present, the association between inflammatory markers and physical activity remains controversial.

Our hypothesis is that an increase in aerobic capacity may have cardiovascular protective effects by inhibiting inflammatory processes. This study was designed to investigate the relationship between aerobic capacity and inflammatory markers such as white blood cell count and hs-CRP in overweight women.

Subjects and methods

Subjects

The study involved enrollment of 90 overweight female patients [age, 51.5 ± 10.7 yrs; BMI, 28.1 ± 2.5 kg/m²] with coronary risk factors, including hypertension, impaired glucose tolerance, or dyslipidemia. A breakdown of patients' coronary risk factors was as follows: 33 hypertension; 20 impaired glucose tolerance; and 49 dyslipidemia. Patients taking cardioactive drugs such as anti-hypertensive drugs, statins, or hypoglycemic agents, or patients with a history of cerebrovascular disease, CAD, or inflammatory diseases and habitual smokers were excluded from the study. The local institutional review board approved the study protocol, and written informed consent was obtained from each patient prior to commencement of the study.

Blood sampling and anthropometric measurements

Blood samples were collected early in the morning by venipuncture from an antecubital vein, after at least 12 h fasting. The fasting blood samples were used to measure the following parameters: monocyte and neutrophil counts measured by the mirror examination method, hs-CRP levels by the high sensitivity latex photometric immunoassay method, high-density lipoprotein cholesterol (HDL-C) levels by the direct method, low-density lipoprotein cholesterol (LDL-C), triglyceride, and plasma glucose levels by enzymatic methods, serum insulin level by the enzyme immunoassay method, and hemoglobin A1c (HbA1c) by high performance liquid chromatography. BMI was calculated as the ratio of body weight (kg) to height squared (m²). Waist circumference was measured at the level of umbilicus.

Exercise stress test

A multistage graded submaximal exercise stress test was performed on each subject using an electric bicycle ergometer (75XL-II COMBI, Tokyo, Japan). The workload was increased every 4 min, depending on their daily activity levels. A CM5-lead ECG (ML-1800, Fukuda Denshi, Tokyo, Japan) was recorded continuously during exercise testing. The following parameters were measured at rest and during the last 1 min of each stage: the rate of perceived exhaustion; blood pressure (FB-300, Fukuda Denshi); and blood lactate concentration (Lactate Pro, Arkray, Kyoto, Japan). Maximal oxygen uptake (VO₂ max) was estimated by the nomogram of Åstrand and Ryhming [17], using the heart rate measured at three different
Monocytes, neutrophil count, CRP and VO2 max

Results

Table 1 summarizes baseline characteristics of the low, moderate, and high VO2 max groups. Among the 3 groups, significant differences were seen in the levels of HDL-C, fasting triglyceride and fasting insulin, and BMI and waist circumference. Monocyte counts were 278 ± 102, 278 ± 62 and 221 ± 82/μl in the low, moderate, and high VO2 max groups, respectively, and higher in low and moderate VO2 max groups, compared to high VO2 max group (p < 0.05), respectively. Neutrophil counts were 3097 ± 972, 2859 ± 816, and 2587 ± 853/μl in the low, moderate, and high VO2 max groups, respectively, and higher in low VO2 max group (p < 0.05). The hs-CRP levels were similar among the 3 groups (1.15 ± 1.02, 1.25 ± 1.75, 0.92 ± 1.37 mg/l in low, moderate, and high VO2 max groups, respectively) (Fig. 1).

Table 2 shows the associations of monocyte counts, neutrophil counts, and hs-CRP levels with other CAD risk factors, determined by univariate regression analysis. Monocyte counts were correlated positively with fasting triglyceride level (p < 0.0001), HbA1c, fasting glucose level, fasting insulin level (p < 0.01), BMI, waist circumference (p < 0.05), and negatively with VO2 max (p < 0.05). Similarly, we found neutrophil counts were correlated positively with fasting triglyceride level, fasting insulin level (p < 0.01), BMI (p < 0.05), and negatively with VO2 max (p < 0.05). The hs-CRP levels were positively correlated with fasting triglyceride levels (p < 0.001), waist circumference (p < 0.01), HbA1c, fasting glucose level, fasting insulin level, BMI (p < 0.05), and negatively with HDL-C level (p < 0.01).

In the stepwise multivariate regression analysis, either monocyte count, neutrophil count, or hs-CRP levels were entered as the dependent variable. The following factors were entered as independent variables: fasting triglyceride; HbA1c; fasting insulin; waist circumference; and VO2 max. This analysis showed that monocyte counts were associated independently with fasting triglyceride and VO2 max (r² = 0.260, p < 0.0001).
Figure 1 Differences in monocyte, neutrophil counts, and hs-CRP in the low (n = 30, 15.5–24.5 ml/kg/min), moderate (n = 30, 24.7–28.3 ml/kg/min), and high VO₂ max groups (n = 30, 28.4–50.9 ml/kg/min). The results are shown as the means ± S.D. NS, not significant.

Table 2 Association between monocyte, neutrophil counts, hs-CRP, and other coronary risk factors calculated by simple regression analysis

<table>
<thead>
<tr>
<th></th>
<th>Monocyte counts</th>
<th>Neutrophil counts</th>
<th>hs-CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-C</td>
<td>0.057</td>
<td>0.074</td>
<td>0.125</td>
</tr>
<tr>
<td>HDL-C</td>
<td>−0.166</td>
<td>−0.185</td>
<td>−0.277</td>
</tr>
<tr>
<td>Fasting triglyceride</td>
<td>0.510**</td>
<td>0.337*</td>
<td>0.388**</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.320†</td>
<td>0.126</td>
<td>0.231†</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>0.292**</td>
<td>0.119</td>
<td>0.245†</td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>0.310†</td>
<td>0.297†</td>
<td>0.210†</td>
</tr>
<tr>
<td>Resting systolic blood pressure</td>
<td>0.015</td>
<td>0.046</td>
<td>0.098</td>
</tr>
<tr>
<td>Resting diastolic blood pressure</td>
<td>0.025</td>
<td>0.047</td>
<td>0.011</td>
</tr>
<tr>
<td>BMI</td>
<td>0.210†</td>
<td>0.237†</td>
<td>0.210†</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.217†</td>
<td>0.186</td>
<td>0.337**</td>
</tr>
<tr>
<td>VO₂ max</td>
<td>−0.251†</td>
<td>−0.235†</td>
<td>−0.035</td>
</tr>
</tbody>
</table>

Data are expressed as the coefficient of correlation. *p < 0.05, †p < 0.01, ‡p < 0.001, §p < 0.0001. LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; BMI, body mass index; VO₂ max, maximum oxygen uptake; NS, not significant.

Table 3 Association between monocyte, neutrophil counts, hs-CRP, and the other coronary risk factors calculated by stepwise multiple regression analysis

<table>
<thead>
<tr>
<th></th>
<th>Monocyte counts</th>
<th>Neutrophil counts</th>
<th>hs-CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.063</td>
<td>0.139</td>
<td>0.069</td>
</tr>
<tr>
<td>HDL-C</td>
<td>−0.088</td>
<td>−0.010</td>
<td>−0.077</td>
</tr>
<tr>
<td>Fasting triglyceride</td>
<td>0.491</td>
<td>0.275</td>
<td>0.333</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.198</td>
<td>0.002</td>
<td>0.125</td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>0.151</td>
<td>0.220</td>
<td>0.041</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.043</td>
<td>0.037</td>
<td>0.268</td>
</tr>
<tr>
<td>VO₂ max</td>
<td>−0.206</td>
<td>−0.169</td>
<td>−0.216</td>
</tr>
<tr>
<td>r²</td>
<td>0.260</td>
<td>0.114</td>
<td>0.151</td>
</tr>
</tbody>
</table>

β; standard regression coefficient. In the stepwise multiple regression analysis either the monocyte, neutrophil counts, or hs-CRP were entered as dependent variables. The following factors were entered as independent variables: age, high-density lipoprotein cholesterol (HDL-C), fasting triglyceride, hemoglobin (Hb) A1c, fasting insulin, waist circumference and maximum oxygen uptake (VO₂ max).
Neutrophil count was also found to be associated with fasting triglyceride and fasting insulin \( (r^2 = 0.114, p < 0.0001) \), while hs-CRP was associated with fasting triglyceride and waist circumference \( (r^2 = 0.151, p < 0.0001) \) (Table 3).

**Discussion**

The major finding of our study was that monocyte and neutrophil counts were higher in the low VO\(_2\) max group compared to the high VO\(_2\) max group. In addition, our univariate regression analysis showed that monocyte and neutrophil counts, but not hs-CRP levels, were related to VO\(_2\) max. Furthermore, the stepwise multivariate regression analysis showed the independent associations of monocyte count with fasting triglyceride and VO\(_2\) max, neutrophil count with fasting triglyceride and fasting insulin, and hs-CRP with fasting triglyceride and waist circumference.

Several studies have demonstrated an association between inflammatory markers and the level of physical activity \([13–15]\). Mora et al. \([14]\) reported that increased BMI or decreased physical activity levels in middle-aged women were associated with increased levels of CRP, fibrinogen, and soluble intracellular adhesion molecule-1 (sICAM-1). Similarly, in a study on the relationship between inflammatory markers and aerobic capacity in apparently healthy Japanese subjects, Ichihara et al. \([15]\) observed that elevated CRP levels and white blood cell counts were associated with lower VO\(_2\) max levels. Another study also confirmed that moderate exercise was effective for improving immune function such as neutrophil function and the activity of natural killer cells \([19]\).

However, despite this evidence, the mechanism linking VO\(_2\) max and inflammation in relation to atherosclerosis remains unknown.

VO\(_2\) max is determined by the product of the arteriovenous oxygen difference and cardiac output. VO\(_2\) max is not merely an index of physical fitness, but also reflects the function of the cardiopulmonary system and the prevalence of risk factors for atherosclerosis \([20]\). Cade et al. \([21]\) demonstrated that VO\(_2\) max and arteriovenous oxygen difference were lower in subjects with human immunodeficiency virus infection compared with controls. Possible mechanisms to account for these changes were the influence of highly active antiretroviral therapies or the effects of inflammatory responses such as interleukin-1 and tumor necrosis factor-\( \alpha \). A longitudinal study in patients with chronic heart failure demonstrated that the plasma levels of granulocyte-colony stimulating factor, monocyte chemoattractant protein-1, sICAM-1, and soluble vascular cell adhesion molecule-1 (sVCAM-1) were decreased by bicycle exercise training for 12 weeks at 70–80% of maximal heart rate intensity, and that the resulting increase in VO\(_2\) max was associated with a reduction in the levels of sICAM-1 and sVCAM-1 \([22]\). The adhesion of leukocytes including neutrophils and monocytes to the endothelium induced by cell adhesion molecules, and also leukocyte migration across the endothelium into the vessel wall, are characteristic steps in the inflammatory processes involved in the various stages of atherosclerosis \([4–9]\). Adamopoulos et al. \([22]\) suggested that increased VO\(_2\) max resulting from endurance training was effective in inhibiting monocyte infiltration into the vascular wall. Therefore, increases in VO\(_2\) max may be a sensitive marker of cardiovascular protective effects as a consequence of its potential to inhibit some inflammatory processes in obese subjects.

In our study, fasting triglyceride levels were also related to the monocyte and neutrophil counts and hs-CRP. Increased triglyceride levels are well known to be associated with the development of CAD \([24]\). Furthermore, several studies have reported that hypertriglyceridemia is associated with endothelial dysfunction and levels of inflammatory markers \([5,23,24]\). Our finding of an independent association of monocyte and neutrophil counts and hs-CRP with fasting triglyceride in the multivariate analysis supports the findings of these studies and suggests that increased serum triglyceride may be a predictive factor for vascular infiltration.

Although prior research has focused on monocytes as the predominant inflammatory cell type involved in atherosclerosis \([4,5,9]\), there is increasing evidence that neutrophil activation is also a major participant in the inflammatory process of ischemic cardiovascular disease, particularly in the acute inflammatory reaction \([6,8]\). In addition, a study in a large patient cohort with a high risk of CAD showed that of the various types of leukocytes, the neutrophil count was the best predictor of future cardiovascular events \([10]\). In our study, both the monocyte and neutrophil counts were associated with VO\(_2\) max in the univariate analysis. However, the results of our multivariate analysis suggest that monocyte counts may have a greater effect on aerobic capacity.

Our data showed no association between hs-CRP levels and VO\(_2\) max. At present, the association between hs-CRP and physical activity levels is controversial. Although several previous studies reported an independent association between physical activity and hs-CRP levels \([13–15]\), Rawson et
al. [16] showed that hs-CRP correlated with BMI levels, but not with physical activity levels. More recently, Verdaet et al. [25] also reported that leisure time physical activity was associated with general risk factors for CHD, such as obesity indices and serum lipid profile, but not with the levels of CRP, serum amyloid A, or fibrinogen levels. These contradictory results may have been caused by differences in study population, study sample size, and assessment of aerobic capacity. Irrespective of these earlier findings, our results suggest that monocyte and neutrophil counts contribute to aerobic capacity to a greater extent than hs-CRP, at least in overweight women.

Study limitations

There were several potential limitations in our study. Firstly, the limited study population resulted in a small number of subjects, with this group being restricted to middle-aged women. Secondly, as the study was a cross-sectional design, we were unable to clarify the causative role of the inflammatory markers on VO2 max. Thirdly, VO2 max in our study was estimated using the nomogram of Åstrand and Ryhming. Finally, it may be acceptable to generalize our results to obese subjects for both men and women, although our study was only in women. Therefore, further research is required to clarify more precisely the association between inflammation and aerobic capacity following exercise therapy.

Conclusions

Our study demonstrated that monocyte and neutrophil counts were higher in the low VO2 max group compared to the high VO2 max group. In addition, univariate regression analysis showed that VO2 max was related to monocyte and neutrophil counts, but not with hs-CRP levels. These results suggest that VO2 max is a sensitive factor that reflects inflammatory status and might support cardiovascular protective effects of aerobic exercise in overweight women.

References

Monocytes, neutrophil count, CRP and VO2 max


