Association of Inflammatory Marker and Highly Sensitive C-Reactive Protein With Aerobic Exercise Capacity, Maximum Oxygen Uptake and Insulin Resistance in Healthy Middle-Aged Volunteers

Noriyasu Kondo, MD; Masahiro Nomura, MD*; Yutaka Nakaya, MD**; Susumu Ito, MD*; Takashi Ohguro, MD

Background Increased levels of inflammation markers, such as C-reactive protein (CRP) and tumor necrosis factor-\(\alpha\), have been found in insulin resistance syndrome. Those with elevated levels of high-sensitive CRP (hs-CRP) are at a higher risk for coronary heart disease. In the present study, we evaluated whether maximum oxygen uptake and insulin resistance are related to hs-CRP for the primary prevention of coronary heart disease.

Methods and Results The subjects were 50 subjects who did not have diabetes mellitus. A multi-step treadmill exercise test was performed to obtain the maximum oxygen uptake when assessed by computerized breath-by-breath analysis. As an index of insulin resistance, the homeostasis model insulin resistance index (HOMA-R; fasting glucose\(\times\)fasting insulin/405) was used. In addition, bodyweight, body mass index, subcutaneous fat thickness, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride were measured. Multivariate analysis revealed that hs-CRP was significantly correlated with HDL-cholesterol, uric acid, \(\gamma\)-glutamyl transpeptidase and maximum oxygen uptake. The maximum oxygen uptake showed the smallest odds ratio was in terms of the relationship with hs-CRP.

Conclusions The present study suggests that the development of exercising habits increases the maximum oxygen uptake. Furthermore, an elevated maximum oxygen uptake decreases HOMA-R and reduces the inflammatory marker CRP, suggesting that exercising habit plays an important role in the primary prevention of coronary heart disease. (Circ J 2005; 69: 452–457)

Key Words: Exercising habits; High-sensitive C-reactive protein; Insulin resistance; Maximum oxygen uptake

Inhibiting the initial development and advancement of cardiovascular disease by controlling the risk factors is important, but the involvement of various factors including improved lipid levels, has also been suggested. Attention has recently been focused on the inflammatory reaction as a factor for predicting the risk to the cardiovascular system.\(^1\)\(^-\)\(^3\) It has been reported that the use of highly sensitive C-reactive protein (hs-CRP), an inflammatory marker, facilitated the quantitative measurement of CRP at a higher sensitivity, even if the CRP levels measured by using conventional methods appeared to remain within the normal range.\(^4\)\(^-\)\(^9\) In addition, the risk of initially developing myocardial infarction has been reported to increase significantly with the elevation of the hs-CRP levels.\(^10\)\(^-\)\(^12\)

Previous studies in animals and humans have suggested that inflammation in the coronary artery is closely associated with the formation and advance of plaque.\(^13\)\(^-\)\(^14\) Clinical evaluations suggest that patients with vascular inflammation have a higher risk of coronary arterial events. Furthermore, Ridker reported that the initial development of myocardial infarction, stroke, and obstructive arteriosclerosis can be predicted by hs-CRP in healthy subjects.\(^15\) There is increasing evidence to suggest that insulin resistance is a chronic, low-grade inflammatory state.\(^16\) Physical activity is associated with a lower risk of cardiovascular disease, and inflammatory markers have been linked to the risk of cardiovascular disease.\(^17\)\(^-\)\(^18\) However, few systematic evaluations regarding the relationship between hs-CRP and exercising habits have been reported to date. We therefore evaluated the relationship between hs-CRP and maximum oxygen uptake, which is mainly associated with physical activity.

Methods

The subjects were 50 healthy workers (36 men and 14 women, mean age: 45.6\(\pm\)15.3 years) who underwent health examinations at workers’ health promotion service centers in accordance with the Total Health Promotion Plan (THP) of Japan.\(^19\) The subjects showed a fasting blood glucose level of 109 mg/dl or below, and persons with diabetes mellitus were excluded.

In accordance with the THP, health examinations were performed. These examinations consisted of medical examinations, the administration of a questionnaire on physical activity, and an exercise test to evaluate aerobic exercise capacity. Based on the results of these evaluations, indus-
trial physicians, in cooperation with other health promotional staff members, gave the workers instructions and information on how to promote their mental and physical health. The THP was performed by industrial physicians, health-care trainers, industrial health instructors, industrial nutrition instructors and psychological consultants. The health examination items consisted of an interview, in which the subjects were questioned about their lifestyle and daily habits, consultations, medical examinations, and an examination of motor function.

The propriety of the exercise tolerance test was determined by industrial physicians and was based on the results of the interview, consultation, medical examinations, and ECG findings at rest. The exercise tolerance test was not performed in patients with heart disease, uncontrolled hypertension, acute active medical diseases, or severe physical handicaps. The multi-step exercise tolerance test was performed by gradually increasing the intensity of exercise by using a Combi’s ergometer and a multi-exercising system (ML1800, FUKUDA DENSFI Co, Ltd, Tokyo, Japan). Because the protocol of the exercise tolerance test consisted of 4 stages (1–4 stages, each stage lasting for 4 min), the total duration of the exercise tolerance test was 16 min.

The target heart rate was established at \((220 - \text{age (years)}) \times 85\%\), and the exercise tolerance system was programmed to automatically establish the intensity of exercise based on heart rates during the exercise tolerance test, allowing subjects to reach their target heart rate in 16 min. During the exercise tolerance test, monitoring of the CMs led to ECG and blood pressure measurement by the use of an automatic sphygmanometer (NIPPON COLIN Co, Ltd, Tokyo, Japan), which was performed at 1-min intervals.

However, the exercise tolerance test was discontinued before the end of the protocol when the target heart rate was achieved within 16 min, when patients complained of symptoms such as dyspnea, chest pain, or other symptoms, developed severe arrhythmias such as frequent ventricular arrhythmia, showed a reduction of the ST segment of over 1.5 mm, or when patients showed elevated blood pressure (systolic blood pressure \(\geq 230\) mmHg, diastolic blood pressure \(\geq 120\) mmHg).

By using the estimation method developed by Astrand and Kodahl, the maximum oxygen uptake was estimated by obtaining a regression equation based on the heart rates obtained at each stage (1–4 stages) over a period of 3 min and 50 s, and the intensity of exercise (W)\(^20\) When the exer-
Exercise tolerance test was discontinued during the course of the third and fourth stages, the maximum oxygen uptake was calculated by using the values of the heart rates obtained up until the second stage of the exercise tolerance test. When the exercise tolerance test was discontinued during the course of the second stage, such cases were excluded from analysis to avoid errors in the estimation of the maximum oxygen uptake.

Hs-CRP and subcutaneous fat thickness were measured, and measurements were also obtained during the general health examination for height and bodyweight. Hematological and biochemical examinations were also conducted, as were examinations of respiratory function, a urinalysis, and a chest X-ray. Furthermore, as an index of insulin resistance, the homeostasis model insulin resistance index (HOMA-R) was obtained by using the following equation:

\[ \text{HOMA-R} = \frac{\text{fasting glucose (mg/dl)} \times \text{fasting insulin (U/ml)}}{405} \]

In each respective health examination item, logistic multivariate analysis for hs-CRP was performed.

A follow-up study was performed by distributing a questionnaire survey to the 50 subjects 1 year after the initial study had been performed. The correlation between the HOMA-R and changes in the maximum oxygen uptake was evaluated in 39 subjects, in whom health promotion over the year since the study had been performed was confirmed by a follow-up study. The follow-up study confirmed that 39 of the 50 subjects had followed the health promotion recommendations of the physicians and staff of the health promotion team. The correlation between the HOMA-R and changes in the maximum oxygen uptake was evaluated in these subjects.

**Statistical Analysis**

All values were expressed as the mean±standard deviation, and statistical analysis was performed by using StatView 5.0 (SAS Institute Inc, USA). The correlation between the 2 groups was evaluated by a single regression analysis. In addition, the influence of the respective factors on hs-CRP was evaluated by calculating 95% confidence intervals in the logistic regression model using multivariate analysis. P-values of less than 0.05 were considered to be significant.

**Results**

**Relationship Between hs-CRP and Body Composition**

Fig 1 shows the correlation between hs-CRP and subcutaneous fat thickness and body fat. There was a significant positive correlation between hs-CRP and subcutaneous fat thickness at the upper and lower extremities. In addition, hs-CRP was significantly correlated with body fat.

**Measurement of the Maximum Oxygen Uptake by Exercise Tolerance Test Using an Ergometer, and the Relationship With hs-CRP**

Fig 2 shows an example of the maximum oxygen uptake.

Fig 3. Correlation between high-sensitive C-reactive protein (hs-CRP) and the maximum oxygen uptake.

![Image](image1)

**Fig 4.** Correlation between high-sensitive C-reactive protein (hs-CRP) and the total cholesterol, triglyceride, high-density lipoprotein (HDL)-cholesterol, and uric acid levels.

![Image](image2)
obtained by the exercise tolerance test using an ergometer. The correlation between the amount of exercise and heart rates was obtained before the maximum oxygen uptake was calculated. In this case, the maximum oxygen uptake was calculated to be 39.1 ml·kg\(^{-1}\)·min\(^{-1}\) as the exercise loads were 28, 83 and 102 W at 70, 94 and 110 beats/min of the heart rate after the initiation of the exercise tolerance test, respectively. As shown in Fig 3 there was a significant negative correlation between hs-CRP and the maximum oxygen uptake.

**Relationship Between hs-CRP and Blood Chemical Data**

**Relationship Between hs-CRP and Total Cholesterol, Triglyceride and High-Density Lipoprotein (HDL)-Cholesterol, and Uric Acid Levels**

Fig 4 shows the correlation between hs-CRP and total...
Fig 6. Correlation between the maximum oxygen uptake and homeostasis model insulin resistance index (HOMA-R) before and after exercising. $\Delta$HOMA-R = (HOMA-R before therapeutic exercising) – (HOMA-R after therapeutic exercising). $\Delta$VO2max (ml·kg$^{-1}$·min$^{-1}$) = (VO2max after therapeutic exercising) – (VO2max before therapeutic exercising).

Discussion

Patients showing elevated hs-CRP levels are reported to have a higher risk factor for coronary arterial disease. In the present study, we evaluated the relationship between hs-CRP and maximum oxygen uptake or insulin resistance. Logistic multivariate analysis showed that maximum oxygen uptake, $\gamma$-GTP, HDL-cholesterol and uric acid levels, were the important independent factors for hs-CRP: the maximum oxygen uptake being the most important among these items. In addition, exercise training improved not only the maximum oxygen uptake, but also insulin resistance and hs-CRP. These results indicate that increased physical activity, represented by maximum oxygen uptake, might improve the risk for cardiovascular disease, at least by alleviating inflammation.

hs-CRP and Primary Prevention of Coronary Artery Disease

Previous studies in animals and humans have suggested that inflammation in the coronary artery is closely associated with the formation and development of plaque. Clinical evaluations have also suggested that patients with vascular inflammation are at a higher risk for coronary arterial events. The greatest attention is currently being paid to the blood levels of CRP, as measured by a highly sensitive procedure (hs-CRP), as a marker of vascular inflammation.

The hs-CRP is an acute phase reactant for trauma and infections, and is mainly produced in the liver in response to systemic inflammations, resulting in increased blood CRP levels. Because Berk et al reported higher hs-CRP levels in patients with unstable angina pectoris compared to those with stable angina, the association of hs-CRP with coronary artery disease has been aggressively studied. Although the inhibition of the initial development and advances of cardiovascular diseases by controlling risk is reportedly important, the involvement of various factors, including improved lipid levels, has also been suggested. Ridker paid particular attention to inflammation as a factor for predicting the risk to the cardiovascular system. He reported the risk of ischemic heart disease, and stated that the use of hs-CRP facilitated the quantitative measurement of CRP with more sensitivity, even if the CRP levels appeared to be within the normal range. He also evaluated healthy middle-aged male subjects using the quartile of hs-CRP, and reported that the risk of initially developing myocardial infarction was significantly increased with the elevation of hs-CRP levels.

Furthermore, even in studies involving low-risk, healthy postmenopausal women without hypertension, hyperlipemia, smoking habits, diabetes mellitus, or any particular family history, the frequency of cardiovascular events increased with the elevation of hs-CRP levels at the quartile. In addition, there was a markedly positive correlation between the risk of initially developing myocardial infarction and the total cholesterol/HDL-cholesterol ratio. Moreover, Ridker et al reported that the initial development of myocardial infarction, stroke and obstructive arteriosclerosis can be predicted by hs-CRP.

Although the detailed physiological role of CRP remains unclear, several histological studies have demonstrated that part of the CRP that accumulates in the arteriosclerotic site was absorbed by foam cells. Therefore, the CRP is likely to become a target of macrophages by labeling the tissue as a necrotic cell marker after inflammation. That is, maintaining CRP at a lower level is important in preventing the development of coronary heart disease. However, the results of the present study suggest that the elevation of maximum oxygen uptake by routine exercise is also important for decreasing CRP levels in individuals, in addition to maintaining the $\gamma$-GTP, HDL-cholesterol and uric acid levels within the normal range.
Exercise Performed and Prevention of Coronary Heart Disease

In the present study, there was a significant negative correlation between maximum oxygen uptake and hs-CRP. Thus, the maintenance of hs-CRP at a lower level may inhibit the development of arteriosclerosis. The present study revealed that an elevated maximum oxygen uptake, that is, an increased tolerance, decreased hs-CRP levels. Maintaining hs-CRP levels below a specified level may contribute to the primary prevention of coronary heart disease. In the present study, we could not demonstrate the upper limit of hs-CRP levels for preventing the development of coronary heart disease. However, the elevation of maximum oxygen uptake by exercise as a therapy while maintaining the $\gamma$-GTP, HDL-cholesterol and uric acid levels at normal ranges may be useful for preventing the development of vascular inflammation and subsequent arteriosclerosis. Based on these findings, it was concluded that monitoring the hs-CRP levels may play an important role in the primary prevention of coronary heart disease.

Study Limitation

In the present study, the number of subjects was small for multiple regression analysis and in terms of speculation and drawing accurate conclusions. Future analysis using a large number of subjects should be conducted.

References


