High-sensitive C-reactive protein level and oxidative stress-related status in former athletes in relation to traditional cardiovascular risk factors

E. Pihl a,∗, K. Zilmer a, T. Kullisaar a, C. Kairane a, A. Pulges b, M. Zilmer a

a Department of Biochemistry, University of Tartu, Ravila 19, Tartu 50411, Estonia
b Department of Cardiovascular and Thoracic Surgery, University of Tartu, Puusepa 8, Tartu 51014, Estonia

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Abstract

Objective: To analyze systemic and cellular oxidative stress-related indices as well as C-reactive protein level in former top-level athletes in relation to traditional cardiovascular risk factors. Methods: A cross-sectional study was performed in 53 former male athletes and 25 sedentary controls (age range: 39–59 years). We measured anthropometric factors (BMI, fat percentage, WHR), resting blood pressure (SBP, DBP), serum cholesterol (CHOL), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG), total antioxidant status (TAS), oxidized LDL-C (oxLDL), diene conjugates (DC), glutathione redox status, high-sensitive C-reactive protein (hsCRP), and leisure-time physical activity. Results: Physically active former athletes had significantly lower mean overweight (BMI, fat percentage, WHR), better spectrum of atherogenesis indicators (CHOL, HDL-C, TG, TG:HDL-C ratio) and lower oxidative stress (oxLDL, oxLDL:LDL-C ratio, DC) values than sedentary ex-athletes. No significant differences in these variables were found between the sedentary ex-athletes and control group. Significant associations were found between physical activity (METs), SBP, DBP, hypertension, CHOL, HDL-C, TG, TG:HDL-C ratio, oxLDL, oxLDL:LDL-C ratio, DC and hsCRP. Conclusions: A physically active lifestyle is related to a lower cardiovascular disease (CVD) risk profile including a substantially lower systemic and cellular oxidative stress status as well as C-reactive protein level in middle-aged men.

Keywords: Lipoproteins; Hypertension; Oxidized LDL; Glutathione; hsCRP; Exercise; Former athletes

1. Introduction

Former top-level athletes represent a special group of individuals who have exercised several years with heavy training loads. Most studies in former athletes have shown lower mortality rates and longer life expectancy [1,2]. The existing data reveal that former top-level athletes have a decreased prevalence of diabetes, hypertension, ischaemic heart disease, as well as a lower prevalence of cancer mortality in comparison with the general population [3,4]. However, it remains unclear which biochemical indices or their combinations (high-density lipoprotein-cholesterol (HDL-C), oxidized LDL (oxLDL), high-sensitive C-reactive protein (hsCRP), etc.) describe more precisely the cardiovascular health in former athletes and to what extent the lower cardiovascular disease (CVD) prevalence in ex-athletes depends on the previous athleticism.

Although the relationship between the classical cardiovascular disease risk factors and cardiovascular mortality is widely known, the latest summarized information has shown that less than 50% of cardiac patients present classical risk factors [5,6]. At the same time, prolonged high-grade oxidative stress-caused damage is increasingly recognized as playing an important role in the pathogenesis of several disorders, including CVD [7]. Exhaustive exercise generates excess of free radicals followed by increased lipid peroxidation and oxidative damages of other biomolecules [8]. However, it has been suggested that regular adequate physical activity might maintain and promote the antioxidant defense capacity against oxidative stress [9]. The existing data on the effects of exercise on the grade of oxidative stress and antioxidant defenses in middle-aged and older subjects are inconsistent [9,10]. Thus, much more attention should be paid to the biomarkers of oxidative stress, together with
other principal biomarkers, when cardiovascular risk is assessed. No complex study considering such circumstances, including the evaluation of systemic and cellular oxidative stress, in the former athletic population is available.

Recent findings have shown that high-sensitive C-reactive protein, the inflammatory marker associated with high risk of coronary heart disease, is decreased with regular physical exercise [11,12]. However, there is not enough data indicating the effect of long-term intensive training on the oxidative stress status and the antioxidant defense capacity as well as on the hsCRP level or to what extent this could depend on the current lifestyle. Consequently, our study analyzed the status of oxidative stress and hsCRP level in former top-level athletes in relation to other cardiovascular risk factors according to the physical activity level during the post-competitive period.

2. Methods

2.1. Subjects

The study population consisted of 78 males (48.0 ± 6.1 years; 87.5 ± 12.9 kg; 180.1 ± 6.6 cm) who were randomly recruited from the previous study held in 1993–1994 [13]. The inclusion criteria for former athletes were their previous participation in endurance sports events and sports games at international or national level at least 15 years ago (mean duration of the post-competitive period of ex-athletes was 23.1 ± 6.3 years). The control group consisted of fellow workers of the ex-athletes who had no competitive sports history and who had similar age distribution. All the subjects were apparently healthy and without regular medication. Two subjects with cardiac problems were excluded from the study.

All the males were white and belonged to the middle-to-high socio-economic class. The local Medical Ethics Committee of the University of Tartu approved the protocol and all the participants signed an informed consent document. All study subjects were prohibited from participating in vigorous exercise and from smoking at least 24 h before the examination.

2.2. Lifestyle variables

The general health status and lifestyle parameters (smoking, alcohol consumption, dietary habits, etc.) of the study subjects were estimated by the modified questionnaire by Sarna et al. [2]. Smoking was classified as never, quit smoking, smokes cigars or a close family member smokes, smokes fewer than 15 cigarettes per day or smokes more than 15 cigarettes daily. In addition, the subjects were asked to specify the number of cigarettes per day. Alcohol intake was analyzed as grams per day. Consumption of vegetables and fruits was expressed as ≥200 or ≥200 g per day. Oil, butter, high-fat milk, and salt consumption were recorded as ordinal variables.

Additionally, the subjects recorded their competitive athletic history and the current sports activity during the past 12 months in detail (mode, weekly frequency, mean duration, intensity). The repeatability of the reported exercise of this questionnaire was described previously [13]. According to their physical activity data, the ex-athletes were divided into two categories—"physically active" (those exercising regularly three or more times per week) (PAEA); the ones who did not meet these criteria were transferred into the "sedentary" group (SEA). Additionally, on the basis of this information, the score of leisure-time physical activity was calculated as MET-hours per week (MET is the ratio of the work metabolic rate to the resting metabolic rate). MET was calculated as a product of intensity × duration × frequency. The scoring of METs was based on the data of Ainsworth et al. [14], where 4 MET corresponded to walking, 7 MET to jogging, and 12 MET to running. The physically active study subjects were mainly engaged in aerobic activities (jogging, swimming, cycling, and sports games).

2.3. Anthropometric measurements

Subjects’ height and weight were determined by the Martin metal anthropometer (±0.1 cm) and clinical scales (±0.05 kg), respectively. The body mass index (BMI) was calculated (kg/m²). Body fat percentage was assessed by the dual energy X-ray absorptiometry method (Lunar, DPX-IQ, USA). For the evaluation of fat distribution, waist and hip circumferences were measured, and the ratio of waist and hip circumferences (WHRs) was calculated.

2.4. Blood pressure measurements

Sitting blood pressure (BP) was measured using a mercury sphygmomanometer after a 5 min rest. Systolic (Korotkoff phase 1) and diastolic (Korotkoff phase 5) blood pressures (SBP, DBP) were measured twice on the left upper arm, and the average was used for the analysis. According to the hypertension criteria published by the National High Blood Pressure Education Program Working Group [15], a systolic blood pressure ≥140 mmHg and diastolic blood pressure ≥90 mmHg is defined as mild arterial hypertension.

2.5. Laboratory procedures

Blood samples were obtained in the morning after a 12 h fast. During 4 weeks before the study the subjects were advised to avoid the use of vitamin supplements. Serum glucose (GL) was determined enzymatically using glucose-oxidase method (Human, Germany). From serum, total cholesterol (CHOL) (Human, Germany), low-density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein-cholesterol (HDL-C) (Roche Diagnostics, Germany), and triglycerides (TG) (Roche Diagnostics, Germany) were measured. hsCRP was determined by latex particle-enhanced immunoturbidimetric assay (Roche
Diagnostics, Germany) with an automated analyzer Hitachi 912.

Blood samples for diene conjugates (DC), total antioxidant status (TAS), oxidized LDL (oxLDL), reduced and oxidized glutathione (GSH, GSSG, respectively) were stored at −70 °C until the analysis. In this study, DC were measured according to the methods previously described [16], with minor modifications. Briefly, samples (150 μl) + (150 μl) of 0.9% NaCl (reagent blank contains only isotonic saline) were incubated at 37 °C for 25 min, 0.25% butylated hydroxytoluene (15 μl) was added and the lipids were extracted by heptane/isopropanol (1:1). Then the samples were acidified by 5 mol l\(^{-1}\) hydrochloric acid and extracted by cold heptane (1600 l\(^{-1}\)). After centrifugation (for 5 min at 3000rpm), absorbance of heptane fraction was measured spectrophotometrically at absorbance maximum at 234 nm. Oxidized LDL levels were measured using enzyme-linked immunosorbent assay (ELISA) kit (Mercodia, AB, Up- psala, Sweden). TAS was assessed by the TAS method (TAS, Randox Laboratories Ardmore, UK). The method is based on the inhibition of absorbance of ferrylmyoglobin radicals of 2,2′-azinobis(ethylbenzothiazoline-6-sulphonate (ABTS\(^{+}\)) generated by activating metmyoglobin perox-
didase with \(H_2O_2\). The suppression of the absorbance of ferrylmyoglobin radicals (TAS) generated by activating metmyoglobin perox-
didase with \(H_2O_2\). The suppression of the absorbance of ferrylmyoglobin radicals (TAS) by the sample under investigation [17]. The assessment of GSH and GSSG was described by Kullisaar et al. [18]. The glutathione system redox potency was expressed as the glutathione redox ratio (GSSG/GSH). Also, TG, HDL-C [19] and oxLDL/LDL-C [20] ratios were calculated.

2.6. Statistical analysis

The results are presented as a mean ± standard deviation. The Pearson product moment or Spearman correlations were used to determine the relationships between variables. Partial correlation analysis was used to eliminate the effects of age, smoking and body weight. One-way analysis (ANOVA) followed by Tukey’s post hoc comparison was used for multiple comparisons between the groups. The \(\chi^2\)-test was used to determine the between-group differences in categorial vari-
able (smoking, dietary habits, etc.). The independence and significance of variables was tested by multiple regression analysis based on the correlation analysis. Calculations were performed with the SPSS (SPSS Inc, Chicago, IL) statistical package. Statistical significance was defined as \(P<0.05\).

3. Results

Table 1 provides descriptive information for anthropo-
metric, blood pressure, and physical activity (METs) PAEA were characterized by significantly lower values in all mea-
sured anthropometric characteristics in comparison with SEA and controls. Statistically significant differences in SBP and DBP were also found between the groups, show-
ing lower values in PAEA. No significant differences in these variables were found between the SEA and controls.

Table 2 shows the biochemical parameters of the blood of the investigated groups. Comparison of the groups indi-
cated that PAEA had a significantly lower CHOL, HDL-C, TG, TG-HDL-C ratio, oxLDL, oxLDL/LDL-C ratio, and DC values in comparison with SEA. In addition, the values of CHOL, oxLDL, and the oxLDL/LDL-C ratio differed also significantly from the PAEA and the controls. The glu-
tathione redox ratio showed a significantly higher value in SEA in comparison with the controls. No statistically sig-
nificant differences were found between the groups with re-
gard to LDL-C, TAS, and hsCRP. However, only in PAEA the level of hsCRP was lower than 1 mg l\(^{-1}\). The prevalence of current smokers among the study groups was relatively low (21.5%), and there were no signifi-
cant differences in the smoking habits between the PAEA, SEA, and controls (six, five and five current smokers, re-
spectively). Smokers had a higher oxLDL level than the reference value (129 and 117U l\(^{-1}\)), respectively, \(P>0.05\). The \(\chi^2\)-test did not reveal any significant differences be-
tween the groups in regard to alcohol consumption (grams per day) and dietary habits.

Correlation analysis showed significant relationships be-
tween hsCRP, DC, oxLDL, TG, and TG-HDL-C ratio (\(r=0.315-0.579\), \(P=0.05-0.001\)). Significant inverse corre-
lations were detected between physical activity (METs), SBP, DBP, hypertension (≥140/90 mmHg), CHOL, TG, TG-HDL-C ratio, oxLDL, oxLDL/LDL-C ratio, DC, and hsCRP (\(r=-0.245\) to \(-0.405\), \(P=0.05-0.001\)). Positive association was found between METs and HDL-C (\(r=0.297\), \(P<0.01\)). After adjustment for age and smoking, most associations stayed significant but disappeared between physical activity, DBP, hypertension, and hsCRP.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PAEA (n = 29)</th>
<th>SEA (n = 24)</th>
<th>Controls (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47.5 ± 7.2</td>
<td>48.6 ± 6.9</td>
<td>47.7 ± 6.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>181.0 ± 6.6</td>
<td>179.0 ± 7.1</td>
<td>181.2 ± 6.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>81.1 ± 10.9</td>
<td>93.4 ± 13.0</td>
<td>89.8 ± 12.6</td>
</tr>
<tr>
<td>BMI (kg m(^{-2}))</td>
<td>25.3 ± 2.4</td>
<td>29.1 ± 3.2</td>
<td>27.3 ± 3.1</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>15.9 ± 5.1</td>
<td>25.3 ± 4.6</td>
<td>22.8 ± 5.9</td>
</tr>
<tr>
<td>WHR</td>
<td>0.86 ± 0.04</td>
<td>0.94 ± 0.04</td>
<td>0.90 ± 0.05</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>127.2 ± 9.8</td>
<td>138.2 ± 14.6</td>
<td>136.7 ± 17.9</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>83.5 ± 9.8</td>
<td>90.9 ± 9.7</td>
<td>87.3 ± 9.3</td>
</tr>
<tr>
<td>DC</td>
<td>48.4 ± 23.4</td>
<td>14.5 ± 6.7</td>
<td>78.6 ± 6.7</td>
</tr>
<tr>
<td>METs</td>
<td>48.4 ± 23.4</td>
<td>14.5 ± 6.7</td>
<td>78.6 ± 6.7</td>
</tr>
<tr>
<td>TG</td>
<td>138.2</td>
<td>136.7 ± 17.9</td>
<td>136.7 ± 17.9</td>
</tr>
<tr>
<td>HDL-C</td>
<td>129</td>
<td>117U l(^{-1})</td>
<td>117U l(^{-1})</td>
</tr>
<tr>
<td>LDL-C</td>
<td>138.2</td>
<td>136.7 ± 17.9</td>
<td>136.7 ± 17.9</td>
</tr>
<tr>
<td>CHOL</td>
<td>138.2</td>
<td>136.7 ± 17.9</td>
<td>136.7 ± 17.9</td>
</tr>
</tbody>
</table>

Note: PAEA: physically active ex-athletes; SEA: sedentary ex-athletes; SBP: systolic blood pressure; DBP: diastolic blood pressure; METs: score of leisure-time physical activity (MET-hours per week).

\* \(P<0.05\) values statistically significantly different from controls.

\# \(P<0.01\) values statistically significantly different from controls.

### \(P<0.001\) values statistically significantly different from controls.

## \(P<0.05\) values statistically significantly different from SEA.

### \(P<0.01\) values statistically significantly different from controls.

#### \(P<0.001\) values statistically significantly different from controls.

### \(P<0.001\) values statistically significantly different from SEA.
It is widely accepted that the development of cardiovascular disease has also an inflammatory background. Considering this information it is noteworthy that oxLDL is recognized also as an inflammatory marker [7], and we established its lower levels in PAEA. In addition, recent studies provide compelling evidence that elevated value of hsCRP (an inflammatory marker) also play a role in the pathogenesis of cardiovascular disease [24]. According to the latest findings, there is a strong relationship between hsCRP and the prevalence of cardiovascular diseases. It is reported that any manipulation
that lowers hsCRP has a potential to lower cardiovascular events [25]. It is noteworthy that only physically active ex-athletes had mean hsCRP value under 1.0 mg l\(^{-1}\), which refers to only a mild clinical level of hsCRP [25].

Our data suggest that current recreational physical exercise is more closely associated with oxidative stress markers than the long-term physical loading several decades ago. Thus, our research supports previous findings that cardiovascular risk level in middle-age appears to be more strongly associated with current physical activity than with the sports history about 10–25 years ago [26]. Furthermore, several other lifestyle factors, such as cigarette smoking and dietary habits may also have an impact on oxidative stress [7,27]. High dietary intake of fruit and vegetables are associated with a higher plasma antioxidant concentration and a lower blood pressure [27]. A Finnish study among top-level athletes revealed that “mixed” and “power” athletes consumed less fat and more vegetables and fruits than the reference group, but the dietary habits of endurance athletes did not differ from the reference group [28]. However, our study did not find any significant differences in the consumption of fat, vegetables, and fruit between the groups. The percentage of current smokers in our study population was relatively low and this could explain the fact that smoking did not affect significantly the biochemical and blood pressure data. Thus, we assume that smoking and dietary habits did not confound the interpretation of physical activity with regard to the level of oxidative stress.

We found that indices of systemic oxidative stress (DC, oxLDL, oxLDL/LDL-C) were significantly lower in physically active ex-athletes. However, the intracellular glutathione system also participates in regulating the production of reactive oxygen species. The lower glutathione redox ratio refers to a lower level of cellular oxidative stress [7]. The existing data are inconsistent with regard to the glutathione system in relation to long-term exercise training [29]. Our cross-sectional study showed that the baseline glutathione redox ratio was significantly higher in sedentary ex-athletes than in sedentary controls while the mean ratio values were relatively low.

Our previous study of former athletes stressed the importance of a physically active lifestyle during the post-competitive period in relation to cardiovascular health and weight regulation [13]. After the adjustment of body weight there were significant associations between METs, CHOL, oxLDL, and oxLDL/LDL-C ratio, showing that there exists an independent relationship between physical activity, and the profile of atherogenic lipid and oxidative stress. There is growing evidence that exercise intensity more than 4 MET is strongly associated with lower cardiovascular mortality than less intense activity [30]. Moreover, exercise training with a higher intensity seems to be associated with a lower level of CRP [11]. Our data suggest that the intensity of physical exercise plays an important role in the level of oxidative stress (judging by the multiple regression analysis). On the other hand, the total amount of exercise is also important because there were significant between-group differences depending on the number of physical activity engagements per week and independent associations between MET-hours per week, CHOL, and oxLDL. Thus, our data are in accordance with the ACSM recommendation, where physical exercise three or more times per week with a duration of >20 min per session with moderate to vigorous intensity is recommended for better cardiovascular health [31].

In conclusion, the present study showed that physically active male former top-level athletes are characterized by significantly better biochemical indices (including oxidative stress-related markers) compared both with the sedentary ones and non-athletic controls. A physically active lifestyle is related to a lower CVD risk profile including a substantially lower systemic and cellular oxidative stress status as well as C-reactive protein level in middle-aged men.

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References


