Effects of training on resting plasma levels of homocysteine and C-reactive protein in competitive male and female wrestlers

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Summary

Study aim: To assess the effects of training on resting plasma levels of homocysteine (Hcy), C-reactive protein (CRP), folic acid, and on the activity of creatine kinase (CK) in competitive male and female wrestlers.

Material and methods: Polish elite wrestlers, male MW; n = 11) and female (FW; n = 11), as well as corresponding numbers of untrained, control subjects (MC and FC, respectively), participated in the study. Blood for assays was withdrawn from the antecubital vein in the morning, in pre-prandial state. Homocysteine (Hcy), C-reactive protein (CRP), folic acid and creatine kinase (CK) activity were assayed in plasma.

Results: Mean concentrations of Hcy and CRP were in the control groups significantly higher and those of folic acid – lower than in the respective groups of wrestlers. Folic acid levels were negatively correlated with Hcy, especially in wrestlers (r = -0.540; p<0.01). Mean CK activity was significantly (p<0.001) higher in male wrestlers than in male controls or female subjects. No significant correlation between CK and CRP was found.

Conclusions: Strength-speed training practiced by elite wrestlers, associated with significantly lower values of Hcy and CRP in them compared with the untrained subjects, may reduce the risk of cardiovascular diseases at later age, like in case of endurance training.

Key words: Homocysteine – Folic acid – C-reactive protein – Creatine kinase – Competitive training

Introduction

Homocysteine and C-reactive protein have been recently classified as the non-lipid, independent risk factors of cardiovascular diseases including atherosclerosis and coronary heart disease [6,25]. The sulphur-containing amino acid homocysteine (Hcy), a product of intracellular methionine metabolism in the presence of vitamins B6, B12 and folic acid [5], is protein-bound in circulation in the oxidised form. Homocysteine catabolism disorders lead to its accumulation in blood and, in effect, initiate inflammatory processes and accelerate atherosclerotic changes. Concentrations of Hcy in blood exceeding 16 µmol/L are considered abnormal and called hyperhomocysteinaemia [35], its aetiology being associated with deficiencies of vitamins B6, B12 and folic acid in the diet [17], but also with life style (consumption of alcohol and large amounts of coffee, smoking, etc.) and physical activity [5]. Plasma levels of Hcy were shown to depend on the dietary supply of folic acid; supplementation with folic acid was demonstrated to reduce the risk of cardiovascular diseases in men [26] and women [34].

The mechanisms of adverse effects of Hcy on the circulatory system have not been fully explained. Homocysteine is known to undergo auto-oxidation in plasma thus enhancing the oxidative stress by intensifying the generation of reactive oxygen species which damage endothelial cells and oxidise low-density lipoproteins (LDL). The latter are scavenged by macrophages foam cells being formed which participate in the generation of atheromatous plaques [4]. It was demonstrated [18] that an increase of Hcy concentration by 5 µmol/L was associated with a marked increase of the risk of cardiovascular diseases – by 60% in men and by 80% in women.

The C-reactive protein (CRP), a constituent of plasma [11], is synthesised in the liver and its increased generation is a non-specific response to infections, inflammations or tissue damages. Its concentrations were increased in patients with coronary heart disease. This suggests that inflammatory states may be substantial in atherosclerosis pathogeny, albeit the specific role of CRP in that process has not been fully clarified [27]. It was reported [8] that CRP concentrations were significantly higher in patients with cardiovascular diseases.
than in the healthy subjects; the risk of infarction was three times as high in subjects, in whom CRP levels exceeded 2.11 mg/L, compared with those in whom those levels did not exceed 0.55 mg/L [30] and this made CRP another (besides Hcy) non-lipid marker of risk of cardiovascular diseases. Physiologic concentration of CRP in plasma ought not to exceed 2 mg/L but in response to infection and inflammation may increase as much as thousandfold [11]. Even a slight increase of CRP may be indicative of prospective cardiovascular diseases in presently healthy men [30] and women [3]. Regular physical activity was shown to affect resting CRP levels – these were lower in physically active than in sedentary men [7].

Competitive athletes are prone to exercise-induced damages of skeletal muscle cells [12] often followed by inflammations. Damaged muscles may produce cytokines which stimulate CRP synthesis in the liver [31]. Liberation of intramuscular proteins (including enzymes) into blood is an indirect symptom of muscle cell damages [16]. One of those enzymes, the creatine kinase (CK), attracted particular attention and many authors considered CK a sensitive and specific marker of muscle cell damages (cf. [15]).

The reports on the effects of competitive training on resting CRP and Hcy concentrations in plasma are scarce. Since Hcy levels may depend on folic acid and B-vitamins supplied in diets, the aim of this study was to assess the effects of training on resting concentrations of C-reactive protein, homocysteine and folic acid, as well as on creatine kinase activity, in plasma of elite male and female wrestlers.

Material and Methods

Male and female elite wrestlers, as well as male and female untrained subjects, n = 11 each, volunteered to participate in the study and submitted their written consents to blood withdrawal. Study protocol was approved by the local Committee for Ethics. Blood for assays (about 4 ml) was sampled from the antecubital vein into heparinised tubes in the morning, in pre-prandial state. The following data were recorded: body height and mass, the accuracies amounting to 0.1 cm and 0.1 kg, respectively; body fat content from 4 skinfolds measured on the left side of the body, in standing position, according to Durnin et al. [10]; in plasma: creatine kinase (CK) activity, at 37ºC and wavelength equal to 340 nm, using commercial kits (Alpha Diagnostics, Poland); concentration of C-reactive protein (hsCRP) by high-sensitivity latex method using commercial kits (Pointe Scientific, USA); concentration of homocysteine (Hcy) – immunochemically, using commercial kits (Abbot, Great Britain); concentration of folic acid – by radioisotope-based using commercial kits (MP Biomedicals, USA).

Between-group differences were assessed using Student’s t-test for independent data. Pearson’s coefficients of correlation were computed from the combined residual sums of squares and products. The level of p≤0.05 was considered significant.

Table 1. Mean values (±SD) of basic characteristics recorded in elite wrestlers and in untrained subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>MC (n = 11)</th>
<th>MW (n = 11)</th>
<th>FC (n = 11)</th>
<th>FW (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td>21.5 ± 1.2</td>
<td>21.7 ± 1.6</td>
<td>21.5 ± 1.3</td>
<td>22.3 ± 10.0</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td></td>
<td>179.2 ± 5.6</td>
<td>174.3 ± 5.4*</td>
<td>164.4 ± 4.1</td>
<td>165.7 ± 7.0</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td></td>
<td>73.1 ± 6.7</td>
<td>77.1 ± 10.3</td>
<td>54.7 ± 3.1</td>
<td>60.9 ± 8.9*</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td>22.7 ± 1.3</td>
<td>25.3 ± 2.1***</td>
<td>20.2 ± 0.7</td>
<td>22.1 ± 2.1*</td>
</tr>
<tr>
<td>Body fat content (%)</td>
<td></td>
<td>17.4 ± 4.7</td>
<td>10.8 ± 1.7***</td>
<td>26.0 ± 3.0</td>
<td>20.2 ± 2.3***</td>
</tr>
<tr>
<td>Training experience (years)</td>
<td>–</td>
<td>11.1 ± 2.4</td>
<td>–</td>
<td>–</td>
<td>9.5 ± 2.7</td>
</tr>
</tbody>
</table>

Legend: M – Male subjects; F – Female subjects; C – Untrained subjects (controls); W – Elite wrestlers

Significantly different from the respective control group: * p<0.05; ** p<0.01; *** p<0.001

Results

General characteristics of the studied subjects are presented in Table 1. Both male and female wrestlers had significantly (p<0.001) lower body fat content than their untrained mates and significantly (p<0.05) higher BMI, which was indicative of higher muscle mass.

Plasma concentrations of Hcy, folic acid and CRP, as well as creatine kinase activities, are shown in Table 2. The levels of Hcy were significantly (p<0.001) higher in untrained subjects than in the respective groups of wrestlers and, in addition, untrained male subjects exhibited a much higher scatter of values (SD) than the wrestlers and the same was true for CRP (p<0.05 – 0.01).
Interestingly, significantly (p<0.01) higher Hcy levels were noted in the female than in male wrestlers. Mean concentrations of folic acid mirrored those of Hcy, were highest in male wrestlers and significantly (p<0.05) higher than in the female ones. That relation was reflected by correlation coefficients; when both male or female subgroups were combined, the r values amounted to -0.628 (p<0.01) and -0.442 (p<0.05), respectively. Correlation coefficients were non-significant in individual subgroups due to small numbers of subjects. The activities of creatine kinase were significantly (p<0.01) highest in male wrestlers and significantly (p<0.05) higher than in the female ones. That relation was reflected by correlation coefficients; when both male or female subgroups were combined, the r values amounted to -0.628 (p<0.01) and -0.442 (p<0.05), respectively. Correlation coefficients were non-significant in individual subgroups due to small numbers of subjects. The activities of creatine kinase were significantly (p<0.01) highest in male wrestlers than in all other subgroups and were not significantly correlated with CRP concentrations.

Discussion

Concentrations of homocysteine in plasma are considered to depend, to a high degree, on folic acid, and vitamins B₆ and B₁₂ intakes. Appel et al. [1] reported increases in plasma folic acid and decreases in Hcy concentrations following an augmented consumption of fruits and vegetables rich in folic acid. Similar effect was observed by Ubbink et al. [33] who supplemented diet with a preparation containing vitamins B₆, B₁₂ and folic acid.

Homocysteine concentrations were reported to be higher in sedentary men than in women in a wide age range [29]; in this study no such difference was observed but the numbers of subjects and age ranges were small. Yet, the levels of folic acid were significantly higher and those of Hcy — lower in wrestlers than in the untrained subjects while Hermann et al. [13] found also lower Hcy values in endurance athletes but those of folic acid were unchanged compared with controls. Konig et al. [20] reported that Hcy concentrations decreased following an endurance training lasting 30 days but increased following an isolated, intense exertion. In another study [14], however, a 3-week training brought about increases in plasma Hcy irrespectively of training intensity.

In this study, female wrestlers had significantly higher concentrations of folic acid than their untrained mates but lower than in male wrestlers, the levels of Hcy being alike but higher than in male athletes. These results are difficult to discuss as no reports on the effects of competitive training on folic acid and homocysteine in women were found in the available literature and, moreover, no data pertaining to the intake of those compounds with the diet were available. It could be, however, presumed that female wrestlers were less strict than their male mates in observing the rules of weight reduction. The report of de Bree et al. [9] who found lower consumption of folic acid by Danish women than men might support our view.

The negative relationship between Hcy and folic acid concentrations was clearly visible in all subgroups but the coefficients of correlation were non-significant due to small numbers of subjects but attained high significance after having combined the results within genders. Such a negative relationship was found also in patients with coronary heart disease and in healthy ones [26].

Resting levels of plasma CRP in athletes depend on two processes opposite to one another: intense exertions induce microdamages of muscle fibres and local inflammatory responses, thus increasing CRP concentration, while regular training reduces such responses and brings about CRP decreases [28]. On the other hand, isolated physical exertions may induce CRP increases in plasma; in a study on Ironman triathletes, Suzuki et al. [32] found significant increases in CRP and in creatine kinase activity 24 h post-exercise. In addition, plasma levels of CRP and CK activities were significantly correlated 24 h following a 32-km mountain race [23]; however, CK activities steadily increased throughout 96 h following repeated eccentric exertions, CRP levels remaining unchanged [24]. No significant correlation between those variables was found in this study.

The reports on the effects of sport training on CRP levels in plasma are equivocal; Marcell et al. [21] found

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**Table 2.** Mean values (±SD) of studied plasma constituents in elite wrestlers and in untrained subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>MC (n = 11)</th>
<th>MW (n = 11)</th>
<th>FC (n = 11)</th>
<th>FW (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hcy (µmol/L)</td>
<td></td>
<td>11.12 ± 2.88</td>
<td>6.14 ± 1.52***</td>
<td>9.84 ± 1.58</td>
<td>8.01 ± 1.10**</td>
</tr>
<tr>
<td>Folic acid (ng/mL)</td>
<td></td>
<td>7.10 ± 2.60</td>
<td>12.20 ± 3.50***</td>
<td>6.80 ± 1.70</td>
<td>9.20 ± 3.10*</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td></td>
<td>1.36 ± 0.84</td>
<td>0.36 ± 0.18**</td>
<td>0.86 ± 0.45</td>
<td>0.50 ± 0.17*</td>
</tr>
<tr>
<td>CK activity (U/L)</td>
<td></td>
<td>120 ± 30</td>
<td>241 ± 94***</td>
<td>108 ± 45</td>
<td>119 ± 57</td>
</tr>
</tbody>
</table>

Legend: M – Male subjects; F – Female subjects; C – Untrained subjects (controls); W – Elite wrestlers; Hcy – Homocysteine; hsCRP – C-reactive protein; CK – Creatine kinase

Significantly different from the respective control group: * p<0.05; ** p<0.01; *** p<0.001; Significantly different from the respective male group: º p<0.05; ºº p<0.01
no effect of 16-week training of moderate or high intensity while Mattusch et al. [22] observed significant decrease following endurance training lasting 9 months and suggested that such kind of training reduced inflammatory responses by augmenting the antioxidative potential. Our results, showing significantly lower CRP levels in plasma of elite wrestlers than in untrained subjects, supported that latter report.

The activity of creatine kinase is low under resting conditions and lower in women than in men due to protective action of estrogens on cell membranes [2]. That activity may dramatically increase following a strenuous exercise and physical training may induce persistently elevated activity of that enzyme [16]. Endurance athletes were reported to have higher resting CK activity compared with untrained subjects [19]. The differences observed in this study might have been related to gender and to the training history; some carry-over effects of strenuous training could be more expressed in male wrestlers who had highest levels of CK activity.

References


