Investigation of possible changes to biochemical indices regarding specific forms of exercise (soccer, swimming etc) in childhood

Bekris E.1, Giovanis V.1, Anagnostakos K.2, Dafoopoulos G.1, Souglis A.1, Sotiropoulos A.1
1National and Kapodistrian University of Athens – Department of Physical Education and Sport Science
2General Hospital of Karpenissi – Resident Physician Orthopedics

Address for correspondence: Bekris E., E-mail: bekris@med.uoa.gr

Abstract

The classical danger factors (high blood pressure, diabetes, smoking, hyperlipidemia, obesity) remain the basic causes of cardiovascular disease, but its increased incidence nowadays cannot be totally attributed to them. Over the last few years, researches have been investigating “newer” cardiovascular disease factors, such as Fibrinogen, reactionary protein (CRP) and total antioxidant capacity (TAC). Many researchers argue that Fibrinogen is instrumental in causing atherosclerosis and inflammation [73,30,71]. Reactionary protein has been involved in all stages of atherosclerosis, atherosclerosis and inflammation [73,30,71]. Reactionary protein has been involved in all stages of atherosclerosis, atherosclerosis and inflammation [73,30,71]. Reactionary protein has been involved in all stages of atherosclerosis, atherosclerosis and inflammation [73,30,71]. Reactionary protein has been involved in all stages of atherosclerosis, atherosclerosis and inflammation [73,30,71]. Reactionary protein has been involved in all stages of atherosclerosis, atherosclerosis and inflammation [73,30,71].

Keywords:
biochemistry of exercise, football, swimming, reactionary protein (CRP), fibrinogen (fib), total antioxidant capacity (TAC).

Introduction

One of the most important problems plaguing developed societies is cardiovascular diseases, namely coronary disease, strokes and peripheral occlusive heart disease. According to Murray & Lopez [57], coronary disease (CVD) is the first cause of death in developed countries, while according to Kochanek et al. [40], cardiovascular disease is the main cause of death among men in the USA.

The classical danger factors (high blood pressure, diabetes, smoking, hyperlipidemia, obesity) remain the basic causes of cardiovascular disease, but its increased incidence nowadays cannot be totally attributed to them. Over the last few years, researches have been investigating “newer” cardiovascular disease factors, such as Fibrinogen, reactionary protein (CRP) and total antioxidant capacity (TAC). Many researches argue that Fibrinogen is instrumental in causing atherosclerosis and inflammation [73,30,71]. Reactionary protein has been involved in all stages of atherosclerosis, from the initial intake of inflammatory cells inside the artery wall to the rupture of the plaque. CRP not only appears to be an important factor in predicting cardiovascular disease (CVD), [69], but also, increased levels of it point to potential cardiovascular incidents [49]. Atherosclerosis is an example of nosological procedure in which there is tell-
ing evidence regarding the participation of oxidative stress. Drastic oxygen radicals affect the vessel function with a series of pathogenic mechanisms [84,87]. Oxidative damage on the vessel wall leads to atherosclerosis when the balance / equilibrium between its pro-oxidant and antioxidant substances is disrupted [83]. According to Giannakopoulos [25] and Osganian [61], antioxidants protect the cells’ membranes by neutralizing free oxygen roots, maintaining redox balance (homeostasis) and protecting the heart since they increase the resistivity of the vessels and limit inflammatory factors.

Although the factors of the risk of developing cardiovascular disease clinically appear in adulthood, those that affect coronary disease originate in childhood [81] and according to Colin Boeham et al. [14], the benefits stemming from adequate physical activity during childhood, such as a healthier cardiovascular profile, are multifarious and are also enjoyed in adult life.

Many researches have proven the positive influence of exercise on the prevention of cardiovascular complaints [4,5,86,78], on the minimized likelihood of developing metabolic and heart diseases and its contribution to longevity [62,41,67,86]. Physical activity influences the cardiovascular, neuro – hormonal and muscular structures, the coagulation and fibrinolysis mechanisms, while it helps the atherosclerosis regression and it promotes the endothelial function [75,29,31,51,79,45]. In a number of researches studying adults or children, fibrinogen is inversely related to physical fitness [46,18,17,9], while other studies (on children) have not reached the same conclusion [76,77]. In certain bibliography reviews, it is observed that CRP decreases with regular exercise in adults, whereas, equivocal results are confirmed in another review, regarding children [76]. In recent researches, it is observed that regular exercise improves antioxidant capacity in teenage athletes [2], in adult soccer players after a preparatory program [52] or after a routine of practice and games [23] as well as in mice, after a short exercise program [88]. Nevertheless, there is no bibliographical data considering the effect of exercise on antioxidant capacity in children with different physical activity. The aim of the present study is to investigate the possible influence of different forms of physical activity on cardiovascular biochemical indices, such as reactionary protein (CRP), total antioxidant capacity (TAC) and Fibrinogen in boys aged 9 – 14 years old.

**Methodology**

**Sample**

The study was based on 168 boys classified into four groups with diverse physical activity – exercise patterns.

The first group (Group 1) comprised 41 boys aged 10.33±0.88 years old with a Low Physical Activity Level (L.P.A.L.) for whom the only form of exercise was P.E. (Physical Education) at school, which took place twice a week and lasted 45 minutes each time.

The second group (Group 2) comprised 42 boys aged 10.62 ± 0.21 years old with a Moderate Physical Activity Level (M.P.A.L.) who, apart from P.E. classes participated in 90 – minute soccer practice twice a week.

The third group (Group 3) comprised 45 boys aged 11.68±0.11 years old with a High Physical Activity Level (H.P.A.L.) who, apart from P.E. classes participated in 90 – minute soccer practice three times a week.

The fourth group (Group 4) comprised 40 boys aged 10.71±0.24 years old with a Very High Physical Activity Level (V.H.P.A.L.) who, apart from P.E. classes participated in 90 – minute swimming training five times a week.

The planning of the survey was such so as to minimize any differentiation among the four Groups regarding environmental factors. The subjects (boys) were Athens residents since birth. The selection was based on random sampling among a number of volunteers after lots were drawn.

**Measurement procedure**

The individuals of all four Groups underwent a routine clinical examination test (cardiograph, chest x ray, systolic and diastolic pressure) which did not reveal any obvious pathological condition. Moreover, the same individuals did not have any health problems and were not using any medication during blood taking. At the same time, their eating habits medical history was taken. The boys and their parents were informed on the purpose, the procedure and the planning of the survey in detail. All parents signed a written consent form, allowing their children’s participation in the study.

**Measurements**

**a). Anthropometric.** The anthropometric parameters that were examined were height, body fat percentage and body mass index. For the estimate of body composition (fat mass and body fat percentage), the Meyhew method was applied [54]. This method is based on measuring the fat between two skin folds, middle femoral and hyper iliac (table 1).

**b). Maximum oxygen intake.** To measure maximum oxygen intake (VO2max), the retrograde run endurance test (recurrent 20m running) was used (table 2-3). The test is based on a recorded sound which is heard [39].

**c). Blood taking.** Blood was taken from all subjects in the morning after a 12 hour fasting and a 36 hour abstinence from exercise. During the donation, the subjects were sitting down. The samples were taken from the Royal Bonanza vein. 9 ml of blood were taken with a Cliss syringe without anti-freezing for serum taking. Reactionary protein (CRP) was determined through the method of anosofelometria in special, automatic Dade Behring reagents. Fibrinogen was determined through the method of anosofelometria in special, automatic Dade Behring reagents. Total antioxidant capacity (TAC) was measured with an Olympus Au – 600 automatic biochemical analyzer (table 2 – 3).

**Statistical analysis**

The SPSS 15 statistical program was used to statistically process the finds, while one way anova variance analysis was used for the comparisons of different forms of exercise among all four Groups.

The post hoc test – Bonferroni was used for the differences of each variable between any two Groups. All statistical significances were controlled in the entire probability level α = 0.1. Parametrical correlations between physiological characteristics and biochemical indices were made through the linear correlation technique and the Pearson (r) coefficient correlation balance on a
Table 1

Means and standard deviations of boys (n = 168) for the age (years old), training age (years old), height (cm), body weight (kg), body fat percentage (%) and body mass index (kg / m2).

<table>
<thead>
<tr>
<th>GROUPS WITH DIVERSE PHYSICAL ACTIVITY PATTERNS. N=168</th>
<th>AGE</th>
<th>TRAINING AGE</th>
<th>HEIGHT</th>
<th>BODY WEIGHT (KG)</th>
<th>BODY FAT PERCENTAGE (%)</th>
<th>BODY MASS INDEX (KG / M2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (L.P.A.L.) N=41</td>
<td>10,33±0,88</td>
<td>0</td>
<td>151,33±8,11</td>
<td>44,93±6,86</td>
<td>10,75±2,06</td>
<td>19,32±1,25</td>
</tr>
<tr>
<td>Group 2 (M.P.A.L.) N=42</td>
<td>10,62±0,21</td>
<td>2±0,8</td>
<td>144,90±1,95</td>
<td>40,02±1,28</td>
<td>12,79±1,06</td>
<td>19,02±0,57</td>
</tr>
<tr>
<td>Group 3 (H.P.A.L.) N=45</td>
<td>11,68±0,11</td>
<td>3±0,7</td>
<td>154,94±3,15</td>
<td>46,27±2,40</td>
<td>11,89±2,48</td>
<td>19,30±0,98</td>
</tr>
<tr>
<td>Group 4 (V.H.P.A.L) N=40</td>
<td>10,71±0,24</td>
<td>4±1,2</td>
<td>148,04±2,94</td>
<td>41,46±3,02</td>
<td>13,65±1,74</td>
<td>18,67±0,82</td>
</tr>
</tbody>
</table>

Table 2

Means and typical errors in the 4 Groups variables of different forms of exercise

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total antioxidant capacity (TAC)</td>
<td>1,30±0,09</td>
<td>1,02±0,06</td>
<td>1,38±0,07</td>
<td>1,32±0,11</td>
</tr>
<tr>
<td>Fibrinogen (Fib)</td>
<td>340,33±28,061</td>
<td>377,33±30,76</td>
<td>288,66±15,45</td>
<td>267,00±49,64</td>
</tr>
<tr>
<td>Reactionary protein (CRP)</td>
<td>4,52±0,92</td>
<td>4,41±0,32</td>
<td>4,57±0,53</td>
<td>1,97±0,20</td>
</tr>
<tr>
<td>Maximum oxygen intake (VO2max)</td>
<td>43,58±0,58</td>
<td>48,76±1,28</td>
<td>54,94±1,02</td>
<td>48,94±1,11</td>
</tr>
</tbody>
</table>

Table 3

One way anova variance analysis on the 4 Groups regarding the TAC, Fib, CRP, VO2max variables which presented statistically significant differences.

<table>
<thead>
<tr>
<th>ANOVA</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum oxygen intake (VO2max)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>176,179</td>
<td>3</td>
<td>58,73</td>
<td>3,890</td>
<td>0,017</td>
</tr>
<tr>
<td>Within Groups</td>
<td>498,188</td>
<td>33</td>
<td>15,10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>674,367</td>
<td>36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total antioxidant capacity (TAC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>0,770</td>
<td>3</td>
<td>0,26</td>
<td>4,661</td>
<td>0,007</td>
</tr>
<tr>
<td>Within Groups</td>
<td>2,202</td>
<td>40</td>
<td>0,06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2,972</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (Fib)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>96,977,841</td>
<td>3</td>
<td>32,325,95</td>
<td>3,568</td>
<td>0,023</td>
</tr>
<tr>
<td>Within Groups</td>
<td>344,230,731</td>
<td>38</td>
<td>9,058,70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>441,208,571</td>
<td>41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactionary protein (CRP)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>55,768</td>
<td>3</td>
<td>18,589</td>
<td>14,279</td>
<td>0,000</td>
</tr>
<tr>
<td>Within Groups</td>
<td>41,661</td>
<td>32</td>
<td>1,302</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>97,429</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The differences of antioxidant capacity levels observed among the Groups seem to be affected by a number of various factors, such as the kind and frequency of training stimuli [52], the duration [6], the quantity and vigor [27] of the applied training program when measurements are taken, the subjects’ age, their diet as well as their physical fitness before the application of a specific workout protocol [21].

**Regarding reactionary protein (CRP) results**

The favorable influence of exercise on the reduction of CRP levels has been proven by many scholars / researchers [26,54,1,41,66]. This survey’s results agree with those of the aforementioned researches, since Group 4 (VHPAL) shows significantly the lowest levels of CRP in relation to the other Groups, thus affirming Kasapis & Thompson’s [36] view that there is an inverse relationship between CRP serum levels and physical activity level. The same view has also been expressed by King et al. [38], who observed that the significant superiority of those exercising – regardless of form of exercise – in comparison to the control group (sedentary lifestyle) was independent of age. Reinforcing the significance of regular exercise in children’s health [55], realized that children of normal weight with dyslipidemia (low levels of HDL-C and increased cholesterol) who participate in regular exercise do not show – as would be expected – high levels of CRP, thus sustaining their health on non-dangerous / risk – free levels. According to Moldoveanu et al. [54], moderate to high vigor exercise causes a transient increase in pro-inflammatory cytokines as well as in acute phase proteins, like CRP, whereas long-term and systematic physical activity is linked to lower pro-inflammatory cytokines and acute phase proteins readings in these subjects’ blood. Reactionary protein (CRP) is mainly produced by the liver in response to a variety of stimulators and increases the inflammatory response in all those factors that commence acute phase cataract. Acute phase is a common reaction to a variety of dangers that disrupt homeostasis, one of which is extended exercise [19]. Physical activity seems to cause many of these changes in the subject’s organism, which is mainly attributable the acute response following vigorous and exhausting exercise. This response includes metabolic changes, such as change in the nitrite balance, changes in the metabolism of lipids, changes in the concentration of cations, changes in the metabolism of iron, activation of the complement, leukocytosis and increased liver – originating protein production that participate in and orchestrate acute phase response. Respectively, albumin, a2 glycoprotein, a fetoprotein and transferrin are reduced, thus reflecting the change of the liver’s functional equilibrium [43]. According to Pedersen et al. [63], CRP’s source is liver-induced production with IL-6 as the major stimulator, which seems to be produced in the highest percentage by the exercised muscle. One of the ways in which exercise can reduce the risk of cardiovascular disease is through inflammation reduction. However, the mechanisms responsible for the protective effect of physical activity are not perfectly clear yet. According to Okita et al. [60], the beneficial effects of regular exercise on CRP levels mainly stem from changes in the fat tissue, while, at the same time, Pitsavos et al. [64] observe that regular exercise greatly reduces the levels of inflammatory
Fig. 1. Total antioxidant capacity means in Groups with different forms of exercise.

Fig. 2. Fibrinogen means in Groups with different forms of exercise.

Fig. 3. CRP means in Groups with different forms of exercise.
Fig. 4. Maximum oxygen intake (VO2 max) means in Groups with different forms of exercise.

Fig. 5. Correlation between body fat percentage and fibrinogen in the four experimental groups.

Fig. 6. Correlation between body mass index and fibrinogen in the four experimental groups.
indices contributing to atherosclerosis, causing a 33% reduction in CRP levels.

Regarding fibrinogen results

The present study realized statistically significant differences in fibrinogen levels between Groups 1 and 4 (p – value = 0.070 < 0.10) as well as between Groups 2 and 4 (p – value = 0.071 < 0.10). Moreover, Group 4 (VHPAL) shows the lowest fibrinogen levels, followed by Group 3 (HPAL). The results are consistent with the conviction that regular exercise reduces fibrinogen levels [15,17,9] and is related with a better cardiovascular function since it seems to intervene in the fibrinogen regulation mechanism [11]. The parameters involved in all these procedures are not always clear but it seems that action on endothelial level plays a pivotal role.

Apart from the quantity of exercise (frequency per week), the level of physical fitness (ergo, the intensity of exercise) seems to play a significant role in fibrinogen levels, as estimated from maximum oxygen intake (VO2 max). According to this study’s results, maximum oxygen intake (VO2 max) is negatively correlated to fibrinogen (correlation coefficient = -0.415). These conclusions have also been reached by various researchers [9,35,58,33], who mention that there is a gradually inverse relationship between fibrinogen and physical activity as well as physical fitness level as estimated from maximum oxygen intake, noting that physical activity and cardiopulmonary condition are independent prognostic indices of fibrinogen levels. The present study believes that this inverse relationship between fibrinogen and maximum oxygen intake may also be valid for children (boys). It also agrees with Blair et al. view [5] that cardiovascular benefits can be unexpectedly big even with a small increase in physical fitness level.

An important find in this research is the fact that body fat percentage is positively correlated to fibrinogen (correlation coefficient = 0.398) and that body mass index (BMI) is positively correlated to fibrinogen (correlation coefficient = 0.462). Other surveys also reached the same conclusions [8,10,3,22,74], reinforcing the view that anthropometric characteristics, such as body fat percentage and body mass index, which are largely dependent on workout patterns, affect fibrinogen levels in children.

It seems that the favorable influence of exercise on a better cardiovascular function may also be mediated by the influence of exercise on the coagulation system [12]. More specifically, systematic exercise helps in the secretion of NO [13]. According to surveys, NO is the main vasodilation factor and is produced by the vessel endothelium [80,32], while its secretion is related to good endothelium function. Endothelium dysfunction is a basic parameter of atherosclerosis, whose major characteristic is inadequate NO secretion, while at the same time, the substances and procedures that are related to increased inflammation and resulting vessel contraction thrive [16,70,72,20]. NO activates t-PA secretion by inhibiting PAI – I, a molecule which gets involved in blood coagulation and blood clot formation, thus increasing the fibrinogen situation [50]. The generated NO reduces the basic fibrinogen levels, which is why there are lower fibrinogen levels in the plasma of athletes and generally, in the plasma of people with higher levels of physical activity. Thus, there is better cardiovascular function [37]. Moreover, regular exercise contributes to low platelet activation, reduced tendency towards their accumulation and fibrinogen sticking to their surface, resulting in lower concentration of this factor in the plasma of regularly exercising individuals [85].

Conclusions

From the results of the present survey, the protective role of regular exercise and physical fitness level against cardiovascular complaints in children is clear. The beneficial advantages of various forms of physical activity seem to differentiate according to their characteristics such as frequency, intensity, duration and kind as well as the physical fitness level of the exercising children. According to the results, Group 4 (VHPAL), working out 5 times a week, swimming for a total of 7.5 hours, presents the most favorable levels of cardiovascular biochemical indices, since it shows the lowest levels of inflammatory indices contributing to atherosclerosis, such as fibrinogen and CRP, in relation to other forms of exercise, and it also shows the highest antioxidant capacity levels after Group 3 (HPAL).
It is therefore concluded that very frequent weekly exercise through swimming is particularly effective in shielding children’s health. An important find of this survey is the realization that the better the physical activity level and especially the improvement of maximum oxygen intake, the lower the fibrinogen levels, so children are less at risk of cardiovascular complaints. This result denotes the importance of the qualitative characteristics of physical activity (intensity) and not just the quantitative ones (duration). Finally, a particularly important conclusion of this survey is the fact that body fat percentage and body mass index are positively correlated to fibrinogen, proving the emphasis that should be laid to all of children’s normal weight gaining habits, which affect their cardiovascular function irrespective of their physical activity level.

To summarize, the present survey concludes that regular exercise in children is beneficial against atherosclerosis and coronary disease. However, to maximize these benefits, exercise should be combined with a high physical activity level and a proper diet.

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