Original Article

Evaluation of biochemical parameters in the blood of adolescent boys and girls

KAKAVAS ATHANASIOS¹; SIOKOU EVAGELIA¹; SOTIROPOULOS ANTONIOS²; SOUGLIS ATHANASIOS¹

¹ Department of Physical Education and Sport Science, National and Kapodistrian University of Athens, GREECE

² Faculty of Sports and Physical Education, Transilvania University of Brasov, ROMANIA

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Abstract:

A great amount of research shows the correlation of lipids and other biochemical indicators with the appearance of cardiovascular and other diseases. The purpose of this project is to investigate the effect of exercise on the biochemical indicators that relate to the appearance of cardiovascular diseases on young athletes and children having physical activity. 233 children participated in the project, which were separated in two groups, the control group with 120 non athletic children (60 boys and 60 girls) and the experimental group with athletic children (55 boys and 58 girls). During measurement, anthropometric elements and blood tests results were taken and the levels of :T-C, TG, LDL-C, HDL-C, CK-MB, CRP, BNP, NT-proBNP, fibrinogen, Hb, PEROX AND IMANOX were determined. The results prove that boy – athletes showed statistically significant lower concentrations of T-C and CRP in comparison to the control group. A similar difference, albeit statistically insignificant, was also noticeable in girls. Likewise, the boys as well as the girls of the experimental group showed statistically significant lower values in LDL-C, IMANOX, PEROX, BNP, NT-proBNP in comparison to their non-athlete peers. Regarding fibrinogen values, they were lower in the control group girls than in the experimental group girls, a discrepancy not observed in boys. Finally, there were no discrepancies regarding TG, HDL-C, Hb and CK-MB. In conclusion, we can say that exercise during childhood and teenage years contributes to the reduction in factors which lead to cardiovascular and other metabolic ailments in adult life, improving the level of life.

Key words: cardiovascular disease, exercise, children, biochemical markers, lipoprotein.

Introduction

A great amount of researches indicate the correlation of lipoprotein and other biochemical indicators, such as the C-reactive protein with the appearance and prevention of cardiovascular diseases (CVD) (Baigent et al, 2010; Bittner et al 2002). The cardiovascular diseases that connect to the lipid profile of each person is the most common condition leading to the death in America (Gao & Yuan 2010). The beginning of that pathogenic situation begins from childhood (Bahas-Nakash et al 2010; Chae et al 2010). However, it is highly recommended that prevention should start from that age (Bao et al 1994). More specifically, it appears that lack of exercise or decreased physical activity is the main reason for the appearance of cardiovascular and other diseases in childhood (Despres et al 1990; Hernandez et al 1999; Jago et al 2010). As a person grows, these ten indicators become more aggravating (Bittner et al 2002; Kelley & Kelley 2008). That is the reason why many centers around the world recommend preventive exercising programs for children to achieve the decrease and control of these biochemical indicators (lipoproteins of high and low frequency, C-reactive protein, triglycerides, natriuretic peptide and more) that are responsible for the appearance of various diseases, during childhood as well as during adolescence when the danger increases (Torrance et al 2007; Kelley & Kelley 2008). There is a reverse relation between normal activity and the factors that are responsible for the appearance of the coronary disease (Blair 1997; Lee et al 2001).

Therefore, in bibliography there are many controversial researches, regarding with physical exercise and its contribution in the decrease of LDL-C, obesity, cardiovascular diseases, triglycerides, and other factors (Kelley & Kelley 2005; Twisk et al 2000). In many cases aerobics are highly suggested and in some others a way of exercise that combines aerobics and weight-lifting is suggested (Chae et al 2010; Krämer et al 2009; Lee et al 2010). Simultaneously, there are many referenced on the appropriate time and frequency of exercise especially for kids. Particularly, it is mentioned that the appropriate time is 40 minutes or more and the appropriate frequency is 5 times per week (Bahas-Nakash et al 2010) so that hard exercise can contribute in the prevention of the factors that were mentioned before. Furthermore, it is mentioned that 30 minutes of physical exercise per day significantly contributes in the prevention of the previously mentioned diseases for persons over 2 years old (Epstein et al 2001). Finally it is marked that physical activity during school has no contribution in

that prevention, maybe because of the short duration, small intensity and the low frequency (Dobbins et al 2009; Pate et al 2006). For the reasons above, it is thought necessary to examine the effect of the exercise on the biochemical indicators that are related with the cardiovascular and other metabolic diseases, on kids during the transitive period of adolescence. Regarding with the comparison of athletic activity during school and intense athletic activity on high level there will be an appropriate separation in order to examine that factor as well.

According to the above, the purpose of that research is the evaluation and examination of the biochemical indicators that are related with the other diseases during adolescence and their relation with the exercise.

Method

Participants

At this research participated 233 children in total, that were separated in two groups: A) The control group was consisted of 120 children (60 boys and 60 girls), at the age of $14,5 \pm 2,3$ and $13,8 \pm 1,9$ years old. The members of this group did not perform any kind of exercise on regular bases (sedentary life), except from physical exercise during school. B) The experimental group was consisted from 113 children (55 boys and 58 girls) at the age of $14,4 \pm 1,9$ and $13,2 \pm 2,1$ years old). The members of this group were football and rhythmic gymnastics athletes, who worked out on regular bases from the age of 6-7 years old. The selection of the participants was made through the method of random sampling among many volunteers. The differences between those two teams were minimized during the designing of the research based on the environmental factors. All the participants were born and live in Athens.

Procedure:

The parents and the participants (children) were informed in details for the purpose, read and signed written informed consent from consistent with the principles outlined in the Declaration of Helsinki. Before the measurement procedure, the members of both teams took routine clinic tests (cardiogram, chest x-ray, systolic and diastolic blood pressure) which did not uncover any obvious pathological situation. At the same time, individual background about eating habits was taken and abstinence from drugs was ensured. The measurement procedure was consisted of 3 parts:

1. Human-based characteristics: Firstly height, body weight and the percentage of body fat were measured (Table 1). The calculation of body fat was made through the two skin fold method (femoral and loin) [29].

Table 1. Age, Height, body weight and percentage of body fat in the Experimental and Control groups

Research Groups	Age	High	Weight	Body Fat
Experimental Group	♂ $14,4 \pm 1,9y$	$162 \pm 4cm$	$50,02 \pm 4,54kg$	$12,5 \pm 3,06\%$
	♀ $13,2 \pm 2,1y$	$154 \pm 2,1cm$	$39,72 \pm 3,32kg$	$8,95 \pm 3,02\%$
Control Group	♂ $14,5 \pm 2,3y$	$160 \pm 3,8cm$	$52,5 \pm 4,9kg$	$15,02 \pm 4,95\%$
	♀ $13,8 \pm 1,9y$	$151 \pm 1,7cm$	$44,61 \pm 4,8kg$	$13,47 \pm 4,8\%$

2. Blood tests: Blood sampling were made during morning hours following 12 hours fasting. A quantity of great blood was taken with type Cliss needle from the royal and middle vein. The samples were put in glass tubes. Centrifugation took place half or one hour from when the sample was taken, for 10 minutes at 2.500 spins per minute. The analysis of the samples was done in the laboratory of the university of biological chemistry of Athens and the biochemical department of the hospital "Evangelismos" in Athens.

3. Definition of parameters: In the end, the definition of the parameters that were to be analyzed was made. More specifically, total cholesterol (T-C) and triglycerides (TG) were identified enzymatic (BIOSIS, KIT, No 2403 and No 2101). The definition of lipoproteins of high density (HDL-C) was made through the phosphorous tungsten method (BIOSIS, KIT No 2501) and through BIOSIS, KIT No 2403 enzymatically. For the isoenzyme of the creatine kinase (CK-MB) an ROCHE /HITACHI analyzer was used (KIT No 917), whereas for the c-reactive protein (CRP) an nepelometric analyzer was used (BNII, Dade, Behring Diagnostics). The levels of sodium peptides (BNP, NT-proBNP) were measured with the immunoassay method ELISA. The Fibrinogen was measured with the Clauss method. The definition of hemoglobin (HB) was made with Sysmex analyzer. Finally, they studied the antioxidant ability in order to define the indicator of oxidative stress PEROX (active oxygen compounds) and the indicator of total antioxidant ability (IMANOX).

Statistic analysis

For the definition of the differences of the two groups for every sex (boys experimental and control group, girls experimental and control group). A t-test was used for independent samples for all the dependent variables. Statistical significance was established at $p < 0,05$ (two-side control).

Results

The averages, the standard deviations and the standard errors of the averages of the experimental and the control group of the dependent variables for gils and boys are displayed at tables 2 and 3.

Table 2. Averages, standard deviations and standard errors of the averages of the experimental and the control group of the dependent variables for girls.

<i>Depended Variables</i>	<i>Girls</i>	N	Average	<i>Standard Deviation</i>	<i>Standard Error of Average</i>
T-C	Control group	60	175.50	28.21	5.15
	Experimental group	58	165.00	16.89	3.19
TG	Control group	60	60.60	21.00	3.83
	Experimental group	58	53.58	13.67	2.58
HDL-C	Control group	60	65.13	16.12	2.94
	Experimental group	58	69.92	6.00	1.13
LDL-C	Control group	60	98.23	24.17	4.41
	Experimental group	58	84.36	17.09	3.23
CK-MB	Control group	60	18.21	4.08	0.75
	Experimental group	58	19.65	2.36	0.45
CRP	Control group	60	0.127	0.07	0.01
	Experimental group	58	0.114	0.04	0.01
Pro BNP	Control group	60	79.82	74.24	13.55
	Experimental group	58	45.15	23.20	4.39
Fibrinogen	Control group	60	316.53	55.67	10.17
	Experimental group	58	289.48	25.54	4.83
Hb	Control group	60	13.11	0.64	0.12
	Experimental group	58	12.91	0.97	0.18
IMANOX	Control group	60	312.78	35.63	6.50
	Experimental group	58	256.88	13.75	2.60
PEROX	Control group	60	314.11	99.60	18.19
	Experimental group	58	250.86	43.63	8.25

Table 3. Averages, standard deviations and standard errors of the averages of the experimental and the control group of the dependent variables for boys

<i>Depended Variable</i>	<i>Boys</i>	N	Average	<i>Standard Deviation</i>	<i>Standard Error of Average</i>
T-C	Control group	60	184.63	23.20	4.10
	Experimental group	55	158.72	19.66	3.59
TG	Control group	60	65.84	25.86	4.57
	Experimental group	55	63.47	23.95	4.37
HDL-C	Control group	60	63.81	13.21	2.34
	Experimental group	55	61.60	9.27	1.69
LDL-C	Control group	60	107.63	27.00	4.77
	Experimental group	55	84.43	15.32	2.80
CK-MB	Control group	60	20.78	4.16	0.74
	Experimental group	55	23.20	7.46	1.36
CRP	Control group	60	0.200	0.150	0.03
	Experimental group	55	0.130	0.070	0.01
Pro BNP	Control group	60	60.44	34.05	6.02

	Experimental group	55	38.56	35.75	6.53
Fibrinogen	Control group	60	327.55	59.06	10.44
	Experimental group	55	308.39	48.75	8.90
Hb	Control group	60	13.56	0.75	0.13
	Experimental group	55	13.63	1.15	0.21
IMANOX	Control group	60	299.87	45.31	8.01
	Experimental group	55	266.20	26.76	4.89
PEROX	Control group	60	353.29	111.97	19.79
	Experimental group	55	274.71	175.29	32.00

Total cholesterol (T-C): Regarding with the averages of T-C, no statistically significant difference was observed between both teams of girls. However, as far as the boys as concerned, the experimental group (athletes) showed statistically significant less concentration of T-C than the control group ($p<0.001$). Triglycerides: When we compare the averages of TG we observe that there isn't a significant difference between the girls. Lipoprotein of high density (HDL-C): The same was observed for HDL-C. No statistically significant differences were observed in both groups. Lipoprotein of low density (LDL-C): Regarding with LDL-C significant differences appear to exist between the two groups. More specifically, the girls that did not exercise on regular bases (control group) showed greater concentration of LDL-C than the girls that worked out (experimental group), ($p<0.05$). The difference between the averages is much higher for the boys of the control group, which showed higher concentration of LDL-C than the boys in the experimental group (athletes, $p<0.001$). Isoenzyme of the creatine kinase: No statistically significant differences was observed between the experimental and control group of girls and boys. C-reactive protein (CRP): When we compare the averages of CRP in girls, we don't observe any statistically significant difference between the experimental and the control group ($p>0.05$). However, when comparing the averages in boys, the experimental group (athletes) showed statistically significant less concentration of CRP than the control group ($p<0.009$). Natriuretic peptides: When we compare the averages of the levels natriuretic peptides (BNP, NT-proBNP), we find statistically significant differences in the groups of girls ($p<0.03$) as well as in the groups of boys, where the control group had higher concentration of PNP and NT-proBNP than the experimental group ($p<0.02$). IMANOX-PEROX: Similar results appear for IMANOX. When we compare the averages, we observe statistically significant higher values in the values control group of girls ($p<0.001$), as well as in the control group of boys ($p<0.001$), than the corresponding experimental groups. The same is observed for PEROX, where the boys of the control group have higher concentration, than the experimental group ($p<0.05$). The girls of the control group have higher concentration of PEROX than the experimental group ($p<0.005$). Fibrinogen: When comparing the averages of fibrinogen in girls, the experimental group (athletes), we observe statistically significant lower values of fibrinogen than the girls of the control group ($p<0.03$). On the other hand, no statistically significant difference between the control and the experimental group for boys ($p>0.05$). Hemoglobin(HB): Finally, no statistically significant differences were observed between the experimental and the control group, when comparing the averages of hemoglobin, for girls as well as for boys.

Discussion and conclusion

The purpose of this research was to study the lipid profile and other biochemical indicators of children (boys and girls), which were separated in two groups according to their occupation or their non contending athletics.

From the consideration of the bibliography we concluded that, the appearance of cardiovascular diseases has significantly high correlation with the high level of cholesterol (T-C), triglycerides (TAG), lipoproteins, reactive protein (CRP) and other factors. Comparing the averages of total cholesterol for girls, no statistically significant differences were observed between the two groups. However for boys, this gap was more significant. The control group had higher levels of total cholesterol than the athletes. This fact may be due to the low percentage of body fat of athletes both boys and girls, than the control group. Other researches (Jago et al 2010; Twisk et al 2000) were led to similar results. Twisk et al (2000) mention that sedentary life relates instantly with increased body fat and therefore with factors that burden the cardiovascular function. In general, the children that work out on regular bases appear to have better lipid profile than their peers, who choose sedentary life (Chae et al 2010; Lee et al 2001). A possible explanation for the lower concentration of cholesterol in the experimental group (athletes) could be the theory of the enzymatic hydrolysis of cholesterol esters, as well as the presence of hormone-sensitive enzymes (lipase/cholesterol esterase) which must be considered as responsible for a part of the total hydrolysis. During this procedure, we should not leave out the concentration of a key-enzyme to the bio-composition of cholesterol (HMGCoA- reductase).

As far as low density lipoprotein (LDL-C) is concerned the results are similar with the results of researches that involve regular aerobics exercise and combined exercise, showing that exercising in early age

contributes in decreasing LDL-C and therefore minimizes the danger of cardiovascular and other diseases (Kelley & Kelley 2005; Pitsavos et al 2009). It is known that high levels of LDL-C accelerate the procedure of atherosclerosis by placing cholesterol on the walls of the vein and creating atherosclerotic plaques, which can create an independent predisposed factor of developing cardiovascular diseases. The decreased levels of LDL-C in athletes could be explained either from the act of lipoprotein lipase (LPL) and the liver lipase (HL) in the lipoprotein particles, or the increased number of LDL-C receptors, that contribute in removing the lipoprotein from the circulation and reducing their concentration from the blood serum (Holvoet et al 2007) or decreased composition of particles that contain Apo-B, the main structural unit of LDL-C (Vermulen 1990). No differences were observed in the girls' group while the C-reactive protein (CRP) was examined, but only the experimental group of boys (athletes) showed statistically significant less concentration. However from the bibliography we observe that the aerobic and compound exercise both in children and adults contributes effectively at the decrease of the CRP levels (Krämer et al 2009). One of the ways that exercise can change the danger of cardiovascular disease is by reducing the inflammation. However the responsible mechanisms for the protective effect of exercise are completely clear. The beneficial effects on the levels of CRP come mainly from the alteration of the adipose tissues (Okita et al 2004). Regular exercise decreases significantly the level of the inflammatory indicators that contribute in the atherosclerosis by reducing by 33% the levels of CRP (Pitsavos et al 2003). However, no differences were observed in the study of Lee et al (2010) at the values of CRP. It is very important to emphasize on the fact that CRP concentration is a significant preventive indicator for the appearance of cardiovascular diseases (Lee et al 2010).

As far as it concerns the sodic peptides (BNP, NT-proBNP) higher values appeared in the control group of girls and boys. It is known that peptides relate to heart failure and have been recognized as an independent danger factor. However, researches that have studied the effect of exercise on BNP, NT-proBNP levels of people that already suffer of heart failure showed no correlation and the values stayed exactly the same. This fact shows that if these values can be reduced only in childhood, then prevention should start from early age (Nilsson et al 2010; Saura et al 2009). Furthermore a way to explain this fact is the low intensity of exercise that the people who suffer, accomplish. This issue is very interesting to study. It is known that the antioxidant mechanisms act protectively for the heart, increase the endurance of the vascular, reduce the inflammatory factors, prevent the oxidation of LDL cholesterol and contribute to the control of the levels of the arterial pressure and the homocysteine (Giannacopoulou 2009).

When analyzing the oxidant ability of IMANOX and PEROX for boys, as well as for girls, we observe statistically significant higher values in the control groups, as it is known that exercise contributes in reducing oxidative stress (Muhammad et al 2011). This fact is based on the high ability of the enzymatic and non-enzymatic antioxidant defensive system, since a regular exercise can reduce the oxidative stress and temper the muscular damage. Regular training can reduce the appearance factors that relate to the appearance of drastic kinds of oxygen and nitrogen. On the other hand the hard phase of exercise develops oxidative stress (Leeuwenburgh et al 1994).

Fibrinogen, which poses an important and independent danger to coronary disease, took statistically significant lower values in the experimental group girls than in their control group peers. The same occurred in the boys, although their discrepancies were statistically insignificant. It seems then that exercise contributes to the reduction of fibrinogen levels in children, something that also researches in adults corroborate (Guidi et al 2010; Krämer et al 2009). Additionally, it seems that the beneficial effect of exercise on cardiovascular function may probably be due to the effect of exercise on coagulation (Chen & Li 1993). More specifically, regular exercise assists in NO secretion, and, according to studies, NO is the major vasodilation factor and it is generated in the endothelium of the vessels, while its excretion is related to proper endothelial function. Regular bases exercising also provides us with the low activation of platelets, lower tension for their accumulation and prevents the attachment of the fibrinogen to their surface in order to reduce that factor on the plasma of people that do exercise regularly (Wang et al 1994). Finally, as far as the other indicators are concerned (TG, HDL-C, CK-MB, Hb), no statistically significant gaps were shown.

In conclusion, exercising during childhood and adolescence contributes in preventing the factors that lead to cardiovascular and other diseases during the coming of ages, increasing the level of health and the quality of life. More specifically, regular bases exercising not only heals but prevents as well during childhood obesity and other metabolic diseases that appear later (Jago et al 2010; Lee et al 2010). Furthermore, the study of natriuretic peptides should go to a higher level, since their values can change during childhood (whereas cannot be changed for the already adult sufferers) through exercise and can provide us with a great health advantage during adulthood.

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