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CHANGES IN HORMONAL AND LIPID PROFILE AFTER A SOCCER MATCH IN MALE AMATEUR PLAYERS

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Abstract This study examined the effects of a soccer match on plasma lipid profile, testosterone, cortisol and creatine kinase. Venous blood samples were taken before and after an official soccer match from twenty amateur soccer players (age: 24.5 ± 3). Blood was analyzed for total Cholesterol (T-C), high- (HDL) and low-density lipoproteins (LDL), serum triglycerides (TG), apolipoprotein B (APO-B) and AI (APO-AI), serum creatine phosphokinase (CPK) activity, testosterone (T), cortisol (C). CPK ($p<0.001$), C ($p<0.05$), APO-A ($p=0.004$) and HDL ($p=0.009$) level increased significantly after the match. Also, there was a significant decrease in LDL ($p=0.002$), APO-B ($p= 0.000$), TG ($p=0.000$), T-C ($p= 0.000$), LDL/HDL ratio ($p= 0.001$), T-C /HDL ratio ($p=0.000$), APO-A/APO-B ratio ($p=0.019$) and LDL/HDL ratio ($p=0.000$). T concentration decreased, while C concentration increased significantly, resulting in a >50% drop of T to C ratio ($p=0.000$). This data suggest that intermittent exercise of long duration, such as a soccer match, results in an acute antiatherogenic modification of lipid profile, possibly due to the high aerobic energy expenditure. The increase in C and decrease in T and the consequent large decrease of the T to C ratio, suggest that a soccer match places considerable stress on the endocrine system.

Key words: testosterone, cortisol, LDL, HDL, cholesterol, football

INTRODUCTION

Soccer is a high-intensity, intermittent exercise that relies predominantly on aerobic energy pathways, but also places considerable demands on the neuromuscular and hormonal systems due to the considerable high intensity exercise component (e.g. sprints, jumps, etc) [5, 25, 37, 41]. Prolonged acute submaximal exercise bouts (i.e. those exceeding 1-2 hours) may be associated with complex changes in circulating androgen. Plasma levels of testosterone (T) and cortisol (C) have been proposed as indicators of anabolic and catabolic activities, respectively [8]. In addition T to C ratio has been suggested as a useful marker for assessing both acute and longer-term training load as well as overtraining [22, 43, 47]. Elevation in resting total CPK may reflect muscle damage due to repeated explosive muscle actions with a high eccentric component performed during a soccer match [9, 10, 11].

Prolonged continuous and intermittent exercise has been shown to influence lipid metabolism [32]. Exercise exerts an independent effect on cardiovascular risk factors through a modification of plasma lipid levels. Decreases in plasma triglyceride levels as well as increases in plasma high density lipoprotein cholesterol and apolipoprotein A-I levels have been reported in both longitudinal and cross-sectional studies in which exercise effects have been studied [29, 36, 42, 46]. This effect may last only for 1-2 days after exercise [20] and thus regular training may be necessary in order to sustain the positive effects of exercise on lipid metabolism.

The present study was undertaken to examine the effects of a soccer match on plasma lipids, testosterone, cortisol and creatine kinase in amateur soccer players. Furthermore our purpose was to demonstrate the possible protective effect of this type of exercise on risk factors of cardiovascular disease and the degree of physiological stress associated with intermittent exercise.

MATERIAL AND METHODS

SAMPLES

Twenty amateur soccer players (age 24.5 ± 3) were recruited (Table 1). Measurements were conducted before and after an official match of the Amateur's league during the year 2004/2005. Soccer match began at 15:45 and finished at 17:30, while the environmental temperature ranged from 19 to 21 ^{o}C. All players were amateurs and their habitual training regiment consisted of 4-5 times a week for about 75-90 min per training session. All participants had a soccer experience of 6 ± 2 years. None of the subjects had any evidence of cardiovascular disease, diabetes (fasting glucose > 7 mmol/liter) or hypertension (blood pressure >160/90 mm Hg) and they were free of steroid medication. Each participant was informed of the experimental procedures and a written informed consent was obtained.

Table 1. Anthropometric and physical characteristics of soccer players.

Age (years)	Weight (kg)	Height (cm)	Fat (%)
24.5 ± 3	71.4 ± 4.8	177.6 ± 5.7	11.2 ± 2.31

DIET

To avoid any confounding effects of individualized nutrition, subjects followed a balanced diet containing 55 % carbohydrates, 15 % protein and 30 % lipids (average daily energy intake: 2784±150 Kcal) for six weeks before the study.

PROCEDURE

Two venous blood samples were taken from the right arm. The first sample was taken after a 12-h overnight fast, in the morning of the match day and without any previous training for at least 42h. The second sample was taken immediately after the end of the match. Blood was placed in tubes and immediately centrifuged for 10 min at 4000 x g. Serum was collected and stored at -20^{o}C before analysis.

BLOOD ANALYSIS

Measurement of total Cholesterol (T-C) and high density lipoprotein (HDL) was performed enzymatically by the oxidase-phenol aminopyrine (CHOD-PAP) method (Boehringer-Manheim) and serum triglycerides (TG) were determined by the Boehringer enzymatic peridichrome method. The lipid component of HDL was determined in the supernatant obtained from the whole plasma after precipitation of very low density lipoprotein (VLDL), and low density lipoprotein (LDL) by $MnCl_2$ (1mol/L) and dextran sulfate (0.4 mol/L, pH 7.7). LDL cholesterol was calculated using the following formula: LDL = TC – HDL (triglycerides/5).

Estimation of HDL_2 and HDL_3 concentrations was performed as previously described by Eyre et al. [17]. The apoprotein B contained in lipoproteins was precipitated from plasma samples with 20 ml heparin (5000 U/ml) and 50 ml MnCl2 (1mol/L). The supernatant (120µl) was adjusted to a density of 1.125 g/mL with a 1.351 g/mL density aqueous NaCl-NaBr solution and centrifuged for 3.5 h at 160.000 x g in an air-driven bench-top ultracentrifuge. The supernatant (containing HDL_2) was isolated from the infranatant (containing HDL_3 and other plasma proteins) in the tubeslicer made up to a final volume of 120 µl with 0.15 mol/l NaCl (using a Hamilton syringe) and assayed for cholesterol. Plasma HDL_2 cholesterol concentration was then calculated and multiplied by the dilution factor 1.163 HDL_3 cholesterol was either quantified as the difference between total HDL cholesterol and HDL_2 cholesterol or measured directly. Serum cortisol (C) was determined by Fluorescence Polarization Immunoassay-FPIA of the Abbott Company, while total testosterol (T) also was determined by using an immunoassay method of ROCHE.

Total serum creatine phosphokinase (CPK) activity was determined according to the method of the Boehringer Manheim Company. Serum apolipoprotein AI (APO-AI) and apolipoprotein B (APO-B) were both determined with single radial immunodiffusion–SRID from BEHRING. All samples were analyzed in the same assay in duplicate.

ANTHROPOMETRIC MEASUREMENTS

Body mass (wearing only sport clothes) and height were recorded to one decimal place (Seca, model: 7701321004, Vogel & Hamburg, Germany). Body fat was estimated using the equation developed by Durnin and Womersley based on the measurement of four skinfolds [16]. All measurements were made by the same investigator. All analyses were performed at the Department of Biological Chemistry of the University of Athens.

STATISTICAL ANALYSIS

Statistical analysis was carried out by SPSS 11.0. All measures are expressed as mean ±SD. Paired t-test was used to identify differences between the dependent variables. The level of significance was set at $P<0.05$.

RESULTS

Total CPK ($p<0.001$), Cortisol ($p<0.05$), APO-A ($p=0.004$) and HDL ($p=0.009$) levels increased significantly post exercise (Table 2). Moreover there was a significant decrease after the match in LDL ($p=0.002$), APO-B ($p= 0.000$), TG ($p=0.000$), T-C ($p= 0.000$), at LDL/HDL ratio ($p= 0.001$), T-C /HDL ratio ($p=0.000$), APO-A/APO-B ratio ($p=0.019$), LDL/HDL ratio ($p=0.000$). No significant differences were seen between pre and post exercise for HDL_2, HDL_3 and for HDL_2/HDL_3 ratio.

Testosterone concentration decreased, while cortisol concentration increased significantly (Table 2), resulting in a >50% drop of T to C ratio ($p=0.000$).

Table 2. Lipid profile, cortisol (C), testosterone (T) and creatine phosphokinase (CPK) in soccer players before and after a soccer match. Values are presented as mean ± SD.

Parameter	Pre match	Post match	P
CPK (U/L)	233.8± 79.4	361.3±100.2	P=0.000
C (μg/dl)	10.60±3.11	18.84±4.58	P=0.000
T (ng/dl)	5.65±1.74	4.66±1.76	P=0.002
T/C ratio (ng/μg)	0.56±0.23	0.27±0.15	P=0.000
HDL (mg/dl)	48.5±11.1	54.1±7.5	P=0.009
HDL_2 (mg/dl)	7.6±5.3	10.9±5.0	P=0.110
HDL_3 (mg/dl)	40.8±11.8	42.7±6.6	P=0.490
LDL (mg/dl)	88.4±16.4	78.0±14.9	P=0.000
APO-AI (mg/dl)	1.54±0.29	1.80±0.50	P=0.004
APO-B (mg/dl)	1.06±0.32	0.94±0.32	P=0.002
TG (mg/dl)	67.7±14.7	60.6±16.6	P=0.000
T-C (mg/dl)	155.9±17.8	144.5±16.7	P=0.000
LDL/HDL	1.92±0.57	0.49±0.32	P=0.001
T-C/HDL	3.38±0.7628	2.60±0.61	P=0.000
HDL_2/HDL_3	0.22±0.20	0.26±0.14	P=0.543
APO-A/APO-B	1.53±0.55	2.15±1.08	P=0.0019

HDL: High density lipoprotein, LDL: Low density lipoprotein, APO: apolipoprotein, TG: triglycerides, T-C: Total cholesterol

DISCUSSION

The present study was undertaken to quantify changes in C, T, CPK and lipid profile after a typical soccer match at amateur level. Circulating T and C levels were proposed as markers for estimation of anabolic/catabolic state and as an index of endocrine system exercise adaptations. Frequently T to C ratio was suggested as a marker of the overtraining – overreaching syndrome [22, 34, 43].

Prospective studies have confirmed that endurance training and other forms of strenuous exercise can result in reductions of total T and biologically available T levels [14]. Testosterone reduction may be an adaptation to stress associated to endurance exercise. During stressful circumstances, it may be beneficial to reduce functions that could detract survival [3]. The exact mechanism of decreasing resting total T in endurance athletes has not been clearly elucidated. Hackney et al suggested a reduction in testicular function [33]. Wheeler et al implicated hypothalamic dysfunction which was in accordance to the findings of Mac Connie et al who observed a reduction in the amplitude and frequency of luteinizing hormone

pulses in highly trained male runners [19, 21]. Another factor may be an increase in the clearance rate of testosterone due to exercise training [44]. Elevations in resting hepatic and renal blood flow have been observed in trained versus untrained subjects. In accordance with Wheeler et al a fall in serum testosterone levels must result from a decreased production, decreased binding or increased clearance rate [19, 30, 40, 45].

Elevated C levels may also contribute to lowering T concentrations. Cumming et al observed a suppression of T when circulating levels of C were elevated from 13μg/dl to 25μg/dl [13]. Mac Connie et al suggested that elevation of stress hormones caused by exercise may contribute to suppressing hypothalamic release of gonadotropin-releasing hormones. Cortisol values in our study were significantly increased. Our results may suggest that low T levels may be associated with the elevated C levels. Cortisol levels similar to our values have been found in similar studies [4, 23] .

T to C ratio has been used to reveal an imbalance between the anabolic and catabolic state of an athlete. Furthermore it has been suggested that this ratio should be a useful index of the overall exercise stress and the early detection of an imbalance between anabolic and catabolic metabolism. A drop of 30% or more in T to C ratio reflects a state of catabolism [4, 23, 12]. In our study we found a >50 reduction in T to C ratio. This is an interesting finding because a decrease in the T to C ratio has been associated with an increased exercise stress, a disturbance in the anabolic-catabolic balance which may result in a decreased athletic performance [18, 27]. Therefore, it can be argued that a soccer match places considerable stress on the endocrine system.

Elevated blood lipids, physical inactivity and male sex are well recognized risk factors for atherosclerotic heart disease. Androgens are important in regulating blood lipid levels. Blood levels of high density lipoprotein cholesterol (HDL) in men are significantly lower compared to women. Since HDL cholesterol has a cardio protective effect, the risk of atherosclerosis in men is increased. In contrast to endurance training, prolonged exercise and intermittent exercise have been associated with lower overall cholesterol, lower low density lipoprotein (LDL) cholesterol and higher HDL cholesterol [7, 15].

An inverse relationship should be expected between circulating T and HDL cholesterol levels [26]. In our study we found a significant decrease in LDL and total cholesterol. Moreover APO-B levels (the main apolipoprotein of LDL) were significantly decreased. On the other hand there was a significant increase post exercise in plasma HDL cholesterol and APO-AI lipoprotein (the main apolipoprotein of HDL) levels. Apolipoproteins are considered more sensitive indicators of cardiovascular risk than lipid and lipoprotein levels [2]. In our study the APO-A/APO-B ratio was significantly higher post exercise, confirming the presence of an anti-atherogenentic modification of the lipoprotein profile in athletic population.

Post exercise increase in plasma HDL cholesterol maybe attributed to both delayed clearance and increased synthesis of HDL constituents. It is well known that post heparin lipoprotein lipase activity is an important determinant of TG levels [28]. Plasma APO-AI levels are determined mainly by alterations in their fractional catabolic rate. The later phenomenon is directly related to plasma TG concentrations. Exercise induces acute increases in post heparin LPL which in turn leads to enhanced TG clearance and probably decreases plasma clearance of HDL constituents [35]. In the present study we found a decrease of plasma TG. Both enhanced peripheral tissue clearance of plasma TG and decreased liver secretion of VLDL have been associated with decreased plasma TG concentrations. Lipoprotein lipase (LPL) activity is the major enzyme involved in the catabolism of plasma TG and has been found to be increased in a skeletal muscle and adipose tissue as well as in plasma of trained men compared to untrained men [35]. The latter is in agreement with the negative correlation found postexercise between the change in plasma HDL cholesterol levels, APO-AI and plasma TG levels.

HDL cholesterol is composed of a heterogeneous mixture of particles with differences in shape, size, density and lipid and protein composition. HDL subtractions surface charge can be separated according to density or size into HDL_2 and HDL_3 and play an important role in the function of the HDL molecule. HDL_3 is a small, lipid-poor particle that promotes cholesterol efflux in vitro, while HDL_2 being a larger, sterol-rich particle, delivers cholesterol to steroid-producing cells. Case control studies have shown that the larger HDL_2 rather than HDL_3 are responsible for the inverse relationship between HDL levels and CAD. However, longitudinal data from the Physicians Heart Study involving over 14.000 male physicians demonstrate that both HDL_2 and HDL_3 appear to be protective against CAD [38].

Additionally the atheroprotective effects of HDL cholesterol is well documented and has been exerted through various functions like anti-inflammatory action, anti-oxidative properties against LDL cholesterol and the ability to inhibit cytokine-induced expression of endothelial cell adhesion molecules in addition to enhanced reversed cholesterol transport [1, 6, 39].

One of the mechanisms that may explain the improvement of lipid profile after a soccer match is the high aerobic demand placed on the players. The average aerobic oxygen uptake during a match is around 70% VO2max [5] and players may cover a total distance of 8-12 km [33]. Although there are differences in running distance and intensity due not only to the positional and tactical role of the player and his physical capacity, but also to the importance of the match as well as seasonal variations, we can argue that the duration of the match (90 min) and the demands placed on the player ensure that there is a considerable loading of the aerobic as well as the anaerobic system.

Unfortunately, due to the difficulties in measuring physiological variables during an official match, we could not quantify and relate the individual physiological stress with changes in lipid profile. However it may be argued that the beneficial effect of soccer on lipid profile will be greater in the players who run more and at higher intensity during the game.

CONCLUSIONS AND PRACTICAL APPLICATION

In conclusion the present study showed an acute improvement of the lipid profile after a soccer match. The increased levels of APO-A, HDL and APO-A/APO-B ratio together with the decreased levels of APO-B, LDL, TG, T-C, LDL/HDL, T-C/HDL ratio suggest that intermittent exercise of long duration, such as a soccer match, results in an *antiatherogenic modification of lipid profile*, possibly due to the high aerobic energy expenditure. The increase in C and decrease in T and the consequent large decrease of the T to C ratio, suggest that a soccer match places considerable stress on the endocrine system.

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