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Full Length Research Paper

Leptin, high-sensitivity C-reactive protein and malondialdehyde concentrations in elite adolescent soccer players and physically active adolescents

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Leptin is the product of the *ob* gene and circulates as a 16 kDa protein hormone. High-sensitivity C-reactive protein (hs-CRP) is known to be a sensitive marker of inflammation and cardiovascular risk factor and malondialdehyde (MDA) is a direct marker of oxidative stress and naturally occurring product of lipid peroxidation. The resting serum leptin, hs-CRP and MDA levels of elite adolescent soccer players and physically active adolescents were investigated. Eighteen elite adolescent soccer players aged 14.3±0.3 years and eighteen physically active subjects aged 14.6±0.4 years participated in the study. Resting serum leptin levels were not different, but hs-CRP and MDA levels were higher in adolescent elite soccer players compared to physically active adolescents. Therefore, participation in prolonged soccer training increased resting serum hs-CRP and MDA levels but had no effect on resting serum leptin compared to participation in sports activities/classes in adolescents.

Key words: Soccer, training, leptin, high-sensitivity C-reactive protein (hs-CRP), malondialdehyde (MDA), biochemistry.

INTRODUCTION

Leptin is the product of the *ob* gene and circulates as a 16 kDa protein hormone (Zhang et al., 1994). Leptin is primarily synthesized and secreted from the adipose tissue but it is also produced in small amounts in a variety of tissues including the hypothalamus, pituitary, skeletal muscle, bone, arterial endothelium, intestines and placenta (Moran and Phillip, 2003; Speakman, 2004; Considine and Caro, 1999; Hulver and Houmard, 2003; Hickey and Calsbeek, 2001). Leptin plays an important role in the regulation of energy balance (Hulver and Houmard, 2003), as a function of the energy stores and its concentration reflects the amount of energy stored in body fat (Klok et al., 2007). Exercise is a physiological stress that may alter leptin production (Sandoval and Davis, 2003). As the type of exercise affects leptin response with exercise (Olive and Miller, 2001), leptin's response may depend on duration and intensity of exercise (Sandoval and Davis, 2003). Many studies have investigated the serum leptin levels in response to different types of exercises, short-term exercises (Bouassida et al., 2009; Lau et al., 2010; Nindl et al., 2002), long-term exercises (Jurimae et al., 2003; Bouassida et al., 2009; Ramson et al., 2008), after a single bout of exercise.

Resting levels of leptin may generally decline with training (McMurray and Hackney, 2005), therefore circulating leptin concentration has been reported to be low at rest in trained subjects, and even at biologically extreme low levels of body fat (Hickey and Calsbeck, 2001; Bouassida et al., 2009). High-sensitivity C-reactive protein (hs-CRP) is known to be a sensitive marker of inflammation and increased levels have also been related to increased risk of vascular disease (Martins et al., 2010; Ridker et al., 2003). Accordingly, factors that may interfere with hs-CRP levels, such as physical activity, an exercise session, long-term training, require further investigation in athletes of different sports. King et al. (2003) showed that jogging and aerobic dancing were associated with lower CRP levels when compared with other forms of activity including swimming, cycling and weightlifting and Huffman et al. (2006) found out no change in hs-CRP in response to exercise training. In addition, Siahkouhian and Esmaeilzadeh (2011) reported that long-term soccer training has no significant effect on serum hs-CRP concentration in soccer players. Malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation and a direct marker of oxidative stress (Zanella et al., 2011; Yang et al., 2008). Plasma MDA concentrations increased during intensive exercise (Xiao and Li., 2006) and after submaximal resistance exercise (Ramel et al., 2006) as well as after eccentric exercise (Childs et al., 2001).

Despite that numerous studies on leptin, hs-CRP and MDA responses to different bouts of exercises, it has become of interest to examine the effects of training status on resting levels of leptin, hs-CRP and MDA of adolescent elite soccer players. Although there were some investigations in the acute responses of serum leptin, hs-CRP and MDA to different types of exercises (Bouassida et al., 2009; Sandoval and Davis, 2003; King et al., 2003; Xiao and Li, 2006; Ramel et al., 2006), limited data were available on acute responses of serum leptin, hs-CRP and MDA to soccer training (Unal et al., 2005; Siahkouhian and Esmaeilzadeh, 2011; Zanella et al., 2011). In addition, it gains importance to determine the resting serum levels of leptin, hs-CRP and MDA of adolescent soccer players who have been training regularly and to compare that data with physically active adolescents who have not been training but not sedanters too.

Therefore, the aim of the present study was to investigate and compare the resting serum leptin, hs-CRP and MDA concentration levels of elite adolescent soccer players and physically active adolescents.

MATERIALS AND METHODS

Subjects

Eighteen elite adolescent soccer players participating in the champion team of the regional league at U-15 age group and competing at the regional and country league of Turkey, aged 14.3±0.3 years, along with eighteen physically active subjects aged 14.6±0.4 years, used as control group, participated in the study. Soccer players were training on average of 6 to 9 $h \cdot week^{-1}$ for more than the last 3 years. They were examined during the mid-season of the regional league. At the time of the measurements, the players were in the competitive period of the season, performing 4 to 5 training sessions per week.

Physically active groups consisted of students of sports-oriented high-school at the same age level of 15 years. They had sports classes for the last one year but did not perform any regular training individually or in a team on a daily basis (exercising three or more times a week). All participants and their parents provided written informed consent. The physical characteristics of the subjects are shown in Table 1.

Body composition

Body weight was measured using an electronic body weight scale with a precision of 0.1 kg and height was measured to the nearest 0.1 cm on the same equipment with a perpendicular scale (Seca; Seca GmbH&Co. Hamburg, Germany). Body mass index (BMI) was calculated as weight (kg) / height^2 (m^2).

Biochemical analysis

Resting blood samples were drawn at the same time of day for each group throughout the study and 22 h after the last exercise session for soccer players. Blood samples were obtained from an antecubital vein into 10-ml vacutainer tubes with the participant sitting in the upright position. Plasma was separated by centrifugation and stored at -80℃ for subsequent analysis which was performed within 2 weeks after collection of bloods. Serum leptin was determined in duplicate using an enzyme-linked immunosorbent assay with commercial Human Leptin kit (ELISA) (DRG, Germany). The leptin assay had an intraassay coefficient of variation as < 4%. High-sensitivity C-reactive protein (hs-CRP) was measured using a high-sensitivity immunoturbidimetric assay with commercial kits on the Architect C8000 analyzer (Abbott Lab. Canada).

MDA concentration was measured in terms of thiobarbituric acid reactive substances, spectro-photometrically. Samples (0.125 ml) were mixed with 20% trichloroacetic acid (1.25 ml) and 0.67% thiobarbituric acid (0.5 ml). Mixture was then boiled at 95℃ for 30 min, followed by cooling on ice. Reaction mixture was then vortexed, following the addition of n-butanol (2 ml). All vials were then centrifuged at 1,000×g for 10 min. Absorbance of the supernatant was then measured at 535 nm. Concentration of lipid peroxidation products was calculated as MDA concentration using the extinction coefficient for MDA-thiobarbituric acid complex of 1.56×10^5 mol^{-1}/cm^{-1}.

Statistical analysis

The biochemical and body composition data were expressed as mean±SD. The relationship between variables was determined using Pearson correlation analysis. The normality of distribution was verified for all parameters with the Kolmogorov–Smirnov test. The independent samples t-test was used to compare differences in leptin, hs-CRP, MDA and body composition data between soccer players and control group. The level of significance was set at $p < 0.05$ for all analyses.

RESULTS

The physical and biochemical characteristics of the soccer players and physically active participants are presented in Table 1. Although soccer players had lower mean leptin level, there was no statistically significant

Table 1. Physical characteristics of the population of the present study and levels of the biochemical parameters monitored.

Characteristics	Soccer players (n = 18) Mean±SD	Physically active group (n = 18) Mean±SD	p
Height (cm)	170.7±5.3	170.9±7.3	0.938
Body mass (kg)	57.5±6.5	61.2±10.5	0.208
BMI ($kg \cdot m^{-2}$)	19.7±2.0	20.9±2.7	0.161
Leptin (ng/ml)	2.04±1.61	2.44±2.02	0.514
Hs-CRP (mg/L)	0.54±0.15	0.40±0.12	0.003*
MDA (µmol/ml)	2.10±0.59	1.45±0.58	0.002*

*Significant differences of soccer players versus physically active group (p<0.05).

difference in leptin levels between the groups (p>0.05). At rest, serum hs-CRP and MDA concentrations were significantly lower in physically active groups than in the soccer players (p<0.05). There were no significant correlations between serum leptin and hs-CRP, MDA, BMI within each group (p>0.05).

DISCUSSION

The present study investigated the resting serum leptin, hs-CRP and MDA concentrations of elite adolescent soccer players and physically active adolescents. It has also showed the effects of long-term regular training experience on resting serum leptin, hs-CRP and MDA concentrations in adolescent elite soccer players trained at least three years. Resting serum leptin concentration did not differ, but hs-CRP and MDA concentration levels higher in adolescent elite soccer players compared to physically active adolescents. Leptin is believed to play a crucial role in energy balance (Campfield et al., 1995). Many previous studies have discussed the effects of regular physical activity on leptin concentrations (Ara et al., 2006; Polak et al., 2006; Lau et al., 2010) and the effects of a training period or a single bout of exercising on circulating leptin. These studies ranged from short duration exercises at varying degrees of intensity to very long duration bouts of considerably high volume (Hulver and Houmard, 2003). It was observed that, trained athletes had lower resting and exercising leptin levels, independently of the type of training (Bouassida et al., 2009).

In the present study, leptin concentrations did not differ significantly, although soccer players had a lower mean level of leptin. Moreover, no correlation was observed between resting leptin and BMI, hs-CRP and MDA. These results were in accordance with the study carried out by Yamaner et al. (2010) who examined the resting leptin and lipopretion levels of forty-five (45) Turkish national wrestlers and 43 sedentary students. The resting serum leptin was 2.3±4.0 ng/ml for wrestlers and 3.0±27.6 ng/ml for controls. No significant difference was observed among the means of leptin levels between the elite young wrestlers and controls. They also found out no significant correlation between leptin and other parameters. They concluded that wrestling training had no effect on resting leptin level of elite wrestlers. Serum leptin levels show great variability in healthy adolescents even at the same age and the same gender (Ahmed et al., 1999). Unal et al. (2005) reported that leptin is lower in football players with respect to untrained subjects.

Similarly, Bouassida et al. (2009) reported that trained volleyball players had lower leptin concentration than untrained controls. At rest, mean leptin level was significantly lower for volleyball players than for controls (p<0.05). Additionally, Yang et al. (2009) recently found out that trained dancers had higher leptin level than controls. In that study, sixty dancers aged 15 to 17 years (mean 16.4±0.6 years) had a mean serum leptin level of 5.28±2.48 ng/ml, and seventy-seven age-matched controls (mean 16.5±0.7 years) as 2.51±1.96 ng/ml. The soccer players who participated in this study were actively training for a long period. Participating in soccer training did not differ the leptin levels with respect to participating in physical activities/classess. Recently, hs-CRP has been reported to be a useful marker for atherosclerosis. Prolonged and moderate intensity exercise may prevent atherosclerotic cardiovascular disease (Ridker et al., 2002). Although some researches showed an inverse relationship between physical activity, cardiovascular fitness and hs-CRP concentration (Huffman et al., 2006; Ridker et al., 2002; Martins et al., 2010), some researches did not find relationships between physical activity level and hs-CRP concentration (Mazurek et al., 2011; Kelly et al., 2006; Marcell et al., 2005; Rawson et al., 2003).

In the present study, physically active participants had significantly lower mean hs-CRP value than soccer players. Intense training may not decrease the resting hs-CRP levels in adolescent soccer players. Similarly, Siahkouhian and Esmaeilzadeh (2011) showed that resting hs-CRP were 0.33±0.13 mg/dl for soccer players and 0.34±0.19 mg/dl for controls and concluded that long-term soccer training may have no significant effect on the CRP level of a soccer player. Similarly, Kelley and Kelley (2006) stated that positive changes in BMI, body fat and VO_{2max} achieved through aerobic exercise were not followed by significant lowering of hs-CRP. The authors suggested that intense physical activity probably does not considerably affect low (<1 mg/L) and moderately increased (1.0 to 3.0 mg/L) hs-CRP values. Moreover, Rawson et al. (2003) reported that physical activity is not associated with C-reactive protein. They longitudinally examined the effects of BMI and both current and previous-year physical activity on hs-CRP in healthy men and women. Despite the increase in physical activity, hs-CRP was unchanged over the course of the study. In another study, there was no significant change in hs-CRP after either 10-weeks exercise program of aerobic and combined exercise groups of obese children aged 12 to 14.

MDA is a naturally occurring product of lipid peroxidation and a direct marker of oxidative stress (Yang et al., 2008; Zanella et al., 2011). Hoffman et al. (2007) indicated an increase in the concentration of MDA as a result of resistance exercise. Similarly, Alessio et al. (2000) stated an elevation in MDA concentrations following endurance exercise. On the contrary, Leelarungrayub et al. (2011) showed a decrease in MDA levels after aerobic dance exercise of a 6-week intervention and Zanella et al. (2011) reported an association between endurance exercise training and lower levels of MDA in professional footballers. In this study, soccer players had a significantly higher serum MDA level than the physically active group. This elevated MDA levels in adolescent soccer players may have resulted from the regular intensive soccer training.

Conclusion

The results of the present study indicated that elite young soccer players had higher resting serum hs-CRP and MDA levels and not different serum leptin level compared to physically active adolescents. Participation in prolonged soccer training increased resting serum hs-CRP and MDA levels but had no effect on resting serum leptin compared to participation in sports activities/ classes which was not regular training in adolescents. That physical activity participation background of the control group may be an explanation for the resting levels of leptin, hs-CRP and MDA concentrations in those adolescents. It is suggested that coaches of adolescent soccer teams pay particular attention to monitor the serum leptin, hs-CRP and MDA of players to bring these blood biomarkers to desired level. The coaches may also plan the intensity and volume of the training to have the resting levels of serum leptin, hs-CRP and MDA at the optimal level.

Therefore, the current findings should be viewed as a preliminary study for the resting serum leptin, hs-CRP and MDA of elite adolescent soccer players. Further researches are needed on the other blood biomarkers at rest for adolescent soccer players, to determine the effects of long-term soccer training. This may give a view about the influence of soccer training on these blood biomarkers. Moreover, the comparison of blood bio-markers with physically active adolescents should be provided to determine the effects of training in adolescents. Nevertheless, adult soccer players and physically actives may be included in some researches to investigate the differences with age.

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