Original Article

Time-course of IL-6 in amateur soccer players after an official soccer match

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Abstract:

The purpose of this study was to investigate the influence of an official soccer match on the time-course of interleukin-6 (IL-6) in amateur male soccer players. The study consisted of 104 participants, 54 of which were amateur male soccer players (age 24.48 ± 2.53), constituting the experimental group, while 50 subjects (age 25.2 ± 4.1) were non active healthy individuals defined as the control group. The study was carried out during the season 2013-14 in official games and in high level games (e.g. importance of match etc.). All the games were carried out under the same conditions as far the weather and the field is concerned. The athletes had a long lasting period of avocation with soccer, with training age 11.23 ± 2.54 years. Blood samples were taken before, immediately after, 24, 48, 72, 96, 120, and 144 hours after the game. The results of the Bonferroni post hoc analysis between groups for each measure for the significant Groups by Measures interaction in IL-6 concentration (pg/ml) indicated that at post-game soccer players had significantly higher concentrations ($M = 4.61$, $SD = .74$ and than control group ($M = 1.14$, $SD = .40$, $p < 0.001$). Moreover, Bonferroni analysis for groups across measures indicated that soccer players at post-game had significantly higher concentration than all other measures ($p < 0.001$). The rest of the paired comparisons did not reach statistical significance. The results show the significant influence of the soccer game in IL-6 in amateur players immediately after the match. This should be taken into account by those who are involved in soccer training.

Key words: soccer, exercise, inflammation, Interleukin-6

Introduction

Coronary artery disease is the leading cause of mortality in Western societies, while it is becoming a growing problem of health care systems in several developing countries (Leeder et al., 2004; WHO, 2002). It is well established that atherosclerosis is a complex inflammatory process, in which several cytokines play a central role in the initiation and progression of the disease (Ross, 1999). On the other hand, exercise mediates several beneficial effects in all cofounders of atheromatosis, being capable of modifying the risk of suffering from a future cardiovascular event (Bauman, 2004; Ignarro et al., 2007; Prasad & Das, 2009) however, an acute bout of exercise is correlated with certain immune responses and activation of specific molecules that lead to enhanced inflammatory processes (Pedersen, 2007). Nowadays, it has become clear that the most significant part of this immune response is mediated by the secretion of certain cytokines, with interleukin-6 (IL-6) being the most important of them (Pedersen, 2007; Pedersen & Nieman, 1999; Steensberg, 2003; Suzuki et al., 2002). Plethora of studies has examined the impact of exercise on the serum response of IL-6 in athletes.

The increase of the molecule in response to a bout of exercise, often in a dramatic pattern, has been confirmed by several studies. More specifically, Northoff et al., (1991) were the first who examined the immune parameters after an acute type of exercise, while another research group has demonstrated a statistically significant increase of IL-6 after marathon (Ostrowski et al., 1999), or after 2.5 hours treadmill running (Ostrowski et al., 1998). Moreover, IL-6 has also been found to be significantly increased after exhaustive exercise, representing a pattern of excessive response of immune system after extravagant stress (Margeli, et al 2005). Soccer is the most popular kind of sport played worldwide by adults of any age, teens and children. It has been established that soccer playing, even in the form of recreational football, is largely characterized by periods of high aerobic demands proceeding maximum heart rate periods, involving often jumps, turns and sprints representing an intense muscle and bone overwork (Souglis et al. 2013; Rampinini et al., 2007; Krstrup et al., 2010; Pedersen et al., 2009). At the same time, it is characterized by long periods of standing and walking with no or minor impact on physical fitness and load on muscles and bones (Krustrup et al., 2010; Krustrup et al., 2009, Pedersen et al., 2009).

However, in general, soccer is a demandive type of sport with clear effects on fitness and immune system, especially after an acute bout of exercise. Importantly, the impact of soccer playing in the IL-6 response in the serum of athletes has not been fully studied and the data on this field is quite inadequate. The aim of this

study was to evaluate the immune response by serum determination of the proinflammatory cytokine of IL-6 after an acute bout of exercise as well as to examine the impact of a soccer game on the dearrangement of homeostasis of an athlete, implying the need for the establishment of a certain period of rest after an acute stress for all soccer players.

Method

Participants. The study consisted of 104 participants, 54 of which were amateur male soccer players (age 24.48 ± 2.53), constituting the experimental group, while the resting 50 (age 25.2 ± 4.1) subjects were non active defined as the control group. The study was carried out during the season 2013-14 in official games and in high level games (e.g. importance of match etc.). All the games were carried out under the same conditions as far the weather and the field is concerned. The athletes had a long lasting period of avocation with soccer, with training age 11.23 ± 2.54 years.

The professional athletes were subjected to almost (4 to 5 times/week) daily training. The training involved running exercises with or without the ball, exercise for strength, exercise of technique and tactics of variable intensity lasting 75 to 90 minutes/training time. The selection of the athletes was done with random sampling between the volunteers. The control group consisted of subjects with average level of physical activity and the selection was done with random sampling. All participants did not smoke (or smoked least) and did not drink alcohol.

All the participants were advised to have a balanced diet during the week before the participation with daily consumption of 55% carbohydrates, 30% fat and 15% protein. All the participants were subjected to routine clinical assessment (Electrocardiogram, measurement of arterial pressure, chest ray, blood tests), which showed no evidence of any pathological condition. All participants were informed of the aim of the study and written consent was given.

Anthropometric characteristics. In terms of anthropometric measurements, the following factors were determined: height, weight, fraction of visceral fat (Table 1). Height was determined in terms of centimeters, weight was determined in terms of kilograms, while the visceral fat was determined using the Mayhew Method (1981).

Table 1. Means and Standard deviations ($\pm$) of height (cm), weight (kg), age (yr), percent body fat and training age (yr) for male soccer players (n = 54), male control (n = 50).

	Height (cm)	Weight (kg)	Age (yr)	Body fat (%)	Training age (yr)
Male Soccer Players n = 54	178.98 ± 5.64	73.64 ± 5.07	24.48 ± 2.53	11.04 ± 0.71	11.23 ± 2.54
Male Control n = 50	177.09 ± 5.43	78.07 ± 6.05	25.2 ± 4.1	21.28 ± 3.64	—————

Blood Samples: Eight blood samples were taken from soccer players as well as from the control group. The first sample was taken in the morning of the game day after fasting of more than 12 hours and without prior exercise for at least two days. The second sample was taken 8 hours after the first and immediately after the soccer game (5.5-6 h after lunch). The 3rd – 8th sample took place 24, 48, 72, 96, 120, and 144 hours after the game respectively. All participants were sitted, the samples were taken from the basilic and mesobasilic vein, while the amount of blood taken was 5 ml and placed in vacutainers without anticoagulant for the isolation of serum. The centrifugation took place for 10 min in 2500 rads/min. The storage of serum was made in -70°C until the day of determination. The determination of IL-6 was made with an immunoabsorbent assay supplied by the BIOSIS manufacturer.

Statistical analysis

Univariate procedures were used to analyze IL-6 concentrations for soccer palyers and control Groups. The experimental design used was a 2 (Groups) by 8 (Measures) with Groups as a between factor and Measures as the within subjects factor. The degrees of freedom for significant main effects, interactions and error terms were corrected according to the Greenhouse-Geisser method (Geisser & Greenhouse, 1958) when the assumption of sphericity was violated. Partial Eta squared (η^2) was estimated for significant main effects and interactions (Gamst, Meyers, & Guarino, 2008). Bonferroni post hoc analyses were conducted to identify statistically significant differences (Keppel, 1991). The alpha level of significance was set at .05 for ANOVAs and post hoc analyses. Results are presented as means (*M*) and standard deviations (*SD*). Data were analyzed using SPSS 13.0 (SPSS, Chicago, IL).

Results

The 2 by 8 (Groups x Measures) mixed ANOVA for IL-6 concentration showed statistical significances for Groups ($F_{1, 102} = 66.57, p < 0.001$, partial $\eta^2 = .395$) and Measures main effects ($F_{7, 714} = 852.84, p < 0.001$, partial $\eta^2 = .893$) and Groups by Measures interaction ($F_{7, 714} = 815.18, p < 0.001$, partial $\eta^2 = .889$).

Bonferroni *post hoc* analysis between groups for each measure for the significant Groups by Measures interaction in IL-6 concentration (pg/ml) indicated that at *post-game* soccer players had significantly higher concentrations ($M = 4.61, SD = .74$) than control group ($M = 1.14, SD = .40, p < 0.001$). Moreover, Bonferroni analysis for groups across measures indicated that soccer players at *post-game* had significantly higher concentration than all other measures ($p < 0.001$). The rest of the paired comparisons did not reach statistical significance. The means and standard deviations of IL-6 for soccer players and control Groups at pre-game, post-game, 24, 48, 72, 96, 120 and 144 hours after the game measures are presented in Figure 1.

Fig. 1. Means and SD for soccer players and control groups at 8 measures (pre-game, post-game, 24h, 48h, 72h, 96h, 120h, and 144h after the game) for IL-6 concentration (pg/ml).

Discussion

The results of this study are in accordance with bibliographic data concerning the impact of exercise on the release of the cytokine under study (Ullum et al., 1994; Drenth et al., 1995; Nehlsen-Cannarella et al., 1997; Rohde et al., 1997; Hellsten et al., 1997; Bruunsgaard et al., 1997). The observation that the contracting muscle is capable of secreting plausible amounts of the cytokine in systemic circulation, has rendered IL-6 as the most known myokine, which is thought to mediate most of the effects of exercise in a molecular and biochemical level (Fischer et al., 2004; Steensberg et al., 2000; Plomgaard et al., 2005; Penkowa et al., 2003; Hiscock et al., 2004). IL-6 seems to be accumulated in the skeletal muscle before its secretion in circulation (Penkowa et al., 2003; Hiscock et al., 2004), where it can be detected and determined with specific immunoassays.

In the present study, there was a statistically significant difference between the basal levels and those after the game and 24 hours after the game in soccer players. This implies a quick rearrangement of homeostasis of immune system, lasting at least 24 hours in soccer players. However, it seems that the contracting muscle is not the only source of IL-6 after exercise. For example, a minor release from the internal jugular vein represents an attribution of central nervous system (CNS) in the secretion of IL-6 during exercise (Nybo et al., 2002). Under normal conditions, IL-6 levels are almost undetectable in CNS, but in pathological situations, such as trauma, hypoxia or ischemia IL-6 can be derived by activated astrocytes (Van Wagoner & Benveniste 1999), while it can also be expressed in hypothalamus after long lasting and acute stress (Schobitz et al., 1993), such as intense and durable exercise.

The secretion of IL-6 after exercise follows a quite complex pattern in which several molecules and mechanisms are participating. More specifically, the promoter region of the gene of IL-6 contains sequences, where the nuclear factor κ B (NF- κ B) and the nuclear factor of IL-6 (NFIL-6) can bind leading to enhanced transcription of the gene (Matsusaka et al., 1993). Additional transcriptional factors, such as the nuclear factor of activated T lymphocytes (NFAT) as well as the factors of Heat shock 1 and 2, can induce further the transcription of this gene. In vitro, potassium can activate NFAT as well as NF- κ B, while the specification leads to an increase of IL-6 generation in cultures of muscle cells through the activation of p38 MAPK kinase (Chan et al., 2004). In fact, studies in human muscle cells have demonstrated increased phosphorylated p38 MAPK kinase in muscle biopsies after one hour of vigorous cycling (McGee & Hargreaves 2004). Additionally,

NF- κ B has been found to be activated in experimental animals after exercise (Ji et al., 2004) however, the data are not clear enough. Moreover, NF- κ B is a transcriptional factor that can be activated by reactive oxygen species (ROS) (Schreck et al., 1991). ROS are also elevated in response to exercise not only in animal models but in human environment as well (Davies et al., 1982; Jackson et al., 1985).

The impact of the certain transcriptional factor in the release of IL-6 has also been confirmed by the observation that administration of indomethakin, a well known anti-inflammatory agent with modulating effect on the NF- κ B, suppresses the IL-6 secretion after stimulation with an acute bout of exercise, implying a distinct role of the factor in the IL-6 secretion kinetics (Kopp & Ghosh, 1994; Rhind et al., 2002). Oxidative stress, the poor availability of glucose, the low levels of glycogen, the catecholamines, the increased intracellular levels of potassium, hyperthermia and ischemia are all distinct characteristics in exercise; all these situations provoke the release of heat shock proteins 1 (HSF1) and 2 (HSF2) (Bergstedt et al., 1993; Cavaliere et al., 2001; Febbraio et al., 2002; Paroo & Noble 1999; Wallen et al., 1997), which in turn mediate further secretion of IL-6. All these mechanisms are even more complicated, if one considers that IL-6 is capable of inducing itself transcription through a complex process of self-modulation, an observation that can explain the increasing transcription of the gene over the lasting exercise (Keller et al., 2004). All the aforementioned mechanisms are not thought to act independently; on the other hand, it seems that they act at the same time and synergistically leading to a complex molecular result of IL-6 generation during the exercise period as well as after its end.

The exercise induced increase of IL-6 in serum of athletes is dependent on the type, the intensity and the duration of exercise. The initial view that IL-6 was the result of muscle destruction, which is often accompanying several kinds of sports, has not been confirmed from recent bibliographic data. Croisier et al (1999) demonstrated that IL-6 production was the same in athletes subjected to isokinetic form of exercise independently of their level of fitness. Moreover, IL-6 and creatine kinase, which is a sensitive marker of muscle destruction, were not found to be proportionally elevated after pleiometric type of exercise (Ostrowski et al., 2000).

The type of exercise seems also to be correlated with the pattern of alteration, since eccentric type of exercise is associated with a rather delayed increase of plasma IL-6, which is also accompanied by a more gradual and slower decrease (MacIntyre et al., 2001; Willoughby et al., 2003). IL-6 is not also linearly modified with the duration of the exercise: the repeated measured values show an accelerated increase in the duration of the exercise (Fischer et al., 2004), with the greatest values being observed in the end of the exercise form (Ostrowski et al 1998), which is followed by a rather quick decrease towards the basal levels in the period of rest. Lastly, the relation of IL-6 increase with intensity of exercise seems to be correlated with the muscle mass involved (Bergfors et al., 2005; Nosaka & Clarkson 1996; Hirose et al., 2004; Ostrowski et al., 1998). The duration of exercise is the most important factor affecting the variability of IL-6 values, since more than 50% of the alterability of IL-6 values can be attributed to the exercise duration (Nielsen et al., 1996; Bruun et al., 2006).

As aforementioned, the data concerning the impact of soccer in the secretion of IL-6 is quite incomplete. Bishop et al., (2002) demonstrated statistically significant differences in soccer players after intense exercise periods that were accompanied by periods of less intensive exercise and rest. They also found correlation with the administration of carbohydrates, implying the complex role of IL-6 in the modulation of metabolism in exercise. In another study, IL-6 was found to be significantly increased after the end of a soccer game in the serum and urine of prepubertal children, underlying the potential central role of kidneys in the clearance of enhanced IL-6 after an acute bout of exercise (Scheett et al., 1999). Similarly, Navero et al., (1999) demonstrated significant differences of the IL-6 levels after a game, while in an interesting recent study from Greece by Ispiridis et al., (2008), IL-6 was elevated after a soccer game in professional athletes, with the values returning to their basal levels one day after the occurrence of the game, a pattern similar to that observed in the present study.

Conclusions

In conclusion, IL-6 is an important attributor of the immune response observed after an acute type of exercise. Although the contracting muscle seems to be the main source of IL-6 in the serum of exercising subjects, the mechanisms involved in the evolution of this process are quite complex and in certain fields remain unknown. IL-6 is a molecule with diverse properties and represents the inflammation process as well as the response of the immune system after an acute bout of exercise. IL-6 seems to be an interesting marker for the evaluation of the physical condition of the athletes and may be a sensitive biomarker of fitness of overworked athletes that may be in need of rest or in directing to certain forms of exercise for the regain of their homeostasis. These observations may be useful also in monitoring soccer players routinely as well as after soccer games or exhausting preparation periods. However, more studies are needed in order to certify a certain role for IL-6 in the every day assessment of soccer players.

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