ORIGINAL ARTICLE

# Impact of Loughborough Intermittent Shuttle Test versus soccer match on physiological, biochemical and neuromuscular parameters

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Abstract The aim of the present study was to analyze the impact of Loughborough Intermittent Shuttle Test (LIST) versus soccer match on heart rate (HR), muscle damage, redox status, blood leukocytes and neuromuscular function throughout 72 h recovery. Sixteen male soccer players  $(21.3 \pm 1.1 \text{ years}; 175.0 \pm 6.0 \text{ cm}; 70.7 \pm 6.3 \text{ kg})$  completed LIST and performed a soccer match separated by 2 weeks and data were collected before, 30 min, 24, 48 and 72 h after LIST and match. HR, plasma creatine kinase (CK) activity, myoglobin (Mb), uric acid (UA), protein sulfhydryls (–SH), malondialdehyde (MDA) contents, total antioxidant status (TAS), blood leukocyte counts, delayed onset muscle soreness, 20 m sprint and jump performances,

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Department of Clinical Analysis, Faculty of Pharmacy, Institute for Molecular and Cell Biology, University of Porto, Porto, Portugal and maximal isokinetic knee extension and flexion were analyzed. HR after LIST was significantly lower than after the match. Post-match TAS was lower and UA was higher than after LIST. Thirty minutes and 24 h after soccer MDA was higher and -SH was lower than after LIST (P < 0.05). LIST and soccer match induced elevation in total leukocytes and a reduction in lymphocytes at 30 min. This reduction in blood lymphocytes 30 min after match was lower than after LIST. In conclusion, the impact of both exercises did not differ regarding the observed muscle damage markers and some neuromuscular parameters, although soccer requires higher cardiac demand and induced higher changes on redox status, adenine nucleotide metabolism and on lymphocyte counts than LIST, which should be taken into account when using LIST to simulate a match to study these type of physiological and biochemical-related endpoints.

**Keywords** Intermittent exercise · Muscle damage · Oxidative damage · Antioxidants · Fatigue and recovery

# Introduction

The physiological, biochemical and neuromuscular impact of intermittent multi-sprint sports, including soccer, has been studied through different methodologies. Time-motion analysis of soccer match provides information regarding total distance covered and type of movement, number of physical contacts, tackles, headers and kicks performed by players. Moreover, specific field approaches during soccer matches (Krustrup et al. 2006), tentative replications of match demands in laboratory (Drust et al. 2000) or a combination of field and lab tests (Greig et al. 2006) have been designed to monitor heart rate (HR), blood and muscle

metabolite alterations, estimated energy expenditure and oxygen uptake of soccer players. These laboratory and field tests are used to uncover the lack of control of the activity pattern and exercise intensity in a game, resulting from the randomized order of players' actions, thus allowing a standardized analysis of metabolic, biochemical and functional features of intermittent exercise. Among several intermittent field tests, the Loughborough Intermittent Shuttle Test (LIST) has been used to study the effects of the ingestion of carbohydrate-electrolyte solutions or meals (Foskett et al. 2008; Nicholas et al. 1995, 1999), fluid ingestion and gastric emptying (Leiper et al. 2005), muscle metabolism and temperature (Morris et al. 2005), muscle soreness and damage (Thompson et al. 1999), heat acclimatization protocols (Sunderland et al. 2008), cryotherapy treatment against muscle damage (Bailey et al. 2007), as well as the influence of antioxidants (Kingsley et al. 2005; Thompson et al. 2001a) on intermittent exercise performance and recovery with special reference to soccer. In fact, some authors have suggested that LIST is a field test that simulates the activity pattern and the workload imposed by soccer, i.e., was designed to mimic the activities performed and the distance covered in a typical soccer match (Bishop et al. 1999; Nicholas et al. 2000). This was based on data from time-motion analysis and estimated indirect calorimetric variables (Nicholas et al. 2000). However, the analysis of the distance covered by players during the match tends to underestimate the energy expended as many unorthodox modes of motion, such as running backwards and sideways, jumping, decelerating and changing direction, as well as the dribbling and contesting possession accentuate the mechanical and metabolic loading (Reilly 1997). Moreover, even considering the pre-defined turns implying acceleration and decelerations during LIST, the great quantity and variety of soccer actions associated with eccentric contractions during a match are expected to cause additional muscle disturbances in soccer when compared to LIST.

Recent studies from our lab and others have analyzed the response of performance indices, muscle damage, inflammation as well as oxidative stress and damage markers of male and female soccer players during the recovery from a match (Andersson et al. 2008; Ascensao et al. 2008; Ispirlidis et al. 2008), as the recovery is thought to be influenced by changes in these functional and biochemical parameters. Some of these endpoints have also been measured during the recovery period after the LIST (Bailey et al. 2007; Kingsley et al. 2005; Thompson et al. 1999, 2001a, 2003). In addition to the comparison of time-motion analysis and estimated indirect calorimetric variables between LIST versus soccer match (Nicholas et al. 2000), no data had been published comparing the impact of LIST versus soccer on biochemical and neuromuscular parameters. Therefore, it would be of interest to examine whether, and to what extent, muscle damage, plasma oxidative stress and damage and blood inflammatory markers, as well as lower limb neuromuscular variables such as jump, 20 m sprint ability and strength performance, are altered in response to LIST versus soccer. Thus, the present study aimed to comparatively analyze the effect of LIST versus soccer match on muscle damage, plasma antioxidant capacity, oxidative damage, blood leukocyte counts and neuromuscular variables throughout 72-h post-exercise recovery period. Our hypothesis is that soccer induces higher levels of muscle and oxidative damage than LIST.

## Methods

# Subjects

Sixteen male soccer players from 2nd and 3rd Portuguese divisions participated in this study after being informed about the aims, experimental protocol, procedures and after delivering writing consents. At the time of the experiments, the players were in the competitive period of the season, performing 4–5 training sessions per week. The experimental protocol was approved by the Ethical Committee of Faculty of Sport, University of Porto, Portugal, and followed the Declaration of Helsinki of the World Medical Association for research with humans.

#### Experimental design and procedures

The players performed the LIST and one soccer match separated by 2 week (Fig. 1). For 2 weeks prior to data collection and during the protocol period, soccer players were instructed not to change their normal eating habits and to refrain from additional vitamin, antioxidant dietary supplementation or any recovery treatment such as cryotherapy. Subjects were also instructed to abstain from exhaustive exercise during the 72-h pre- and post-LIST and match, with exception of the functional evaluation tests. The overall set-up was performed during a 3-week interruption of the competitive period. During the remaining days, with the exception of the 72 h pre- and post-protocol periods, the players were engaged in normal training routines.

The temperature at the days of field exercise (LIST and soccer match) was around 16°C.

Blood samples and functional data (jump and sprint performance, quadriceps and hamstrings muscle strength) were assessed pre-LIST/match and at 30 min, 24, 48 and 72 h of the recovery period. On the day of the LIST/match, players arrived at the laboratory after an overnight fast between 10.00 and 12.00 h. A resting blood sample was taken after subjects had been standing for at least 15 min, after which subjects consumed a light standardizing meal



Fig. 1 Test schedule for the whole test period. *Downward arrows* denote the time points when sprint and jump performance, isokinetic strength and DOMS were measured. *Drop marks* denote the time points when blood samples were taken. *HR* heart rate

and drink and rested for 2 h. According to Thompson et al. (2003), the meal consisted of 1.7 g/kg white bread and 0.3 g/kg of low-fat spread. Rest muscle strength, jump and sprint performance were assessed during the 2-h period between the consumption of pre-exercise meal and the start of the LIST/match.

For 3 days after the LIST/match, subjects returned to the laboratory. A blood sample was taken from the forearm vein in the same conditions described above. Subsequently, the players performed the sprint and strength tests as outlined below.

#### Preliminary measurements

The players performed an incremental (0.1 m/s increase each 1 min step) treadmill (Quasar-Med, Nussdorf, Germany) test until voluntary exhaustion to determine maximal oxygen uptake ( $VO_{2max}$ ) and maximal HR (HR<sub>max</sub>, Vantage NV, Polar Electro, Finland). Expired respiratory gas fractions were measured using an open circuit breath-bybreath automated gas-analysis system (Cortex, Metalyzer, 3B, Germany). From the  $VO_{2max}$ , running speeds corresponding to 55 and 95%  $VO_{2max}$  were calculated. Thereafter, the subjects performed the LIST for 30 min to familiarize with the test. Jump and 20 m sprint abilities and strength performance were also evaluated at baseline as described below.

#### The LIST

The 90-min shuttle run test was conducted according to Thompson et al. (1999) in a natural green soccer pitch. Briefly, the participants were required to run between two lines, 20 m apart, at various speeds dictated by an audio signal and based on the velocities corresponding to their individual  $VO_{2max}$ . The exercise periods were designed as follows:

- $3 \times 20$  m walking
- 1 × 20 m maximal running sprint
- 4 s recovery
- $3 \times 20$  m at a running speed corresponding to 55%  $VO_{2max}$

•  $3 \times 20 \text{ m}$  at a running speed corresponding to 95%  $VO_{2max}$ 

This pattern was repeated for each 15-min exercise block followed by the corresponding 3-min recovery period for five times.

HR during the LIST was measured and recorded every 5 s.

## Match time-motion analysis

For time-motion analysis, each player was video-filmed close up during the entire match. The videotapes were later replayed for computerized time-motion analyses according to the procedures described by Mohr et al. (2003) The used motor pattern categories included standing ( $0 \text{ km h}^{-1}$ ), walking ( $6 \text{ km h}^{-1}$ ), jogging ( $8 \text{ km h}^{-1}$ ), low-speed running ( $12 \text{ km h}^{-1}$ ), moderate-speed running ( $15 \text{ km h}^{-1}$ ), high-speed running ( $18 \text{ km h}^{-1}$ ), sprinting ( $30 \text{ km h}^{-1}$ ), sideways, and backwards ( $10 \text{ km h}^{-1}$ ) running. The match activities were later analyzed considering standing, walking, jogging, cruising, sprinting, backwards running and sideways running.

HR was also measured during the match as previously described.

Delayed onset muscle soreness (DOMS)

After LIST/match and prior to blood sampling, each subject was asked to complete a leg muscle soreness questionnaire, in which they rated their perceived muscle soreness on a scale from 0 (normal absence of soreness) to 10 (very intense sore).

Blood sampling and preparations

All venous blood samples were taken by conventional clinical procedures as described previously (Magalhaes et al. 2007). An aliquot of the whole blood was used to perform leukocyte counts as indirect markers of muscle damage. The remaining freshly withdrawn blood was immediately centrifuged at 3,000 rpm during 10 min to obtain plasma. Plasma was separated into aliquots and rapidly frozen at -80°C for later biochemical analysis of the muscle damage markers myoglobin (Mb) and creatine kinase (CK), as well as the redox state using total antioxidant status (TAS), malondialdehyde (MDA), protein sulfhydryl groups (SH) and uric acid (UA).

#### **Biochemical** assays

## Muscle damage

Plasma CK activity was determined spectrophotometrically using a commercial kit (ABX A11A01632, Mompelier, FR). Plasma Mb concentration was assessed using a commercial kit (myoglobin bioMerieux 30446, Carnaxide, PT).

Leukocyte count was assessed by an automatic cell counter (Horiba 60; ABX Diagnostics, France). Whole blood smears on glass slides (VBS 655/A Microscope, Biosigma) were used for white blood cell differential analysis. Smears were stained using Wright coloring (Merck) and air-dried. Cell differentials were performed using an Olympus microscope equipped with 1,000× oil immersion lens.

## Redox state

Total antioxidant status was measured spectrophotometrically using a commercial kit (Randox NX2332 Crumlin, UK). Uric acid was determined by an enzymatic method using a commercial kit (Horiba ABX A11A01670, Montpellier, France).

Plasma MDA was assayed according to Rohn et al. (1993) with some modifications and measured by the formation of thiobarbituric acid reactive substances at 535 nm. Plasma SH was spectrophotometrically evaluated at 414 nm according to Hu (1990). Protein content was spectrophotometrically assayed using bovine serum albumin as standard according to Lowry et al. (1951). Samples were analyzed in duplicate and the mean of the two values was used for statistical analysis.

#### Jumping performance

Vertical jumping was evaluated on a Bosco's mat (Ergojump, Globus, Italy). In accordance with Hertogh et al. (2005), free counter-movement jumps with extension of both upper limbs were chosen to simulate spontaneous jumping movements. The depth of the counter-movement was selfselected and represented each players' optimal depth for maximal jump. Each athlete performed three jumps and the best result expressed as jump height was recorded.

## 20 m sprint ability

Sprint ability measurements were carried out using telemetric photoelectric cells placed at 0 and 20 m (Brower Timing System, IRD-T175, USA). The players stood 1 m behind the starting line, started on a verbal signal being time activated when players cross the first pair of photocells, and then ran as fast as they could to complete the 20-m distance. Players completed two runs interspersed by 1-min recovery period and the best time was registered.

#### Strength assessment

In order to evaluate muscle function, subjects were familiarized with the muscle function test on at least two occasions during preliminary visits to the laboratory. Maximal gravity corrected concentric peak torque of quadriceps and hamstrings was measured during isokinetic knee joint movement of dominant leg at an angular velocity of 90 s<sup>-1</sup> (1.57 rad s<sup>-1</sup>) using a isokinetic dynamometer (*Biodex* System 2, USA) as described previously by our group (Magalhaes et al. 2004).

## Fluid loss and intake

In order to determine sweat loss, the players were weighed wearing dry shorts immediately before and after the LIST and match using a digital weight (Tanita Scale BC533). The subjects were allowed to drink water ad libitum during both the LIST and the match, and their water intake was recorded.

## Statistics

Mean, standard deviation and standard error mean were calculated for all variables. A Kolmogorov–Smirnov test was used to test whether physiological, biochemical and neuromuscular-related variables were normally distributed. Twoway analysis of variance (ANOVA) for repeated measures followed by the Bonferroni post hoc test was used to compare variables between LIST and soccer at the analyzed time points (before vs. 30 min vs. 24 vs. 48 vs. 72 h). When there were only single comparisons, a paired sample t test was used to determine whether any differences between LIST and soccer existed. All data analysis was performed using SPSS 17.0 package. The significance level was set at 5%.

## Results

Physiological and anthropometric characteristics of the soccer players are presented in Table 1.

Time-motion analysis showed that players were around 80 min of match time involved in low-intensity activities including standing, walking, jogging and cruising, and 8 min in high-intensity activities including sprinting,

 Table 1
 Anthropometric and physiological characteristics of the subjects before LIST and match

Variables	Before LIST	Before match	
Age (year)	$21.3 \pm 1.1$	$21.3 \pm 1.1$	
Mass (kg)	$70.7\pm 6.3$	$69.8\pm5.3$	
Height (cm)	$175.0\pm6.0$	$175.0\pm6.0$	
Body fat (%)	$8.3\pm1.9$	$7.9\pm2.9$	
$VO_{2max}$ (mL kg <sup>-1</sup> min <sup>-1</sup> )	$55.1 \pm 5.1$	_	

Values are mean  $\pm$  SD; VO<sub>2max</sub> maximal oxygen uptake

backwards and sideways running, corresponding to approximately 92 and 8% of the total match time, respectively (Table 2).

The mean HR during the match was  $173.0 \pm 8.8$  bpm and the peak HR was  $195.6 \pm 6.0$  bpm, which corresponds to  $87.1 \pm 3.2\%$  and  $99.7 \pm 7.0\%$ , respectively, of the maximal HR previously determined. Mean HR during the match (including 1st and 2nd halves) was significantly higher than during the LIST (Fig. 2).

Plasma Mb content increased 30 min after both LIST and match (P < 0.05), returning to baseline at 24, 48 and 72 h recovery. No significant differences were found between LIST and soccer in any analyzed time point (Fig. 3a). Plasma CK activity and DOMS increased at 30 min, 24, 48 and 72 h after LIST and match when compared to pre-exercise values (P < 0.05), but no significant differences were found between protocols at any time point (Fig. 3b, c).

Plasma TAS (Fig. 4a) increased significantly at 30 min, 24, 48 and 72 h recovery time points after both LIST and match. Plasma UA only increased at 30 min after both LIST and match (Fig. 4b). The increases in TAS and UA at 30 min were higher after the match than after the LIST (P < 0.05). Plasma MDA and SH levels (Fig. 4c, d), respectively, increased and decreased at 30 min, 24, 48 and 72 h both after LIST and soccer (P < 0.05). The increase in MDA and the decrease in SH contents 30 min and 24 h after the match were significantly higher than after LIST.

LIST and match increased blood leukocyte counts (Fig. 5a) at 30 min (P < 0.05) which returned to baseline values at 24, 48 and 72 h recovery. No differences were observed between LIST and match for any time point. After

both LIST and match, lymphocyte counts at 30 min were significantly lower than pre-exercise values, returning to baseline values at 24 h. However, match induced a significantly higher lymphopenia than LIST at 30 min.

LIST and match induced significant reductions in jump (Fig. 6a) and sprint (Fig. 6b) abilities as well as in isokinetic peak torques for knee extension (Fig. 6c) and flexion (Fig. 6d) until 72 h recovery. No significant differences were found between LIST and match in the analyzed neuro-muscular parameters with the exception of sprint performance, which was less affected after LIST than after soccer at 30 min and 24 h recovery (P < 0.05).

The fluid loss during LIST versus match was  $0.88 \pm 0.17$  L versus  $0.90 \pm 0.2$  L, or  $1.2 \pm 0.3\%$  versus  $1.2 \pm 0.5\%$  of the body mass, respectively. The fluid intake was  $0.74 \pm 0.1$  L versus  $0.65 \pm 0.1$  L. Thus, the total fluid loss was similar between the two conditions, respectively  $1.62 \pm 0.4$  L versus  $1.55 \pm 0.3$  L, corresponding to  $2.3 \pm 0.3\%$  versus  $2.2 \pm 0.4\%$  of the body mass.

#### Discussion

Intensity of the soccer match

The match examined in the present study was a friendly game played by secondary division players, and it should be thus considered how far the intensity is from games played at an elite level. Nevertheless, the mean and absolute HR values were similar to those reported for Danish soccer players from similar level (Krustrup et al. 2006). Additionally, time-motion analysis showed that the frequency and the percentage of time both at low- and highintensity activities were also similar to that described for players of the same level, although below to those observed in elite players (Mohr et al. 2003). These observations may suggest that the analyzed match intensity was somewhat similar to other non-elite games, and probably lower than the intensity performed by elite soccer players.

#### Muscle damage

The tendency and magnitude of changes induced by both LIST and match in muscle damage-related parameters were

Table 2 Frequency, mean duration and percent of match time spent on the considered motor categories

	Standing	Walking	Jogging	Cruising	Sprinting	Backwards running	Sideways running
Frequency (n)	$114\pm44.8$	$408.6\pm93.8$	$441.9\pm96.2$	$69.6 \pm 10.4$	$41.7 \pm 18.0$	$122.1\pm26.6$	$64.9\pm4.8$
Mean duration (min)	$7.0 \pm 2.5$	$39.5\pm3.6$	$31.8\pm7.4$	$2.9\pm1.5$	$2.2\pm1.5$	$4.3 \pm 1.3$	$1.4 \pm 0.4$
Total time (%)	$7.8\pm3.4$	$43.8\pm7.9$	$35.3\pm5.6$	$5.8\pm2.3$	$2.5\pm1.3$	$4.8\pm1.9$	$1.6\pm0.6$

Values are mean  $\pm$  SD



Fig. 2 Mean heart rate (bpm) values observed during LIST and soccer (1st and 2nd halves and overall). Values are mean and SD. *Asterisk* versus LIST

somewhat expected and in the range of similar exercise protocols (Andersson et al. 2008; Bailey et al. 2007; Kingsley et al. 2005; Thompson et al. 1999, 2001b, 2004), being the significant alterations observed after the match in plasma CK, Mb, DOMS and leg strength close to some reported after LIST protocol (Kingsley et al. 2005; Thompson et al. 1999, 2001a, 2003).

Although the activity pattern of LIST is representative of the typical activities of soccer, there are activities such as jumping, running backwards and time in possession of ball that are not included. Some of these unorthodox activities, together with tackles and sudden direction changes rely greatly on eccentric contractions, probably increasing the neuromuscular demands imposed by match when compared with LIST. The considerable amount of this type of lengthening-based contractions characteristic of soccer were initially expected to induce additional signs of muscle injury in match when compared to LIST. However, no differences in plasma CK and Mb, as well as in DOMS levels and lower limb strength were observed between the match and the LIST during recovery. One hypothetical reason to explain this absence of differences might be the number of turns, including accelerations and decelerations during LIST.

As reported after other types of exercise (Ascensao et al. 2007; Magalhaes et al. 2007) and also after match (Ascensao et al. 2008; Ispirildis et al. 2008), data showed that LIST and match induced a leukocytosis. This can be ascribed to the mobilization of blood cells from marginal pools by hemodynamic redistribution and augmentation that resulted from exercise-related metabolic conditions, such as enhanced catecholamine secretion (Bangsbo 1994). Our results also reported a marked lymphocytopenia during the subsequent period after both exercise protocols. Nevertheless, lymphocyte counts differed significantly between LIST and match at 30 min recovery being lower in match



**Fig. 3** Plasma myoglobin content (**a**), creatine kinase activity (**b**) and perceived delayed onset muscle soreness (**c**) during the 72-h recovery following LIST (*triangles*) and soccer (*squares*). Values are mean and

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SEM. Asterisk versus pre-exercise for both exercises (LIST and soccer); no significant differences were found between LIST and soccer



В mg.dL<sup>-1</sup> + UST 3 2 0 Pre 30 min 24 h 48 h 72 h D µmol.g<sup>-1</sup> 9 ---LIST Soccer 8,5 8 7,5 7 6,5 6 5,5 5 72 h Pre 30 min 24 ł 48 h

**Fig. 4** Plasma total antioxidant status (**a**), uric acid (**b**), malondialdehyde (**c**) and sulfhydryl (**d**) contents during the 72-h recovery following LIST (*triangles*) and soccer (*squares*). Values are mean and SEM.

Asterisk versus pre-exercise for both exercises (LIST and soccer); #LIST versus soccer



Fig. 5 Blood leukocytes (a) and lymphocytes (b) counts during the 72-h recovery following LIST (*triangles*) and soccer (*squares*). Values are mean and SEM. *Asterisk* versus pre-exercise for both exercises (LIST and soccer); #LIST versus soccer

than in LIST. In comparison to concentric exercise, eccentric activity has been shown to result in a greater release of immune system modulators such as proinflammatory cytokines, acute phase proteins, and the recruitment of phagocytic cells with the potential to release ROS (Malm et al. 2000). These signals may favor additional oxidative stress and damage, as well as apoptosis in several tissues and cells, including lymphocytes. However, the hypothesis that a higher apoptosis-induced lymphocytopenia observed after match than after LIST can be attributed to the referred unorthodox eccentric activities during match is unlikely, as Simpson et al. (2007) reported that the levels of blood lymphocytopenia apoptosis observed after downhill running were not different compared with intensive running. The precise reasons for the lower lymphocyte counts after match should be further investigated.

#### Redox status

Match induced higher increases in UA (30 min) and MDA (30 min and 24 h) contents than LIST. Moreover, TAS (30 min) and SH (30 min and 24 h) were lower after match than after LIST. These results suggest that match may induce higher changes on redox status and on adenine nucleotide metabolism than LIST. Given that around 1-5% of the total oxygen uptake results in the generation of



3.4

3,3

3,1

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Socce

3 2,9 2,8 2.7 24h 48h Pre 30 min 72h D Nm 150 - IIST 140 Soccer 130 120 110 100 90 80 Pre 30 min 24h 48h 72h

**Fig. 6** Jump performance (**a**), sprint performance (**b**), isokinetic peak torque for knee extension (**c**) and flexion (**d**) during the 72-h recovery following LIST (*triangles*) and soccer (*squares*). Values are mean and

SD. Asterisk versus pre-exercise for both exercises (LIST and soccer); #LIST versus soccer

superoxide radical and given the elevated oxygen consumption accompanying both LIST and match, it is not surprising its impact on these biomarkers of oxidative stress and damage. Furthermore, other sources of free radicals can influence cellular and blood antioxidant status. For example, stress hormones undergoing autoxidation (Cooper et al. 2002) and circulating neutrophils-induced oxidative burst (Quindry et al. 2003) can contribute to blood oxidative stress and damage. The influence of eccentric exercisemediating muscular damage-like events on the formation of free radicals has also been reported (Lee and Clarkson 2003). Considering the specific physiological demands imposed by the intermittent exercise models used, none of these potential-free radical sources should be ruled out, although we cannot conclusively demonstrate a causal link between any of those potential sources and the increased plasma oxidative stress and damage.

This study also shows that plasma TAS and UA increased at 30 min after both LIST and match. The observation that plasma UA levels increased in response to LIST and match is consistent with the findings from other studies (Ascensao et al. 2007; Magalhaes et al. 2007). However, match induced a higher increase in UA levels than LIST (at 30 min), which should probably due to an enhanced contribution of purine metabolism during match than during LIST. Recent data from Krustrup et al. (2006) showed a significant decrease in muscle ATP levels after an intense

exercise period in the second half and after the entire soccer match, as well as significant increase in muscle inosine monophosphate content after an intense exercise period in the second half. Moreover, increased blood ammonia, plasma UA and hypoxanthine contents were earlier reported (Bangsbo 1994). Therefore, it is likely that the observed increased oxidative stress and damage during the intense exercise periods comprised during the match might have a higher contribution from xanthine oxidase-free radical generating system than during LIST.

The enhanced oxidative damage induced by the match compared to LIST can also be observed by the accumulation of lipid peroxidation by-products, measured as plasma MDA. Accordingly, the match also induced a significant decrease in plasma SH, suggesting increased disulphide linkages (–S–S–) from both proteins and reduced glutathione (GSH).

Since both LIST and match did not seem to represent sufficient severe muscular stimuli to cause leukocyte infiltration, as shown by the maintenance of blood leukocyte counts from 24 to 72 h recovery period compared to baseline, the possible effects of neutrophils-related oxidative burst on muscle damage induced by match should probably be ruled out. However, other immune cell-mediated free radical production during the post-exercise periods might be considered, such as monocyte and macrophage oxidative burst (MacIntyre et al. 1995). It is possible that a delayed and continuous monocyte mobilization from bone narrow, thus compensating infiltration of these cells into muscle after damaging exercise (MacIntyre et al. 1995) had occurred masking leukocyte count changes in blood.

#### Neuromuscular function

In accordance with previous reports (Andersson et al. 2008; Bailey et al. 2007; Kingsley et al. 2005; Krustrup et al. 2006; Nicholas et al. 2000; Thompson et al. 1999, 2001b, 2003, 2004), both LIST and match impaired neuromuscular parameters assessed through measurements of sprint, jump and strength performance. Interestingly, only sprint ability was more affected by match than by LIST. Accordingly, Mohr et al. (2005) observed that soccer players decreased their ability to sprint after intense periods of the game, as well as after the end of the first and second halves. This temporary fatigue-related impairment should also be expected in jump and strength performance. In fact, considering that, at least in part, similar involvements of metabolic pathways should occur in energy turnover during these maximal power-elicited running and jumping tests, these distinct results were unexpected. An hypothetical explanation based on the presumable changes in the forcevelocity relationship as a result of selective damage to type II muscle fibers induced by the great amount of eccentric exercise during match is truly appealing (Twist and Eston 2005). In fact, a greater reliance on intra-muscular highenergy phosphates in the countermovement jump instead of glycogen was likely to occur. The relatively longer duration of the 2- to 3-s sprint test would rely more heavily on glycogen metabolism, which may be affected by the presumable eccentric exercise-induced muscle-damage and could, at least partially, explain differences between the two tests (Twist and Eston 2005). In fact, both LIST and match induce significant glycogen depletion in skeletal muscle fibers (Krustrup et al. 2006; Nicholas et al. 1999), although the effects of glycogen depletion on high intensity/short duration performance should only occur below critical levels. Alternatively, considering the distinct levels of motor coordination involved in the actions of jumping and sprint running, the repeated eccentric actions performed during the match might cause disturbances in movement control (Bottas et al. 2005) affecting more pronouncedly the running performance, which likely rely to a greater extent on muscular coordination than a single jump or leg curl and extension.

In summary, the impact of LIST and match did not differ regarding the observed muscle damage markers and some neuromuscular parameters, although match requires higher cardiac demand and induced higher changes on redox status, adenine nucleotide and on lymphocyte metabolism than LIST, which should be taken into account when using LIST to simulate a soccer match to study these type of physiological and biochemical-related endpoints.

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