ORIGINAL ARTICLE

Effect of Short-Term Maximal Exercise on Biochemical Markers of Muscle Damage, Total Antioxidant Status, and Homocysteine Levels in Football Players

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Abstract

Purpose: **Prolonged physical exercise results in transient elevations of biochemical markers of muscular damage. This study examined the effect of short-term maximal exercise on these markers, homocysteine levels (Hcy), and total antioxidant status (TAS) in trained subjects.**

***Methods*:** **Eighteen male football players participated in this study. Blood samples were collected 5-min before and 3-min after a 30-s Wingate test.**

***Results*:** **The results indicated that plasma biochemical markers of muscle injury increased significantly after the Wingate test ($P<0.05$). Moreover, significant increase of white blood Cells and their main subpopulations (i.e. monocytes, neutrophiles, and lymphocytes) ($P<0.001$) has been observed. Likewise, uric acid, total bilirubin, and TAS increased significantly after exercise ($P<0.05$). However, Hcy levels were unaffected by the Wingate test (for 3-min post-exercise measurement).**

***Conclusions*:** **Short-term maximal exercise (e.g. 30-s Wingate test) is of sufficient intensity and duration to increase markers of muscle damage, and TAS; but not Hcy levels. Increases in the selected enzymes probably come primarily from muscle damage, rather than liver damage. Moreover, increase of TAS confirms the Wingate test induced oxidative stress.**

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INTRODUCTION

Regular and moderate exercise has been shown to provide various beneficial health effects, including reduced risk of cardiovascular diseases, certain cancers, osteoporosis, and obesity [1]. Nevertheless, some forms of exercise and particularly maximal exercise are also accompanied by some deleterious effects. In this context, muscle damage after exercise results in a substantial increase in myocellular protein levels in the blood [2,3]. It has been shown that prolonged physical exercise results in transient elevations of biochemical markers of muscular damage such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), and lactate deshydrogenase (LDH) [2].

Moreover, in team sports (e.g. rugby), significant elevations in muscle enzyme activities and cortisol concentrations, which are indicative of muscle tissue damage and stress, have been observed after competitions [4]. However, it was noted that there have been no published data about the effect of short-term maximal exercise on these markers of cellular damage [5]. In this context, maximal exercise which is primarily anaerobic in nature, in particular sprint and resistance exercise, is commonly used by athletes who regularly train in an attempt to improve athletic performance [5]. Nevertheless, although this form of exercise is useful for these goals, it often results in an acute state of oxidative stress [5,6].

Short-term maximal exercise (e.g. 30-s Wingate test) has been associated with a substantial lactic

acidosis both in blood and muscle [7] and also with a major increase in plasma catecholamine levels [8]. Moreover, such exercise stimulates the catabolism of purines to xanthine and urate, as evidenced by plasma urate accumulation [9]. Regarding the health related effects of exercise, the importance of elevated homocysteine (Hcy) as a possible risk-marker for cardiovascular disease is continuously under debate [10]. There is evidence that physical activity may also alter Hcy metabolism by increasing protein and/or methyl group turnover [11]. However, there are contradictory data available concerning the associations between Hcy and exercise [12,13]. Recent animal research concluded that acute maximal anaerobic exercise induced an increase in plasma Hcy concentration [13] and support a relationship between high blood levels of Hcy and oxidative stress signal-transduction pathways leading to inflammation and apoptosis activation [14]. In this context, it was noted that the effects of short-term maximal exercise on Hcy levels in humans has not yet been investigated [13,14].

Thus, the aim of the present study was to investigate the effect of short-term maximal exercise on biochemical markers of muscle damage, Hcy levels and total antioxidant status in football players.

METHODS AND SUBJECTS

Subjects:

Eighteen male football players aged 17.5 ± 0.4 (mean ± S.D.) years, VO2max 3.41 ±1.09 l.min^{-1}, weight68.2 ± 4.4 kg and height, 178.7 ±3.3 cm, volunteered to participate in this study. At the time of the experiment they were training for at least 4 days per week for an average of 2-h per day. None of the participants was taking any vitamin or antioxidant supplements. None had suffered from any illness for at least two weeks before the study. The procedures of the study were explained to the subjects and all gave their written informed consent knowing that they could withdraw from the study at any time without any penalty. The study protocol and procedures that were in accordance with the Declaration of Helsinki, were approved by the Clinical Research Ethics Committee of the National Centre of Medicine and Science of Sports of Tunis.

Experimental Design:

Before beginning the study, subjects were familiarized with the high-velocity cycling exercises, in particular the 30-s Wingate test [15,16,17]. After warming-up at a low intensity (100 W) for 5-min and 3-min of subsequent rest they performed the test. After 10 hours of fasting, 12 ml of blood was collected from a forearm vein at 5-min of seated rest and 3-min after the test. To avoid the effects of circadian rhythm on the measured biological parameters, the experiment was performed at the same time-of-day (i.e. 09:00 h). Throughout the experimental period, subjects were requested to maintain their habitual nutritional habits and physical activity and to avoid strenuous activity during the 36-h before the test. They were requested to report to the laboratory in a fasted state. Laboratory temperature was maintained at a constant temperature of 20.4 ± 1.1 °C.

Wingate Test:

As previously described by Chtourou et al. [18, 19, 20], the Wingate test was conducted on a friction-loaded cycle ergometer (Monark model 864 Crescent AB, Varberg, Sweden) interfaced with a microcomputer. The seat height and handlebars were adjusted appropriately for each subject. The Wingate test consisted of a 30s maximal sprint against a constant resistance related to body mass (0.087 kg · kg^{-1} body mass). The Wingate test began from a rolling start, at 60 rpm against minimal resistance (weight basket supported). When a constant pedal rate of 60 rpm was achieved, a countdown of "3–2-1-Go!" was given, and the test resistance was applied. Then, subjects were required to pedal as fast as they could. They were verbally encouraged to avoid pacing and to sustain a maximal effort throughout the test. Every second, power output was calculated by the computer and stored. The highest power output over 1s (P_{peak}) and the mean power (P_{mean}), corresponding to the ratio between total work done and time to do it (i.e. 30s), were recorded at the end of the test. The Fatigue Index (i.e. the percentage of decrease in power output) was equal to the difference between the highest (P_{peak}) and lowest power

(P_{low}) divided by the highest power:

$$\text{Fatigue Index} = (P_{peak} - P_{low})/P_{peak}$$ [21]

Dietary Records:

To assess the adequacy of nutrient intake, a 7-day consecutive dietary record was completed. All players received a detailed verbal explanation and written instructions on data collection procedures. Subjects were asked to continue with their usual dietary habits during the period of diet recording, and to be as accurate as possible in recording the amount and type of food and fluid consumed. A list of common household measures, such as cups and tablespoons, and specific information about the quantity in each measurement (grams, etc) were given to each participant. Each individual's diet was calculated using the Bilnut 4 software package (SCDA Nutrisoft, Cerelles, France) and the food composition tables published by the Tunisian National Institute of Statistics in 1978. Estimated nutrient intakes were referred to reference dietary intakes (RDI) for physically active people [22,23]. The data about the daily nutriment intake are presented in Table 1 and showed that total calorie, macronutrient, and micronutrient intakes are situated in the interval of the RDI. Only vitamin E and folate intakes are below this interval.

Blood sampling and analysis:

Four distinct tubes were used in this study. The first heparinized tube was used to determine plasma creatinine (CRE), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Lactate Dehydrogenase (LDH), Creatine Kinase (CK) activities, glucose (GLC), uric acid (UA), and total bilirubine (TBIL). The second tube, without anticoagulant, was used to determine total antioxidant status (TAS). The third EDTA tube was used to determine Hcy and hematological parameters (i.e. red blood cells (RBC), w hite blood cells (WBC) and principal derivative sub-populations such as monocytes (MO), neutrophiles (NE) and lymphocytes (LY)). The fourth tube with Sodium fluorure was used to determine plasma lactate levels (Lac). Samples were placed in an ice bath and centrifuged immediately at 2500 × g and 4 °C for 10-min. Aliquots of the resulting plasma were stored at -80 °C until analyzed. Venous samples were corrected for plasma volume changes, using the equations of Dill and Costill [24].

All biological tests were performed as previously detailed [3,25]. CRE was measured by the compensated alkaline picrate assay. AST and ALT activities were determined by the enzymatic rate method. GLC levels were measured with glucose oxidase method and Lac concentrations were measured by the lactate oxidase peroxidase method. Moreover, magnesium (MG) was measured by a colorimetric assay and inorganic phosphate (PO_4^{3-}) was assayed spectrophotometrically with Briggs' method. The coefficients of variation (CV) for these parameters were <8%.

CK activity was determined spectrophotometrically by measuring NADPH formed by hexokinase and the D-glucose-6-phosphate dehydrogenase coupled

Table 1: Dietary record of the subjects (mean ± standard deviation)

Nutriments	Daily Intake	Reference Dietary Intake
Kilocalorie	3302.0 (709.0)	(2300-3450) [a]
Carbohydrate (g)	432.1 (144.0)	(400-500) [a]
Protein (g)	101.2 (21.0)	(70-110) [a]
Fat (g)	107.6 (63.0)	(100-140) [a]
Carbohydrate (%)	52.3 (6.6)	(45–65) [b]
Protein (%)	12.2 (1.1)	(10–30%) [b]
Fat (%)	29.2 (5.7)	(25–35) [b]
Cholesterol (mg.d^{-1})	357.2 (264.0)	< 350
Vitamin C (mg.d^{-1})	46.6 (30.0)	(40-70) [a]
Vitamin E (mg.d^{-1})	4.0 (2.0)	(11-30) [b]
Vitamin A (ER)	1300.0 (268.0)	(900-3000) [b]
Folate (µg. d^{-1})	341.0 (58.0)	(400-1000) [a]
Vitamin B12 (µg. d^{-1})	7.3 (1.5)	(2-6) [a]

a: RDI for Tunisian adult men [22]; b: (RDIs) acceptable macronutrient distribution range [23]

enzymatic system. The intra-assay CV for the CK kit was 1.85%. LDH activity was determined by measuring NADH consumption using the reagent kits. The intra-assay CV for the LDH kit was 2.61%.

TBIL measurements were performed by means of a diazo reaction. UA was determined by an enzymatic method at 550 nm using a Randox kit (Randox, Antrim, UK). The CV for UA was < 1.9%.

All these measures were done as adapted for the auto-analyzer by Synchron CX PRO systems (Beckman Coulter, Brea, CA, USA). All reagents employed in biochemical tests were obtained from Randox Laboratories, Ltd. (Crumlin, Co, Antrim, UK). To eliminate inter-assay variance, all samples were analyzed in the same assay run.

Hcy was determined by immunoassay using the IMX Analyzer (Abbott Laboratories, Lake Bluff, Illinois. Intra- and inter-assay imprecision CV were 2.3% and 3.2%, respectively at 8.0 µmol/L.

The TAS analyses were performed on a new-generation Daytona (RX) automatic chemical analyzer according to instructions provided by the Randox Co and using a kit purchased from Randox Laboratories (Crumlin, Northern Ireland). The kit is based on the principle of inhibition of the oxidation of ABTS 2,2'-azinodi-[ethylbenzthiazoline sulfonate] to radical cation $ABTS^{\cdot+}$ by the antioxidants present in the sample. The $ABTS^{\cdot+}$ was produced by reacting ABTS with potassium persulfate (K2S2O8). In this assay, a peroxidase (metmyoglobin) reacts with H_2O_2 to form the radical species ferrylmyoglobin. Detection limit for the TAS kit was 0.21 µmol/L and the intra-assay CV was 2.77 %.

Hematological parameters were generally performed within 3-h in a multichannel automated blood cell analyzer Beckman Coulter Gen system-2 (Coulter T540). RBC, hemoglobin (HGB), hematocrit (HCT), WBC, and principal derivative sub-populations were all simultaneously measured. All the analyzing devices were properly calibrated according to the manufacturers' guides.

Statistical Analyses:

Statistical tests were processed using STATISTICA Software (StatSoft, France). All values are expressed as mean ± SD. Following normality confirmation using the Shapiro-Wilk *W*-test, biological parameters data were analyzed using a paired student T-test. A probability level of 0.05 was selected as the criterion for statistical significance.

RESULTS

Statistical analysis indicated significant increase of RBC, HGB, WBC, and main derivatives' subpopulations (i.e. NE, LY, MO) ($P<0.05$) after the 30-s Wingate test (Table 2). However, HCT didn't change significantly. Moreover, UA, TBIL, and TAS increased significantly after exercise ($P<0.05$) (Table 3). Data indicated also a significant increase of CRE, GLC, MG, PO_4^{3-} ($P<0.05$), and Lac ($P<0.001$). While Hcy didn't change significantly, biochemical markers of muscle injury increased significantly 3-mins after the Wingate test ($P<0.05$) (Table 4).

DISCUSSION

The aim of the present study was to investigate the effect of a single bout of high-intensity exercise on biochemical markers of muscle and hepato-cellular

Table 2: Values (mean ± standard deviation) of hematological parameters before and after the Wingate test

	WBC (10^3/µl)	Neut (10^3/µl)	Lym (10^3/µl)	Mono (10^3/µl)	RBC (10^6/µl)	Hgb (g/dl)	Hct (%)
Before	8.89 (1.75)	5.05 (1.39)	3.11 (0.81)	0.81 (0.28)	5.06 (0.31)	14.36 (0.85)	44.97 (2.41)
After	10.52 (1.68)	5.58 (1.31)	4.15 (1.32)	0.99 (0.23)	5.29 (0.52)	14.91 (1.08)	46.44 (4.01)
***P.* Value**	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	>0.05

WBC: White Blood Cells, Neut: Neutrophils, Lym: Lymphocytes, Mono: Monocytes, RBC: Red Blood Cell, Hgb: Hemoglobin, Hct: Hematocrit

Table 3: Values (mean ± standard deviation) of biochemical parameters before and after the Wingate test

	Lac (mmol/L)	CRE (µmol/L)	GLC (mmol/L)	MG (mmol/L)	$PO4^{3-}$ (mmol/L)	TBIL (µmol/ L)	UA (µmol/ L)	TAS ($\mu mol \cdot L^{-1}$)
Before	1.14 (0.39)	81.39 (21.34)	4.71 (0.69)	0.92 (0.13)	1.31 (0.26)	16.52 (3.52)	253 (60.04)	1.25 (0.19)
After	9.68 (0.77)	90.4 (14.31)	5.12 (0.49)	0.98 (0.07)	1.46 (0.27)	17.89 (4.06)	269.53 (61.05)	1.30 (0.18)
***P.* Value**	0.001	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Lac: Lactate, CRE: Creatinine, GLC: Blood Glucose, MG: Magnesium, $PO4^{3-}$: inorganic phosphate, TBIL: Total Bilirubin, UA: Uric Acid, TAS: Total antioxidant status

damage, Hcy levels, and TAS in trained subjects. Excepting Hcy levels, the data indicated that 30-s of maximal cycling exercise are sufficient to affect the studied parameters even as soon as 3-min post-exercise. A transient leucocytosis as well as a mobilisation of the main WBC subpopulations (i.e. MO, LY, and NE) were also observed after this exercise. The present results showed that the levels of CK, LDH, AST, and ALT increased after the Wingate test. Recent research confirmed that these latter molecules are the most useful serum markers of muscle injury [2, 26]. Although Takahashi et al [4] showed that only LDH increased after a Rugby sevens match lasting 10-min without significant change of CK, AST, and ALT, many previous studies found that maximal physical exercise (e.g. running) induced transient elevation of hepatic and muscle enzymes [27]. When the exercise loading exceeds a certain limit of muscle ability, CK and LDH leak into the interstitial fluid and are taken up by the lymphatic system and returned into the circulation [2]. Moreover, in well-trained athletes there is a positive correlation between mean sprint velocity and LDH 4 and 5 isoenzyme activities [28]. AST is contained in striated muscle and increases with ALT as well as CK after exercise [29]. The simultaneous change of these parameters shown in the present investigation could be due to muscle damage rather than hepatic injury. Indeed, it has been identified that elevated ALT levels in the absence of other evidence of liver disease should lead to consideration of muscle injury, which is confirmed by the observed elevation of CK and LDH levels [26]. While only few studies focused on the effect of anaerobic exercise on biomarkers of muscle injury [3], it has been shown that a single bout of Wingate test induced lipid peroxidation and lead to oxidative stress [5]. Additionally, a positive correlation between increased lipid peroxidation (i.e. TBARS, isoprostane) on one hand, and CK and AST on the other hand was found in previous animal studies during high-intensity exercise [30]. This positive correlation is consistent with the hypothesis that free radicals produced during exercise alter muscle cells' membrane permeability [5] and that biomarkers of muscle damage during exercise are indicative of oxidative stress [31]. According to recent research findings [3], the present study showed that CRE and GLC increased significantly after exercise. The greater post exercise GLC levels result from an increase in hepatic glucose production which exceeds the concomitant rise in peripheral glucose uptake, as shown during high-intensity exercise [32]. Furthermore, these results also indicate a strong mobilization of creatine which is recycled or converted to creatinine during anaerobic exercise [10]. Hence, the Wingate test strongly stimulates both the ATP-PCr and glycolytic systems [33] and thus activates purine catabolism [9] and

Table 4: Values (mean ± SD) of Hcy and biochemical markers of muscle injury before and after the Wingate test

	ALT (IU/L)	AST (IU/L)	CK (IU/L)	LDH (IU/L)	Hcy (µmol/ L)
Before	22.89 (7.28)	29.28 (11.3)	180.78 (80.79)	438.39 (116.94)	16.87 (3.46)
After	26.54 (8.03)	32.23 (9.86)	201.08 (75.55)	496.12 (76.63)	17.15 (4.53)
***P.* Value**	<0.05	<0.05	<0.05	<0.05	>0.05

ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, CK: Creatine Kinase, LDH: Lactate Dehydrogenase, Hcy: Homocysteine

lactic acid production [7]. Moreover, after the Wingate test HCT of the athletes was virtually not altered, indicating no changes in plasma volume. In agreement with previous research [27], it seems that elevated plasma enzymes showed in the present study is essentially due to muscle damage or muscle glycogen depletion rather than an altered hemoconcentration. Furthermore, Hcy levels were not affected 3-mins after the 30-s Wingate test in the present study. Similar results were found during a sub-maximal exercise protocol [12]. However, a recent animal study concluded that acute maximal anaerobic exercise induced an increase in plasma Hcy concentrations that was not found in Wistar rats subjected to acute moderate aerobic exercise [13]. It has been suggested that high-intensity long-duration physical activity, which increases the demand for creatine, increases Hcy production compared with less-intense short-duration physical activity [10]. However, the exact mechanism by which plasma Hcy concentrations increase after acute exercise is unknown [34]. Furthermore, the timing of sampling in the present study could have missed any further increase of Hcy. In this context, further studies are needed to check if there is any delayed increase of Hcy after such a short-intensive exercise.

After the Wingate test, the present study results also showed an increase of RBC and HGB, as well as WBC and their subpopulations (i.e. LY, NE, MO). Similarly, Yalcin et al [7] detected a transient significant increment of RBC, WBC and granulocytes activation immediately after the same test. Moreover, a significant increase of WBC, LY, MO, and NE has been shown immediately after 60-s of exercise at 150 % of maximal aerobic power [35]. Recruitment of leucocyte subpopulations was of 25 to 43 % from basal values after 3 sets of 6 maximal repetitions of leg flexion-extension [36]. The most likely cause of the inflammatory responses after strenuous exercise is generalized muscle damage [37]. In addition, a recent review about exercise induced leukocytosis [38] proposed a model of the possible roles of stress hormones (specifically the catecholamines, growth hormone, and cortisol) in exercise-induced immune changes [38]. Actually, it has been shown that catecholamines increase significantly after a Wingate test confirming that this test is a highly stressful effort [8].

A significant impairment of RBC deformability and mechanical deterioration resulting from a decreased elongation index has been reported to occur immediately after a Wingate test [7]. As previously shown [7], the present study showed that Lac levels increased significantly after the Wingate test. This increase might be an induced witness of the high-intensity of this exercise as well as altered RBC deformability and rigidity [7].

Nevertheless, in agreement with previous reports [6, 39,40], the present results indicated a significant increase of TAS, TBIL, and UA after the Wingate test. From the dietary analyses, although Vit A and C showed normal values, daily Vit E intake are below the RDI. Insufficient dietary Vit E intake could affect antioxidant system. Therefore, the modification of TAS, TBIL, and UA due to the Wingate test could be influenced by inadequate dietary intake. Increases in TBIL and UA are thought to reflect imbalances in protein metabolic homeostasis and an increased protein catabolism that are associated with tissue damage [41]. It is well established that UA is a powerful antioxidant [42]. In agreement with previous research [6,39], the increase in TAS observed in this study could be explained by a possible increase in UA and/or endogenous peroxidases. In this context, it has been reported that oxidative stress response and countermeasure against the generation of reactive oxygen species indicate an adaptative response to exercise induced oxidative stress, as manifested by the increase in TAS [39]. On the other hand, an exercise bout such as the Wingate test strongly stimulates purine catabolism, as evidenced by the large plasma increase of both hypoxanthine and UA [9]. Indeed, these findings confirmed the increase of oxidative stress via xanthine oxidase activation during such high intensity exercise [5].

A limitation of this type of field study is the indirect effects of the selected markers on oxidative stress. Future studies should focus upon the specific markers of oxidative stress (e.g. malonyldialdehyde, carbonylated protein, glutathione, etc.). Another limitation of the present study is that we didn't use a control group to investigate the effect of training status on the biochemical responses during the anaerobic exercise test.

CONCLUSION

In conclusion, the present study shows for the first time that a single bout of 30-s maximal exercise is sufficient to increase muscle damage enzymes and TAS, and that WBC were selectively mobilized, and such changes were even observed as early as 3-mins post-exercise. Moreover, Hcy levels were not affected 3-mins after the intensive exercise. The present study suggests that the increase in the selected enzymes comes primarily from muscle damage rather than liver damage. The underlying mechanisms of asymptomatic elevations of clinical chemistry parameters caused by muscular exercise are to a large extent unknown and need to be further explored. Indeed, to ascertain whether the Wingate test causes hepatocyte damage requires further studies using more specific markers such as glutathione S-transferase or cholinesterase. Lastly, the increase of muscle damage and antioxidant status parameters confirms that the 30-s maximal exercise (Wingate Test) induces oxidative stress.

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