Asian Journal of Sports Medicine

ORIGINAL ARTICLE

Biochemical Responses to Level-1 Yo-Yo Intermittent Recovery Test in Young Tunisian Football Players

Omar Hammouda*[1ABCDG], PhD; Hamdi Chtourou[1,5ABCEG], PhD; Anis Chaouachi[1EG], PhD; Henda Chahed[2EG], PhD; Nidhal Zarrouk[3EG], PhD; Abdelhedi Miled[2BEG], MD; Karim Chamari[1,4EG], PhD; Nizar Souissi[1,4AEG], PhD

Authors' Affiliation:

1. Research Laboratory ''Sport Performance Optimisation'', National Center of Medicine and Science in Sport, Tunisia
2. Laboratory of Biochemistry, CHU Farhat Hached, Sousse, Tunisia
3. Neurophysiologie de la Vigilance, de l'Attention et des Performances, Service d'Explorations Fonctionnelles du Système Nerveux, CHU Sahloul, Sousse, Tunisia
4. High Institute of Sport and Physical Education, Ksar-Saïd, Manouba University, Tunisia
5. Research Unit (EM2S), High Institute of Sport and Physical Education, Sfax University, Tunisia

Authors' Contribution

- A Concept / Design
- B Acquisition of Data
- C Data Analysis / Interpretation
- D Manuscript Preparation
- E Critical Revision of the Manuscript
- F Funds Collection
- G Approval of the Article

*** Corresponding Author;**

Address: National Center of Medicine and Science in Sports, Bp263, Ave Med Ali Akid, 1004 El Menzah, Tunis, Tunisia

E-mail: omar_hammouda86@yahoo.fr

Received: *Mar* 31, 2012
Accepted: *Aug* 26, 2012
Available Online: *Sep* 29, 2012

Abstract

Purpose: **The aim of this work was to investigate the metabolic and muscle damage responses after the level-1 Yo-Yo intermittent recovery test (YYIRT) in young football players.**

***Methods*:** **Fifteen male football players (17.42 ± 0.2 yrs, 69.91 ± 4.4 kg, 178.64 ± 3.8 cm; mean ± SD) participated in this study. Fasting blood samples for various biochemical parameters (i.e. lactate (Lac), glucose (GLC), triglycerides (Tri), creatine kinase (CK), uric acid (UA)) collected from a forearm vein after 5-min of seated rest and 3-min after the test. Moreover, rating of perceived exertion (RPE) and maximal heart rate during and after the YYIRT were recorded.**

***Results*:** **Mean levels of the selected biochemical markers were raised after the YYIRT exercise (*P*<0.001 for the other markers). Moreover, lipid parameters increased significantly after the test (*P*<0.01 for Tri and *P*<0.001 for HDL).**

***Conclusion*:** **These findings confirm the higher metabolic demand of aerobic as well as anaerobic metabolism and reflect a significant mobilization of purine cycle during the YYIRT. The increase of muscle damage markers also reflects the higher anaerobic solicitation. From these findings, we can conclude the importance of aerobic and anaerobic metabolism during soccer-specific endurance performance (i.e. YYIRT, soccer match).**

Key Words: Metabolic Responses; Intermittent Recovery Test; Lactate; Lipid Profile

Asian Journal of Sports Medicine, Volume 4 (Number 1), March 2013, Pages: 23-28

INTRODUCTION

Soccer is an intermittent sport characterized by sustained movement incorporating frequent bursts of high-intensity activity interspersed with regular recovery periods [1,2]. Heart rate recordings and analyses of muscle and blood samples obtained during match-play suggest that the aerobic load is high throughout matches and that the anaerobic energy turnover is extensive during intense periods of the game [1,3-5].

Particularly, the level-1 Yo-Yo intermittent recovery test (YYIRT) was specifically designed to evaluate the ability to perform high-intensity intermittent exercise [4]. This test is extensively utilized by scientists and coaches in monitoring cardio-respiratory fitness of football players since it correlates with match performance [4,6]. Indeed, the physiological measurements showed that aerobic energy turnover reached maximal values and that the anaerobic energy system was highly taxed toward the end of the test [4,6]. However, very few studies have been reported on the metabolic response to the YYIRT [4,7]. Moreover, only restricted a number of biochemical markers has been measeured (i.e. lactate, hydrogen, bicarbonate). In this context, a dispersed relationship has been observed between muscle and blood lactate when subjects performed repeated intense exercise during the YYIRT

[4]. The authors reported also a significant relationship between the level of blood Lac accumulation during the execution of the YYIRT and the final performance [4]. More recently, Rampinini et al [7] detected a significant increase in blood Lac, hydrogen as well as bicarbonate levels which confirms the important anaerobic contribution in this test. Nevertheless, biochemical measures of muscle damage and lipid profile were not yet evaluated during the YYIRT in young Tunisian football players. On the other hand, it has also been shown that physical exercise results in transient elevations of biochemical markers of muscular damage such as creatine kinase (CK), and lactate deshydrogenase (LDH) [8-10].

Indeed, the YYIRT could impact the biomarkers of muscle injury since the increase of these parameters has been related to both the intensity and duration of exercise, with intensity playing the main role [8]. Thus, it seems logical to assess metabolic and muscle damage response to the YYIRT. Based on the rationale that existing research lacked full monitoring of biochemical modifications after soccer-specific endurance test (i.e. YYIRT), we measured in the present study biochemical responses of various markers (i.e. Lac, lipid profile, uric acid, and muscle damage) in Tunisian footballers. These markers are indicative of metabolic responses and muscle damage during exercise.

METHODS AND SUBJECTS

Participants:

Fifteen male football players from Tunisian junior football squads affiliated with professional clubs volunteered to participate in this study.

They usually trained at least 4 d/wk for an average of 2 h daily with one match weekly. The experimental protocol has been done in the summer. After receiving a thorough explanation of the possible risks and discomforts associated with the experimental procedures, they provided written informed consent. No subject reported tobacco use within the 6 months prior to the study, and none were taking antioxidant compounds, including vitamins and medications (e.g. anti-inflammatory agents). The experimental design of the present study was approved by the Clinical Research Ethics Committee of the National Centre of Medicine and Science of Sports (CNMSS), Tunisia and met the ethical standards of the Declaration of Helsinki.

Experimental Design:

Participants were already familiar with the testing procedures as it was part of their usual fitness assessment program. Upon arrival for their first test session, participants' body mass and height were recorded using an electronic scale (Tanita, Tokyo, Japan). Participants were asked to maintain, as closely as possible, their usual sleeping habits, with a minimum of 7-h of sleep taken on the night preceding each test session [11-13]. Throughout the experimental period, participants were requested to maintain their habitual physical activity and to avoid strenuous activity during the 24 h before the test sessions [14,15]. Fasting blood samples were collected from a forearm vein after 5-min of seated rest and 3-min after the test. To avoid the effects of circadian rhythm on the measured biological parameters [16,17], the experiment was performed at the same time of the day (i.e. in the morning).

The yo-yo intermittent recovery test:

As previously described by Hamouda et al [18] and Chtourou et al. [19,20] the YYIRT was performed according to the procedures suggested by Krustrup et al [4]. The reliability of YYIRT level 1 was established in a previous study [21]. The test consisted of 20-m shuttle runs performed at increasing speeds with 10 s of active recovery in a distance of 5-m between runs until exhaustion. Audio cues of the YYIRT were recorded on a CD (www.teknosport.com, Ancona, Italy) and broadcasted using a portable CD player (Philips, Az1030 CD player, Eindhoven, Holland). The end of the test was considered when the participant twice failed to reach the front line in time (objective evaluation) or he felt unable to complete another shuttle at the dictated speed (subjective evaluation). The total distance (TD) covered during the YYIRT level 1 was considered as the test score. Before the test, all participants carried out a warm-up period consisting of the first four running bouts in the test. The total duration of the test was 6–20 min. Heart rate was recorded during the YYIRT, using a Polar heart rate monitor (Polar Electro Oy, T61-coded, Hungary) and only heart rate max (HRmax) was presented in this work.

***Rating of perceived exertion scale (RPE)*: [22]**

The RPE scale allows participants to give a subjective exertion rating for the physical task. The participants were familiarized to the use of the RPE scale. The scale presents a 15-point scale ranging from 6 (very very light) to 20 (very very hard) [23]. The RPE scale is a reliable indicator of physical discomfort, has sound psychometric properties, and is strongly correlated with several other physiological measures of exertion.

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 [24,25]. The data about the daily nutriment intake are presented in table 1 and showed that total calorie, macronutrient, and micronutrient intakes are whithin the range of the RDI for healthy Tunisian adults.

Blood Sample Variable Analysis:

Fasting blood samples (12 ml) were collected from a forearm vein after 5-min of seated rest and 3-min after the test. GLC levels were measured with the glucose oxidase method, and Lac concentrations were measured, as previously shown [9,10], by the lactate oxidase peroxidase method. The coefficients of variation (CVs) for these parameters were <8%. CK activity was determined spectrophotometrically by measuring nicotinamide adenine dinucleotide phosphate formed by hexokinase and the glucose-6-phosphate dehydrogenase coupled enzymatic system [9,10]. The intra-assay CV for the CK kit was 1.85%. LDH activity was determined by measuring nicotinamide adenine dinucleotide consumption using the reagent kits. The intra-assay CV for the LDH kit was 2.61% [9,10]. UA was determined by an enzymatic method at 550 nm using a Randox kit (Randox, Antrim, UK) [9,10]. The CV for UA was <1.92%. Moreover, total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL) were estimated by standard enzymatic analysis using reagents, standards, and controls from Randox Laboratories Ltd. (Antrim, UK) [9,10]. (LDL) was calculated by the Friedewald formula for TG levels below 400 mg/dL [26]. The coefficients of variation (CVs) for these parameters were <7%. All the above measures were done as adapted for the autoanalyzer by Synchron CX systems (Beckman Instruments, Danville, California, USA). All reagents employed in the biochemical tests were obtained from Randox Laboratories. Venous samples were corrected for plasma volume changes, using the equations of Dill & Costill [27]. Haematocrit was measured on the same day as the experiment by microcentrifugation [9].

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. Effect sizes, d, were analyzed to determine the magnitude of an effect independent of sample size. A probability level of 0.05 was selected as the criterion for statistical significance.

RESULTS

Mean age of 15 male football players was 17.43 (±0.2) years and mean weight and height of them was 69.92 (±4.4) (kg) and 178.61 (±3.8) (cm), respectively.

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

Nutriments	Daily Intake Mean (SD)	Reference Dietary Intake (RDI)
Kilocalorie	3302 (709)	(2300-3450)[a]
Carbohydrate (g)	432.12 (144)	(400-500)[a]
Protein (g)	101.16 (21)	(70-110)[a]
Fat (g)	107.61 (63)	(100-140)[a]
Carbohydrate (%)	52.33 (6.6)	(45–65)[b]
Protein (%)	12.23 (1.09)	(10–30%)[b]
Fat (%)	29.16 (5.7)	(25–35)[b]
Cholesterol ($mg.d^{-1}$)	357.21 (264)	< 350

SD: Standard Deviation; a: RDI for Tunisian adult men[24]. b: (RDIs) acceptable macronutrient distribution range[25]

Dietary Records:

The mean daily calories, protein, carbohydrate, fat, cholesterol, vitamin E, and vitamin A intakes were in the normal ranges. However, the percentage of protein intake is relatively small. These data are presented in table 1.

Physical performance, HRmax, and RPE:

The total distance covered during the YYIRT was of an average of 1763.64 (±482.48) m. The HRmax recorded during the YYIRT was of an average of 190.94 (±5.1) batt/min. The RPE scores recorded at the end of the YYIRT were of average 14 (±1.5).

Biochemical parameters:

Table 2 shows the mean values for the selected biochemical parameters before and after the YYIRT as well as the effect size results. As this table indicates, the Lac increased between before and after the exercise. Likewise, all the other biochemical variables were raised after the exercise.

DISCUSSION

The principal aim of the present study was to investigate the biochemical monitoring of soccer players after a specific-endurance test (YYIRT). The results indicated that lipid profile, GLC as well as Lac levels increased significantly following the YYIRT. Likewise, purine metabolite (i.e. UA) increased significantly after the YYIRT.

According to previous studies [4,7], the present findings indicate that Lac levels increased significantly after the YYIRT. A significant relationship has been reported between blood Lac accumulation and the final performance after the YYIRT [4]. In this context, elite soccer players typically perform up to 250 brief intense actions and have blood lactate values of 2–14 mmol L soccer players typically perform up to 250 brief intense actions and have blood lactate values of 2–14 mmol L during a soccer match [2,3,28]. Moreover, GLC levels are significantly greater following the exercise in the present work. This increase in GLC levels is in

Table 2: Selected biochemical parameters before and after YYIRTing

Parameter	Before YYIRT Mean (SD)	After YYIRT Mean (SD)	d	*P* Value
Total Cholesterol (mmol·L−1)	3.21 (0.66)	4.0 (0.7)	1.05	<0.001
High Density Lipoprotein (mmol·L−1)	1.14 (0.13)	1.42 (0.12)	2.48	<0.001
Low Density Lipoprotein (mmol·L−1)	1.33 (0.21)	1.39 (0.20)	0.3	<0.01
Triglyceride (mmol·L−1)	1.19 (0.32)	1.73 (0.43)	1.47	<0.01
Blood Glucose (mmol·L−1)	4.62 (0.1)	5.69 (0.43)	3.55	<0.001
Lactate Level (mmol·L−1)	1.05 (0.29)	9.83 (0.65)		<0.001
Lactate Dehydrogenase (IU·L−1)	396.82 (25.87)	542.91 (40)	4.49	<0.001
Creatine Kinase (IU·L−1)	173.73 (14.48)	219.27 (27.74)	2.13	<0.001
Uric Acid (µmol·L−1)	245.11 (22.48)	318.57 (13.14)	4.13	<0.001

YYIRT: Yo-Yo intermittent recovery test; SD: Standard Deviation accordance with previous results during exercises of similar intensities [29] and during soccer games [30] but not after a YYIRT. These findings suggest that the rate of glucose release from the liver is high enough to compensate for the use of blood glucose throughout a game [1]. Therefore, Lac and GLC mobilization after the YYIRT indicate also the high anaerobic energy production after the Level-1 YYIRT, which confirms the findings of Krustrup et al [4].

Considering the effects of the YYIRT on lipid profile, the authors' findings indicated that this exercise is of sufficient intensity to induce a significant increase in TC, TG, HDL and LDL levels. In this context, the physiological measurements showed that aerobic energy turnover reached maximal values in this test [4], which could explain the TG mobilisation after the exercise. To the best of the authors' knowledge, there seems to be no study upon the acute effects of exercise on these biomarkers.

These findings showed that YYIRT could induce a high rate of lipolysis. Similar findings have been shown during soccer games indicated by the increase of the free fatty acids (FFA) and glycerol essentially during the second half of a soccer match [5,28]. Hormonal changes also might play a major role in the increase in FFA. Indeed, insulin concentrations are lowered and catecholamine levels are progressively elevated during a soccer match [28], stimulating a high rate of lipolysis and, thus, release of FFA into the blood. From the dietary analyses of macronutrient intakes indicated that carbohydrate, protein as well as fat intakes are within the reference dietary intakes (RDI) [24,25] (Table 1), which indicates that the present findings on biochemical responses are not affected by dietary nutrient intakes.

Concerning UA, this study also shows that plasma levels increased after the YYIRT. These findings are consistent with other studies during soccer matches [31,32]. An increase in UA after the YYIRT should be probably due to an enhanced contribution of purine metabolism. In this context, recent data from Krustrup et al. [5] 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 of the match. Moreover, increased blood ammonia, plasma UA and hypoxanthine contents were reported in soccer players [1,28].

On the other hand, the present findings indicate that CK and LDH levels increased significantly after the YYIRT. These markers are essentially indicative of muscle damage [8]. The extent of muscle damage has been related to both the intensity and duration of exercise, with intensity playing the main role [8,9,10]. Recent researches have indicated also an increase in muscle damage markers after a soccer match in male and female soccer players [33].

CONCLUSION

It is suggested that a single YYIRT is of sufficient intensity to induce a significant increase of biochemical response in soccer players. In fact, lipid profile, GLC, Lac and UA responses are significantly higher after the test. These findings confirm the higher metabolic demand of aerobic as well as anaerobic metabolism and reflect a significant mobilization of purine cycle following this type of exercise. The increase of muscle damage markers also reflects the higher anaerobic solicitation. From these findings, we can conclude that there is a concomitant solicitation of aerobic and anaerobic metabolism after soccer-specific endurance performance in Tunisian soccer players (i.e. YYIRT, soccer match).

For practical application, this research focuses on the biochemical responses of various markers of metabolic profiles and inflammatory status after the YYIRT in young soccer players. Many of these markers are measured for the first time and confirm the solicitation of aerobic as well as anaerobic metabolism during the YYIRT.

ACKNOWLEDGMENTS

This study was supported by the Ministry of Higher Teaching and Scientific Research, Tunisia. We are grateful to all of the players who participated so willingly in the study. The protocol of the study was approved by the Clinical Research Ethics Committee of the National Centre of Medicine and Science of Sports of Tunis (CNMSS).

Conflict of interests: None

REFERENCES

[1] Bangsbo J, Marcello IF, Krustrup P. Metabolic Response and Fatigue in Soccer. *Int* J *Sports Physiol Perform*, 2007;2:111-27.

[2] Krustrup P, Christensen JF, Randers MB, et al. Muscle adaptations and performance enhancements of soccer training for untrained men. *Eur J Appl Physiol* 2010;108:1247-58.

[3] Aslan A, Açıkada C, Güvenç A, et al. Metabolic demands of match performance in young soccer players. *J Sports Sci Med* 2012;11:170-9.

[4] Krustrup P, Mohr M, Amstrup T, et al. The Yo-Yo intermittent recovery test: physiological response, reliability, and validity. *Med Sci Sports Exerc* 2003;35:697-705.

[5] Krustrup P, Mohr M, Steensberg A, et al. Muscle and blood metabolites during a soccer game: implications for sprint performance. *Med Sci Sports Exerc* 2006;38:1165-74.

[6] Bangsbo J, Iaia FM, Krustrup P. The Yo–Yo intermittent recovery test: a useful tool for evaluation of physical performance in intermittent sports. *Sports Med* 2008;38:37-51.

[7] Rampinini E, Sassi A, Azzalin A, et al. Physiological determinants of YoYo intermittent recovery tests in male soccer players. *Eur J Appl Physiol* 2010;108:401-9.

[8] Brancaccio P, Lippi G, Maffulli N. Biochemical markers of muscular damage. *Clin Chem Lab Med* 2010;48:757-67.

[9] Hammouda O, Chtourou H, Chahed H, et al. Diurnal Variations of Plasma Homocysteine, Total Antioxidant Status, and Biological Markers of Muscle Injury During Repeated Sprint: Effect on Performance and Muscle Fatigue-A Pilot Study. *Chronobiol Int* 2011;28:958-67.

[10] Hammouda O, Chtourou H, Chahed H, et al. High Intensity Exercise Affects Diurnal Variation of Some Biological Markers in Trained Subjects. *Int J Sports Med* 2012;33:886-91.

[11] Chtourou H, Zarrouk N, Chaouachi A, et al. Diurnal variation in Wingate-test performance and associated electromyographic parameters. *Chronobiol Int* 2011;28:706-13.

[12] Chtourou H, Chaouachi A, Driss T, et al. The effect of training at the same time of day and tapering period on the diurnal variation of short exercise performances. *J Strength Cond Res* 2012;26:697-708.

[13] Chtourou H, Driss T, Souissi S, et al. The effect of strength training at the same time of the day on the diurnal fluctuations of muscular anaerobic performances. *J Strength Cond Res* 2012;26:217-25.

[14] Chtourou H, Chaouachi A, Hammouda O, et al. Listening to Music Affects Diurnal Variation in Muscle Power Output. *Int J Sports Med* 2012;33:43-47.

[15] Chtourou H, Hammouda O, Chaouachi A et al. The effect of time-of-day and Ramadan fasting on anaerobic performances. *Int J Sports Med* 2012;33:142-47.

[16] Hammouda O, Chahed H, Chtourou H, et al. Morning to evening difference of biomarkers of muscle injury and antioxidant status in young trained soccer players. *Biol Rhythm Res* 2012;43:431-8.

[17] Chtourou H, Souissi N. The effect of training at a specific time-of-day: a review. *J Strength Cond Res* 2012;26:1984-2005.

[18] Hamouda O, Chtourou H, Farjallah MA, et al. The effect of Ramadan fasting on the diurnal variations in aerobic and anaerobic performances in Tunisian youth soccer players. *Biol Rhythm Res* 2011;43:177-90.

[19] Chtourou H, Hammouda O, Souissi H, et al. The effect of Ramadan fasting on physical performances, mood state and perceived exertion in young footballers. *Asian J Sport Med* 2011;2:177-85.

[20] Chtourou H, Hammouda O, Souissi H, et al. Diurnal variations in physical performances related to football in young soccer players. *Asian J Sport Med* 2012;3:139-44.

[21] Castagna C, Impellizzeri I, Cecchini E, et al. Effects of intermittent-endurance fitness on match performance in young male soccer players. *J Strength Cond Res* 2009;23:1954-9.

[22] Borg, GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 1982;14:377-81.

[23] Chtourou H, Jarraya M, Aloui A, et al. The effects of music during warm-up on anaerobic performances of young sprinters. *Sci Sports* 2012; 27:85-8.

[24] Aounallah-Skhiri H, Traissac P, El Ati J, et al. Nutrition transition among adolescents of a south-Mediterranean country: dietary patterns, association with socio-economic factors, overweight and blood pressure. A cross-sectional study in Tunisia. *Nutr J* 2011;10:38-55.

[25] Otten JJ, Hellwig JP, Meyers LD. Dietary reference intakes: The essential guide to nutrient requirements. Washington, DC: *National Academy Press* 2006.

[26] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499-502.

[27] Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 1974;37:247-8.

[28] Bangsbo J. The physiology of soccer—with special reference to intense intermittent exercise. *Acta Physiol Scand* 1994;151:1-156.

[29] Marliss EB, Kreisman SH, Manzon A, et al. Gender differences in glucoregulatory responses to intense exercise. *J Appl Physiol* 2000;88:457-66.

[30] Bangsbo J, Graham T, Kiens B, et al. Elevated muscle glycogen and anaerobic energy production during exhaustive exercise in man. *J Physiol (Lond)* 1992;451:205-27.

[31] Magalhaes J, Ferreira R, Marques F, et al. Indoor climbing elicits plasma oxidative stress. *Med Sci Sports Exerc* 2007;39:955-63.

[32] Magalhães J, Rebelo A, Oliveira E, et al. Impact of Loughborough Intermittent Shuttle Test versus soccer match on physiological, biochemical and neuromuscular parameters. *Eur J Appl Physiol* 2010;108:39-48.

[33] Ascensao A, Rebelo A, Oliveira E, et al. Biochemical impact of a soccer match—analysis of oxidative stress and muscle damage markers throughout recovery. *Clin Biochem* 2008;41:841-51.