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

Omar Hammouda\textsuperscript{1ABC}DG, PhD; Hamdi Chtoureou\textsuperscript{1,5ABC}EG, PhD; Anis Chaouachi\textsuperscript{1EG}, PhD; Henda Chahed\textsuperscript{2EG}, PhD; Nidhal Zarrouk\textsuperscript{1EG}, PhD; Abdelhedi Miled\textsuperscript{2EG}, MD; Karim Chamari\textsuperscript{1,4EG}, PhD; Nizar Souissi\textsuperscript{1,4AE}, PhD

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 increased significantly after the test ($P<0.001$ for the other markers). Moreover, lipid parameters increased significantly after the test ($P<0.01$ for Tri and $P<0.001$ for HDL).

Conclusions: 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

INTRODUCTION

Soccer is an intermittent sport characterized by sustained movement incorporating frequent bursts of high-intensity activity interspersed with regular recovery periods \cite{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 \cite{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 \cite{4}. This test is extensively utilized by scientists and coaches in monitoring cardio-respiratory fitness of football players since it correlates with match performance \cite{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 \cite{4,6}. However, very few studies have been reported on the metabolic response to the YYIRT \cite{4,7}. Moreover, only restricted a number of biochemical markers has been measured (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.
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 (CNSSS), 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 5-3 min of seated rest and 3-5 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
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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 nutrient intake are presented in table 1 and showed that total calorie, macronutrient, and micronutrient intakes are within 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)                   |
| Carbohydrate (g)       | 432.12 (144)           | (400-500)                     |
| Protein (g)            | 101.16 (21)            | (70-110)                      |
| Fat (g)                | 107.61 (63)            | (100-140)                     |
| Carbohydrate (%)       | 52.33 (6.6)            | (45-65)                       |
| Protein (%)            | 12.23 (1.09)           | (10-30%)                      |
| Fat (%)                | 29.16 (5.7)            | (25-35)                       |
| 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.

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)           | 0.28 | <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)           | 0.54 | <0.001   |
| Blood Glucose (mmol·L⁻¹)            | 4.62 (0.1)             | 5.69 (0.43)           | 1.05 | <0.001   |
| Lactate Level (mmol·L⁻¹)            | 1.05 (0.29)            | 9.83 (0.65)           | 8.78 | <0.001   |
| Lactate Dehydrogenase (IU·L⁻¹)      | 396.82 (25.87)         | 542.91 (40)           | 4.49 | <0.001   |
| Creatine Kinase (IU·L⁻¹)            | 173.75 (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

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⁻¹ 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
According to 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 Krstrup 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 mobilization 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 [5,26]. 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 Krstrup 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 player (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.

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Conflict of interests: None
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