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The Yo-Yo IE2 Test: Physiological Response for Untrained Men versus Trained Soccer Players

The Yo-Yo IE2 test: Physiological response for untrained men vs trained soccer players

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Running title: The Yo-Yo Intermittent Endurance level 2 test

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ABSTRACT

PURPOSE: To examine the physical capacity and physiological response to the Yo-Yo Intermittent Endurance level 2 test (IE2) for untrained individuals and trained male soccer players, and to investigate the determinants of intense intermittent exercise performance. METHODS: Thirty-four healthy untrained males (UTR) and fifteen age-matched trained soccer players (TR) performed a maximal incremental treadmill test (ITT) and a Yo-Yo IE2 test. Muscle biopsies and blood samples were obtained and HR were measured before, during and after tests. RESULTS: UTR had a 67% lower ($P<0.01$) Yo-Yo IE2 performance (665±271 vs. 2027±298 m; ES:4.8), 34% lower ($P<0.01$) VO_2max and 19% lower ($P<0.05$) resting muscle glycogen than TR. Blood lactate and heart rates during the first 560 m of the Yo-Yo IE2 test were higher ($P<0.01$) in UTR than TR (560 m: 7.4±2.8 vs. 2.4±0.8 $mmol\cdot L^{-1}$; ES:1.7-2.8; 188±11 vs. 173±8 bpm, ES:0.9-1.5) with no differences at exhaustion. Time >95%HRmax was lower ($P<0.01$) in UTR than TR (1.0±1.1 vs. 6.3±2.9 min, ES:3.1). Mean rate of muscle creatine phosphate utilization (16.5±9.5 vs. 4.3±2.7 $mmol\cdot kg^{-1}d.w\cdot min^{-1}$), muscle lactate accumulation (16.8±9.1 vs. 4.2±2.9 $mmol\cdot kg^{-1}d.w\cdot min^{-1}$), and glycogen breakdown (29.6±14.2 vs. 7.7±5.4 $mmol\cdot kg^{-1}d.w\cdot min^{-1}$) were 4-fold higher ($P<0.01$,ES:1.4-1.7) in UTR than TR. For UTR, correlations ($P<0.01$) were observed between Yo-Yo IE2 performance and VO_2max ($r=0.77$), ITT performance ($r=0.79$) and muscle citrate synthase activity ($r=0.57$), but not for TR ($r=-0.12$-0.50; $P>0.05$). CONCLUSION: The Yo-Yo IE2 test was shown to possess high construct validity by showing large differences in performance, heart rates and anaerobic metabolism between untrained individuals and trained soccer players. Additionally, VO_2max appeared to be important for intermittent exercise performance in untrained individuals, but not for trained soccer players. Key words: MUSCLE LACTATE; BLOOD LACTATE; HEART RATE; VO_2MAX; INTENSE INTERMITTENT EXERCISE PERFORMANCE; RECOVERY.

INTRODUCTION

Paragraph 1

Over the last decade a high number of studies have applied intermittent field testing modes such as the Yo-Yo Intermittent Recovery tests (Yo-Yo IR1 and IR2) and the Yo-Yo Intermittent Endurance tests (Yo-Yo IE1 and IE2) in order to examine the importance of training status (27), tactical role (21), seasonal period (26), heat acclimatization (35), nutritional supplementation (29,409) and different training regimes (10,13,15,19,28) for soccer players and other athletes in intermittent sports. In contrast to standard exercise laboratory tests consisting of continuous or incremental treadmill and cycling exercise with concomitant monitoring of the maximum oxygen uptake (VO_2max), submaximal heart rates and blood lactate responses, the Yo-Yo tests have shown to possess construct and ecological validity for elite team sport performance (1,4-6,38) and to be sensitive to detect training-induced changes in intermittent exercise performance (10, 15, 23, 37). Specifically, it has been observed that the Yo-Yo IE2 test performance is highly correlated to running performance in competitive elite soccer matches for men and women (4,5) and that the aerobic response to the test is altered according to the training-induced changes in soccer-specific fitness for elite male and female players during a competitive season (4,10).

Paragraph 2

Invasive studies have been conducted examining the physiological response to the Yo-Yo IR1 (20) and Yo-Yo IR2 tests (22,28). These studies demonstrated moderate to large magnitude correlations between the heart rate and blood lactate response during the first part of the tests and test performances and also that short-term high-intensity aerobic training as well as anaerobic training can lower the heart rate, muscle lactate and blood lactate concentration during the first part of the Yo-Yo IR tests and that there was a markedly higher anaerobic energy production determined as greater lactate production and creatine phosphate (CP) breakdown in the Yo-Yo IR2 test compared to the Yo-Yo IR1 test (1,20,22,28). Furthermore, these studies revealed a poor relationship between changes in VO_2max and changes in Yo-Yo IR performance after short-term training and showed moderate correlations between VO_2max and Yo-Yo IR performances for a large group of subjects with a large range in fitness level (1). However, invasive studies examining the muscle and blood metabolite response during the Yo-Yo IE2 test have not yet been conducted for trained soccer players, and likewise it is yet to be investigated whether the importance of VO_2max and other potential determinants of the Yo-Yo IE2 performance differ between untrained individuals and trained soccer players.

Paragraph 3

The Yo-Yo Intermittent Endurance test level 2 (Yo-Yo IE2) has a speed progression comparable to the Yo-Yo IR1 test (1), but the recovery periods between the shuttle runs are only 5 s, which is half the duration in comparison to the recovery periods in the Yo-Yo IR1 test (10 s). It is well-known that trained individuals have higher VO_2max values as well as lower heart rates and blood lactate concentrations during standardized submaximal continuous exercise, and that these variables are largely correlated to endurance exercise performance for untrained as well as trained endurance athletes (17). However, it is has not been examined to what extent the physiological response to a team sports specific intermittent exercise protocol such as the Yo-Yo IE2 test differs between untrained subjects and well-trained soccer players and whether there are differences in the determinants of intermittent exercise performance of the two groups. The latter can be investigated by correlating the VO_2max, the time to exhaustion in an incremental treadmill test, the muscle oxidative capacity and the anaerobic energy production to the Yo-Yo IE2 performances of the two distinct groups. Likewise it would be of interest to evaluate whether the physiological response to the first part of the test is correlated to the Yo-Yo IE2 running distance at exhaustion for trained soccer players that are familiar with the exercise mode in the test as well as for untrained individuals. This can be investigated by correlating the heart rate and the blood lactate values at fixed times in the first part of the Yo-Yo IE2 test to the Yo-Yo IE2 test performance.

Paragraph 4

Thus, the aim of the present study was to examine heart rate , blood and muscle metabolite responses during the Yo-Yo IE2 test in untrained men and trained soccer players and to study the determinants of intermittent exercise performance for these subject groups.

METHODS

Paragraph 5

Subjects

Thirty-four healthy untrained males (UTR) and fifteen trained soccer players (TR) participated in the study (age: 30±6 vs. 25±7 yr, body mass: 84.8±13.1 vs. 75.7±9.4 kg, and $\dot{V}O_{2max}$: 40.0±6.2 vs. 60.9±5.5 mL·kg·min^{-1}). None of the untrained subjects had been involved in regular physical activity for a least 2 yr. The TR participated in 3-8 h of training per week and 1-2 matches per week and had been regularly involved in soccer for at least 8 yr. All subjects were fully informed of the risks associated with the experimental procedures, and all provided written consent. The study conforms to the code of ethics of the World Medical Association and was approved by the appropriate institutional ethical committees.

Paragraph 6

Experimental design

Within a 14 day period, the subjects carried out the Yo-Yo intermittent endurance – level 2 (Yo-Yo IE2) test (see below) as well as a laboratory treadmill test protocol with submaximal running and incremental maximal test. Heart rate was measured in 5-s intervals and blood samples were collected frequently during the two protocols. In addition, muscle biopsies were collected from m. vastus lateralis before and after the Yo-Yo IE2 test. All subjects had been familiarized to the Yo-Yo IE2 test prior to the testing period, by carrying out the full test including warm-up twice 2 to 6 wks prior to the main experimental day.

Paragraph 7

The Yo-Yo intermittent endurance test – level 2 (Yo-Yo IE2)

The Yo-Yo IE2 test was performed in accordance with previous studies (4,5). The participants performed repeated 20-m shuttle runs, back and forth between the starting line and finishing line marked by cones, at progressively increasing speeds dictated by an audio bleep emitted from a CD player. Between each shuttle the participants had a 5-s period of slow jogging around a cone placed 2.5 m from the starting line. Failure to achieve the shuttle run on two successive occasions resulted in termination of the test and the distance covered represented the test result. All testing sessions were performed indoors on 2 × 20 m running lanes marked by cones. After 15 min of rest prior to the beginning of the test protocol, the subjects had a heart rate monitor (Polar Electro Oy, Kempele, Finland) placed around the chest for continuous recordings throughout the warm-up, the test and during the first 15 min of recovery. A cannula was placed in a forearm vein and covered by a supporting bandage. Saline was used to flush the cannula at rest, after the warm-up and in the recovery period after the test, but not during the test due to the frequent blood sampling. In preparation for obtainment of needle biopsies in the m. vastus lateralis, an incision was made through the skin and muscle fascia under local anesthesia (20 $m \cdot g \cdot L^{-1}$ lidocain). The incision was covered by a sterile strip and thigh bandage. Resting blood samples were obtained before the warm-up which consisted of 2 × 2 min of the Yo-Yo IE1 test. Subsequently, the subjects rested for 4 min before they performed the Yo-Yo IE2 test. Blood samples were obtained immediately before, during (240, 400, 560, 720, 880, 1040 m etc.) at exhaustion and during recovery from the test (1, 3, 5, 10 and 15 min). During the test, blood samples were taken in the 5 s recovery periods between the shuttle-runs. When these blood samples were collected, the participants were asked to stand still at the finishing line, rather than jogging around the cone placed behind the finishing line. Muscle biopsies were obtained from the m. vastus lateralis before the warm-up, at exhaustion and after 3 min of recovery using the Bergstrom technique (3). Suction was applied to maximize the tissue sample size (3)

Paragraph 8

The laboratory treadmill test

For UTR the laboratory treadmill test consisted of 6 min bouts of 6.5, 8.0, 9.5 and 11.0 $km \cdot h^{-1}$ separated by 2 min intervals of rest as previously described (19, 21). The protocol for TR comprised of 6 min bouts of 8.0, 9.5, 11.0 and 13.5 $km \cdot h^{-1}$ separated by 2 min intervals of rest, the variation in starting speed was based on the training status of the subjects. After 15 min of recovery an incremental maximal test was performed. The test started at running speed of 9.5 and 11.0 $km \cdot h^{-1}$ for 2 min for untrained and trained subjects, respectively. This then continued at 11.0 and 13.5 $km \cdot h^{-1}$for 60 s for the untrained and trained subjects respectively, followed by stepwise 1.0 $km \cdot h^{-1}$ speed increments every 60 s until exhaustion. Time to exhaustion was recorded. Heart rate was recorded every 5 s during the test using Team 2 heart rate monitoring belts (Polar Electro Oy, Kempele, Finland). Pulmonary oxygen uptake was measured during each sub-maximal running speed and during the maximal test using a breath-by-breath gas analyser (MedGraphics CPX/D, Saint Paul, MN, US). This system was calibrated before trials with gases of known concentrations and the tube flow meter was calibrated using a 3 L syringe. This was done in accordance with the calibration procedure recommended by the manufacturer. Individual values for $\dot{V}O_{2max}$ and HR_{max} were determined as the peak values reached in 15 and 5 s periods, respectively.

Paragraph 9

Blood analysis

Approximately 10 s after the 2 mL venous blood sample was obtained, 200 µL of blood was hemolyzed in an ice cold 200 µL Triton X-100 buffer solution and was later analysed for lactate and glucose concentration using an automated analyser (YSI Model 2300 STAT PLUS, Yellow Spring Instruments, Ohio, USA), with a CV value of 5% for duplicate measurements (9). The remainder of the blood collected was immediately centrifuged and the plasma removed and stored at -20 ºC. Plasma potassium concentration was measured using flame photometry (Radiometer FLM3, Copenhagen, Denmark) with lithium as internal standard. Plasma ammonia (NH_3) was determined spectophotometrically (11). Plasma free fatty acid (FFA) concentration was measured using an enzymatic kit (WAKO Chemical, GmbH, Neuss, Germany).

Paragraph 10

Muscle metabolite and enzyme analysis

The muscle tissue (~60 mg wet weight) was immediately frozen in liquid nitrogen and stored at -80ºC. The frozen sample was weighed both before and after freeze-drying to determine water content. After freeze-drying, the muscle sample was dissected free of blood, fat and connective tissue, and about ~1 mg·d.w. of muscle tissue was extracted in solution of 0.6 M of perchloric acid (PCA) and 1 mM of EDTA, neutralized to a pH of 7.0 with 2.2 M of $KHCO_3$, stored at -80 ºC until it was analysed for CP and lactate by a fluorometric assay (24), with CV values for duplicate measurements of 5 and 6%, respectively (28). Another 1-2 mg·d.w. of muscle tissue was extracted in 1 M of HC1 and hydrolyzed at 100ºC for 3 h, and the glycogen content was determined by the hexokinase method (24). Muscle pH was measured by a small glass electrode (Radiometer GK2801, Copenhagen, Denmark) after homogenizing a freeze-dried muscle sample of about 2 mg·d.w. in a non-buffered solution containing 145 mM of KCI, 10 mM of NaC1, and 5 mM of iodoacetic acid. For muscle enzyme analysis, ~3 mg·d.w. of muscle tissue was homogenized (1:400) in a 0.3-M phosphate buffer adjusted to a pH of 7.7 and containing 0.5 mg·mL^{-1} of bovine serum albumin. Citrate synthase (CS) was determined by the flourometric method with NAD-NADH coupled reactions (24).

Paragraph 11

Statistical analysis

All statistical analyses were conducted using the appropriate software (SPSS Inc., Chicago, USA). Differences in physiological responses between trained and untrained to the Yo-Yo IE2 test and the laboratory treadmill test were evaluated using a two-way analysis of variance (ANOVA). In the event of a difference occurring, univariant Tukey's post-hoc tests were used to identify any localized effects. The effect size (ES) was calculated to determine the meaningfulness of the difference (7). The magnitude of the ES was classified as trivial (<0.2), small (>0.2-0.6), moderate (>0.6-1.2), large (>1.2-2.0) and very large (>2.0-4.0) based on guidelines (2). Relationships between selected performance variables were evaluated using Pearson's product moment test. The magnitudes of the correlations were considered as trivial (<0.1), small (>0.1-0.3), moderate (>0.3-0.5), large (>0.5-0.7), very large (>0.7-0.9), nearly perfect (>0.9) and perfect (1.0) in accordance with Hopkins et al. (12). Statistical significance was set at $P<0.05$. Values are presented as means±SD unless otherwise stated.

RESULTS

Paragraph 12

Performance and VO_2max

Performance in the Yo-Yo IE2 test was 67% lower ($P<0.01$) for UTR compared to TR (665±271 vs. 2027±298 m; ES: 4.8) with test duration being 4.3±1.7 and 12.6±1.8 min, respectively (ES: 4.8). Incremental treadmill test performance was also lower ($P<0.01$) for UTR than TR (6.8±0.9 vs. 9.8±0.7 min; ES: 4.7) with peak running velocities being 30% lower for UTR than TR (14.3±2.9 vs. 20.3±2.0 $km\cdot h^{-1}$; ES: 3.8). UTR had a 34% lower ($P<0.01$) VO_2max than TR (40.0±6.2 vs. 60.9±5.5 $mL\cdot kg^{-1}\cdot min^{-1}$; ES: 3.6).

Paragraph 13

Physiological response to the submaximal treadmill test

During treadmill running at 8.0 and 9.5 $km\cdot h^{1}$ relative heart rate (78±8 vs. 66±3 and 87±6 vs. 73±4%HR_{max}; ES: 2.5-2.8), oxygen uptake (70±9 vs. 51±6 and 81±9 vs. 59±4%$\dot{V}O_{2max}$; ES: 2.5-3.3) and RER (0.97±0.08 vs. 0.87±0.07 and 0.98±0.09 vs. 0.89±0.07; ES: 1.2-1.4) were higher ($P<0.01$) for UTR than TR;; (Table 1). Blood lactate concentrations attained during treadmill running at 8.0 and 9.5 $km\cdot h^{1}$ were higher ($P<0.01$) in UTR than in TR (3.1±1.7 vs. 0.9±0.2 and 4.4±2.6 vs. 1.1±0.3 $mmol\cdot L^{-1}$; ES: 2.2-2.3), with values at exhaustion being similar (9.1±3.0 vs. 8.2±2.3 $mmol\cdot L^{-1}$; ES: 0.4).

Paragraph 14

Physiological Response to the Yo-Yo IE2 Test

Heart rate

Heart rate was higher ($P<0.05$) for UTR than TR immediately before and during the first 560 m of the test (120 m: 157±11 vs. 144±11 bpm or 80±5 vs. 74±6% of HR_{max}, ES: 1.5; Fig 1A). At exhaustion, heart rate was 189±10 and 194±7 bpm for UTR and TR, respectively, or 97±3 and 99±3% of the HR_{max} (ES: 1.0). No group differences were observed in the recovery period (Fig. 1A).

Paragraph 15

Muscle metabolites and pH

Muscle metabolite concentrations and pH before, at exhaustion and after 3 min of recovery from the Yo-Yo IE2 test are presented in Table 2. Muscle CP was similar at rest for UTR and TR (83.3±20.9 vs. 88.0±13.3 mmol·kg^{-1} d.w.; ES: 0.3) and decreased ($P<0.01$) during the Yo-Yo IE2 test to 24.9±22.9 and 38.3±27.6 mmol·kg^{-1}·d.w, respectively, at exhaustion, corresponding to ~30 and 44% of the resting level, respectively (ES: 0.6). The mean rate of muscle CP utilization during the Yo-Yo IE2 test was 4-fold higher ($P<0.01$) for UTR compared to TR (16.5±9.5 vs. 4.3±2.7 mmol·kg^{-1}d.w·min^{-1}; ES: 1.5). Muscle CP increased ($P<0.01$) by 35.5±17.1 and 29.3±27.7 mmol·kg^{-1}·d.w. to ~73 and 77% of resting level during the first 3 min of recovery for UTR and TR, respectively. Muscle lactate for UTR and TR was 8.1±3.0 and 3.7±2.3 mmol·kg^{-1}·d.w., respectively, before the Yo-Yo IE2 test (ES: 1.6) and increased ($P<0.01$) to 68.7±25.4 and 53.3±33.6 mmol·kg^{-1}·d.w. (ES: 0.5) at exhaustion in the Yo-Yo IE2 test for UTR and TR, respectively. The mean rate of muscle lactate accumulation in the Yo-Yo IE2 test was 4-fold higher ($P<0.01$) for UTR compared to TR (16.8±9.1 vs. 4.2±2.9 mmol·kg^{-1}·d.w·min^{-1}; ES: 1.6). Muscle lactate decreased ($P<0.01$) during the first 3 min of recovery in UTR but not in TR. Muscle glycogen content at rest was 19% lower ($P<0.05$) in UTR than TR (434±80 vs. 537±84 mmol·kg^{-1} d.w). It decreased ($P<0.01$) during the Yo-Yo IE2 test by 104±51 and 100±55 mmol·kg^{-1} d.w. in UTR and TR, respectively. The mean rate of glycogen utilization was 4-fold higher in UTR than

TR (29.6±14.2 vs. 7.7±5.4 mmol·kg^{-1} d.w. min^{-1}). Muscle pH at rest was 7.26±0.11 and 7.23±0.07 in UTR and TR, respectively, and decreased ($P<0.05$) to 6.86±0.18 and 6.88±0.17, respectively, at exhaustion (ES: 0.1-0.3). During the first 3 min of recovery, muscle pH increased ($P<0.05$) to 7.01±0.17 and 7.05±0.10 in UTR and TR (ES: 0.3), respectively.

Paragraph 16

Blood metabolites

Blood lactate was higher ($P<0.01$) in UTR than TR after the warm up and during the first 560 m of the Yo-Yo IE2 test (560 m: 7.4±2.8 vs. 2.4±0.8 mmol·L^{-1}; ES: 1.7-2.8) with no significant difference at exhaustion (9.5±2.7 and 10.0±2.3 mmol·L^{-1}; ES: 0.2; Fig 1B). In UTR, blood lactate concentration peaked at 10.9±2.6 mmol·L^{-1} 5 min into the recovery period and decreased ($P<0.01$) to 9.2±2.8 mmol·L^{-1} after 15 min of recovery (ES: 0.7). In TR, blood lactate concentration peaked at exhaustion and decreased ($P<0.01$) substantially after 15 min of recovery reaching 6.0±1.8 mmol·L^{-1} (ES: 1.9).

Paragraph 17

Plasma potassium and ammonia

Plasma K^+ was 3.8±0.3 and 4.1±0.2 mmol·L^{-1} for UTR and TR immediately before the test and increased ($P<0.05$) to 5.1±0.4 and 5.3±0.8 mmol·L^{-1} after 400 m ($P>0.05$, ES: 0.3; Fig 1C). Peak plasma K^+ was reached at exhaustion with lower concentrations ($P<0.05$) for UTR than TR (5.3±0.7 vs. 6.0±0.7 mmol·L^{-1}; ES: 1.0). Plasma K^+ decreased ($P<0.01$) to 4.2±0.5 and 4.2±0.3 mmol·L^{-1} after 1 min and 3.5±0.4 and 3.4±0.2 mmol·L^{-1} after 5 min of recovery for UTR and TR, respectively (Fig. 1C).

Paragraph 18

Plasma FFA concentration was 288±145 and 273±108 μEq·L^{-1} at rest and decreased ($P<0.01$) in UTR but not TR during the test (560 m: 135±61 vs. 233±181 μEq·L^{-1}; ES: 0.4-0.8), and it increased to 308±182 vs. 395±250 μEq·L^{-1} (ES: 1.4-1.7), respectively, after 5 min of recovery, with no significant differences between UTR and TR (Fig. 2A).

Paragraph 19

Plasma NH_3 concentration was markedly higher ($P<0.01$) for UTR than TR at all time points (ES: 2.0-3.8; Fig 2B). For UTR, plasma NH_3 concentration peaked at 269±94 mmol·L^{-1}, which was higher ($P<0.01$) than for TR (105±17 µmol·L^{-1}; ES: 7.4).

Paragraph 20

Blood glucose concentration was similar in UTR and TR at rest and during the first 400 m of the Yo-Yo IE2 test (400 m: 4.3±0.8 vs. 3.9±0.4 mmol·L^{-1}; ES: 0.2-0.7; Fig 2C), but was higher ($P<0.05$) in UTR compared to TR at 560 m of the test, at exhaustion and during the first 10 min of recovery (10 min: 5.9±1.3 vs. 4.9±1.3 mmol·L^{-1}; ES: 0.8-1.3).

Paragraph 21

Correlations

For UTR correlations were observed between Yo-Yo IE2 test performance and time to fatigue on the incremental running test ($r=0.79$; $P<0.01$) and $\dot{V}O_{2max}$ ($r=0.77$, $P<0.01$), but not for TR ($r=0.50$, $P>0.05$) and ($r=0.30$, $P>0.05$; Fig. 3A-D). A correlation was observed between Yo-Yo IE2 test performance and muscle CS ($r=0.57$, $P<0.01$) for UTR, but not for TR ($r=-0.12$, $P>0.05$).

Paragraph 22

Large to very large correlations were observed between performance of the Yo-Yo IE2 test and %HRmax reached at 2 min for UTR (r=-0.53, P<0.01), and for %HRmax reached at 2, 3, 4, 5 and 6 min for TR (r=-0.73, r=-0.62, r=-0.60, r=-0.62 and r=-0.64, P<0.05). No relationships were observed between Yo-Yo IE2 test performance and $\%HR_{max}$ after 1, 3, 5 min of the recovery in both populations. The blood lactate concentrations on completion of the warm up and after 240, 400 and 560 m were inversely correlated to Yo-Yo IE2 test performance in UTR (r =-0.52, -0.43, -0.42 and -0.70; P<0.05) but this was only evident for TR after 400 m (r =-0.62; P<0.05). No correlations were found between Yo-Yo IE2 test performance and blood lactate concentration after 1, 3 and 5 min of recovery in neither UTR nor TR (r =-0.06 vs. 0.02, -0.04 vs. 0.21 and -0.19 vs. -0.16; P>0.05). No correlation was observed for UTR and TR between peak blood lactate concentration and Yo-Yo IE2 test performance. Plasma K^+, FFA and NH_3 concentration during the test were not correlated to Yo-Yo IE2 test performance in UTR or TR.

DISCUSSION

Paragraph 23

The present study demonstrates that the Yo-Yo IE2 test can clearly separate performance and metabolite response between untrained participants and trained soccer players. Additionally, it was revealed that intermittent exercise performance of the untrained individuals correlated to maximal oxygen uptake and muscle CS activity, whereas no such correlations were for the trained soccer players.

Paragraph 24

Positive moderate to large magnitude correlations were observed between the Yo-Yo IE2 test performance and VO_2max as well as muscle CS activity for the untrained group, suggesting that the test can evaluate the aerobic capacity of untrained individuals. The test duration for the untrained subjects was around 4 min, which is similar to the duration observed when trained individuals performed the Yo-Yo intermittent recovery level 2 test (Yo-Yo IR2) (15,22). The mean rate of CP utilization was slightly higher for the untrained subjects in the present study compared to the trained individuals during the Yo-Yo IR2 test (16.5 vs. 12.8 $mmol \cdot kg^{-1}$ $d.w \cdot min^{-1}$), whereas the rate of muscle lactate accumulation (16.8 vs. 16.2 $mmol \cdot kg^{-1}$ $d.w \cdot min^{-1}$) was of similar magnitude (22). Also the heart rate response reaching maximum heart rate at the end was the same. Thus, the metabolic response to the Yo-Yo IE2 test for untrained subject correspond to that observed in the Yo-Yo IR2 test for trained subjects, and although not originally designed for untrained subjects, the test can therefore also be used to examine the anaerobic capacity of untrained men.

Paragraph 25

For the trained soccer players the duration of the Yo-Yo IE2 test was around 13 min corresponding to a performance of 2027 m, which is similar to performance of central defenders in the English Premier League but 10-30% lower than for Premier League players of other outfield playing positions (4) and for Danish Premier League players (10). For the trained subjects both the mean rate of CP utilization and muscle lactate accumulation was about 4-fold lower than observed for a similar group of soccer players performing the Yo-Yo IR2 test (22). Furthermore, the rate of rise in heart rate was observed to be significantly slower in the Yo-Yo IE2 test, as 90% of individual maximal heart rate was reached after about 7 min on average in The Yo-Yo IE2 test compared to 3-4 min in Yo-Yo IR2 test (14, 15, 22). Thus, although reaching the same muscle CP and lactate as well as heart rate at exhaustion during the tests, the metabolic response to the two tests are clearly different. It is also confirmed by the lower blood lactate levels obtained after the Yo-Yo IE2 test compared to the Yo-Yo IR2 test (Exh: 9.8 vs. 13.6 mM; 15 min Rec: 6.0 vs 10.4 mM) (14, 22) and the rather low plasma ammonia levels observed at the end of the Yo-Yo IE2 test. Thus, the anaerobic loading appear to be markedly lower during the Yo-Yo IE2 than the IR2 test for trained individuals suggesting that the test can be labeled as a predominately aerobic intermittent test for team sport athletes.

Paragraph 26

The present study revealed that the physiological response to the Yo-Yo IE2 test is different for untrained than for trained soccer players. The heart rates were 15-20 bpm higher for the untrained subjects at any given time from 1.5 min to the time of exhaustion and the time with heart rates above 90% of maximal heart rate was only 15% of the time achieved by the trained soccer players. As previously observed in studies using the Yo-Yo IR1 and Yo-Yo IR2 test (1,20,22), we also found the that heart rate values after 2 min for the untrained and after 2-6 min for the trained was correlated to end performance in the test. Also blood lactate responses were markedly different between the untrained and the trained individuals with 3-4 fold higher values for the untrained at any given time during their test. However, in contrast to previous findings in Yo-Yo IR1 and Yo-Yo IR2 studies (1,5,20,22), no significant correlations were observed between sub-maximal blood lactates and the performance in the Yo-Yo IE2 test. The end-exercise heart rate and blood lactate as well as the exercise-induced changes in muscle CP, lactate and glycogen were not different between the untrained and trained. However when expressed in relation to the exercise time (4 vs. 13 min) muscle and blood lactate accumulation and the muscle CP and glycogen breakdown were 4-fold greater in the untrained compared to the trained participants. In the present study it was observed that the peak heart rate reached during the Yo-Yo IE2 test were 189±10 and 194±7 bpm for untrained and trained, respectively, corresponding to 97±3 and 99±3% of the HR_{max} attained during the incremental treadmill test to exhaustion. The peak heart rate values obtained for the trained subjects in the present study were similar to values obtained for trained subject during the Yo-Yo IR1 and Yo-Yo IR2 tests confirming that that the Yo-Yo IE2 test can be used as an easy and time-efficient method to determine maximal heart rates of all the players in a team. However, when the exercise time is short, as observed for the untrained subjects in the present study, it appears that not all individuals reach their maximal heart rate using the Yo-Yo tests as also observed in a previous Yo-Yo IR2 study (1).

Paragraph 27

The finding of correlations between VO_2, muscle CS activity and performance in the Yo-Yo IE2 test for the untrained, but not the trained group suggest that the main determinants of intense intermittent exercise performance differ between the trained soccer players and the untrained individuals. Thus, the aerobic energy production appears to play a more important role for fatigue resistance in the untrained group during intense intermittent exercise compared to the trained group. While blood and lactate concentrations, as well as CP levels and muscle pH at exhaustion were not different between the untrained and the trained, it was observed that the plasma K^+ concentration at exhaustion was higher in the trained soccer players compared to the untrained. Fatigue during intense exercise has been linked to depolarization of the muscle membrane resting potential due to disturbances in the muscle ion homeostasis (8,25), where extracellular K^+ accumulation has been proposed as a key factor (18,29,31). In other studies using intense intermittent exercise protocols venous plasma K^+ concentrations between 6-7 mM were reached at the point of exhaustion (20,22,28,30), which is similar to the level observed for the trained group in the present study. However, the untrained participants in the present study had a 12% lower plasma potassium concentration at exhaustion. Thus, the higher venous plasma K^+ levels at exhaustion in the trained group in the present study may indicate that trained individuals are capable of exhausting the muscle ion homeostasis to a greater degree than the untrained subjects, who due to a low aerobic capacity may fatigue before fully exhausting the muscle ion balance.

Paragraph 28

Venous plasma ammonia was markedly higher in the untrained individual during the entire test. Ammonia has been suggested as a cause of fatigue during intense exercise through centrally mediated mechanisms (32,39). At exhaustion the untrained reached concentrations above 200 $\mu mol \cdot L^{-1}$ which is of similar magnitude to findings in other comparable intermittent protocols (20,22,29,30). In contrast the trained group display very low concentrations even at exhaustion (~100 $\mu mol \cdot L^{-1}$). Ammonia is produced in the muscles during breakdown of AMP or during oxidation of branch-chained amino acids, but neither adenine nucleotide turnover nor protein metabolism were assessed in the present study. However, an increased ammonia production has been linked to low muscle glycogen levels (33). In the present study the trained group had markedly higher muscle glycogen values before the test and at exhaustion and it has been shown after the Yo-Yo IR1 test that several muscle fibers displayed low glycogen values (20). In the present study the untrained individuals also showed a markedly greater glycogen oxidation and higher blood lactate levels at submaximal running speeds during the treadmill running in comparison to the trained group and a much greater muscle glycogen utilization rate during the Yo-Yo IE2 test. Therefore, it cannot be ruled out that a fraction of the muscle fibers were low on glycogen during the test in the untrained individuals, which may partly have contributed to the higher degree of hyperammonemia in the untrained group. In addition to a lower ammonia production in the exercising muscles, a more efficient systemic clearance in the trained group may also have played a role. Ammonia is mainly cleared via the uric cycle and a lower cardiovascular loading in the first part of the test for the trained group may well have resulted in a higher renal blood flow during the test compared to the untrained group. Also this mechanism may have contributed to a better ammonia clearance. We did not determine the catecholamine response to the test in the two groups in order to evaluate indications of between-group differences in vasoconstriction in internal organs during the tests. Altogether, elevated ammonia production may also have contributed to the early fatigue development in the untrained group potentially via central mechanisms (30).

Paragraph 29

In summary, the Yo-Yo IE2 test can clearly separate performance and metabolic response between untrained individual and age-matched trained soccer players, with 4-fold higher rate of muscle CP and glycogen breakdown and rate of muscle lactate accumulation in the untrained. The present study reveals that the Yo-Yo IE2 test stimulates the aerobic and anaerobic energy systems differently for untrained individuals and trained soccer players. In addition, maximal oxygen uptake appeared to be important for intermittent exercise performance in the untrained individuals, but not for the trained soccer players. Furthermore, the present study show that heart rate and blood lactate concentration during a submaximal version of the Yo-Yo IE2, i.e. a 400 m version lasting about 4 min, can be used as an indicator of intermittent exercise performance for untrained individuals as well as trained soccer players.

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Legends to tables and figures

Table 1: Cardio-pulmonary variables and blood lactate after submaximal and maximal treadmill running untrained individuals and trained soccer players. Values are means±SD. Different from untrained: ΔΔP<0.01.

Table 2: Muscle variables at rest, at exhaustion and 3 min into recovery of the Yo-Yo Intermittent recovery test for untrained individuals and trained soccer players. Values are means±SD. Different from untrained: $^{\Delta\Delta}$P<0.01. Different from rest: *P<0.05; **P<0.01. Different from exhaustion: †P<0.05; ††P<0.01.

Figure 1:

Heart rate (A), blood lactate (B) and plasma potassium (C) before, during and in recovery from the Yo-Yo Intermittent recovery test for untrained individuals (closed circles) and trained soccer players (open circles). Values are means±SD. Different from untrained individuals: *P<0.05; **P<0.01.

Figure 2:

Plasma Free Fatty Acids (A), ammonia (B) and blood glucose (C) before, during and in recovery from the Yo-Yo Intermittent recovery test for untrained individuals (closed circles) and trained soccer players (open circles). Values are means±SD. Different from untrained individuals: *P<0.05; **P<0.01.

Figure 3:

Correlations between Yo-Yo IE2 test performance and VO_2max (A, B) and incremental treadmill test performance (C, D) for untrained individuals and trained soccer players. Solid lines denote linear trends and dashed lines denote the 95% confidence intervals.