©Journal of Sports Science and Medicine (2014) **13**, 51-58
http://www.jssm.org

Research article

Comparing Fat Oxidation in an Exercise Test with Moderate-Intensity Interval Training

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Abstract
This study compared fat oxidation rate from a graded exercise test (GXT) with a moderate-intensity interval training session (MIIT) in obese men. Twelve sedentary obese males (age 29 ± 4.1 years; BMI 29.1 ± 2.4 kg·m^{-2}; fat mass 31.7 ± 4.4 %body mass) completed two exercise sessions: GXT to determine maximal fat oxidation (MFO) and maximal aerobic power (VO_{2max}), and an interval cycling session during which respiratory gases were measured. The 30-min MIIT involved 5-min repetitions of workloads 20% below and 20% above the MFO intensity. VO_{2max} was 31.8 ± 5.5 ml·kg^{-1}·min^{-1} and all participants achieved ≥ 3 of the designated VO_{2max} test criteria. The MFO identified during the GXT was not significantly different compared with the average fat oxidation rate in the MIIT session. During the MIIT session, fat oxidation rate increased with time; the highest rate (0.18 ± 0.11 g·min^{-1}) in minute 25 was significantly higher than the rate at minute 5 and 15 ($p \le 0.01$ and 0.05 respectively). In this cohort with low aerobic fitness, fat oxidation during the MIIT session was comparable with the MFO determined during a GXT. Future research may consider if the varying workload in moderate-intensity interval training helps adherence to exercise without compromising fat oxidation.

Key words: Interval exercise, Fat_{max}, maximal fat oxidation, obesity .

Introduction

With the growing prevalence of obesity and metabolic disorders such as T2D (Gastaldelli, 2008), there is increasing interest in determining factors that maximise fat oxidation during exercise training to induce weight loss in obese adults (Corpeleijn, 2009). Aerobic exercise is commonly advocated to improve whole body fat oxidation (Zarins et al., 2009), with the highest rates of fat oxidation repeatedly seen with moderate-intensity aerobic exercise (Melanson et al., 2002; Romijn et al., 2000; Saris et al., 2003). Moderate-intensity exercise training is commonly undertaken as a continuous bout at a constant mechanical workload. However, for some individuals constant load work can be difficult to perform for the duration required to attain an adequate training dose. Therefore, participation in multiple short bouts of 10-15 mins was advocated in sedentary individuals who perceive barriers to continuous exercise (Jakicic et al., 2001; Jakicic et al., 1995; Murphy et al., 2009). Recently, there has been growing interest in using shorter interval stages in the diabetic and obese populations (Boutcher, 2011; Earnest, 2008; Hansen et al., 2010), which involves repeated bouts between 30 secs and 5 mins and interspersed by similar durations of rest or low-intensity bouts (Laursen and Jenkins, 2002). Obese women perceived moderate-intensity interval exercise (i.e. alternated 80 and 120%VT every 2 mins for 32 mins) as being less hard than continuous exercise (i.e. 100%VT for 32 mins) (Coquart et al., 2008). Therefore, obese individuals could use moderate-intensity interval exercise instead of moderate-intensity continuous exercise to improve compliance to training.

The intensity (FAT_{max}) that elicits maximal fat oxidation (MFO) during a graded exercise test (GXT) has been suggested as a reference method to prescribe exercise training where optimising fat oxidation is the goal (Achten et al., 2002). The main advantage of the FAT_{max} test is that the MFO is determined with the use of a single GXT protocol, rather than undertaking several constant load tests performed with different workloads on different days (Meyer et al., 2007). Given the interest in moderate-intensity interval training as a strategy for improving exercise compliance (Coquart et al., 2008), it would be valuable to know if the FAT_{max} test is a valid means of prescribing moderate-intensity interval training. Moderate-intensity interval training may not induce exercise duration-related drift in fat oxidation, seen in continuous exercise (Cheneviere et al., 2009; Meyer et al., 2007). It may be more closely related to the fat oxidation values during 3-5 min stages of GXT protocols.

There is some evidence that the impact of moderate-intensity continuous and interval training on fat oxidation are different. Venables and Jeukendrup (2008) investigated the effect of four weeks of moderate-intensity interval training which consisted of 5-min at 20% above FAT_{max}, alternated with 5-min at 20% below FAT_{max} on fat oxidation in obese men, compared with moderate-intensity continuous training at the level of FAT_{max}. In this study interval training did not increase fat oxidation during a 30-min constant-load test compared with the baseline although the same participants were able to increase fat oxidation by 44% after continuous training.

The mechanical workload for continuous aerobic training is commonly prescribed by the relationships between physiological variables such as oxygen consumption (VO_2), heart rate (HR) and the concentration of blood lactate (BLa) and mechanical work (Hofmann and Tschakert, 2011) and between the rating of perceived exertion (RPE) and mechanical work (Chen et al., 2002) derived during a GXT. There is support for the use of GXT for prescribing workloads for exercise training as the physiological responses (VO_2, HR, BLa) to constant-load moderate-intensity exercise relate well to the re-

Received: 05 July 2013 / Accepted: 05 September 2013 / First Available (online): 26 December 2013 / Published (online): 20 January 2014

sponses in the GXT (Salvadego et al., 2010; Steed et al., 1994). It is important to confirm whether the relationship between mechanical work and physiological variables (VO_2, HR and BLa) and between mechanical work and RPE during an exercise session of moderate-intensity interval training relate to the physiological-mechanical work and psychological-mechanical work relationships at MFO in the GXT.

The protocol of GXT to determine MFO was modified in terms of initial and incremental workloads among untrained obese individuals to meet their fitness levels (Bircher et al., 2005; Haufe et al., 2010; Perez-Martin et al., 2001). For example, Bircher and Knechtle (2004) started the test with 100 W in the athletes and 40 W in the obese. Roffey (2008) adapted Achten's protocol among obese men to start with 50 W followed by increments of 30 W. Haufe et al. (2010) designed the protocol to start the workload at 25 W, with increments of 25 W every 2 mins. Perez-Martin et al. (2001) suggested a protocol of four 6-min steady-state workloads at 30, 40, 50 and 60%W_{max}-predicted with a warm-up stage at 20%W_{max}. Bircher et al. (2005) compared two protocols; one was defined as 35 W increments for 3 mins and a total of 20 mins and the other increased according to HR and was 26 W increments for 5 mins and a total of 45 mins, and found significant differences in MFO in men.

The current study aimed to compare fat oxidation, physiological variables (VO_2, HR and BLa) and RPE corresponding with FAT_{max} derived from a GXT and during a 30-min moderate-intensity interval exercise training (MIIT) session consisting of 5-min stages at 20% above then 20% below FAT_{max}.

Methods

Participant

Participants included 12 sedentary overweight/obese men. The characteristics of participants were: age (29 ± 4.1 years), BMI (29.1 ± 2.4 $kg \cdot m^{-2}$), fat mass (31.7 ± 4.4 %body mass) and VO_{2peak} (31.8 ± 5.5 $ml \cdot kg^{-1} \cdot min^{-1}$). Participants were recruited from the staff and student population at the Queensland University of Technology (QUT) and the Brisbane metropolitan region via e-mail and flyers posted on community noticeboards. Consent was obtained and prior to undertaking the study, the participant was required to gain medical clearance to perform a maximal exercise test. The study protocol was approved by the Human Research Ethics Committee at QUT (HREC No. 0900000338).

Experimental design

The study was a cross-over design. Each of the 12 participants completed two sessions: a GXT to determine MFO and VO_{2max} and a moderate-intensity interval exercise session. The tests were performed on a braked cycle ergometer (Monark Bike E234, Monark Exercise AB, Sweden). Seat position was adjusted so that the knee was slightly flexed (about 5˚ less than maximal leg extension) with the ball of the foot on the pedal, and the handlebar was adjusted so that the participant was on an upright posture. Cadence was maintained at 70 rpm during all tests. Expired air was collected and heart rate was monitored during tests.

All participants were asked to maintain their normal dietary intake between tests, and to replicate their food intake as closely as possible on the day before the exercise tests. Participants were also asked to abstain from strenuous exercise and the consumption of caffeine and alcohol in the previous 24 h. They were instructed to wear lightweight, comfortable clothing during the tests. All tests were undertaken after an overnight fast and were run in an air-conditioned laboratory with the temperature held constant at 21°C.

FAT_{max} and VO_{2max} protocol

The FAT_{max} graded cycle ergometry protocol was discontinuous, with participants cycling at 35 W for 4 min followed by a 4-min rest interval. Participants remained seated on the cycle ergometer during the rest interval while finger tip blood lactate samples were immediately collected and perceived effort determined using the Borg Scale 6-20. At the end of the rest interval the work rate was increased by 17.5 W and the participant cycled at the new workload for 4 min. The discontinuous sequence of 4-min work-rest stages with 17.5 W increments in workload continued until the workload at which RER reached 1.0 and remained above 1.0 during the final 2 min of exercise. After a 4-min rest, participants commenced the second phase of the test designed to determine maximal aerobic power. Participants cycled for a minute at a workload two increments lower than the intensity at which an RER of 1.0 was reached, after which the mechanical work was increased by 17.5 W every minute until volitional exhaustion. Finger tip blood lactate samples were collected at the end of this period. FAT_{max} was determined for each participant by examining individual relationships between fat oxidation rate ($g \cdot min^{-1}$) and workload. This protocol has been adapted from Achten et al. (2002), which has been used in the obese men population (Roffey, 2008).

Moderate-intensity interval training (MIIT)

The mechanical work during the MIIT session consisted of 5-min stages at 20% above the mechanical work of FAT_{max} alternated with 20% below the mechanical work of FAT_{max} (±20 %FAT_{max}) for 30 min, so that minutes 0-5, 11-15 and 20-25: +20%FAT_{max}, and minutes 6-10, 16-20 and 26-30: -20%FAT_{max}.

Data management

The measurement of respiratory gas exchange was undertaken using a Parvo Medics Analyser Module (TrueOne®2400, Metabolic Measurement System, Parvo Medics, Inc. USA). The calibration of the system was undertaken prior to each test. The configuration of flowmeter calibration was performed using the 5-stroke method to measure ventilation which was verified using a certified 3 L calibration syringe. Calibration was accepted when the average difference was ≤ 2.0% and the difference between low and high volumes was ≤ 5.0%. Oxygen and carbon dioxide gas analysers were calibrated using known standard gas concentrations (4.01% CO_2, 15.99% O_2).

Gas calibration was performed to obtain the new conversion factors for the computer. The TrueOne®2400 is equipped with the Auto-Cal feature.

Expired breaths were collected, and VO_2, The volume of carbon dioxide (VCO_2), RER as well as HR were averaged for every 30 s automatically via the Parvo Medics Analyser; data were then exported to an Excel file. The last 2 min of each exercise stage of the MFO test where RER <1.0 were averaged. Workload, VO_2, VCO_2, HR, RER and blood lactate concentration were calculated at the point of MFO. In addition, the last 30 s of each minute during continuous stage was used to attain VO_2, VCO_2, RER and HR. Workload. During the MIIT session, the last 2 min of each 5-min stage of exercise was averaged.

Five threshold criteria were used to determine if maximal aerobic power was achieved. As defined by Taylor et al. (1955), a plateau in the current study was deemed to have been achieved if the difference in VO_2 between the last two completed incremental stages was lower than 50% of the change in VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$) seen across stages during the MFO test where wattage increased by 17.5 W. The average 50% increment of VO_2 was 1.6 ± 0.9 $ml \cdot kg^{-1} \cdot min^{-1}$ ($r^2 = 0.95 \pm 0.06$). The secondary criteria were HR ±10 beats/min relative to age-predicted HR_{max} (220 – age), RER ≥ 1.10, BLa ≥ 8 $mmol \cdot L^{-1}$ and RPE >18.

Rates of fat and carbohydrate (CHO) oxidation ($g \cdot min^{-1}$) were calculated using stoichiometric equations of the energy equivalents of oxygen for non-protein and percent kilocalories and grams derived from CHO and lipid (VO_2 is in $L \cdot min^{-1}$) developed by Frayn (1983) as follows:

$$\text{Total fat oxidation} = 1.67\ VO_2 - 1.67\ VCO_2$$
$$\text{Total CHO oxidation} = 4.55\ VCO_2 - 3.21\ VO_2$$

Where VO_2 and VCO_2 in litres per minute

The energy expenditure (EE) during MIIT and MFO were calculated using the abbreviated Weir equation (Weir, 1990):

$$EE_{Weir}\ (kcal/min) = ((1.106 \times RER) + 3.941) \times VO_2$$

where VO_2 is in $L \cdot min^{-1}$

Substrate oxidation and physiological variables were expressed as 5-min intervals. In addition, to allow a comparison with studies that used short durations (~15 min), 30-min MIIT was divided into two 15-min durations. Therefore, the first three 5-min stages substrate oxidation and physiological variables were averaged to obtain the average of first half of MIIT (representing 2 × +20%FAT_{max} and 1 × -20%FAT_{max}), and the second three 5-min stages were averaged to obtain the average of second half of MIIT (representing 1 × +20%FAT_{max} and 2 × -20%FAT_{max}).

A scatter plot of the difference in fat oxidation between MIIT and MFO (MIIT – MFO) versus mean fat oxidation of MIIT and MFO was constructed using Bland-Altman method (Bland and Altman, 1986). Mean and standard deviation of difference (SD_{diff}) between MIIT and MFO in fat oxidation was computed, and reference lines of the zero bias line and 95% upper (0 + 1.96 × SD_{diff}) and 95% lower (0 – 1.96 × SD_{diff}) the zero line are identified on the scatter plot.

Statistical analysis

Data are presented as mean values and standard error of mean (SEM), unless otherwise indicated. Paired t-tests were used to compare physiological variables, RPE and substrate oxidation during MIIT and GXT and during first and second half of the MIIT session. One-way repeated-measure ANOVA was used to assess the effect of time on fat oxidation, and post hoc test was used to compare the rate of fat oxidation during the MIIT session with MFO. Statistical analysis was significant when P value ≥ 0.05. Statistical analyses were carried out with SPSS for Windows (version 18.0.1, 2010, PASW Statistics SPSS, Chicago, IL, USA).

Results

Physiological variables were determined at MFO to use in the comparison with MIIT, and were expressed relative to physiological values determined at VO_{2peak}. Workload at MFO was 34 ± 0.02 %VO_{2peak}, 55 ± 0.01%HR_{peak} and 23 ± 2.0 %W_{max}, which was below the workload at lactate threshold (LT) and aerobic threshold (AT) 58 ± 3.0 and 62 ± 4.0 %W_{max} respectively.

Fat oxidation

There was a significant effect of time on fat oxidation during the MIIT session (p ≤ 0.01). The Mauchly test of sphericity for fat oxidation during MIIT (5, 10, 15, 20, 25 and 30 minutes) compared with reference value (MFO) was not significant (p = 0.16; p > 0.05); thus, the sphericity assumption has not been violated. Comparing each of the higher workload blocks (i.e., +20%FAT_{max}), post hoc comparisons revealed a significantly higher rate of fat oxidation at minute 25 compared with minute 5 (p ≤ 0.05) and 15 (p ≤ 0.01). While this difference was no longer evident at minute 30 of MIIT (Figure 1), the workload from 26-30 mins was -20%FAT_{max}. By nature of the design of the MIIT session, the mechanical work in the first half of the session was higher than the second half (i.e., first half: +20%FAT_{max}, -20%FAT_{max}, +20%FAT_{max}; second half: -20%FAT_{max}, +20%FAT_{max}, -20%FAT_{max}). Despite this difference in workload, the average fat oxidation did not differ between the first and second 15-min blocks. Further, the average rate of fat oxidation in the first and second half of MIIT did not significantly differ from MFO. CHO oxidation was significantly higher in the first half of MIIT compared with the second half (p ≤ 0.01) (Table 1), reflecting the higher total work undertaken in this 15-min block. Accordingly, EE in the first half and second half were not different (EE were 4.8, 4.5, 5.0, 4.6, 5.1 and 4.7 at minute 5, 10, 15, 20, 25 and 30 respectively).

A Bland-Altman plot (Figure 2) shows that there was no systematic bias in the difference between MFO and MIIT, such that data shows a random spread of the bias around the zero difference line. The intra-class correlation coefficient between fat oxidation during MIIT and MFO = 0.53.

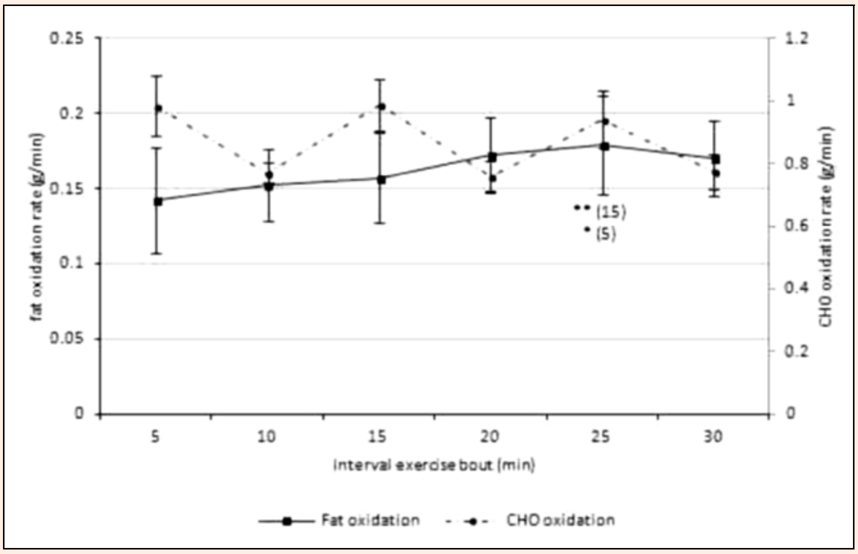

Figure 1. Rates of fat and carbohydrate (CHO) oxidation during the MIIT session. There were significant differences between fat oxidation at minutes 25 and 5 at 0.05 and between minutes 25 and 15 at 0.01. Data were represented as mean ±SEM.

Physiological variables and RPE

VO_2 measured during the first and second half of MIIT were not significantly different from VO_2 at MFO. BLa taken at the end of MIIT also was not significantly different from BLa taken at MFO. HR in the first half of MIIT was not significantly different from HR at MFO, but HR during the second half the MIIT was significantly higher than HR at MFO ($p \leq 0.05$). The change in HR from the first to second half of MIIT did not reach statistical significance.

RPE data is presented both as the average of the three measures taken during the first and second half (RPE_{ave}) of the MIIT session, and as the measure taken the end of the first half (15 min) and the end of the second half (30 min) of the MIIT session (RPE_{end}). RPE (RPE_{ave} and RPE_{end}) increased significantly over the MIIT session ($p \leq 0.01$), and were significantly higher than RPE at MFO ($p \leq 0.01$). Table 2 shows the comparisons between the first and second half of MIIT and MFO in physiological and psychological variables, and 5-min intervals of VO_2, HR and RPE during MIIT are shown in Figure 3.

Discussion

Fat oxidation

The main finding of this study was that while the average fat oxidation during MIIT was not different from MFO, the rate of fat oxidation was higher after the third 5-min interval of $+20\%FAT_{max}$ in the 30-min MIIT session. This indicates that the effect of exercise duration on fat oxidation that has been widely reported to occur during continuous exercise (Capostagno and Bosch, 2010; Cheneviere et al., 2009; Meyer et al., 2007) was also evident when exercise intensity was modulated within the moderate-intensity domain. In the current study we found the increase in fat oxidation during the MIIT session was modest, and was evident only after 15 min of exercise. While the rate of fat oxidation was highest at 25 min of exercise, it is possible that the rate would have continued to increase with further intervals of the 5-min $+20\%FAT_{max}$ efforts. However there was an apparent plateau in fat oxidation over the last 5 min of the exercise session, which was undertaken at the lower work intensity level (i.e., $-20\%FAT_{max}$). This contrasts the upward trend over previous 15 min of exercise which involved two higher intensity bouts ($+20\%FAT_{max}$) separated by one lower ($-20\%FAT_{max}$) intensity workload bout. A longer MIIT session would need to be examined to determine the extent to which fat oxidation increases with time.

The length of exercise required before fat oxidation is seen to increase significantly from the rate at the start of the session is contentious. For example, significant increases in fat oxidation have been reported to occur only after 30 min during 60-min steady-state exercise in trained participants (Capostagno and Bosch, 2010; Cheneviere et al., 2009; Meyer et al., 2007). In addition, a recent study in overweight 10-year-old boys who exercised at 40, 45, 50, 55 and $60\%VO_{2peak}$ did not find an increase in fat oxidation during 30-min constant-load exercise compared with the rate measured during the

Table 1. Comparison between substrate oxidation at MFO and MIIT. Data expressed as means (±SEM).

Variables	GXT	MIIT	
	MFO	First half (0–15 min)	Second half (15–30 min)
Fat oxidation ($g \cdot min^{-1}$)	.14 (.08)	.15 (.09)	.17 (.09)
CHO oxidation ($g \cdot min^{-1}$)	.84 (.20)	.91 (.2) ‡	.82 (.20)
EE (kcal/min)	4.5 (1.0)	4.8 (.3)	4.8 ± 0.3

MFO = maximal fat oxidation; MIIT = moderate-intensity interval exercise; CHO = carbohydrate; EE = energy expenditure. ‡ Significant difference between first half and second half of MIIT session ($p \leq 0.01$).

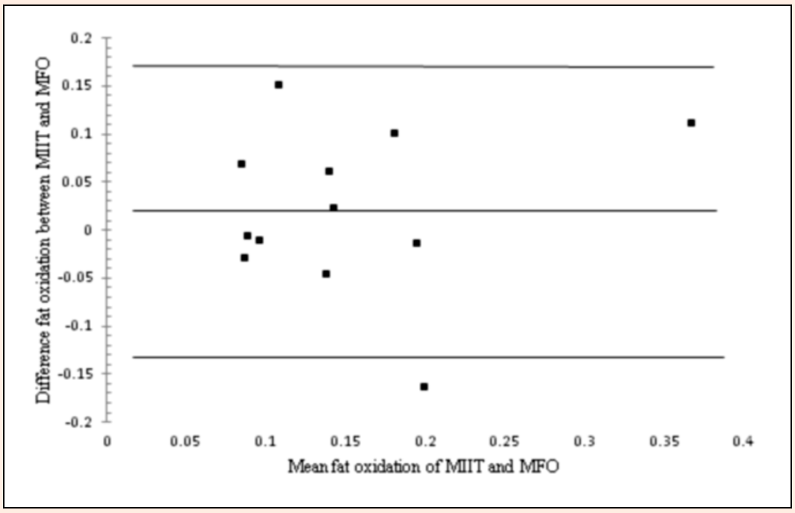

Figure 2. Bland-Altman plot of the mean and difference of fat oxidation during MIIT and MFO.

GXT (Crisp et al., 2012). Other studies have not found significant differences in fat oxidation between the first and second half of a 30-min exercise session. For example, sedentary obese and non-obese individuals significantly increased fat oxidation in the first 15 minutes of a 30-min exercise bout on the treadmill at 70%VO_{2max} compared with rest (i.e., before exercise), but there was no significant change in the rate of fat oxidation between minutes 15 and 30 (Kanaley et al., 2001). A decrease in fat oxidation in the second half compared with the first half of a 30-min moderate-intensity exercise session was also reported in obese children when preformed after 1 and 3 h of consuming a fixed meal (Aucouturier et al., 2011). These data suggested that while most studies found an increase in fat oxidation after 30-min exercise bouts, some studies did not find change in fat oxidation within 30-min exercise bouts.

Fat source contributed 27 ± 14%EE during the first half of the MIIT session and was 32 ± 14%EE during the second half of MIIT although the participants cycled at the level of MFO corresponding to 34%VO_{2max}. Therefore, CHO was the primary mechanism to achieve the energy requirement of the exercise, such that the rate of CHO oxidation was influenced by the change in workload during each of the 5-min intervals. This low level of the contribution of fat to exercise-induced EE at moderate-intensity levels may not be related to body fat as there was a trend for obese individuals to use fat greater than their leaner counterparts who had similar VO_{2max} level (48.2 and 45.5 ml·kg^{-1} FFM/min in lean and obese respectively) (Goodpaster et al., 2002). In addition, sedentary obese and lean children demonstrated a low contribution of fat oxidation to EE (< 20%) during 30-min moderate-intensity exercise (Aucouturier et al., 2011). Physical activity level could explain the low contribution of fat during exercise. This conclusion is supported by the finding of Romijn et al. (1993) that the availability of FFA during exercise training does not proportionally reflect the rate of fat oxidation, and fat oxidation during exercise was limited by the muscle's oxidative capacity and FFA transport capacity.

Bland-Altman plots demonstrated agreement between the rates of fat oxidation in the MIIT and the MFO from the GXT. This finding suggested that training within the FAT_{max} zone (the intensities that elicit a range of fat oxidation within ±5% (Zakrzewski and Tolfrey, 2012), ±10% (Achten et al., 2002; Roffey, 2008) or ±20% (Venables & Jeukendrup, 2008) of MFO) will present the highest rate of fat oxidation. However, the 95% limit of agreement was large (i.e., ±100%MFO). This finding shed lights on the importance of considering the between-subject variation in the FAT_{max} zone. Venables and Jeukendrup (2008) determined the interval training at ±20%FAT_{max}, but they did not mention the between

Table 2. Comparisons between the first and second half of MIIT and MFO in physiological and psychological variables. Data expressed as mean ±SEM.

Variables	GXT	MIIT	
	MFO	First half (0 – 15 min)	Second half (15 – 30 min)
VO_2 (ml·kg^{-1}·min^{-1})	10.8 (.7)	11.1 (1.4)	11.1 (1.4)
HR (beat·min^{-1})	104 (2)	105 (2)	108 (2) *
BLa (mmol·L^{-1})	1.9 (.1)	NA	1.9 (.3)
RPE_{ave} (6-20)	8.0 (.4)	10.0 (.6) **	13.0 (1.0) ** ‡
RPE_{end} (6-20)	8.0 (.4)	11.5 (.7) **	13.2 (1.1) ** ‡

* Significant difference between first and second half at MIIT and GXT ($p \leq 0.05$).
** Significant difference between first and second half at MIIT and GXT ($p \leq 0.01$).
‡ Significant difference between first half and second half during MIIT ($p \leq 0.01$).
VO_2 = oxygen consumption; HR = heart rate; BLa = blood lactate concentration; RPE_{ave} = average of rate of perceived exertion; RPE_{end} = rate of perceived exertion measured at the end of first and second half of MIIT; GXT = graded exercise test; MIIT = moderate-intensity interval training session; NA: not applicable as it was not measured.

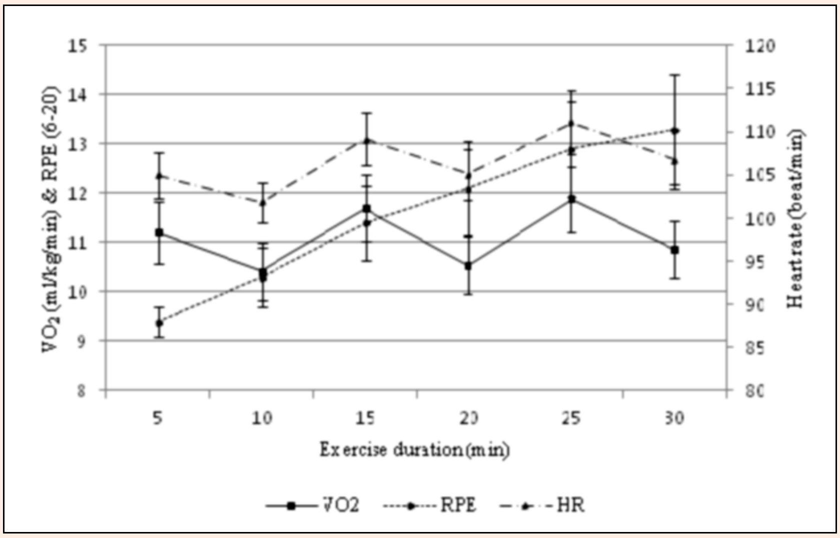

Figure 3. The responses of VO_2, HR and RPE every 5-min intervals during MIIT. Data were represented as mean ±SEM. VO_2 = oxygen consumption; HR = heart rate; RPE = rate of perceived exertion.

subject variation. This could explain why constant-load training was greater than interval training in the improvement of fat oxidation rate. Cheveniere et al. (2009) found that when MFO occurs at higher intensity, the curve of fat oxidation becomes dilated and the FAT_{max} zone tended to be larger. In the current study, MFO occurs at low intensity levels as the participants are untrained obese, and this may explain the large between-subject variation in the rate of fat oxidation at MIIT (±20%FAT_{max}) and FAT_{max} during GXT. The current data suggest that FAT_{max} zone (i.e., work load at ±20%FAT_{max}) does not represent fat oxidation zone (i.e., rate of fat oxidation at ±20%MFO) among obese sedentary individuals.

Physiological variables and RPE

The physiological variables studied (VO_2, HR and BLa) during MIIT were not different to the values found at MFO during GXT. The one exception was the small, but statistically significant increase in HR in second half of the MIIT session. Several previous studies have found that VO_2 is stable during moderate-intensity training in trained and untrained individuals (Meyer et al., 2007; Scott, 2005; Stoudemire et al., 1996). Although VO_2 corresponded to the interval work load in a similar pattern with HR similar to the expected response during interval exercise (Green et al., 2006; Seiler and Sjursen, 2004), it is accepted that HR could increase during exercise training despite stable VO_2 values (Coyle, 1998). The current finding is consistent with other previous studies (Kilpatrick et al., 2009; Kimura et al., 2010).

BLa at the end of MIIT session was not different from BLa at MFO, which was in agreement with the work of Steffan et al. (1999) who found no statistical difference between BLa during GXT and at the end of 15 min of moderate-intensity exercise, which were taken at equivalent intensities at 50%VO_{2max}. The mean value of BLa measured at the end of MIIT and at MFO was 1.9 mmol/L, which was in agreement with Bircher et al. (2005) who found strong correlations between workloads at MFO and a fixed value of BLa at 2 $mmol \cdot L^{-1}$. Instead of using fixed values such as 2 $mmol \cdot L^{-1}$, some studies determined the intensity that elicits MFO based on the difference between the onset of blood lactate accumulation (OBLA) and rest values. BLa at the end of a MIIT session was 0.6 $mmol \cdot L^{-1}$ above the rest value. Two studies found no differences between BLa at MFO and OBLA defined as 0.4 and 0.5 $mmol \cdot L^{-1}$ above BLa at rest (Bircher and Knechtle, 2004; Roffey, 2008). When OBLA defined as 1 $mmol \cdot L^{-1}$ above the rest value, the correlation between VO_2 at MFO and VO_2 at OBLA was strong in trained individuals, but was modest in the obese (Bircher and Knechtle, 2004). Therefore, fixed BLa at 2 mmol/L and relative BLa at 0.5 $mmol \cdot L^{-1}$ above resting values, may be the most representative references for the level of MFO when undertaking moderate-intensity interval exercise.

RPE at MFO among the current participants was slightly lower than the same population, which was determined at 9.4 ± 2.5 on Borg Scale (Rynders et al., 2011). RPE at MFO and during the MIIT session were significantly different, which confirmed the previous finding that RPE increased during exercise (Fanchini et al., 2011). The current data suggest that the RPE-MFO relationship cannot be used in the similar way to the RPE-HR relationship and RPE-BLa relationship. The reason could be that MFO occurs at moderate-intensity, and the RPE-HR and the RPE-BLa relationship is influenced by exercise intensity, demonstrating stronger relationship at high-intensity levels (Pires et al., 2011). Further studies are required to determine the relationship between RPE and fat oxidation at different intensities of interval training.

The responses of RPE during MIIT revealed two-important trends. Firstly, RPE increased linearly with time by one unit every 5-min interval during the MIIT session, but started to plateau in the last 5 min such that the increase was only 0.4 unit. Green et al. (2006) investi-

gated interval exercise for 2-min work at LT with 3-min recovery intervals, and found RPE increased by one unit in each work stage except the last stage as the increase was only 0.5 unit. This reflects the functioning of RPE as it does not only reflect the immediate peripheral afferents but also estimates the cessation of exercise (Lambert et al., 2005; St Clair Gibson et al., 2003). Therefore, the difference at the end of training could be attributed to other factors than physiological effort. Secondly, there were comparable outcomes in the value of RPE when using the end of first and second half or using the average of each stage. Kilpatrick et al. (2009) found that RPE taken after the exercise session related well to the finishing minute of the exercise session, and was higher than the first half and the average of the whole 30-min exercise session. While Kilpatrick et al.'s study found the measure of RPE at the end of 30-min exercise session did not reflect the average of the whole session, the results of the current study indicate that RPE at the 15-min point of the session reflected the average RPE.

Conclusion

The main finding of this study was that the average fat oxidation during 30-min MIIT was comparable with MFO measured during the GXT. Further, there was some evidence that fat oxidation increased over time during the 30-min MIIT session. These findings suggest that fat oxidation during MIIT is not compromised by the undulating exercise intensity. Therefore given the potential for interval training to improve compliance to exercise, MIIT may be a useful training strategy when increasing fat oxidation is a goal. This study also demonstrated that physiological measures corresponding with the MFO measured during the GXT correlated to the MIIT. VO_2 and BLa displayed comparable values during MFO and MIIT, HR statistically increased in the second half of the 30-min MIIT session and RPE linearly increased during MIIT. These findings reinforce the validity of exercise intensity markers derived from a GXT to reflect the physiological responses during MIIT. Further investigation is needed to determine whether fat oxidation during longer sessions of MIIT equate that of continuous exercise training, and the extent to which MIIT may improve compliance to exercise in individuals who find continuous exercise sessions challenging.

Acknowledgments

I acknowledge the great support that I had from my supervisors Professor Nuala Byrne, Professor Andrew Hills and Professor Neil King. Clinical assistance was kindly provided by Dr Rachel Wood and Connie Wishart. The cost of publication was kindly funded by De'aam Medical Equipment in Saudi Arabia.

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Key points

- Fat oxidation during interval exercise is not compromised by the undulating exercise intensity
- Physiological measures corresponding with the MFO measured during the GXT correlated well to the MIIT
- The validity of exercise intensity markers derived from a GXT to reflect the physiological responses during MIIT.

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