

## A NEW METHOD FOR THE MEASUREMENT OF MAXIMAL FAT OXIDATION: A PILOT STUDY

PRIIT PURGE<sup>1</sup>, PRIIT LEHISMETS<sup>2</sup>, JAAK JÜRIMÄE<sup>1</sup>

<sup>1</sup>*Institute of Sport Pedagogy and Coaching Sciences, University of Tartu, Estonia*

<sup>2</sup>*Centre of Behavioral and Health Sciences, University of Tartu, Estonia*

### ABSTRACT

Exercise intensity is one of the main factors determining the rate of fat oxidation during exercise. The 3-min incremental protocol is desirable for practical reasons and allows for the estimation of fat oxidation across a wide range of exercise intensities. However, the use of a small number of exercise intensities traditionally used to estimate fat oxidation does not allow for the precise estimation of exercise intensity at which the rate of fat oxidation is maximal ( $Fat_{max}$ ). The purpose of this study was to examine the validity of the determination of  $Fat_{max}$  adopting 1-min step incremental bicycle ergometer protocol. In this study we also compared  $Fat_{max}$  determined by 3-min step incremental bicycle ergometer test with  $Fat_{max}$  measured by 1-min step incremental bicycle ergometer test. The average peak oxygen consumption ( $VO_{2peak}$ ) for the entire group of subjects was  $48.85 \pm 7.58$  ml/min/kg. The  $Fat_{max}$  occurred at  $47.2 \pm 4.9\%$  of  $VO_{2peak}$  in Test 1 and at  $51.3 \pm 7.4\%$  of  $VO_{2peak}$  in Test 2, which corresponded to  $35.8 \pm 7.4\%$  and  $36.1 \pm 8.0\%$  of the maximal workload ( $W_{max}$ ) in Tests 1 and 2, respectively. Heart rate (HR) in  $Fat_{max}$  was at  $63.9 \pm 6.8\%$  and  $62.1 \pm 6.2\%$  from maximal HR in Tests 1 and 2, respectively. There were no significant differences ( $p < 0.05$ ) between work rate (WR), respiratory coefficient (RER) and HR at  $Fat_{max}$  during both tests. Similarly, the values of  $Fat_{max}$  and  $VO_{fatmax}$  were not significantly different in both tests. It appeared that the proposed 1-min step incremental exercise protocol can be used for the measurement of  $Fat_{max}$  in physically active males as the obtained results were not different from previously used methods to calculate  $Fat_{max}$ . However, the  $Fat_{max}$  results are different as fat oxidation rate is affected by endurance, sex, age, body composition, exercise type and exercise duration.

**Keywords:** *soccer, fat oxidation, aerobic capacity,  $Fat_{max}$*

## INTRODUCTION

It is known that exercise intensity is one of the main factors determining the rate of fat oxidation during exercise [2]. Absolute fat oxidation rates increase during low-to-moderate exercise intensities and then markedly decline at high intensities, implying that there is an exercise intensity at which the rate of fat oxidation is maximal ( $Fat_{max}$ ) [15]. The highest rate of fat oxidation during submaximal exercise has been reported to occur between 40 and 65% of peak oxygen consumption ( $VO_{2peak}$ ) [1]. Exercise intensity that promotes the maximum fat oxidation rate has been termed as  $Fat_{max}$  and is typically expressed as a percentage of  $VO_{2peak}$  [14]. It has been suggested that training at this intensity may have utility for endurance performance [2] and body mass loss [3]. In addition, it has been suggested that high fat oxidation rates can be beneficial for a large variety of individuals [2].

Most studies have only investigated three or four different intensities to determine  $Fat_{max}$ . Achten et al. [1] developed a specific test to determine the exercise intensity which elicits maximal fat oxidation (MFO). This  $Fat_{max}$  consisted considered of a graded exercise test to exhaustion on a bicycle ergometer, starting at 95 W with 35 W increments every 3 min during which gas exchange measurements were performed. Using this protocol,  $Fat_{max}$  occurred at 64%  $VO_{2peak}$  [2]. The 3-min incremental protocol is desirable for practical reasons and allows for the estimation of fat oxidation rate across a wide range of exercise intensities. Consequently, this protocol has been adapted for other studies in adults [16]. However, the use of a relatively small number of exercise intensities traditionally used to estimate fat oxidation does not allow for the precise estimation of  $Fat_{max}$  [14].

A number of studies have used a progressive exercise test lasting 8–12 min to determine  $VO_{2peak}$  when assessing  $Fat_{max}$  [8,12], while others have determined both fat oxidation and  $VO_{2peak}$  during the same test lasting around 30 min and longer [14]. Bircher et al. [4] reported that the protocol that was more time-consuming indicated a higher  $Fat_{max}$  and MFO than those done by a shorter protocol, although the increments for both protocols were small. These data indicated that  $Fat_{max}$  may be influenced by exercise duration, probably due to changes in substrate concentrations and hormonal responses [15]. In contrast, Achten et al. [1] concluded that exercise duration did not affect  $Fat_{max}$  determination. It is possible that the use of an incremental protocol to determine both MFO and  $VO_{2peak}$  does not provide a valid measure of  $VO_{2peak}$  as lower  $VO_{2peak}$  values were observed from protocols lasting 20–28 min compared with 8–12 min in adults [17].

The purpose of this pilot study was to examine the validity of the determination of  $\text{Fat}_{\text{max}}$  adopting 1-min step incremental bicycle ergometer protocol. In this study we compared  $\text{Fat}_{\text{max}}$  during traditional 3-min step incremental bicycle ergometer test with  $\text{Fat}_{\text{max}}$  measured by 1-min step incremental bicycle ergometer test. It was hypothesised that  $\text{Fat}_{\text{max}}$  determined at both protocols are similar but a fat oxidation rate is higher using longer 3-min step protocol.

## **MATERIALS AND METHODS**

### **Participants**

Thirteen soccer players aged 18–30 years volunteered to participate in this study. They were recruited from local training groups and had a training history of  $4.5 \pm 1.5$  years and had trained regularly for minimal 3 times per week for at least last two years. None of the participants was receiving any medications or had any disease. All procedures were approved by the Medical Ethics Committee of the University of Tartu.

### **General design**

All subjects performed two graded exercise tests to exhaustion on a bicycle ergometer (Lode, Groningen, The Nederland). Test 2 was performed one week after Test 1. The results of these tests were used to determine the exercise intensity that elicits maximal fat oxidation.

### **Anthropometry and body composition assessment**

Body height (cm) was measured with the use of a Martin metal anthropometer to the nearest 0.1 cm according to the standard technique, and body mass was measured with minimal clothing to the nearest 0.05 kg with a medical electronic scale (A&D Instruments, Abingdon, UK). BMI was calculated as body mass (kg) divided by body height ( $\text{m}^2$ ). Dual-energy X-ray absorptiometry (DXA) scans of the total body were performed using a QDR Discovery scanner (Hologic, Waltham, MA, USA). Total body scans were analyzed for body fat mass (FM) and fat free mass (FFM) values using Hologic APEX software version 3.3.0.1.

### **Experimental design**

The Test 1 was performed on an electronically braked bicycle ergometer (Lode Corival, Netherlands). Participants performed an initial work rate at

60 W with an increments of 20 W after every 1 min. Participants cycled at a cadence of  $70 \pm 5$  rpm, and they were actively encouraged to continue until volitional exhaustion. Heart rate was recorded every 5 s during the test using a commercially available HR monitor (Polar RS400, Polar Electro, Kempele, Finland). Gas exchange variables were measured throughout the test in a breath-by-breath mode and data were stored in 10 s intervals. Oxygen consumption ( $\text{VO}_2$ ), carbon dioxide output ( $\text{VCO}_2$ ) and minute ventilation ( $\text{V}_E$ ) were continuously measured using portable open spirometry system (MetaMax 3B, Cortex, Leipzig, Germany). The analyser was calibrated with gases of known concentration before each test according to the manufacturer's guidelines. All data were calculated by means of computer analysis using standard software (MetaMax-Analysis 3.21, Cortex, Leipzig, Germany). Peak oxygen consumption was achieved when two of the following three criteria were fulfilled: 1)  $\text{VO}_2$  plateau defined as a failure of  $\text{VO}_2$  to increase by greater than 2.0 ml/min/kg with increase of test load; 2)  $\text{HR} \geq 95\%$  from the predicted individual maximum (formula  $220 - \text{age}$ ); and/or 3)  $\text{RER} \geq 1.05$ .

Test 2 was performed one week after Test 1 on an electronically braked cycle ergometer (Lode Corival, Netherlands). Participants performed an initial work rate at 95 W with an increments of 35 W after every 3 min. Both position on the bike and cadence was kept similar during both trials. Heart rate was recorded every 5 s during the test using a commercially available HR monitor (Polar RS400, Polar Electro, Kempele, Finland). Gas exchange variables were measured throughout the test in a breath-by-breath mode and data were stored in 10 s intervals. Oxygen consumption,  $\text{VCO}_2$  and  $\text{V}_E$  were continuously measured using portable open spirometry system (MetaMax 3B, Cortex, Leipzig, Germany). The analyser was calibrated with gases of known concentration before each test according to the manufacturer's guidelines.

All data were calculated by means of computer analysis using standard software (MetaMax-Analysis 3.21, Cortex, Leipzig, Germany). Aerobic threshold ( $\text{AeT}$ ), anaerobic threshold ( $\text{AnT}$ ),  $\text{VO}_{2\text{peak}}$ ,  $\text{V}_E$ , and maximal work rate ( $\text{WR}_{\text{max}}$ ) were calculated using MetaMax-Analysis 3.21 software (Cortex, Leipzig, Germany) in each subject.

Fat oxidation rates were determined from  $\text{VO}_2$  and  $\text{VCO}_2$  values averaged over the last minute of each stage using the following equation [6]:

$$\text{Fat oxidation} = 1.67 \times \text{VO}_2 - 1.67 \times \text{VCO}_2$$

$$\text{Carbohydrite oxidation} = 4.55 \times \text{VCO}_2 - 3.21 \times \text{VO}_2$$

Fat oxidation rates were calculated for all stages in which  $\text{RER} < 1.00$ . The exercise intensity (W) associated with the highest recorded fat oxidation rate was selected as  $\text{Fat}_{\text{max}}$ .

## Statistical analysis

Statistical analysis was performed with SPSS 17.0 for Windows (Chicago, IL, USA). Means and  $\pm$ SD were determined. Evaluation of normality was performed with the Shapiro-Wilk statistical method.. The difference of the performance data between the two incremental cycling tests and Fatmax were tested with a *paired sample t-test*. The level of significance was set at  $p < 0.05$ .

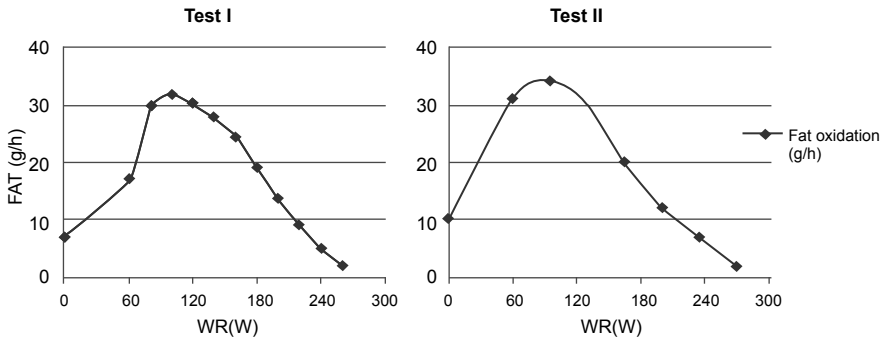
## RESULTS

The average  $VO_{2peak}$  for the entire group was  $48.85 \pm 7.58$  ml/min/kg (Table 1). Figure 1 shows the relationship between fat oxidation rate and exercise intensity expressed in W at every workstage. With increasing exercise intensities the fat oxidation rate increased to  $31.78 \pm 10.46$  g/h in Test 1 and to  $37.15 \pm 7.16$  g/h in Test 2 at  $121.54 \pm 25.12$  W in Test 1 and  $121.92 \pm 29.12$  W in Test 2 beyond which the oxidation rate decreased.

**Table 1.** Anthropometrical and functional parameters of the subjects.

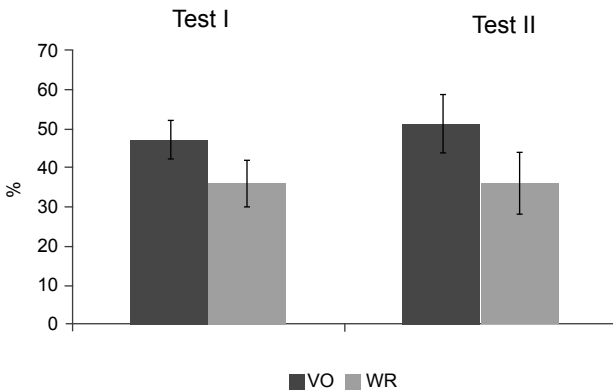
n=13	X $\pm$ SD	Minimum	Maximum
Age (yrs)	23.77 $\pm$ 2.74	21.0	30.0
Height (cm)	184.54 $\pm$ 7.20	175.0	198.0
Weight (kg)	85.82 $\pm$ 12.05	69.2	111.9
AeT (l/min)	139.92 $\pm$ 6.85	127.0	152.0
AeT (W)	186.72 $\pm$ 21.75	159.7	233.3
AnT (l/min)	170.62 $\pm$ 6.95	157.0	181.0
AnT (W)	275.96 $\pm$ 27.65	225.0	336.0
$VO_{2max/kg}$ (ml/min/kg)	48.85 $\pm$ 7.58	34.0	58.0
$VO_{2max}$ (l/min)	4.13 $\pm$ 0.49	3.4	5.0
$VO_{2max}$ (W)	338.90 $\pm$ 33.99	280.3	383.0
$V_E$ (L/min)	149.19 $\pm$ 26.27	105.4	189.6
$WR_{max}$ (W)	351.62 $\pm$ 26.73	313.3	388.7
$WR_{max}$ (W/kg)	4.17 $\pm$ 0.66	2.8	5.0
$HR_{max}$ (l/min)	190.85 $\pm$ 7.15	179.0	203.0
Fat mass (kg)	15.88 $\pm$ 3.97	11.4	24.4
Body fat %	18.73 $\pm$ 2.72	14.1	24.6
Lean body mass (kg)	68.17 $\pm$ 8.76	56.4	88.3

AeT – aerobic threshold; AnT – anaerobic threshold;  $VO_{2max/kg}$  – maximal oxygen consumption scale to body mass;  $VO_{2max}$  – maximal oxygen consumption;  $V_E$  – maximal ventilation;  $WR_{max}$  – maximal work rate;  $HR_{max}$  – maximal heart rate.



**Figure 1.** Fat oxidation in two different incremental workload tests.

The  $Fat_{max}$  occurred at  $47.2 \pm 4.9\%$   $VO_{2peak}$  in Test 1 and at  $51.3 \pm 7.4\%$   $VO_{2peak}$  in Test 2, which corresponds to  $35.8 \pm 7.4\%$  and  $36.1 \pm 8.0\%$  of  $WR_{max}$  in Tests 1 and 2, respectively. The HR in  $Fat_{max}$  occurred at  $63.9 \pm 6.8\%$  in Test 1 and in  $62.1 \pm 6.2\%$  in Test 2 from  $HR_{max}$  (Figure 2).



**Figure 2.**  $Fat_{max}$  at  $VO_{peak}\%$  and  $WR_{max}\%$  in two different workload protocol in healthy active men.

The values for the Fat oxidation in  $Fat_{max}$  intensity obtained during Tests 1 and 2 in bicycle ergometer are presented in Table 2. There were no differences ( $p < 0.05$ ) between WR, RER and HR during both tests. Similarly, the values of MFO and  $VO_2$  compared in both tests were not significant different, however, the values were higher in Test 2 than in Test 1.

**Table 2.** Results of  $Fat_{max}$  in two different test methods.

n=13	Test I	Test II	p
MFO (g/h)	31.78±10.46	37.15±7.16	0.08
CHO (g/h)	69.89±24.17	68.64±32.90	0.87
VO <sub>2</sub> (L/min)	1.95±0.29	2.11±0.37	0.08
VCO <sub>2</sub> (L/min)	1.63±0.24	1.74±0.37	0.16
RER	0.84±0.05	0.82±0.04	0.23
WR (W)	121.54±25.12	121.92±29.12	0.95
HR (l/min)	122.00±14.54	118.77±14.21	0.30

MFO– maximal fat oxidation; CHO–maximal carbohydrate oxidation; VO– oxygen consumption; VCO– carbon dioxide consumption; WR– work rate; HR– heart rate

## DISCUSSION

The aim of the present study was to investigate whether it is possible to measure  $Fat_{max}$  in healthy active males with 1-min long stage incremental exercise protocol in bicycle and compare the results with the suggested 3-min long stages incremental exercise protocol [1]. The first test allows to measure in same time fat oxidation parameters and determine VO<sub>2</sub> peak. We found no differences ( $p>0.05$ ) in  $Fat_{max}$  results in both tests and in this case it is possible to measure  $Fat_{max}$  in healthy active males using the suggested shorter 1-min incremental exercise protocol.

Fat oxidation during exercise has typically been assessed using a small number of prolonged steady state exercise bouts to ensure the valid use of indirect calorimetry in adults [7, 13]. Several studies have measured fat oxidation in different populations generally using 3–6 min long incremental exercise protocols [14]. Achten et al. [1] developed and validated a protocol to identify  $Fat_{max}$  using a 3-min long and wide range of exercise intensities in trained adult males. Fat oxidation was estimated from the final 2 min of each 3-min stage of the incremental exercise test [1]. It was concluded that an incremental exercise test on a bicycle ergometer starting at 95 W with 35W increments every 3-min to exhaustion can be used for the determination of  $Fat_{max}$  and the rate of MFO in trained adult males [14]. A further issue surrounding the use of incremental protocols is that fat oxidation during the later exercise stages may be influenced by the earlier stages, i.e. a residual or carry-over effect [5], although Achten et al. [1] reported that previous stages had no influence on measured fat oxidation.

The studies that are available indicate that  $\text{Fat}_{\text{max}}$  generally occurs between 30 and 60% of  $\text{VO}_{2\text{peak}}$  in young people [14]. Previous studies with healthy active males have found that  $\text{Fat}_{\text{max}}$  occurred at 64%  $\text{VO}_{2\text{peak}}$  [2] and 61–64%  $\text{VO}_{2\text{peak}}$  [10]. In our study the  $\text{Fat}_{\text{max}}$  was  $47.16 \pm 4.88\%$  and  $51.31 \pm 7.37\%$  of  $\text{VO}_{2\text{peak}}$  in Tests 1 and 2, respectively. In the second test, the used 35 W incremental step was considered to be high to determine exactly the  $\text{Fat}_{\text{max}}$  intensity [2]. Accordingly, it can be suggested that the use of lower stage (20 W) increases the number of stages participants are able to complete and increase the precision with which  $\text{Fat}_{\text{max}}$  is estimated. Achten et al. [1] reported that with lower incremental stage the test will be more longer. However, they found no differences in two different workload protocols and suggested to use 35 W stage increments.

While in our first test we used 20 W with 1-min stage increments, the test duration was not longer as usually, however, the participants did not reach steady-state at 1-min stage time [9], which can affect the results. The reason why Achten et al. [1] had higher  $\text{Fat}_{\text{max}}$  results compared to our study was because the participants in Achten et al. [1] study were endurance-trained cyclists and runners with higher  $\text{VO}_{2\text{peak}}$  values. Although when compared our results with results in Achten and Jeukendrup [2] and Lima-Silva et al. [10] studies, there were also higher results in maximal fat oxidation. In our study, maximal fat oxidation was  $0.53 \pm 0.17$  g/min and  $0.62 \pm 0.12$  g/min in Test 1 and 2, respectively. In Achten et al. [1] study maximal fat oxidation was  $0.60 \pm 0.07$  g/min. Achten and Jeukendrup [2] investigated endurance-trained athletes and found similar fat oxidation results ( $0.52 \pm 0.15$  g/min) as in our first test. Many authors have found that maximal fat oxidation is higher in endurance-trained participants [10, 11], however, the results of our study are similar to other studies [2, 3] or even lower [10].

While our  $\text{Fat}_{\text{max}}$  results compared in two tests were not different, it is possible that the suggested exercise test protocol with 1-min stages can be used to evaluate  $\text{Fat}_{\text{max}}$  parameters and also to determine  $\text{VO}_{2\text{peak}}$ . It was probably possible because the physical activity level in our participants was relative high, although the same test could not be suitable for measure  $\text{Fat}_{\text{max}}$  in overweight or physically inactive people.

It was concluded that the proposed 1-min step incremental exercise protocol can be used to measure  $\text{Fat}_{\text{max}}$  in physically active males, because the results between two tests were not significantly different.



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**Correspondence to:**

Priit Purge, PhD

Institute of Sport Pedagogy and Coaching Sciences

University of Tartu

Jakobi 5, 51014, Tartu, Estonia

Telephone: +372 7 37 53 72

E-mail: priit.purge@ut.ee