©Journal of Sports Science and Medicine (2019) **18**, 44-51
http://www.jssm.org

Research article

A Comparison of the Maximal Fat Oxidation Rates of Three Different Time Periods in The Fatmax Stage

Kerem T. Özgünen [1]✉, Çiğdem Özdemir [1], Selcen Korkmaz-Eryılmaz [2], Abdullah Kılcı [2], Özgür Günaştı [1] and Sanlı S. Kurdak [1]

[1] Çukurova University, Medical Faculty, Department of Physiology, Division of Sports Physiology and [2] Department of Physical Education and Sports, Adana, Turkey

Abstract

This study aimed to compare the maximal fat oxidation (MFO) rates obtained from the stage average, last 2 min average, and highest value in the Fatmax stage determined with a 6 min step protocol. A total of 35 overweight, sedentary healthy men (age: 25.4 ± 0.7 years, body mass index: 26.0 ± 0.6 kg/m^2) participated in the study. Substrate oxidation was calculated using breath-by-breath gas exchange data for each stage. When the change in the fat oxidation rate for every min throughout the Fatmax stage was evaluated, the average value of the 4th min was significantly lower than that of the 2nd and 3rd min ($p < 0.01$). In addition, the 5th and 6th min fat oxidation rates were significantly lower than the rates of the 1st, 2nd, 3rd, and 4th min (0.30 ± 0.01 and 0.29 ± 0.01 g/min for the 5th and 6th min, respectively, vs. 0.35 ± 0.02, 0.34 ± 0.02, 0.33 ± 0.02, and 0.31 ± 0.01 g/min for the 1st, 2nd, 3rd, and 4th min, respectively; $p < 0.01$). Most of the participants had MFO rates in the 1st min of the stage (16/35 participants), and the MFO rates of the remaining participants were observed in the 2nd, 3rd, and 4th min (7/35, 4/35, and 8/35 participants, respectively). None of the participants had MFO rates in the 5th or 6th min. The individual MFO rate (highest fat oxidation rate during Fatmax) was significantly higher than the fat oxidation rate calculated with the last 2 min average values (0.36 ± 0.02 and 0.30 ± 0.01 g/min, respectively; $p < 0.05$). In conclusion, the calculation of the fat oxidation rate by averaging the last portion of the Fatmax stage data may cause the underestimation of the MFO rate, which probably occurs earlier in the Fatmax stage.

Key words: Exercise intensity, substrate oxidation, indirect calorimetry, oxygen consumption, respiratory quotient.

Introduction

In recent years, there has been increased interest in fat metabolism in the field of both sports sciences and medicine. During long-distance events, increasing the reliance on fat can help athletes save glycogen reserves for high-intensity situations later in the events. The impairment of fat oxidation has been associated with the development of obesity and type 2 diabetes, and endurance training has been reported to improve fat oxidation and reduce body weight (Rosenkilde et al., 2015). With the aim of utilizing as much fat as possible in a certain period of time, the main strategy should be to exercise with low-moderate intensity. In particular, professional organizations such as the American College of Sports Medicine and American Medical Association recommend regular aerobic activity as part of a weight loss program with a special emphasis on low-intensity exercises for individuals interested in weight loss or control (Haskell et al., 2007).

It is well known that energy production shifts from fat to carbohydrates with an increase in the intensity of physical activity (Christensen and Hansen, 1939; Maunder et al., 2018). The specific intensity at which the fat oxidation rate is maximal (commonly presented as a percentage of the maximal oxygen uptake) is defined as Lipoxmax, Fatoxmax, or Fatmax by different researchers (Brun et al., 2011) and provides a measure of the maximal fat oxidation (MFO; the highest rate of fat oxidation observed at various intensities) (Randell et al., 2017). The determination protocol is often called the Fatmax test; exercise intensity could be individualized with the Fatmax test, and a suitable exercise prescription could be prepared according to the subjects' metabolic responses (Besnier et al., 2015; Dumortier et al., 2003; Tan et al., 2016). Athletic training programs, programs for weight or body fat loss, and exercise programs that treat or prevent welfare diseases may be the potential applications of Fatmax (Jeukendrup and Achten, 2001).

Lipids are oxidized predominantly at submaximal exercise intensities (< 65% $\dot{V}O_{2max}$); however, an exercise intensity that exceeds 65% $\dot{V}O_{2max}$ produces a shift in energy contribution favoring carbohydrates (Purdom et al., 2018). Therefore, Fatmax can be achieved at low-to-moderate exercise intensities (35–65% $\dot{V}O_{2max}$) (Jeukendrup and Wallis, 2005). Different approaches have been proposed to determine the MFO. One approach is to perform a single graded exercise test and determine Fatmax. In another approach, four to six continuous prolonged exercise tests are performed on separate days at the exercise intensities used in the graded exercise test (Achten et al., 2002). In the graded tests, the duration of each stage generally varies from 2 to 6 min, whereas the duration of the stages in the prolonged exercise tests is much longer (Achten et al., 2002; Alkahtani, 2014; Croci et al., 2014; Haufe et al., 2010; Meyer et al., 2007; Marzouki et al., 2014). Although the relatively shorter protocols make the Fatmax test more practical, 6 min intervals are recommended especially for sedentary individuals because of their delay in reaching a steady state (Bordenave et al., 2007).

Depending on the approach of researchers, a specific time period of the Fatmax stage data is typically used to calculate substrate oxidation. In the studies in which 6 min intervals were chosen, investigators have used the last 30 s (Schwindling et al., 2014), 1 min (Isacco et al., 2015), 2 min (Besnier et al., 2015; Bordenave et al., 2008; Borel et al., 2015), or 3 min (Gmada et al., 2012; Marzouki et al.,

Received: 19 September 2018 / Accepted: 30 November 2018 / Published (online): 11 February 2019

2014) average values to calculate substrate oxidation. Although Achten et al. reported that 3 min intervals are appropriate for well-trained subjects to reach steady state (Achten et al., 2002), interval durations longer than 3 min have been recommended for sedentary individuals to avoid overestimating the fat oxidation rate (Bordenave et al., 2007). Furthermore, 3 min steps in the graded exercise protocol may be too short for obese individuals to reach steady state (Dandanell et al., 2017).

To our knowledge, changes in fat oxidation rates throughout the Fatmax stage have not been evaluated. Therefore, this study aimed to compare the MFO rates obtained from the stage average of Fatmax, last 2 min average of Fatmax, and highest value of Fatmax determined with a 6 min step protocol.

Methods

Participants and intervention content

A total of 35 healthy, sedentary males with an average age of 25.4 ± 0.7 years participated in this study (see Table 1 for anthropometric and physical characteristics). The study was explained to all participants in detail, and informed consent forms were acquired. Measurements were performed following the approval of the Ethics Committee and carried out in accordance with the Declaration of Helsinki. All tests were conducted at the Sports Physiology Research and Analysis Laboratory of the Physiology Department of Çukurova University, Medical Faculty. Participants with a history of any disease or drug use were excluded from the study. Calorie restrictions were not applied in terms of nutrition.

Table 1. Physical characteristics of the participants. BMI, Body mass index. Values are given as the mean ± SEM

Variables	n = 35
Age, y	25.4 ± 0.7
Height, m	1.79 ± 0.01
Body mass, kg	83.2 ± 2.4
BMI, kg/m^2	26.0 ± 0.6
Peak $\dot{V}O_2$, mL/min	2875.7 ± 68.2
Peak $\dot{V}O_2$, mL/min/kg	35.0 ± 0.7
Body fat, %	18.6 ± 0.9
Muscle mass, %	35.9 ± 0.7

Anthropometric measurement

The participants visited the laboratory after 12 h of overnight fasting. Anthropometric measurements were performed before the exercise by the same person. Body mass and height were determined with a scale and a stadiometer. Circumference measurements were performed with a nonelastic measuring tape. Body fat estimates were derived according to Siri (1961). Body density, which was used in the Siri formula, was calculated for the men (Jackson and Pollock, 1978). The Martin formula was used to estimate body muscle mass (Martin et al., 1990).

Exercise protocol

Two separate exercise tests were performed at least 48 h apart. A maximal cardiopulmonary exercise test was performed on the first visit, and the Fatmax test was performed on the second visit. Both tests were performed on a treadmill (HP Cosmos, Nussdorf – Traunstein, Germany). Breath-by-breath gas measurements were taken throughout the exercise using an indirect calorimetric system (PFT Cosmed, Rome, Italy). The volume and gas calibration of the system was performed using a 3 L calibration syringe and calibration gases, respectively (16% O_2 and 5% CO_2). The heart rate was recorded continuously by telemetry using a heart rate monitor (Cosmed, Rome, Italy).

Maximal cardiopulmonary exercise test

The participants started the test at 4 km/h, and the speed was increased by 0.5 km/h every min until exhaustion. The test was terminated if one or more of the following criteria were fulfilled: reaching up to 90% of the maximum heart rate according to the 220-age formula, formation of an oxygen uptake plateau, continuation of a non-protein respiratory quotient (npRQ) value at and over 1.15 (Balady et al., 2010).

Fatmax test

Fatmax was determined with an incremental treadmill walking test after at least 12 h of fasting. The participants performed a 2 min warm-up at 3 km/h and started the test at 4 km/h. The speed was increased by 1.0 km/h every 6 min until the fat oxidation level was decreased to 0 with a npRQ value of 1.01 (Achten et al., 2002). The interval duration in the Fatmax test was selected based on a previous study (Perez-Martin et al., 2001).

Calculation of fat and carbohydrate oxidation

Breath-by-breath data were averaged in 10 s increments for the maximal cardiopulmonary test, and 60 s average values were used to calculate substrate oxidation in the Fatmax test. Substrate oxidation was calculated using the deviation from the stoichiometric equation (Frayn, 1983). Protein oxidation throughout the test was accepted as negligible. Fat and carbohydrate oxidation rates were calculated as follows:

$$\text{Fat oxidation rate (g/min)} = 1.67 \times \dot{V}O_2 - 1.67 \times \dot{V}CO_2$$
$$\text{Carbohydrate oxidation rate (g/min)} = 4.55 \times \dot{V}CO_2 - 3.21 \times \dot{V}O_2$$

By using the last 2 min average data, fat and carbohydrate oxidation rates were calculated for each stage, and the stage with the highest level of fat oxidation was recognized as Fatmax. In addition, the MFO rates were calculated with three different approaches in the Fatmax stage: (1) highest level of fat oxidation in the Fatmax stage (individual MFO rate), (2) 6 min average of fat oxidation in the Fatmax stage, and (3) last 2 min average of fat oxidation in the Fatmax stage. MacRae et al. (1995) proposed reference values to define the steady state level of gas exchange variables. In their study, during the 6 min progressive exercise, $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}E$ were measured for the 5th and 6th min. In this period, $\dot{V}O_2$ and $\dot{V}CO_2$ varied by less than 0.1 L/min, and $\dot{V}E$ varied by less than 0.5 L/min even at the highest (60–70% of the peak $\dot{V}O_2$) exercise intensities (MacRae et al., 1995). The difference between the 6th min and previous min $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}E$ values were calculated and compared with the reference values established by MacRae et al. (1995).

Statistical analysis

The results are presented as the mean ± SEM. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS for Windows Version 22.0, USA). The normality of values was assessed with the Shapiro-Wilk test. ANOVA with repeated measures was performed to evaluate the differences in the mean values of fat oxidation rates for each min during the 6 min interval. The equality of variances between the differences was assessed with Mauchly's test of sphericity. Regarding the violation of sphericity, the Greenhouse-Geisser correction was applied. In the case of any significant differences in the test of within-subject effects, post-hoc comparisons using Bonferroni's method were performed to determine pairwise differences. Wilcoxon signed rank test was used to analyze the differences between the 6th min and previous min with reference values of 100 mL/min for $\dot{V}O_2$ and $\dot{V}CO_2$ and 0.5 L/min for $\dot{V}E$ as reported in the study by MacRae et al. (1995). Statistical significance was set at $p < 0.05$.

Results

The physical characteristics of the participants are shown in Table 1. The cardiopulmonary and metabolic responses of the participants to the Fatmax test are given in Table 2. The mean values of the variables in Table 2 (6 min average) were obtained in the stage where the MFO was determined. The number of participants who achieved Fatmax in the 1st, 2nd, and 3rd stages was 22, 9, and 3, respectively. Only a single participant's Fatmax was observed in the 4th stage.

Table 2. Performance characteristics of the participants during the Fatmax test in the stage where the fat oxidation rate was maximal. Values are the 6 min averages of the Fatmax stage and given as the mean ± SEM

Variables	Fatmax stage
Fat oxidation rate, g/min	0.32 ± 0.02
Carbohydrate oxidation rate, g/min	0.66 ± 0.06
Contribution of fat to energy production, %	57.68 ± 2.38
Contribution of carbohydrates to energy production, %	42.32 ± 2.38
Exercise intensity, % $\dot{V}O_{2peak}$	39.81 ± 1.17
Heart rate, bpm	106 ± 3
Respiratory exchange ratio	0.83 ± 0.01
Energy expenditure, kcal/h	330.7 ± 13.6
Speed, km/h	4.51 ± 0.13

The number of participants who underwent the Fatmax test until the 3rd (6 km/h), 4th (7 km/h), and 5th stages (8 km/h) was 3, 13, and 18, respectively. Only a single participant's Fatmax test lasted until the 6th stage (9 km/h). The cardiopulmonary and metabolic responses of the participants for each of the stages are given in Table 3.

The change in $\dot{V}O_2$ values throughout the Fatmax stage was lower than the previously established reference value of 100 mL/min (MacRae et al., 1995). Moreover, the difference between the values in the 6th min and 2nd, 3rd, 4th, and 5th min was significantly lower than 100 mL/min (70.01 ± 15.56, 51.09 ± 8.92, 49.13 ± 6.05, and 36.98 ± 3.62 mL/min for the 2nd, 3rd, 4th, and 5th min, respectively; $p < 0.05$) (Figure 1).

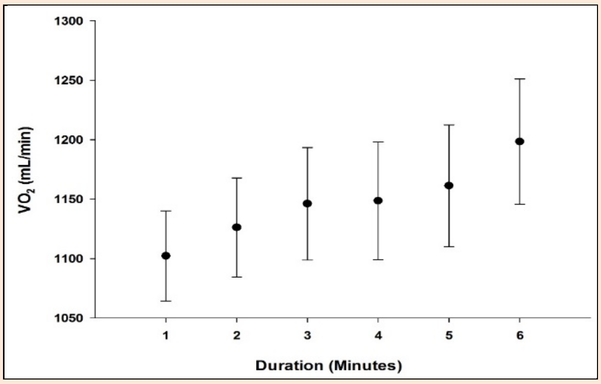

Figure 1. Average $\dot{V}O_2$ values for every min throughout the Fatmax stage.

Similar to $\dot{V}O_2$, the change in $\dot{V}CO_2$ values throughout the Fatmax stage was lower than the reference value of 100 mL/min (MacRae et al., 1995). In addition, the difference between the values in the 6th min and 3rd, 4th, and 5th min was significantly lower than 100 mL/min (71.55 ± 9.15, 61.67 ± 6.51, and 40.36 ± 4.13 mL/min for the 3rd, 4th, and 5th min, respectively; $p < 0.05$) (Figure 2).

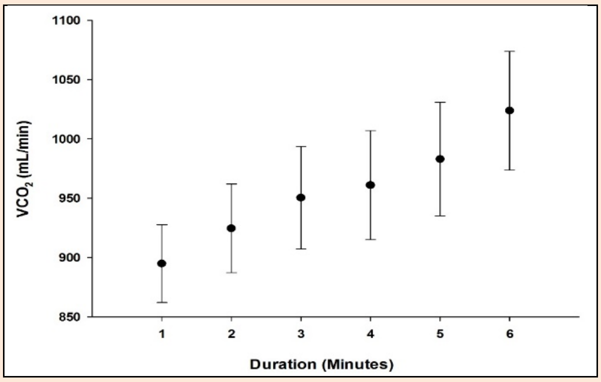

Figure 2. Average $\dot{V}CO_2$ values for every min throughout the Fatmax stage.

Table 3. Metabolic and cardiac responses of the participants for each completed stage. Values are the 6 min averages of each stage and given as the mean ± SEM.

Stage (speed, km/h)	$\dot{V}O_2$ (mL/min)	$\dot{V}CO_2$ (mL/min)	Respiratory exchange ratio	Heart rate (bpm)
1 (4 km/h)	1039.83 ± 26.55	853.98 ± 21.32	0.82 ± 0.01	102 ± 2
2 (5 km/h)	1175.24 ± 30.21	1002.55 ± 25.77	0.85 ± 0.01	107 ± 2
3 (6 km/h)	1459.99 ± 40.34	1314.61 ± 39.91	0.90 ± 0.01	120 ± 2
4 (7 km/h)	1917.92 ± 54.03	1876.26 ± 57.79	0.98 ± 0.01	144 ± 2
5 (8 km/h)	2225.47 ± 63.09	2216.75 ± 64.59	1.00 ± 0.01	158 ± 3
6 (9 km/h)	2916.8	2881.3	0.99	194

In contrast to the consistency of the $\dot{V}O_2$ and $\dot{V}CO_2$ values throughout the Fatmax stage, the change in $\dot{V}E$ was higher than the previously established reference value (MacRae et al., 1995) (Figure 3).

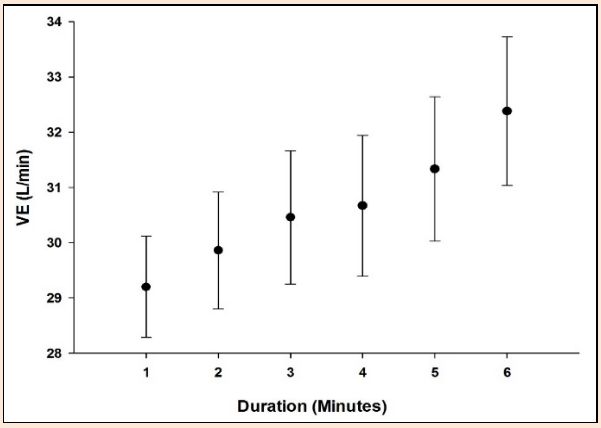

Figure 3. Average $\dot{V}E$ values for every min throughout the Fatmax stage.

The change in the respiratory exchange ratio (RER) throughout the Fatmax stage is shown in Figure 4. The RER was continuously increased from the 1st min (0.81 ± 0.01) to the 6th min (0.85 ± 0.01) (Figure 4).

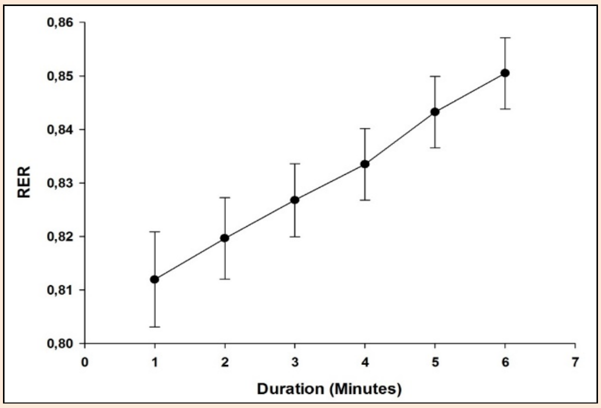

Figure 4. Average respiratory exchange ratio (RER) values for every min throughout the Fatmax stage.

An evaluation of the fat oxidation rate per min revealed that the average value of the 4th min was significantly lower than that of the 2nd and 3rd min (0.31 ± 0.01, 0.34 ± 0.02, and 0.33 ± 0.02 g/min for the 4th, 2nd, and 3rd min fat oxidation rates respectively; $p < 0.01$). In addition, the 5th and 6th min fat oxidation rates were significantly lower than the rates of the 1st, 2nd, 3rd, and 4th min (0.30 ± 0.01 and 0.29 ± 0.01 g/min for the 5th and 6th min, respectively, vs. 0.35 ± 0.02, 0.34 ± 0.02, 0.33 ± 0.02, and 0.31 ± 0.01 g/min for the 1st, 2nd, 3rd, and 4th min, respectively; $p < 0.01$). The difference was approximately 17% between the 1st and 6th min fat oxidation rates (Figure 5).

Most of the participants had MFO rates in the 1st min of the stage (16/35 participants), and the MFO rates of the remaining participants were observed in the 2nd, 3rd, and 4th min (7/35, 4/35, and 8/35 participants, respectively). None of the participants had MFO rates in the 5th or 6th min (Figure 6).

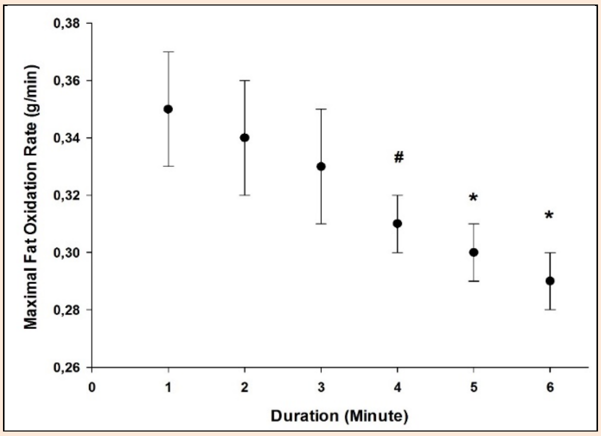

Figure 5. Changes in the maximal fat oxidation (MFO) rate over time. *: significantly lower than the 1st, 2nd, 3rd, and 4th min values ($p < 0.01$) #: significantly lower than the 2nd and 3rd min values ($p < 0.01$).

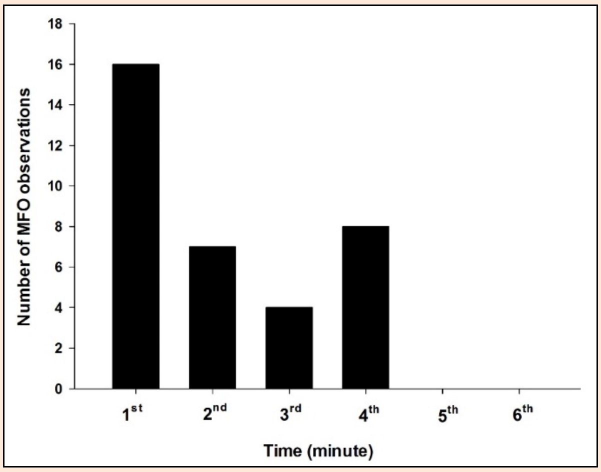

Figure 6. Frequency distribution of the number of participants who reached their individual maximal fat oxidation (MFO) rate with respect to the time in the Fatmax stage.

The MFO rates with respect to the individual maximal values, stage average values (average of 6 min), and last 2 min average values were 0.36 ± 0.02, 0.32 ± 0.02, and 0.30 ± 0.01, respectively. The MFO rate that was calculated from the mean of the measurements taken during the last 2 min of the 6 min stage was significantly lower than that calculated from the individual maximal values ($p < 0.05$) (Figure 7).

Discussion

This study demonstrated that the selection of the last 2 min data for further metabolic analysis in a graded exercise test with 6 min intervals involving young, sedentary, and overweight men could result in the underestimation of the MFO rate. Furthermore, the results showed that participants had MFO rates at different times in the Fatmax stage due to inter-individual variations. Therefore, if the steady state is reached early in the Fatmax test, then it is recommended to select the individual's highest fat oxidation rate rather than the average over a time period.

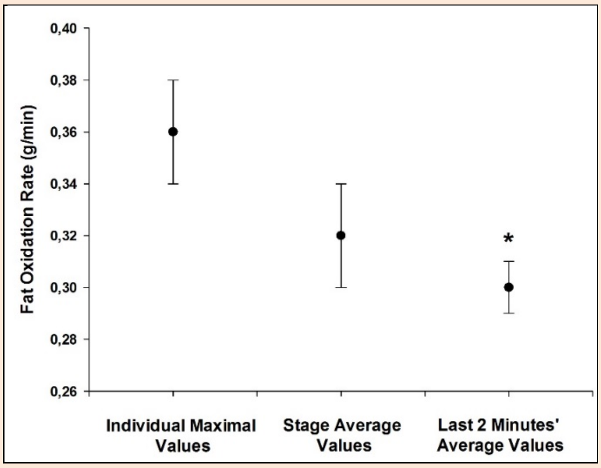

Figure 7. Comparison of fat oxidation rates calculated from the individual maximal, stage average, and last 2 min average values of the 6 min stage. *: significantly different from the individual maximal value ($p < 0.05$).

Changes in gas exchange variables throughout the Fatmax stage

Interestingly, most of the studies that used 6 min intervals have referred to the study by MacRae et al. for their data analysis. MacRae et al. stated a precondition for the measurement of gas exchange variables ($\dot{V}E$, $\dot{V}O_2$, and $\dot{V}CO_2$) during the 5th and 6th min of the graded test, suggesting that $\dot{V}O_2$ and $\dot{V}CO_2$ should vary less than 100 mL/min and that VE should vary less than 0.5 L/min. However, our data revealed that $\dot{V}O_2$ and $\dot{V}CO_2$ had a similar amount of variation not only between the 5th and 6th min but also throughout the whole stage (Figure 1 and 2). On the other hand, the difference in $\dot{V}E$ was well above 0.5 L/min, which was measured as an average of 1–3 L/min throughout the stage (Figure 3). This variation may be attributed to slight changes in the respiratory frequency and tidal volume during exercise. Nevertheless, the selected reference value of 0.5 L/min (variation in the ventilation per min) might be too narrow for graded exercise tests.

MFO intensity and self-determined pace

The participants that were recruited in our study were sedentary, overweight individuals. Their peak oxygen uptake values and MFO rates (35.0 ± 0.7 mL/kg/min and 0.32 ± 0.02 g/min, respectively) were similar to those of other studies (Bogdanis et al., 2008; Nordby et al., 2015). Most of the participants' MFO rates were observed in the first and second stages with an average speed of 4.51 ± 0.13 km/h. A similar observation was made by Chrzanowski-Smith et al. In their study, most of the participants' peak fat oxidation rates were recorded in the first stage of the graded test (Chrzanowski-Smith et al., 2018). In a study by Loftin et al., the energy expenditure on walking a mile for adult normal-weight walkers and overweight walkers was compared. They found that the preferred walking speed for normal weight walkers was 2.94 ± 0.08 mi/h (4.70 ± 0.13 km/h), whereas the preferred walking speed for overweight walkers was 2 .97 ± 0.08 mi/h (4.75 ± 0.13 km/h) (Loftin et al., 2010). In addition, Browning et al. compared the preferred walking speeds of class II obese and normal-weight men and women. Their male normal-weight participants were fitter than our participants (48.6 ± 4.4 mL/min/kg for $\dot{V}O_{2max}$); their preferred speed was 1.41 ± 0.03 m/s (5.08 ± 0.11 km/h), and their speed of minimum energy cost per distance was 1.38 ± 0.01 m/s (4.99 ± 0.04 km/h) (Browning et al., 2005). In a study in which the effect of body weight on the basic spatial and temporal gait measures of healthy lean and obese women walking at their self-selected pace was evaluated, the mean velocity was reported as 1.08 ± 0.20 m/s (3.89 ± 0.72 km/h) for lean women and 1.12 ± 0.20 m/s (4.03 ± 0.72 km/h) for class I obese women (Błaszczyk et al., 2011). Therefore, regardless of gender and physical properties, it is possible to conclude that the preferred walking speed of sedentary individuals is between 4–5 km/h, which is consistent with our results (4.51 ± 0.13 km/h). With increasing workload, the oxidation rate of fat could be reduced. This reduction might begin instantaneously with the application of the load or might occur later in the stage. The walking speed of the subjects who reached their MFO rate may be closely similar to the preferred speed of sedentary individuals (Błaszczyk et al., 2011; Browning et al., 2006; Loftin et al., 2010). Therefore, the participants' MFO rate might decrease when the speed exceeds their metabolically adapted self-preferred speed.

Comparison of three different MFO rates obtained in the Fatmax stage

Previously, investigators have used protocols with different time intervals to calculate substrate oxidation. Although a "short" graded exercise test with 3 min stages is acceptable for the assessment of Fatmax in well-trained athletes (Achten 2002), the interval duration in our study was set at 6 min to ensure that steady states were reached for the gas exchange variables (Dandanell et al., 2016). In our study, the $\dot{V}O_2$ and $\dot{V}CO_2$ values fluctuated in a relatively narrow band in the Fatmax stage. The amount of fluctuation (difference between the end and beginning of the Fatmax stage) was 92.92 ± 23.45 mL/min and 125.51 ± 21.84 mL/min for $\dot{V}O_2$ and $\dot{V}CO_2$, respectively. In addition, although our protocol started from 4 km/h, the participants walked at 3 km/h for 2 min to warm up. Therefore, metabolic adaptation when priming with 3 km/h might be different compared with that when starting from rest. Our participants (young, sedentary, and overweight men) reached a steady state before the last 2 min of the 6 min Fatmax stage.

According to our findings, the MFO rate was decreased throughout the 6 min Fatmax stage (Figure 5). As stated previously, most of the MFO rates were observed in the first 4 min in the Fatmax stage (Figure 6).

When the 1st and 6th min fat oxidation rates were compared, the 1st min rate was 0.35 ± 0.02 g/min, whereas the 6th min rate was 0.29 ± 0.01 g/min. Throughout the stage, both oxygen uptake and carbon dioxide production were increased; however, the increase in $\dot{V}O_2$ was around 8%, whereas $\dot{V}CO_2$ was increased by around 12%. This difference led to the decrease in the MFO rate from the 1st min to the 6th min (Figure 5). Individually determined MFO rates were approximately 17% and 11% higher than the stage average and average values, respectively, calculated using the last 2 min data (Figure 7). Although these three different MFO rate calculation methods gave different results, all values were based on the same stage. In other words, neither of these values changed the prescribed exercise intensity at Fatmax. Nevertheless, a correctly calculated fat oxidation rate is important to elucidate the methodological approaches among different research groups. Furthermore, a correctly calculated MFO rate is vital for evaluating the effect of any kind of intervention at Fatmax. Miscalculations might lead to erroneous conclusions. Our data showed that selecting the average of the last 2 min data, as preferred by different investigators, could risk underestimating the MFO rate when subjects reached the steady state earlier in the Fatmax stage (Bordenave et al., 2008; Isacco et al., 2015; Schwindling et al., 2014) (Figure 7). Different averaging approaches might yield different fat oxidation rates, and gas exchange kinetics should be evaluated before making any decisions.

Conclusion

This study demonstrated that the calculation of the fat oxidation rate by averaging the last portion of the Fatmax stage data may cause the underestimation of the MFO rate, which probably occurs earlier in the Fatmax stage. Furthermore, individual MFO rates might be observed at different times in the Fatmax stage. Therefore, selecting an individual's MFO rate rather than the average over a time period might give better results. The acceptance of an individual's maximal or average fat oxidation rate in the Fatmax stage does not change the prescribed training intensity. Nevertheless, the use of the average of the last 2 min to calculate the fat oxidation rate could risk underestimating the actual MFO rate. The selection of an appropriate fat oxidation rate is important for evaluating pre- and post-training effects.

Acknowledgements

The experiments comply with the current laws of the country in which they were performed. The authors have no conflicts of interests to declare.

References

Achten, J., Gleeson, M. and Jeukendrup, A.E. (2002) Determination of the exercise intensity that elicits maximal fat oxidation. *Medicine and Science in Sports and Exercise* **34**, 92-97.

Alkahtani, S. (2014) Comparing fat oxidation in an exercise test with moderate-intensity interval training. *Journal of Sports Science and Medicine* **13**, 51-58.

Balady, G. J., Arena, R., Sietsema, K., Myers, J., Coke, L., Fletcher, G. F., Forman, D., Franklin, B., Guazzi, M., Gulati, M., Keteyian, S. J., Lavie, C. J., Macko, R., Mancini, D. and Milani, R. V. (2010) Clinician's guide to cardiopulmonary exercise testing in adults: A scientific statement from the American Heart Association. *Circulation* **122**, 191-225.

Besnier, F., Lenclume, V., Gérardin, P., Fianu, A., Martinez, J., Naty, N., Porcherat, S., Boussaid, K., Schneebeli, S., Jarlet, E., Hatia, S., Dalleau, G., Verkindt, C., Brun, J. F., Gonthier, M. P. and Favier, F. (2015) Individualized exercise training at maximal fat oxidation combined with fruit and vegetable-rich diet in overweight or obese women: The LIPOXmax-Réunion randomized controlled trial. *PLoS One* **10**, e0139246.

Błaszczyk, J. W., Plewa, M., Cieślińska-Świder, J., Bacik, B., Zahorska-Markiewicz, B. and Markiewicz, A. (2011) Impact of excess body weight on walking at the preferred speed. *Acta Neurobiologiae Experimentalis*, **71**, 528-540.

Bogdanis, G. C., Vangelakoudi, A. and Maridaki, M. (2008) Peak fat oxidation rate during walking in sedentary overweight men and women. *Journal of Sports Science and Medicine* **7**, 525-531.

Bordenave, S., Flavier, S., Fédou, C., Brun, J. F. and Mercier, J. (2007) Exercise calorimetry in sedentary patients: Procedures based on short 3 min steps underestimate carbohydrate oxidation and overestimate lipid oxidation. *Diabetes & Metabolism* **33**, 379-384.

Bordenave, S., Metz, L., Flavier, S., Lambert, K., Ghanassia, E., Dupuy, A. M., Michel, F., Puech-Cathala, A. M., Raynaud, E., Brun, J. F. and Mercier, J. (2008) Training-induced improvement in lipid oxidation in type 2 diabetes mellitus is related to alterations in muscle mitochondrial activity. Effect of endurance training in type 2 diabetes. *Diabetes & Metabolism* **34**, 162-168.

Borel, B., Coquart, J., Boitel, G., Duhamel, A., Matran, R., Delsart, P., Mounier-Vehier, C. and Garcin, M. (2015) Effects of endurance training at the crossover point in women with metabolic syndrome. *Medicine and Science in Sports and Exercise* **47**, 2380-2388.

Browning, R. C., Baker, E. A., Herron, J. A. and Kram, R. (2005) Effects of obesity and sex on the energetic cost and preferred speed of walking. *Journal of Applied Physiology* **100**, 390-398.

Brun, J. F., Romain, A. J. and Mercier, J. (2011) Lipoxmax ou oxydation maximale des lipides lors de l'exercice: Des mesures physiologiques aux applications cliniques. Réalités et incertitudes. *Science & Sports* **26**, 57-71.

Christensen, E. H. and Hansen, O. (1939) Arbeitsfähigkeit und Ernährung. *Scandinavian Archives of Physiology* **81**, 160-171.

Chrzanowski-Smith, O. J., Edinburgh, R. M., Betts, J. A., Stokes, K. A. and Gonzalez, J. T. (2018) Evaluation of a graded exercise test to determine peak fat oxidation in individuals with low cardiorespiratory fitness. *Applied Physiology Nutrition and Metabolism* **43**, 1288-1297 .

Croci, I., Hickman, I. J., Wood, R. E., Borrani, F., Macdonald, G. A. and Byrne, N. M. (2014) Fat oxidation over a range of exercise intensities: Fitness versus fatness. *Applied Physiology, Nutrition, and Metabolism* **39**, 1352-1359.

Dandanell, S., Boslev Praest, C., Dam Søndergård, S., Skovborg, C., Dela, F., Larsen, S. and Wulff Helge, J. (2016) Determination of the exercise intensity that elicits maximal fat oxidation in individuals with obesity. *Applied Physiology, Nutrition, and Metabolism* **42**, 405-412.

Dandanell, S., Husted, K., Amdisen, S., Vigelsø, A., Dela, F., Larsen, S. and Wulff Helge, J. (2017) Influence of maximal fat oxidation on long-term weight loss maintenance in humans. *Journal of Applied Physiology* **123**, 267-274.

Dumortier, M., Brandou, F., Perez-Martin, A., Fedou, C., Mercier, J. and Brun, J. (2003) Low intensity endurance exercise targeted for lipid oxidation improves body composition and insulin sensitivity in patients with the metabolic syndrome. *Diabetes & Metabolism* **29**, 509-518.

Frayn, K. N. (1983) Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology* **55,** 628–634.

Gmada, N., Marzouki, H., Haboubi, M., Tabka, Z., Shephard, R. J. and Bouhlel, E. (2012) Crossover and maximal fat-oxidation points in sedentary healthy subjects: Methodological issues. *Diabetes & Metabolism* **38**, 40-45.

Haskell, W. L., Lee, I. M., Pate, R. R., Powell, K. E., Blair, S. N., Franklin, B. A., Macera, C. A., Heath, G. W., Thompson, P. D. and Bauman, A. (2007) Physical activity and public health: Updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Medicine and Science in Sports and Exercise* **39**, 1423-1434.

Haufe, S., Engeli, S., Budziarek, P., Utz, W., Schulz-Menger, J., Hermsdorf, M., Wiesner, S., Otto, C., Fuhrmann, J. C., Luft, F. C., Boschmann, M. and Jordan, J. (2010) Determinants of exercise-induced fat oxidation in obese women and men. *Hormone and Metabolic Research* **42**, 215-221.

Isacco, L., Thivel, D., Pereira, B., Duclos, M. and Boisseau, N. (2015) Maximal fat oxidation, but not aerobic capacity, is affected by oral contraceptive use in young healthy women. *European*

Journal of Applied Physiology **115**, 937-945.
Jackson, A. S. and Pollock, M. L. (1978) Generalized equations for predicting body density of men. *British Journal of Nutrition* **40**, 497-504.
Jeukendrup, A. and Achten, J. (2001) Fatmax: A new concept to optimize fat oxidation during exercise? *European Journal of Sport Science* **1**, 1-5.
Jeukendrup, A. E. and Wallis, G. A. (2005) Measurement of substrate oxidation during exercise by means of gas exchange measurements. *International Journal of Sports Medicine, Supplement* **26**, S28-S37.
Loftin, M., Waddell, D. E., Robinson, J. H. and Owens, S. G. (2010) Comparison of energy expenditure to walk or run a mile in adult normal weight and overweight men and women. *Journal of Strength and Conditioning Research*, **24**, 2794-2798.
MacRae, H. S. H., Noakes, T. D. and Dennis, S. C. (1995) Role of decreased carbohydrate oxidation on slower rises in ventilation with increasing exercise intensity after training. *European Journal of Applied Physiology and Occupational Physiology* **71**, 523-529.
Martin, A. D., Spenst, L. F., Drinkwater, D. T. and Clarys, J. P. (1990) Anthropometric estimation of muscle mass in men. *Medicine and Science in Sports and Exercise* **22**, 729-733.
Marzouki, H., Farhani, Z., Gmada, N., Tabka, Z., Shephard, R. J. and Bouhlel, E. (2014) Relative and absolute reliability of the crossover and maximum fat oxidation points during treadmill running. *Science & Sports* 29, e107-e114.
Maunder, E., Plews, D. J. and Kilding, A. E. (2018) Contextualising maximal fat oxidation during exercise: Determinants and normative values. *Frontiers in Physiology* **9**, 1–13.
Meyer, T., Gäßler, N. and Kindermann, W. (2007) Determination of "Fat$_{max}$" with 1 h cycling protocols of constant load. *Applied Physiology, Nutrition, and Metabolism* **32**, 249-256.
Nordby, P., Rosenkilde, M., Ploug, T., Westh, K., Feigh, M., Nielsen, N. B., Helge, J. W. and Stallknecht, B. (2015) Independent effects of endurance training and weight loss on peak fat oxidation in moderately overweight men: A randomized controlled trial. *Journal of Applied Physiology* **118**, 803-810.
Perez-Martin, A., Dumortier, M., Raynaud, E., Brun, F., Bringer, J., Fedou, C., Bringer, J. and Mercier, J. (2001) Submaximal exercise in lean and obese people. *Diabetes & Metabolism* **27**, 466-474.
Purdom, T., Kravitz, L., Dokladny, K. and Mermier, C. (2018) Understanding the factors that effect maximal fat oxidation. *Journal of the International Society of Sports Nutrition* **15**, 1–10.
Randell, R. K., Rollo, I., Roberts, T. J., Dalrymple, K. J., Jeukendrup, A. E. and Carter, J. M. (2017) Maximal fat oxidation rates in an athletic population. *Medicine and Science in Sports and Exercise* **49**, 133-140.
Rosenkilde, M., Reichkendler, M. H., Auerbach, P., Bonne, T. C., Sjödin, A., Ploug, T. and Stallknech, B. M. (2015) Changes in peak fat oxidation in response to different doses of endurance training. *Scandinavian Journal of Medicine & Science in Sports* **25**, 41-52.
Schwindling, S., Scharhag-Rosenberger, F., Kindermann, W. and Meyer, T. (2014) Limited benefit of Fatmax-test to derive training prescriptions. *International Journal of Sports Medicine* **35**, 280–285.
Siri, W. (1961) Body composition from fluid space and density. In: *Techniques for measuring body composition*. Eds. Brožek, J. and Henschel, A. 1st edition. National Academy of Sciences—National Research Council. 223-244.
Tan, S., Wang, J., Cao, L., Guo, Z. and Wang, Y. (2016) Positive effect of exercise training at maximal fat oxidation intensity on body composition and lipid metabolism in overweight middle-aged women. *Clinical Physiology and Functional Imaging* **36**, 225-230.

Key points

- Taking the average of the last 2 min to calculate the fat oxidation rate could risk underestimating the actual MFO rate.
- Individual maximal fat oxidation rates might be observed at different times in the Fatmax stage.
- Selecting an individual's maximal fat oxidation rate rather than the average over a time period might give better results.

AUTHOR BIOGRAPHY

Kerem Tuncay ÖZGÜNEN
Employment
Çukurova University, Medical Faculty, Department of Physiology, Division of Sports Physiology, Adana, Turkey
Degree
PhD
Research interest
Exercise physiology, biological signals, exercise testing and prescription
E-mail: kozgunen@gmail.com

Çiğdem ÖZDEMİR
Employment
Çukurova University, Medical Faculty, Department of Physiology, Division of Sports Physiology, Adana, Turkey
Degree
MD
Research interest
Substrat oxidation and obesity, isokinetic exercise, sports injuries
E-mail: cozdemir@cu.edu.tr

Selcen KORKMAZ-ERYILMAZ
Employment
Cukurova University, School of Physical Education and Sports, Department of Coaching Education, Adana, Turkey
Degree
PhD
Research interest
Exercise physiology, strength and conditioning, exercise testing, sport performance, physical training and adaptations
E-mail: selcen_korkmaz@yahoo.com

Abdullah KILCI
Employment
Research Assistant at Cukurova University, School of Physical Education and Sports, Adana, Turkey
Degree
PhD Student
Research interest
Exercise Physiology, Soccer, Training Sciences, Substrate Oxidation
E-mail: abdullahkilci89@gmail.com

	**Özgür GÜNAŞTI** **Employment** Research asistant at Çukurova University, Medical Faculty, Department of Physiology, Division of Sports Physiology, Adana, Turkey **Degree** MD, PhD **Research interest** Exercise physiology, obesity, exercise prescription, sports injuries **E-mail:** ogunasti@gmail.com
	**Sanlı Sadi KURDAK** **Employment** Çukurova University, Medical Faculty, Department of Physiology, Division of Sports Physiology, Adana, Turkey **Degree** MD **Research interest** Exercise physiology, obesity, exercise prescription, sports injuries **E-mail:** sskurdak@cu.edu.tr

✉ **Kerem Tuncay Özgünen**
Çukurova University, Medical Faculty, Department of Physiology, Division of Sports Physiology**,** Adana, Turkey