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Maximal Fat Oxidation During Exercise in Trained Men

Abstract

Fat oxidation increases from low to moderate exercise intensities and decreases from moderate to high exercise intensities. Recently, a protocol has been developed to determine the exercise intensity, which elicits maximal fat oxidation rates (Fat_{max}). The main aim of the present study was to establish the reliability of the estimation of Fat_{max} using this protocol ($n = 10$). An additional aim was to determine Fat_{max} in a large group of endurance-trained individuals ($n = 55$). For the assessment of reliability, subjects performed three graded exercise tests to exhaustion on a cycle ergometer. Tests were performed after an overnight fast and diet and exercise regime on the day before all tests were similar. Fifty-five male subjects performed the graded exercise test on one occasion. The typical error (root mean square error and CV) for Fat_{max} and Fat_{min} was 0.23 and 0.33 l $O_2 \times min^{-1}$ and 9.6 and 9.4% respectively. Maximal fat oxidation rates of 0.52 ± 0.15 g × min^{-1} were reached at 62.5 ± 9.8% $\dot{V}O_2max$, while Fat_{min} was located at 86.1 ± 6.8% $\dot{V}O_2max$. When the subjects were divided in two groups according to their $\dot{V}O_2max$, the large spread in Fat_{max} and maximal fat oxidation rates remained present. The CV of the estimation of Fat_{max} and Fat_{min} is 9.0 – 9.5%. In the present study the average intensity of maximal fat oxidation was located at 63% $\dot{V}O_2max$. Even within a homogenous group of subjects, there was a relatively large inter-individual variation in Fat_{max} and the rate of maximal fat oxidation.

Key words

Reproducibility · typical error · cycling · indirect calorimetry · exercise intensity

Introduction

It has been known for a long time that exercise intensity is one of the main factors determining the rate of fat oxidation during exercise. Romijn et al. [19] were one of several research groups to show that fat oxidation is lower at a low intensity (25% maximal oxygen uptake ($\dot{V}O_2max$)) compared to a moderate intensity (65% $\dot{V}O_2max$) and is lower again at a high exercise intensity (85% $\dot{V}O_2max$). This implies that there is an intensity at which individuals have maximal fat oxidation rates.

It has been suggested that high fat oxidation rates can be beneficial for a large variety of individuals. Treatments which prevent conditions like overweight and obesity, are of considerable interest both to the general public and health-care professionals [24]. In addition, it has been shown in athletes that after endurance training, fat oxidation at a given intensity is increased which coincides with increases in performance [10,11]. These observations indicate that the ability to oxidise fatty acids is related to improved performance and that the intensity which elicits maximal fat oxidation can potentially be very important.

Until recently, no studies had been performed to determine the intensity at which maximal fat oxidation is reached systematically and accurately. Most studies only investigated three [19,21,25] or four [3] different intensities. In a series of studies in our laboratory, a test has been developed to determine the exercise intensity which elicits maximal fat oxidation (Fat_{max}) [1]. This Fat_{max} protocol consisted of a graded exercise test to exhaustion on a cycle ergometer, starting at 95 W with 35 W increments every 3 min during which gas exchange measurements were performed. The validity of the protocol was tested

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by comparing fat oxidation rates obtained during the graded exercise test with fat oxidation rates acquired during separate exercise trials performed at similar intensities to those used in the Fat_{max} protocol. Furthermore, this protocol was compared to two other graded exercise tests with different stage durations (3 and 5 min) and work rate increments (20 and 35 W). Since these three protocols provided similar results, for practical reasons the protocol with the shortest duration was chosen. With this protocol maximal fat oxidation rates were found at an average intensity of 64 ± 4% $\dot{V}O_2max$ in 11 moderately trained subjects.

Reproducibility or within-subject variation of a variable appears to be the most important type of reliability measure for researchers. This type of variation will affect the precision of estimates of change in the variable of an experimental study. In other words, when the aim of a study is to determine Fat_{max} before and after an intervention (i.e. after a period of high-fat diet or training) it is important to know how much Fat_{max} varies from day to day. Until now, the within-subject variation has not been established for the estimation of Fat_{max} and Fat_{min} (intensity at which RER = 1.0). Therefore, the first purpose of this study was to determine the reproducibility of the estimation of Fat_{max} and Fat_{min} using the Fat_{max} protocol. An additional purpose of the present study was to determine Fat_{max}, Fat_{min} and the Fat_{max}zone using the Fat_{max}-protocol in a larger group of trained cyclists and triathletes with varying levels of fitness.

Material and Methods

Subjects

Sixty-five healthy, moderately trained men participated in this study, which was approved by the South Birmingham Local Research Ethics Committee, UK. Each volunteer gave his written informed consent after explanations of the experimental procedures, and possible risks and benefits. The subjects ranged from club/county standard to elite endurance cyclists with a training background of at least 3 years.

General design

Ten subjects (age 24 ± 6 y; body mass 71.5 ± 2.2 kg; $\dot{V}O_2max$ 60.1 ± 0.3 ml × kg^{-1} × min^{-1}; body fat 14.8 ± 0.9%) performed the same graded exercise test to exhaustion on a cycle ergometer (Lode, Groningen, The Netherlands) on three different occasions. The results of these tests were used to determine the exercise intensity that elicits maximal fat oxidation, the intensity above which fat oxidation becomes negligible and an intensity zone between which fat oxidation remains within 10% of the peak rate.

Furthermore, 55 additional subjects (age 26 ± 8 y; body mass 75.3 ± 0.9 kg; $\dot{V}O_2max$ 64.2 ± 9.0 ml × kg^{-1} × min^{-1}; body fat 12.3 ± 1.0%) performed the graded exercise test to exhaustion on a cycle ergometer once; the results of this test were used to measure fat oxidation over a wide range of intensities for each subject and to determine Fat_{max} and Fat_{min}.

Experimental design

$\dot{V}O_2max$, maximal work rate (Wmax) and variables related to maximal fat oxidation were determined in 55 subjects using a graded exercise test to exhaustion. A further 10 subjects performed the test on three different occasions. The validity of the cycle ergometer protocol has been tested by comparing the fat oxidation rates during each stage of the graded exercise test with fat oxidation rates determined when subjects cycled on each of the intensities on a separate day for a prolonged period of time. It was concluded that the stage duration of three minutes as well as the incremental nature of the test did not affect the maximal rate of fat oxidation or the determination of the intensity at which this occurred [1]. Experiments were always performed in the morning (start of exercise between 8 and 10 am) and under similar environmental conditions (19 °C and 55% relative humidity). The subjects performing the graded exercise test on three occasions were asked to attend the laboratory at the same time of day to avoid circadian variance. All subjects were advised to avoid strenuous exercise the day before the test and to consume a diet high in carbohydrates (CHO) (examples of high-CHO foods were provided to the subjects). The subjects attending the lab on 3 occasions were asked to record their diet the day before the first trial and to repeat this diet before the two subsequent tests. Dietary analysis showed that on average the ten subjects ingested 3497 ± 193 kcal, with 62 ± 4% of the energy coming from CHO, 24 ± 5% from fat and 15 ± 1% from protein. Total CHO intake was 513 ± 50 g, including 33% simple sugars and 67% starch. No significant differences were found between the diets consumed before the first trial and the two subsequent trials.

Subjects reported to the laboratory after a 10 – 12-h overnight fast and body mass and height were determined. Body fat was estimated from skin fold thickness measurements at 4 sites according to the methods of Durnin and Womersley [6]. Subjects started cycling at 95 W and the work rate was increased by 35 W every 3 min until exhaustion. Both position on the bike and cadence was kept similar during all three trials. Heart rate (HR) was recorded continuously during the test using a heart rate monitor (Polar Vantage NV, Polar Electro Oy, Kempele, Finland). Breath-by-breath measurements were performed throughout exercise using an on-line gas analysis system (Oxycon Alpha, Jaeger, Wuerzberg, Germany). The volume and gas analysers of the system were calibrated using a 3 litre calibration pump and calibration gas (15.12% O_2; 5.10% CO_2), respectively. The validity and reliability of the Oxycon Alpha was recently determined using a portable metabolic simulator (Jaeger, Wuerzberg, Germany) [4]. It was found that values for oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and Respiratory Exchange Ratio (RER) were similar to the simulator over a large range of ventilation rates. The coefficient of variation (CV) for $\dot{V}O_2$, $\dot{V}CO_2$ and RER was 1.9 ± 06, 1.3 ± 0.5 and 0.9 ± 0.3%, respectively.

Wmax was calculated from the last completed work rate, plus the fraction of time spent in the final non-completed work rate multiplied by the work rate increment [16]. $\dot{V}O_2$ was considered to be maximal when at least two of the following three criteria were met: 1) a levelling off of $\dot{V}O_2$ with increasing work rate (increase of no more than 2 ml × kg^{-1} × min^{-1}) [23], 2) a HR within 10 beats × min^{-1} of the predicted maximum (220 beats × min^{-1} minus age), 3) RER >1.05. $\dot{V}O_2max$ was calculated as the average oxygen uptake over the last 60 s of the test.

Table **1** Average values, typical error with confidence limits and coefficient of variation for $\dot{V}O_2$, $\dot{V}CO_2$, RER and HR during first 6 stages of the Fat_{max}-protocol

		95 W	***130 W***	***165 W***	***200 W***	***235 W***	***270 W***
$\dot{V}O_2$	AV	1.85	2.20	2.55	2.93	3.34	3.74
	RMSE (conf lim)	0.10 (0.08 – 0.15)	0.11 (0.08 – 0.16)	0.08 (0.06 – 0.12)	0.10 (0.08 – 0.15)	0.10 (0.08 – 0.15)	0.10 (0.07 – 0.14)
	CV	5.4	5.0	3.2	3.4	3.0	2.7
$\dot{V}CO_2$	AV	1.57	1.95	2.33	2.80	3.30	3.86
	RMSE (conf lim)	0.10 (0.08 – 0.15)	0.12 (0.09 – 0.18)	0.13 (0.10 – 0.19)	0.10 (0.08 – 0.14)	0.11 (0.09 – 0.17)	0.09 (0.07 – 0.14)
	CV	6.4	6.2	5.6	3.6	3.0	2.3
RER	AV	0.849	0.887	0.917	0.953	0.987	1.032
	RMSE (conf lim)	0.038 (0.027 – 0.057)	0.032 (0.025 – 0.048)	0.031 (0.023 – 0.046)	0.021 (0.016 – 0.032)	0.026 (0.020 – 0.039)	0.027(0.020 – 0.039)
	CV	4.5	3.6	3.4	2.2	2.6	2.6
HR	AV	104.8	116.5	128.3	141.1	153.7	165.3
	RMSE (conf lim)	5.1 (3.9 – 7.5)	3.84 (2.9 – 5.7)	6.85 (5.2 – 10.1)	5.54 (4.2 – 8.2)	5.1 (3.9 – 7.5)	4.1 (3.1 – 6.0)
	CV	4.9	3.3	5.3	3.9	3.3	2.5

$\dot{V}O_2$ = oxygen uptake ($l \times min^{-1}$), $\dot{V}CO_2$ = carbon dioxide production ($l \times min^{-1}$), RER = respiratory exchange ratio, HR = heart rate (beats × min^{-1}), AV = Overall mean value, RMSE = root mean square error (95 % Confidence limits), CV = Coefficient of variation.

Indirect calorimetry and calculations

Values for $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated over the last 2 min of every stage. Fat and CHO oxidation and energy expenditure were calculated using stoichiometric equations [7] and appropriate energy equivalents, with the assumption that the urinary nitrogen excretion rate was negligible:

Fat oxidation = $1.67 \times \dot{V}O_2 - 1.67 \times \dot{V}CO_2$

CHO oxidation = $4.55 \times \dot{V}CO_2 - 3.21 \times \dot{V}O_2$

The stoichiometric calculations used with indirect calorimetry are based on the assumption that all the CO_2 originates from the oxidation of protein, fat and CHO. However, $\dot{V}O_2$will only be a reliable estimate of tissue CO_2 production in the presence of a stable bicarbonate pool. One could argue that at higher exercise intensities, a shift in the bicarbonate pool could affect the results of the stoichiometric equations and therefore the determination of fat oxidation rates. Romijn et al. [20] tested the validity of indirect calorimetry at a high intensity by comparing it to a breath 13C/12C ratio method with which fat and CHO oxidation can be calculated independent of $\dot{V}CO_2$ measurements. It was shown that the oxidation rates of fat and carbohydrate were the same when using the breath 13C/12C ratio method and indirect calorimetry at 80 – 85 % $\dot{V}O_2$max. The authors concluded that indirect calorimetry can be used to validly determine fuel oxidation even up to intensities of 80 – 85 % $\dot{V}O_2$max [20].

For each individual, the results of the Fat_{max} protocol were used to construct a curve of fat oxidation rate versus exercise intensity, the later expressed as $\dot{V}O_2$ and HR. The curve was used to determine the following variables:

- Fat_{max}: The exercise intensity at which the highest rate of fat oxidation was observed.
- Fat_{min}: The exercise intensity where the fat oxidation rate became negligible (i. e. where RER = 1.0).
- Fat_{max}zone: range of exercise intensities with fat oxidation rates within 10 % of the fat oxidation rates at Fat_{max}.

If the economy of the subjects during a stage of the Fat_{max}-test was below an arbitrary 3.35 $kJ \times l^{-1}$ (average economy of graded exercise-tests minus 2 times the standard deviation), the fat oxidation rates at that stage were not taken into consideration for the Fat_{max} determination [1].

To quantify Fat_{max}, the results of the graded exercise tests were used to compose an average fat oxidation curve. The specific points on the graph were determined for each individual. In addition to the exercise intensity at Fat_{max} and Fat_{min}, exercise intensities for fat oxidation rates 5, 10 and 20 % below the peak rate were determined for each subject.

Statistical analysis

During analysis, all data were checked for non-uniformity of errors. The reliability of the estimation of Fat_{max} and Fat_{min} was determined using a general linear model with subjects and trials as effects and with estimation by analysis of variance. The root mean square error (expressed as absolute value and as a percentage of the mean value) with precision of estimation represented by 95 % confidence limits, served as an indication of the typical error of the measurement [12]. The typical error for $\dot{V}O_2$, $\dot{V}CO_2$, RER and HR were also calculated for each stage of the Fat_{max} protocol.

A general linear model for repeated measures was used to compare mean $\dot{V}O_2$, $\dot{V}CO_2$ and RER and their typical error at different work rates and a Tukey post-hoc test was used to locate the differences. An unpaired t-test was used to compare the maximal rate of fat oxidation between the low and high $\dot{V}O_2$max group. Pearson's correlation coefficient was calculated to study the relation between Fat_{max}or the maximal fat oxidation rate with body mass, body mass index (BMI) and body fat percentage. Values are expressed as mean ± standard deviation unless stated otherwise.

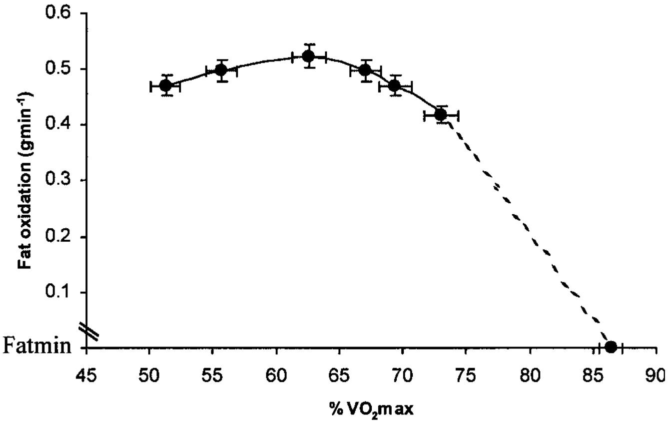

Fig. 1 Fat oxidation rates versus exercise intensity expressed as a percentage of $\dot{V}O_2$max; n = 55; values are mean ± SEM.

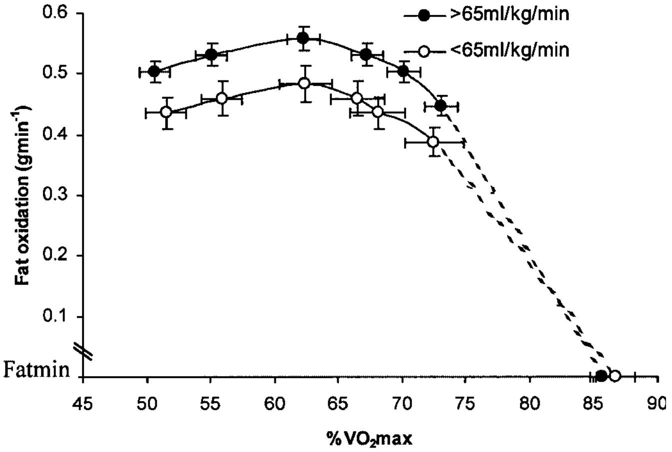

Fig. 2 Fat oxidation rates versus exercise intensity expressed as a percentage of $\dot{V}O_2$max for a low and high $\dot{V}O_2$max group; Low (n = 26), high (n = 27); values are mean ±SEM.

Results

The mean, typical error and corresponding 95 % confidence limits for $\dot{V}O_2$, $\dot{V}CO_2$, RER and HR during the first 6 stages of the three graded exercise tests are displayed in Table **1**. The absolute typical error for all the variables did not change with increasing exercise intensity. When the typical error was expressed as a percentage of the mean, however, a significant decrease was observed from 95 to 270 W ($p < 0.05$). The average CV for $\dot{V}O_2$, $\dot{V}CO_2$, and RER during the graded exercise test was 3.7 %, 4.5 % and 3.2 %, respectively. No order effects were detected for $\dot{V}O_2$, $\dot{V}CO_2$, RER or HR.

In the present group of subjects, Fat_{max} and Fat_{min} were found at a $\dot{V}O_2$ of 2.39 ± 0.35 (56 ± 8 % $\dot{V}O_2$max) and 3.52 ± 0.55 l × min^{-1} (81 ± 9 % $\dot{V}O_2$max). Typical error with 95 % confidence limits for Fat_{max} estimation was 0.23 (0.17 – 0.34) l × min^{-1} and 0.33 (0.25 – 0.49) l × min^{-1} for Fat_{min} estimation. The CV for Fat_{max} and Fat_{min} estimation was 9.6 and 9.4 %. The HR corresponding to Fat_{max} and Fat_{min} was 122 ± 13 and 160 ± 17 beats × min^{-1}. The typical error when HR was used to estimate the intensity of Fat_{max} and Fat_{min} was 9 (7 – 14) and 11 (8 – 16) beats × min^{-1}.

Fig. **1** shows the relationship between fat oxidation rate and exercise intensity, expressed as a percentage of $\dot{V}O_2$max, in moderately to highly trained subjects. With increasing exercise intensities, the fat oxidation rate increased to 0.52 ± 0.15 g × min^{-1} at 62.5 ± 9.8 % $\dot{V}O_2$max beyond which the oxidation rate decreased. The Fat_{max}zone was located between 51.3 ± 8.7 and 69.4 ± 9.5 % $\dot{V}O_2$max, which corresponds to 66.0 ± 13.6 and 78.5 ± 7.7 % HRmax (44 ± 2 and 69 ± 2 % Wmax). It should be noted that some subjects do not reach their actual maximal heart rate during a laboratory based test, which could mean that in the field the Fat_{max}zone percentages are slightly lower than presented here. Fat oxidation rates became negligible from 86.4 ± 6.8 % $\dot{V}O_2$max onwards. The RER was on average 0.89 ± 0.07 at Fat_{max}. At the intensity where absolute fat oxidation rates were maximal, the relative contribution of fat oxidation to energy expenditure was 31.9 ± 1.6 %.

The average $\dot{V}O_2$max for the entire group of subjects was 65.4 ± 9.0 ml × kg^{-1} × min^{-1}. Even though all subjects in the present study were trained triathletes or cyclists, the spread of $\dot{V}O_2$max values was fairly wide. To determine whether the level of fitness in this group of trained athletes was related to the location of Fat_{max} or the maximal rate of fat oxidation, subjects were divided in two groups according their $\dot{V}O_2$max. The subjects were divided in a group with $\dot{V}O_2$max values below 65 ml × kg^{-1} × min^{-1} and one with values above. The average $\dot{V}O_2$max of the low $\dot{V}O_2$max group (n = 26) was 58.6 ± 5.2 ml × kg^{-1} × min^{-1} and 71.9 ± 6.1 ml × kg^{-1} × min^{-1} for the high $\dot{V}O_2$max group (n = 27). Fat_{max} was located at 62.5 ± 10.4 % $\dot{V}O_2$max and 62.3 ± 9.6 % $\dot{V}O_2$max in the low and high $\dot{V}O_2$max group, respectively (Fig. **2**). The maximal fat oxidation rate in the low $\dot{V}O_2$max group was significantly lower than the fat oxidation rate in the high $\dot{V}O_2$max group (0.48 ± 0.15 vs. 0.56 ± 0.14 g × min^{-1}, respectively, $p < 0.05$). The relatively large standard deviations indicate that even within the different subgroups of subjects, there is still a wide spread of maximal rates of fat oxidation. Body mass (74.6 ± 1.1 vs. 71.5 ± 1.1 kg), BMI (22.5 ± 0.4 vs. 21.5 ± 0.3 kg/m^2) and percentage body fat (14.7 ± 0.9 vs. 12.1 ± 0.9 %) were not significantly different between low and high $\dot{V}O_2$max group. Fat_{max} and the maximal fat oxidation rates were not significantly correlated with any of these variables. The maximal fat oxidation rate was, however, significantly correlated with $\dot{V}O_2$max ($r = 0.636$; $p < 0.01$).

Discussion

Recently, a protocol was developed to determine the intensity which elicits maximal fat oxidation (Fat_{max}) [1]. It was found that a cycle-ergometer protocol starting at 95 W with 35 W increments every 3 min can be used to determine Fat_{max}. The main aim of the present study was to determine the reliability of the determination of Fat_{max} using the Fat_{max} protocol [1]. The CV for Fat_{max} and Fat_{min} was 9.6 % and 9.4 %, respectively. When the intensity was expressed as HR, it became apparent that the typical error of the estimation of Fat_{max} and Fat_{min} was approximately 10 beats × min^{-1}. These findings have implications when, for instance, Fat_{max} is used as an outcome variable in an intervention study. The smallest change in Fat_{max}, which can be detected when using a relatively small number of subjects, would be a change from 122 to 132 beats × min^{-1} (mean value + 10 beats × min^{-1}).

Compared to the CV of other physiological variables (typical CV for $\dot{V}O_2$ and $\dot{V}CO_2$ at a given workload are ~4 – 5 % [2,4]), the CV found for Fat_{max} and Fat_{min} is markedly higher. Carter and Jeukendrup [4] have recently determined the CV of $\dot{V}O_2$, $\dot{V}CO_2$ and RER at 100 and 150 W. At 150 W, the CV was 4.5 ± 1.3 %, 5.3 ± 2.0 % and 4.3 ± 1.6 % for these variables. These findings are consistent with the CV for $\dot{V}O_2$, $\dot{V}CO_2$ and RER found in the present study (3.7 %, 4.5 % and 3.2 %, respectively). In the present study, the CV for $\dot{V}O_2$, $\dot{V}CO_2$ and RER decreased significantly when the intensity was increased from 95 W to 270 W. It is likely that at higher exercise intensities, the influence of factors, which can alter $\dot{V}O_2$ and $\dot{V}CO_2$, like small changes in position of the body or cadence, is less important than at the low intensities. While the majority of the variance is due to biological variation, a small part is caused by the equipment used. It was shown by Carter and Jeukendrup [4] that the average CV for $\dot{V}O_2$ and $\dot{V}CO_2$ measured on the gas analysis system used in this study (Oxycon Alpha) using a portable metabolic simulator, thereby excluding any biological error, was 1.9 ± 0.6 and 1.3 ± 0.5 %.

The second goal of the present study was to determine at which intensity trained cyclists/triathletes have maximal fat oxidation. On average, Fat_{max} was located at 62.5 ± 9.8 % $\dot{V}O_2$max, which corresponded to 73.0 ± 6.8 % HRmax. These findings are similar to the values found in a previous study in which Fat_{max} in 11 moderately trained individuals was observed at 64 ± 13 % $\dot{V}O_2$max [1]. In these studies Fat_{max} was determined over a wide range of intensities, whereas most other studies only measured fat oxidation at 3 – 4 exercise intensities [3,19,21,25]. Nevertheless, the findings of the current study are in line with most of those studies. Romijn et al. [19] investigated fat oxidation rates in 5 trained males at 3 different intensities and reported highest fat oxidation rates at 65 % $\dot{V}O_2$max. Van Loon et al. [25] also studied three intensities (44, 57 and 72 % $\dot{V}O_2$max) and found the highest rates of fat oxidation at 57 % $\dot{V}O_2$max.

It is believed that part of the reduction in fat oxidation at high exercise intensities is caused by a reduced availability of fatty acids. Romijn et al. [19] showed that although the lipolytic rate at 85 % $\dot{V}O_2$max is equal to the rate seen at 65 % $\dot{V}O_2$max, the rate of appearance of fatty acids at the high intensity is significantly lower than at 65 %. The decreased Ra of fatty acids has been attributed to a reduction in adipose tissue blood flow. In addition to the reduced fatty acids availability, an increase in the glycolytic flux has been suggested to induce intramuscular changes which will affect fat oxidation. A decreased muscle pH, as a result of increased proton release during anaerobic glycolysis, has been brought forward as a possible mechanism explaining the decreased fat oxidation [22]. Decreased carnitine availability due to an increased acetylation of carnitine [25] or a malonyl-CoA associated decrease in the activity of CPT-I [18] have been brought forward as possible additional mechanisms. The potential mechanisms determining fat oxidation rates during different exercise intensities have been discussed in more detail by Jeukendrup [15].

The results of the present study illustrate that even in a fairly homogenous group of subjects, there is a large inter-individual variation of Fat_{max}. Maximal rates of fat oxidation were observed at 50 % $\dot{V}O_2$max in some athletes, while others could cycle until intensities higher than 85 % $\dot{V}O_2$max before any decrease in fat oxidation occurred. This large inter-individual variation in the intensity of Fat_{max} and Fat_{min} makes it difficult to extrapolate the data of this group to individuals. For example, if the present group of subjects was asked to cycle at Fat_{max} average (~63 % $\dot{V}O_2$max), 67 % of the subjects in this study would be exercising within their own Fat_{max}zone. This means that approximately 33 % (19 subjects) would be performing exercise at an intensity, which induces fat oxidation rates at least 10 % below their maximum rate.

By correlating Fat_{max} with other variables measured in the subjects of the present study, possible factors influencing Fat_{max} could be detected. However, when Fat_{max} was correlated with body mass, BMI, fat percentage or $\dot{V}O_2$max no significant correlations were found. It was found that when the subjects were divided in a low and high $\dot{V}O_2$max group, the low $\dot{V}O_2$max group had maximal fat oxidation at the same relative intensity as the high $\dot{V}O_2$max group (see Fig. **2**). This in contrast with the results from Bergman and Brooks [3] who found that the highest rates of fat oxidation were observed at 40 % $\dot{V}O_2$max in trained (fat oxidation rates 0.25, 0.41, 0.31 and 0.09 g × min^{-1}) and 59 % $\dot{V}O_2$max in untrained subjects (fat oxidation rates 0.12, 0.20, 0.27, 0.06 g × min^{-1}). Although there is no clear explanation for this difference in the outcome of the two studies, this may be partly explained by the relatively small number of subjects in the study by Bergman and Brooks [3]. They compared 7 trained and 7 untrained subjects, while both groups in the present study contained more than 25 subjects. It must also be noted that in the present study we used trained subjects only (even though there was a wide range in $\dot{V}O_2$max values) whereas the study by Bergman and Brooks [3] compared trained ($\dot{V}O_2$max: 58 ± 2 ml × kg^{-1} × min^{-1}) and untrained ($\dot{V}O_2$max: 39 ± 2 ml × kg^{-1} × min^{-1}) subjects. As far as we are aware, there are no other studies that have investigated Fat_{max} in groups with different $\dot{V}O_2$max values.

In addition to the large inter-individual variation in Fat_{max}, the maximal rate of fat oxidation is also very variable between subjects. While some individuals had maximal fat oxidation rates of 0.23 g × min^{-1}, others were able to oxidize fatty acids at 4 times that rate (0.91 g × min^{-1}). These findings are consistent with data from previously performed studies. Goedecke and colleagues [9] studied substrate utilization in 61 trained male and female cyclists at 25, 50 and 70 % Wmax. On these three intensities the RER ranged from 0.78 to 0.94, 0.82 to 0.98 and 0.88 to 1.06. The large ranges in RER reflect the degree of variation in the rate of fat and CHO oxidation between subjects. The high standard deviation of RER at a relative exercise intensity of fairly homogenous subject groups reported in other studies, also indicates that there is much variation in substrate utilization between subjects [13,14,17]. In the study by Goedecke et al. [9] the diet the days before the experimental trials varied markedly and it is likely that these changes in habitual diet at least partly explain the observed variation in RER. Also in the present study, differences in diets between subjects could be responsible for some of the variation in the maximal rate of fat oxidation. Subjects were instructed to consume a diet high in CHO and examples of CHOrich food products were provided. It was shown that in the small subgroup of subjects that performed three trials, these guidelines lead to an ingestion of 7.2 ± 0.7 g CHO × day^{-1}. Even though it is likely that the subjects will have consumed diets with fairly similar compositions, it has been shown that even small changes can influence RER [5]. However, we expect that in this study some but not all of the variations in fat oxidation can be explained by diet.

When the maximal rate of fat oxidation was correlated with body mass, BMI or fat percentage no significant correlations were found. There was, however, a positive correlation between the maximal rate of fat oxidation and $\dot{V}O_2$max. The relationship between maximal fat oxidation rate and $\dot{V}O_2$max was further confirmed when the subject pool was divided into two groups and the group of subjects with the highest $\dot{V}O_2$max values had significantly higher fat oxidation rates than the group with the low $\dot{V}O_2$max values. This finding is in accordance with both cross-sectional [14] and longitudinal [8,17] studies which have looked at the relationship between an individuals $\dot{V}O_2$max and substrate metabolism. In 1987, Jansson and Kaijser [14] studied 5 endurance-trained and 5 untrained volunteers during 60 min of cycling exercise eliciting 65 % $\dot{V}O_2$max. During the last 15 min of exercise, fat oxidation rates were 0.84 g × min^{-1} in the trained and 0.33 g × min^{-1} in the untrained group. The finding that at the same relative intensity trained individuals (i. e. individuals with a higher $\dot{V}O_2$max) have greater rates of fatty acid oxidation, can be explained by the fact that the trained individuals are exercising at a higher absolute work rate. In the present study, the subjects in the low $\dot{V}O_2$max group consumed 36.5 ± 6.9 ml × min^{-1} × kg^{-1} at Fat$_{max}$, while the subjects in the high $\dot{V}O_2$max group consumed 44.8 ± 8.3 ml × min^{-1} × kg^{-1} at this intensity. Whilst the absolute rate of fat oxidation was significantly different between the low and high $\dot{V}O_2$max group at Fat$_{max}$, the relative contribution of fat oxidation to total energy expenditure was equal in both groups (31.3 ± 13.7 % and 32.5 ± 8.8 % for the low and high $\dot{V}O_2$max group respectively).

To summarize, the CV of the estimation of Fat$_{max}$ and Fat$_{min}$ is approximately 9.0 – 9.5 %. The typical error expressed in terms of heart rate was ~10 beats × min^{-1}. In the present study the average intensity of maximal fat oxidation was located at 63 % $\dot{V}O_2$max. Even within a homogenous group of subjects, there was a relatively large inter-individual variation in Fat$_{max}$ and the rate of maximal fat oxidation. It should be noted that these results only apply to moderately trained men and it may be difficult to extrapolate these findings to other groups as it is likely that gender, age and training status affect fat oxidation.

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