

Changes in substrate utilization rates during 40 min of walking within the Fatmax range

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Background and aims: The aim of this study was to evaluate changes in fat oxidation rate during 40 min of continuous exercise and identify the intensity at the highest fat oxidation rate (Fatmax). **Methods:** A total of 14 sedentary males with age, body height, weight, and BMI averages of 29.3 ± 0.7 years, 178.3 ± 1.7 cm, 81.1 ± 3.9 kg, and 25.4 ± 0.9 kg/m², respectively, were included in the study. Fatmax was determined using an indirect calorimeter with an incremental treadmill walking test at least after 12 h of fasting. On a separate day, at least after 12 h of fasting, the participants walked for 40 min within their predetermined individual Fatmax heart rate and speed ranges. **Results:** The initial fat oxidation rate was not sustained within the first 16 min of exercise and was reduced; however, carbohydrate oxidation reached a stable level after nearly 10 min. **Conclusions:** In sedentary individuals, during low-intensity physical activity, fat oxidation rates may not be sustainable as expected from Fatmax testing. Therefore, when exercise is prescribed, one should consider that the fat oxidation rate might decrease in sedentary overweight individuals.

Keywords: exercise, lipid oxidation, carbohydrate oxidation, substrate metabolism, sedentary

Introduction

Carbohydrates and fats are essential sources of energy used during daily activities and exercise. In particular, fat oxidation is a desired process for weight loss and weight maintenance as well as the treatment of related diseases. Fat oxidation is affected by various factors such as nutrition (34, 38), hormones (5, 45), glycogen content of the muscles (45), and exercise duration and intensity (2, 9, 23, 44). Fat oxidation rates have been reported to reach up to the highest levels at low/moderate exercise intensities [approximately 33%–65% of maximal oxygen uptake ($\dot{V}O_{2\max}$)] (2). In addition, the main substrate for energy production shifts from fats to carbohydrates at higher exercise intensities (6, 10).

The Fatmax concept was developed to prescribe exercise according to metabolic responses (2, 24). This specific intensity, commonly presented as a percentage of $\dot{V}O_{2\max}$, is termed Lipoxmax or Fatmax by different researchers (9) and provides a measure of the maximal fat oxidation (the highest rate of fat oxidation observed at various intensities) (37).

On the other hand, the fat oxidation rate following low-intensity exercise (50% of $\dot{V}O_{2\max}$) has been reported to be elevated for 24 h (21). Therefore, it may be important to

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consider the daily changes in fat oxidation rate. In addition, aerobic exercise-induced long-term adaptive responses may enhance resting and exercise fat oxidation rates (18). These responses may include changes in muscle fiber type, upregulation of oxidative enzymes, and increases in capillary density together with pulmonary and cardiovascular adaptations (15). On the other hand, adipose tissue lipolysis, fatty acid transport to the skeletal muscle and its uptake by the tissues, intramuscular lipolysis, mitochondrial uptake of fatty acids, and mitochondrial biogenesis can be counted as training-induced adaptations to maximal fat oxidation rate (18, 31).

Regular exercise around or at Fatmax may be prescribed for weight management, improving the lipid profile, and increasing the oxidative capacity of skeletal muscle in obese and type-2 diabetic patients (7, 9). It should also be kept in mind that most people are able to intuitively self-select an exercise intensity that falls within the Fatmax zone (13). As previously mentioned, fat oxidation may be regulated by many factors including exercise intensity and duration (2, 9, 44). Fat oxidation rate is considered to remain constant when exercising at Fatmax for a long period of time. Meyer et al. (33) reinvestigated the Fatmax testing procedure with constant load tests for 60 min. They discarded the first 10 min of the gas exchange results for the sake of adaptation, and they averaged the next 10 min (duration between 10 and 20 min) (33). By excluding the first 10 min and by averaging longer time intervals after the first 10 min, possible early changes in fat oxidation rate might be overlooked. Thus, there seems to be a lack of studies that evaluate changes in the respiratory exchange ratio and the fat oxidation rate at shorter time intervals throughout the exercise period.

Although fatty acids are the main substrates for energy production during low-intensity exercise, how substrate utilization changes during a single session of 40 min of constant low/moderate-intensity exercise in sedentary overweight individuals has not been clarified. Therefore, we aimed to investigate the substrate utilization and possible substrate shifts during a single exercise session at Fatmax.

Materials and Methods

Participants and intervention content

A total of 14 sedentary males with an average age of 29.3 ± 0.7 years participated in this study. The study procedures and purposes were explained to all participants in detail, and informed consent forms were obtained. Measurements were performed following the approval of the ethics committee and carried out in accordance with the Declaration of Helsinki. Participants were excluded from the study in case of any disease or drug use, which may potentially influence substrate metabolism. There were no calorie restrictions in terms of nutrition. Participants were defined as “sedentary” who were reported to have no physical activity.

Anthropometric measurements

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 using a scale and a stadiometer. Calf, mid-thigh, and forearm circumferences were measured using inelastic tape. Body density, which was used in the Siri formula, was calculated (22). Body fat estimates were derived according to Siri (40). Body muscle mass was estimated using the Martin formula (30).

Exercise protocol and indirect calorimetry

Three different tests including the maximal cardiopulmonary exercise test, the Fatmax test, and the 40-min walking test on a treadmill (HP Cosmos, Nussdorf – Traunstein, Germany) were performed. The tests were performed at least 48 h apart. Breath-by-breath gas measurements were taken throughout the exercise using an indirect calorimetry system (PFT Cosmed, Rome, Italy). The volume and gas of the system were calibrated using a 3-L calibration syringe and calibration gases, respectively (16% O₂ and 5% CO₂). The heart rate (HR) was recorded continuously by telemetry using a HR monitor (Cosmed). Substrate oxidation was calculated using a stoichiometric equation (16); the urinary nitrogen excretion rate was ignored.

Maximal cardiopulmonary exercise test

The participants started the test at 4 km/h. The speed was increased by 0.5 km/h every minute until exhaustion [reaching up to 90% of the maximum HR according to the 220-age formula, achievement of an $\dot{V}O_2$ plateau, or continuation of a non-protein respiratory quotient (npRQ) value at and over 1.15] (3). The anaerobic thresholds of the participants were calculated by the V-slope method (4).

Fatmax test

Fatmax was determined with an incremental treadmill walking test at least after 12 h of fasting. The participants performed a 2-min warm-up at 3 km/h. The speed was increased by 1 km/h every 6 min until the fat oxidation rate decreased to 0 with an npRQ value of 1.01. The last 2 min of every loading stage was used for analysis. The stage at which the fat oxidation rate was maximal was determined, and the last 2 min average HR and $\dot{V}O_2$ were calculated for the 40-min walking test (8).

40-Min walking test

The walking intensity of each participant was determined according to the stage at which their fat oxidation rate in the Fatmax test was maximal. The 40-min walking test was performed at least after 12 h of fasting. The participants performed a 2-min warm-up at 3 km/h before 40 min of walking. $\dot{V}O_2$, HR, and substrate oxidation of the participants were monitored throughout 40 min of walking. The 40-min walking test's data were averaged for every minute. A capillary blood sample of 20 μ l was taken from the fingertips of the subject at rest and at 5 min intervals during the test. Blood lactate and glucose concentrations were

Table 1. Participants' characteristics

Age (years)	29.3 \pm 0.7
Height (cm)	178.3 \pm 1.7
Weight (kg)	81.1 \pm 3.9
Body mass index (kg/m ²)	25.4 \pm 0.9
Fat (%)	18.7 \pm 1.4
Muscle (%)	31.5 \pm 1.0

The values are presented as mean \pm SEM

Table II. Performance values of the participants in the maximal cardiopulmonary exercise and Fatmax tests

	$\dot{V}O_{2max}$ (ml/min/kg)	HR (beats/min)	Rate (km/h)	npRQ	Fat oxidation (g/min)	Carbohydrate oxidation (g/min)
Maximal test	33.53 ± 2.08	180.64 ± 1.95	10.14 ± 0.30	1.04 ± 0.11	0.0 ± 0.0	5.70 ± 0.28
Anaerobic threshold	18.25 ± 1.12	120.0 ± 4.44	5.89 ± 0.20	0.91 ± 0.01	0.22 ± 0.03	1.43 ± 0.17
Fatmax	13.66 ± 0.46* [#]	103.09 ± 4.13* [#]	4.35 ± 0.13*	0.83 ± 0.01*	0.33 ± 0.02 ^{§#}	0.62 ± 0.06 ^{§#}
Power	0.912	0.99	0.99	0.99	0.99	0.99
Effect size	2.82	7.19	2.49	4.45	2.24	4.14
Sample size	2	2	3	2	3	2

Values are presented as mean ± SEM. npRQ: non-protein respiratory quotient.

* $p < 0.001$, significantly different from the threshold and peak values.

[#] $p < 0.05$, significantly different from the threshold values.

[§] $p < 0.001$, significantly different from the peak values

measured using reagents provided by EKF Diagnostics (Germany) and a Biosen S-line lactate analyzer (EKF Diagnostics).

Statistical analyses

The results are presented as the mean \pm SEM. Data distribution was determined by the Shapiro–Wilk test. ANOVA with repeated measures was used to analyze the changes in HR, $\dot{V}O_2$, blood lactate, and glucose concentrations during the 40-min walking test. The Bonferroni *post-hoc* analysis was used to identify differences among trials. Non-parametric data were compared with the Wilcoxon signed-rank test; normally distributed data were compared using repeated-measures ANOVA for Fatmax, peak, and threshold values. Exponential curve fitting for fat oxidation during the 40 min of walking was performed using scientific data analysis and graphing software (SigmaPlot Version 11.0, Germany).

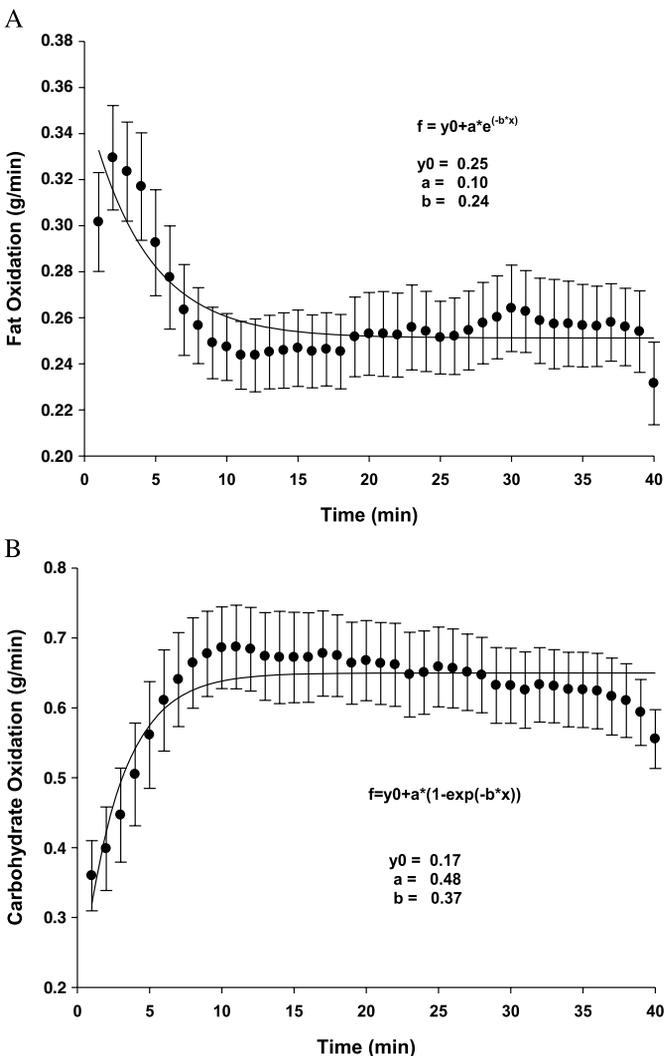


Fig. 1. (A) Exponential decay of fat oxidation rate during the 40-min walking test at Fatmax. Values are given as

mean \pm SEM. (B) Exponential rise of carbohydrate oxidation rate during the 40-min walking test at Fatmax. Values are given as mean \pm SEM

Statistical significance was set at $p < 0.05$. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS for Windows, version 22.0, USA). Effect size was estimated for main effects and interaction by calculating partial eta-squared (η^2) values using the Statistical Package for the Social Sciences (SPSS for Windows, version 22.0). The η^2 value was evaluated after converting to Cohen's d value. Effect size for F -test repeated measured ANOVA was assessed with Cohen's d . Effect sizes were classified as small (0.2), medium (0.5), and large (>0.8).

Results

Participants' characteristics are shown in Table I. The performance values of the participants in the maximal cardiopulmonary exercise and Fatmax tests are shown in Table II. Maximal

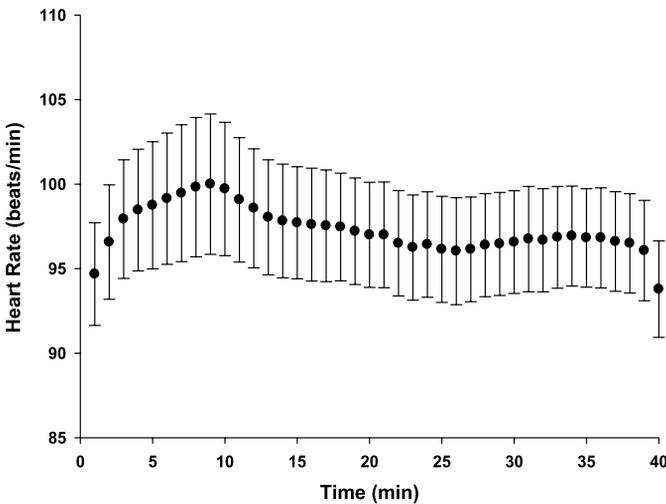


Fig. 2. Heart rate during 40-min walking test at Fatmax. Values are given as mean \pm SEM

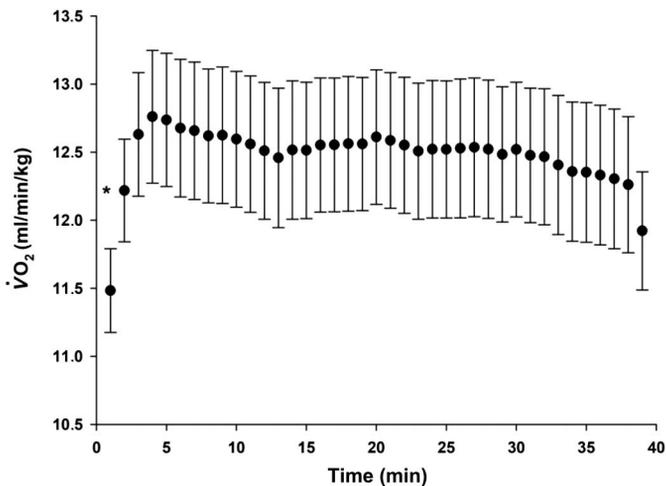


Fig. 3. $\dot{V}O_2$ during 40-min walking test. Values are given as mean \pm SEM. *Significantly lower than the other values

test values and anaerobic threshold values were obtained from the maximal cardiopulmonary exercise test, whereas the Fatmax values were determined from the Fatmax test.

The fat oxidation rate at Fatmax was significantly higher than those at the anaerobic threshold ($p < 0.05$) and maximal test values ($p < 0.001$). The maximal percentage of energy derived from fats and carbohydrates at Fatmax was $57.3\% \pm 3.4\%$ and $42.9\% \pm 3.3\%$, respectively. Carbohydrate oxidation rate at Fatmax was significantly lower than those at anaerobic threshold ($p < 0.05$) and peak values ($p < 0.001$). The $\dot{V}O_2$ and HR values at Fatmax corresponded to $\sim 40.9\%$ of $\dot{V}O_{2\max}$ and $\sim 57.2\%$ of HR. The npRQ value at Fatmax was 0.83 ± 0.0 .

The fat and carbohydrate oxidation rates during the 40-min walking test are presented in Fig. 1A, B, respectively. The fat oxidation rate started to decrease exponentially with the

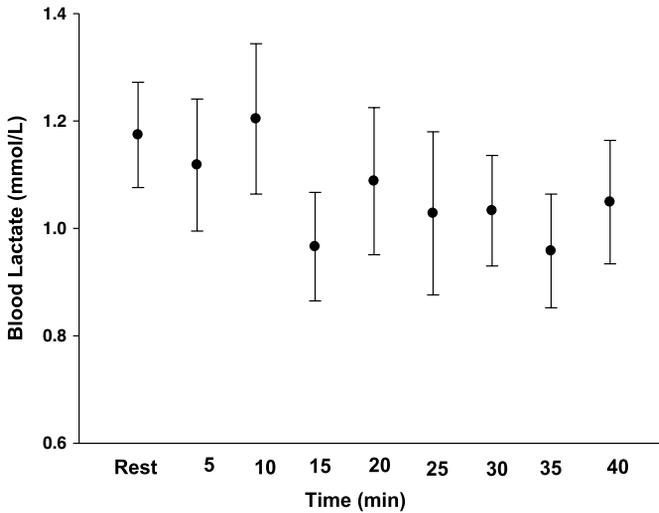


Fig. 4. Lactate concentrations measured during the 40-min walking test at Fatmax. Values are given as mean \pm SEM

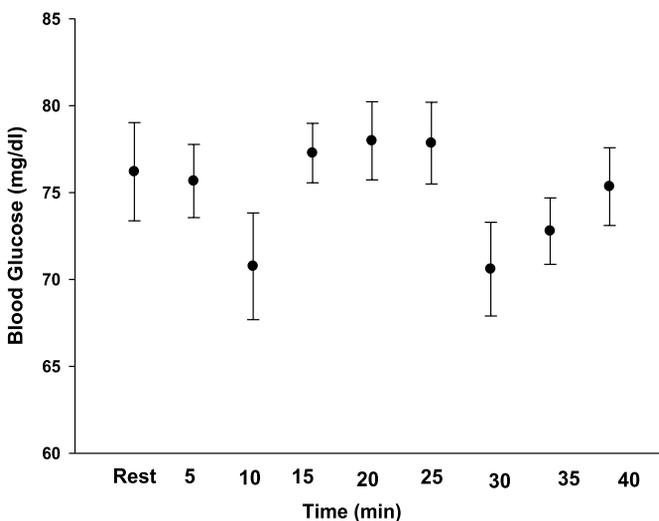


Fig. 5. Blood glucose levels measured during the 40-min walking test at Fatmax. Values are given as mean \pm SEM

onset of exercise and reached a plateau after nearly 16 min (Fig. 1A; Equation 1).

$$f = y_0 + a \exp(-bx). \quad (1)$$

In comparison with the oxidation rate of fats, the carbohydrate oxidation rate increased exponentially and reached a plateau after nearly 10 min (Fig. 1B; Equation 2).

$$f = y_0 + a(1 - \exp(-bx)). \quad (2)$$

Although the fat oxidation rate decreased exponentially, HR did not show significant fluctuations throughout the 40 min of exercise (Fig. 2). $\dot{V}O_2$ increased significantly with the onset of exercise and remained stable throughout the 40-min walking test (Fig. 3).

Neither lactate nor glucose values showed any significant change during the 40-min walking test at Fatmax (Figs 4 and 5).

Discussion

The main finding of this study was that during low-intensity continuous exercise for 40 min, the fat oxidation rate was not sustained and decreased to a plateau level within the first 16 min. On the other hand, the carbohydrate oxidation rate was increased exponentially and reached a stable level after nearly 10 min. This reduction in fat oxidation rate may point out different physiological mechanisms in substrate metabolism during low/moderate-intensity physical activity.

The exercise intensity used in this study was determined by the same method used to define Fatmax (2, 24). The fat oxidation rate has been reported to be maximal during endurance exercise at approximately 33%–65% of $\dot{V}O_{2\max}$ (1), corresponding to mild-moderate exercise (6, 12). Therefore, the exercise intensity at Fatmax used in this study (~40.9% $\dot{V}O_{2\max}$ and 57.2% HR_{max}) is in agreement with previously reported exercise intensities.

In indirect calorimetry systems, the fat oxidation rate is calculated from the $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$) (16). Although the fat oxidation rate is not measured directly with these systems, indirect calorimetry is commonly used to measure substrate metabolism during exercise (25, 32). $\dot{V}O_2$ and $\dot{V}CO_2$ kinetics are important variables that can affect fat oxidation rate calculation. In previous studies, $\dot{V}O_2$ demonstrated faster gas kinetics compared with $\dot{V}CO_2$, and approximately 4 min was required for $\dot{V}CO_2$ to reach a steady state (11, 46). Therefore, the decrease in the fat oxidation rate during the first 10 min may not be attributed to gas “exchange” kinetics. Notably, the mean response time of $\dot{V}O_2$ in low-intensity exercises has been reported to be lower compared with high-intensity exercises (41). Another important finding of this study is that HR and $\dot{V}O_2$ did not show significant fluctuations throughout the 40 min of exercise (Figs 2 and 3). The exercise intensity in this study (4.35 km/h and 40.9% $\dot{V}O_{2\max}$) was lower than the anaerobic threshold intensity (5.89 km/h and 54.5% $\dot{V}O_{2\max}$) (Table II). Since the resting and exercise blood lactate values did not show any significant difference in this study, we did not expect any significant change in blood pH. Therefore, this finding may not explain the observed substrate shift from fatty acids to carbohydrates in this study. These findings suggest that metabolic stress including

lactate accumulation did not occur during the 40-min of exercise. Stable blood glucose levels indicate that substrate supply to active muscle did not change throughout the 40 min of exercise.

The fat oxidation rates calculated in both the Fatmax and 40-min walking tests were in concordance with the literature (6, 29, 31). A study has reported that fat oxidation rates are increased with an improvement in aerobic capacity (29). In addition, the capacity for fat oxidation may be disrupted in sedentary overweight individuals, and the substrate may shift from fats to carbohydrates at lower exercise intensities (35). As the adjustment of fuel oxidation to fuel availability is an indication of “metabolic flexibility,” the fat oxidation rate is not limited during low-intensity exercise in normal individuals. Individuals (including those who are sedentary, obese, or with a poor cardiovascular fitness level) with low-resting metabolic rates and high respiratory quotients may be defined as “metabolically inflexible.” These individuals rely mainly on glucose metabolism with a blunted preference for muscle fat oxidation (17, 26, 39). In this study, the resting metabolic rates of the participants were not evaluated. As the participants were sedentary and overweight with a low aerobic capacity (19, 42), the discontinuation of lipid oxidation with constant low-intensity exercise may be attributed to their metabolic inflexibility. In this context, as an alternative method, the use of the individual ventilatory threshold can be a useful parameter that identifies physical activity that maximizes fat oxidation in obese individuals (14).

Mitochondrial content and/or mitochondrial functional qualities might be a limiting factor in fat oxidation in sedentary/overweight individuals. In sedentary individuals, possible intramyocellular lipid accumulation may impair fat oxidation capacity and reduce lipid turnover (17, 28). Different mechanisms are known to regulate fat oxidation. An early decrease in fat oxidation rate suggests a rapid change in the metabolic pathways of the cell. One of the important pathways that regulate fat oxidation is the carnitine pathway, which plays a role in fatty acid transport. Even though carnitine palmitoyltransferase activity in skeletal muscles is shown to be decreased in sedentary obese individuals (27), any reduction in free carnitine could also lead to decreased fat oxidation rates; at high-intensity exercises, the depletion of free carnitine would prevent fat oxidation (43). Therefore, free carnitine concentration in the cytoplasm is a key determinant of long-chain fatty acid metabolism (36). The decrease in fat oxidation rate during the first 15 min at the beginning of exercise may be attributed to a decrease in the concentration of free carnitine, as demonstrated by a previous study (20); the study reported a decrease (around 10%) in the free carnitine level in the first 10 min of exercise.

This study found that the fat oxidation rate decreased in around 15 min and remained constant for the rest of the exercise duration in sedentary overweight individuals. The identification of the physiological mechanisms associated with the initial reduction in fat oxidation rate may be important for developing various strategies to improve the fat oxidation rate during low-intensity exercise.

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Conflict of interest

The authors declare no conflict of interest.

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