The effect of etomoxir on 24-h substrate oxidation and satiety in humans

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ABSTRACT

Background: The carnitine *O*-palmitoyltransferase I (EC 2.3.1.21) inhibitor etomoxir inhibits fatty acid oxidation, and hepatic fatty acid oxidation has been suggested to be a metabolic satiety signal in subjects who consume high-fat diets.

Objective: We investigated substrate oxidation and satiety after repeated administrations of etomoxir or placebo in subjects who consumed a high-fat diet.

Design: In a randomized crossover design consisting of three 5-d treatments, we fed 10 healthy men [mean ± SE age: 25.6 ± 1.7 y; mean ± SE body mass index (in kg/m^2): 21.8 ± 0.3] a high-fat diet twice and a low-fat diet once. The subjects consumed each diet at home for 3 consecutive days, after which they spent 36 h in energy balance in a respiration chamber. During the chamber stays with the high-fat treatments, etomoxir or placebo was administered in 5 doses (600 mg etomoxir in total). Blood samples were obtained on the mornings of days 4 and 5 of each treatment, and appetite profiles were assessed.

Results: Mean (±SE) 24-h respiratory quotients were significantly ($P < 0.05$) higher with repeated administrations of etomoxir (0.833 ± 0.004) than with repeated administrations of placebo (0.814 ± 0.006), and mean (±SE) 24-h whole-body fat oxidation tended to be less (13.7%, $P = 0.06$) with administration of etomoxir (136.0 ± 5.2 g/d) than with administration of placebo (157.5 ± 5.6 g/d). With the etomoxir treatment, fat balance was positive ($P < 0.0001$) and carbohydrate balance was negative ($P < 0.001$), whereas with the placebo treatment, neither of the balances was significantly different from zero. Hunger and satiety ratings were not affected under these conditions.

Conclusions: Etomoxir decreased whole-body fat oxidation, as indicated by the respiratory quotients in the healthy subjects. With the current protocol, however, hunger and satiety ratings were not affected. *Am J Clin Nutr* 2002;76:141–7.

KEY WORDS Hepatic fatty acid oxidation, respiration chamber, macronutrient composition, obesity, etomoxir

INTRODUCTION

Obesity is a major health concern in industrialized countries. The high fat content of Western diets has been related to increased voluntary energy intake and an increasing body mass index in susceptible individuals (1–3). The high palatability of high-fat foods makes overeating more likely (4), and the high energy density of fat-rich diets has been shown to increase energy intake in animals (5) and humans (6, 7), probably related to decreased total food weight (passive overeating). Furthermore, subjects who are obesity prone seem to have more difficulty than do subjects who are not adjusting their fat oxidation when switched from a low- to a high-fat diet; this difficulty favors fat storage. For example, fat oxidation in formerly obese women failed to increase appropriately after a 3-d adaptation to a 50% fat diet, whereas in normal-weight control subjects, fat oxidation increased sufficiently to match fat intake (8). These results suggest that the partitioning of fat between storage and oxidation is important in the development of obesity in subjects who consume a high-fat diet.

The oxidation of fuels, including fatty acids, has been implicated in metabolic satiety. Evidence for a role of metabolic satiety signals in food intake control comes from animal studies that indicate that eating is inversely related to the rate of fuel utilization (9–12). The liver is one likely location where fuel oxidation is probably monitored and translated into a neural signal for the brain. According to current thinking, high oxidative metabolism in the liver signals the presence of sufficient energy-yielding substrates and suppresses food intake. In particular, fatty acid oxidation in the liver seems to act as a satiety signal. Ingestion or intragastric administration of medium-chain fatty acids, which are easily taken up and rapidly oxidized by liver cells, has been shown to inhibit eating in animals (13, 14) and humans (15, 16). Furthermore, different inhibitors of fatty acid oxidation have been shown to increase food intake in rats and mice adapted to a high-fat diet (17, 18). One way to inhibit fatty acid oxidation is by blocking carnitine *O*-palmitoyltransferase I (EC 2.3.1.21; CPT-I), the rate-limiting enzyme in the transport of long-chain

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fatty acids into the mitochondrion, where β-oxidation takes place. We recently showed that a single dose of the CPT-I inhibitor etomoxir increases food intake in healthy young men who habitually consume a high-fat diet (19). Etomoxir decreased the plasma concentration of β-hydroxybutyrate (BHB) and hence hepatic fatty acid oxidation, but we did not detect a decrease in whole-body fat oxidation, measured during 200 min by indirect calorimetry with the use of a ventilated hood system.

In the present study, we used a respiration chamber to measure 24-h substrate oxidation after adaptation to a high- or low-fat diet. We measured changes in substrate oxidation and satiety ratings in response to repeated administrations of etomoxir in subjects who consumed a high-fat diet. We hypothesized that, under these conditions, administration of etomoxir would decrease whole-body and hepatic fatty acid oxidation and satiety.

SUBJECTS AND METHODS

Subjects

Ten healthy, lean, young men with a mean (±SE) body mass index (in kg/m^2) of 21.8 ± 0.3, a mean (±SE) percentage of body fat of 12.5 ± 1.46%, and a mean (±SE) age of 25.6 ± 1.7 y participated in the study. The men were recruited by advertisements. The Medical Ethics Committee of the University of Maastricht approved the study, and subjects gave their written, informed consent.

Experimental design

Each subject underwent 3 different treatments in randomized order. Each treatment lasted for 5 d. Subjects ate either a low-fat diet (LF-control treatment) or a high-fat diet. We used the low-fat diet as a positive control, because from previous studies we know that substrate oxidation with a low-fat diet is different from that with a high-fat diet. With the high-fat diet, subjects were given either etomoxir (HF-etomoxir treatment) or placebo (HF-placebo treatment). Etomoxir was purchased from HPO Wolf (Projekt Entwicklung GmbH, Allensbach, Germany). All treatments started with a 3-d prefeeding period of the low- or high-fat diet at home (days 1–3). On the evening of day 3, subjects entered the respiration chamber for a 36-h stay. During the stay in the chamber, etomoxir or placebo was given in 5 doses: 1 capsule (75 mg etomoxir) on each of the evenings, 2 capsules (150 mg) on each of the mornings, and 2 capsules (150 mg) on the afternoon of day 4. This resulted in a total etomoxir dose of 600 mg over the 36 h in the respiration chamber. The last dose of 150 mg on the morning of day 5 was administered in accordance with a second part of the experiment, which consisted of a muscle biopsy; the muscle biopsy specimens are still being analyzed.

During the HF-placebo treatment, subjects followed the same time schedule with the same amount of capsules, but the capsules did not contain any drug. During the LF-control treatment, no capsules were given.

During day 4, the subjects completed a 100-mm visual analogue scale (VAS) 10 times: before and after each main meal; in the course of the morning, the afternoon, and the evening; and before going to bed. These scales provided ratings of the subjects' perceptions of hunger and satiety (20). Blood samples were taken in the morning on days 4 and 5. The subjects left the respiration chamber on the morning of day 5.

Diets

The metabolizable energy and macronutrient composition of the diet were calculated by using the Dutch food composition table (21). In this table, metabolizable energy is calculated by multiplying the amounts of protein, fat, and carbohydrate with the respective Atwater factors (16.74, 37.66, and 16.74 kJ/g). The high-fat diet contained 60% of energy as fat, 30% as carbohydrate, and 10% as protein. The low-fat diet contained 10% of energy as fat, 60% as carbohydrate, and 30% as protein (20). Because milk fat contains a high amount medium-chain fatty acids, it was excluded from the high-fat diet. This ensured that mainly long-chain fatty acids contributed to the fat content of the high-fat diet. The high- and low-fat diets provided in the chamber did not differ significantly in mean (±SE) palatability, as measured by the VAS (72.6 ± 2.7 and 71.1 ± 5.2 mm for the high- and low-fat diets, respectively). The 2 diets were closely matched in flavor and did not differ significantly in mean (±SE) energy density (5.4 ± 0.2 and 5.2 ± 0.1 kJ/g for the high- and low-fat diets, respectively). Diets in the chamber were consumed as breakfast, lunch, dinner, and evening snack. The food quotient (FQ) was defined as the ratio of carbon dioxide produced to oxygen consumed during the oxidation of a representative sample of the diet consumed (22).

For days 1 and 2 and the first part of day 3, the diet (either low- or high-fat) was provided for consumption at home. Subjects were given a fixed amount of food (1.65 times the basal metabolic rate, based on the Harris and Benedict equation

$$\mathrm{BMR} = 0.28 + (2.093 \times \mathrm{H}) + (0.058 \times \mathrm{BM}) - (0.028 \times \mathrm{A}) \quad (1)$$

where BMR is the basal metabolic rate in MJ/d, H is height in m, BM is body mass in kg, and A is age in years (23). On the evening of day 3, subjects consumed their dinner and evening snack in the respiration chamber. On day 4, subjects were given an amount of energy equal to 1.55 times the sleeping metabolic rate (SMR), as measured during the preceding night. In a previous study with a comparable activity protocol in the chamber, this same physical activity index of 1.55 was reached (24).

Procedures

Body composition

The subjects' whole-body density was determined by weighing them underwater in the morning while they were fasting. Body weight was measured on a digital balance (E1200; Sauter, Albstadtl-Ebingen, Germany) with an accuracy of 0.01 kg. Lung volume was measured simultaneously by the helium dilution technique with the use of a spirometer (Volugraph 2000; Mijnhardt, Bunnik, Netherlands). Percentage of body fat was calculated by using the equations of Siri (25).

Indirect calorimetry and physical activity

Oxygen consumption and carbon dioxide production were measured in a respiration chamber (26). The respiration chamber is a 14-m^3 room furnished with a bed, chair, television, radio, telephone, computer with internet connection, intercom, wash bowl, and toilet. The room was ventilated with fresh air at a rate of 70–80 L/min. The ventilation rate was measured with a dry gas meter (G6; Schlumberger, Dordrecht, Netherlands). The concentrations of oxygen and carbon dioxide were measured by using a paramagnetic oxygen analyzer (Magnos G6; Hartmann & Braun, Frankfurt, Germany) and an infrared carbon dioxide analyzer

(Uras 3G; Hartmann & Braun). Ingoing air was analyzed once every 15 min and outgoing air once every 5 min. The gas sample to be measured was selected by a computer that also stored and processed the data. Energy expenditure was calculated from oxygen consumption and carbon dioxide production according to the method of Weir (27).

In the respiration chamber, subjects followed an activity protocol consisting of fixed times for breakfast, lunch, and dinner; sedentary activities; and bench-stepping exercise. The bench-stepping exercise was performed 3 times daily for 30 min each time. Each performance of the exercise consisted of 5-min periods of exercise alternated with 5-min periods of rest. The exercises were performed at a rate of 60 steps/min, and the bench height was 33 cm. Thus, subjects exercised for 45 min/d at low-to-medium intensity. Throughout the daytime, no sleeping or other exercise was allowed during the stay in the respiration chamber. All physical activity of the subjects was monitored by means of a radar system based on the Doppler principle.

Urinary nitrogen excretion

During the subjects' stay in the respiration chamber, urine was collected in 2 batches, one from 2000 to 0800 and one over the subsequent 24-h interval. Subjects were requested to empty their bladders at 0800. The urine produced was included in the urine sample of the previous batch. Samples were collected in containers with 10 mL H_2SO_4 to prevent nitrogen loss through evaporation; the volume and nitrogen concentration of the samples were measured; the latter was measured by using a nitrogen analyzer (CHN-O-Rapid; Carlo-Erba, Hanau, Germany).

Twenty-four-hour energy expenditure, substrate oxidation, and sleeping respiratory quotient

Subjects stayed in the respiration chamber for 36 h. For calculating balances, 24-h energy expenditures and 24-h respiratory quotients (RQs) were measured from 0800 on day 4 to 0800 on day 5. The sleeping RQ was defined as the RQ measured from 0030 to 0700. The SMR was defined as the lowest mean energy expenditure measured during 3 consecutive hours between 0000 and 0800 with a minimal activity level as indicated by the radar system. Carbohydrate, fat, and protein oxidation were calculated by using oxygen consumption, carbon dioxide production, and urinary nitrogen losses with the equations of Brouwer (28)

$$\text{Protein oxidation (g/d)} = 6.25 \times \text{N} \quad (2)$$

$$\text{Fat oxidation (g/d)} = (1.718 \times \dot{V}O_2) - (1.718 \times \dot{V}CO_2) - (0.315 \times \text{P}) \quad (3)$$

$$\text{Carbohydrate oxidation (g/d)} = (4.17 \times \dot{V}CO_2) - (2.965 \times \dot{V}O_2) - (0.390 \times \text{P}) \quad (4)$$

where N is the total nitrogen excreted in urine (g/d), $\dot{V}O_2$ is the oxygen consumption (L/d), $\dot{V}CO_2$ is the carbon dioxide production (L/d), and P is protein oxidation (g/d).

Dietary restraint

A Dutch translation of the 3-factor eating questionnaire (TFEQ) of Stunkard and Messick (20, 29) was used to discriminate between "cognitive restrained" and "unrestrained" eaters. If factor 1 of the 3-factor eating questionnaire was not >9, subjects were considered to be unrestrained eaters. We previously showed that the subject population that is used in the Department of Human Biology at the University of Maastricht has a median factor 1 score of 9 (30). According to these criteria, 9 of the 10 subjects who participated in the present study qualified as unrestrained eaters.

Satiety scores

At 10 fixed time points on day 4 (before, after, and in between the main meals), a 100-mm VAS was completed to quantify the perceptions of satiety, hunger, fullness, and desire to eat. After meals, a VAS was included to quantify the palatability of the meal.

From the 10 ratings between 0930 and 2300, the area under the curve was calculated (20). To complete the area under the curve over 24 h, VAS ratings were interpolated from the last measurement at night until the first measurement in the morning (20). One subject who qualified as a restrained eater according to the criteria described above was excluded from the analysis of satiety ratings.

Blood analyses

With all 3 treatments, blood samples were taken on the morning of days 4 and 5 after an overnight fast. For the blood collection on day 4, subjects put their arm through an air lock in the chamber wall, which has a rubber sleeve to fit around the upper arm and is positioned under a window for eye contact. This allowed for blood sampling without disruption of the respiration chamber measurements. Samples of venous blood (10 mL) were obtained in EDTA-coated tubes and immediately centrifuged at high speed for 10 min (1000 × *g*, 4°C). Plasma was frozen in liquid nitrogen and stored at −80°C until analyzed for glucose (hexokinase method, Roche, Basel, Switzerland), fatty acids (Wako NEFA C test kit, Wako Chemicals, Neuss, Germany), lactate (31), BHB (32), and glycerol and triacylglycerols (glycerol kinase-lipase method, Boehringer Mannheim, Mannheim, Germany) on a COBAS BIO analyzer (COBAS FARA semi-automatic analyzer, Roche, Basel, Switzerland).

Statistical analysis

All data are presented as means ± SE. To detect treatment differences, treatments were analyzed pairwise with *t* tests. To compensate for this procedure, *P* values were multiplied by a Bonferroni correction factor of 3.

A two-factor repeated-measures analysis of variance with interactions was used to detect treatment × time interactions in selected variables. When significant differences were found, a Bonferroni-adjusted post hoc test was used to determine the exact location of the difference.

One subject underwent only the HF-etomoxir and the HF-placebo treatments, and thus his data could not contribute to the results analyzed by a two-factor repeated-measures analysis of variance. Therefore, the results that were analyzed with a two-factor analysis of variance are based on only 9 subjects. The analysis of the satiety ratings is also based on 9 subjects.

We used the statistics programs STATVIEW SE + GRAPHICS (Abacus Concepts, Inc, Berkeley, CA) and SPSS for WINDOWS release 10.0.0 (SPSS Inc, Chicago). Outcomes were regarded as statistically significant if $P < 0.05$.

RESULTS

Substrate oxidation

With the HF-etomoxir treatment, 24-h RQs were significantly higher than with the HF-placebo treatment ($P < 0.05$). Twenty-four-hour

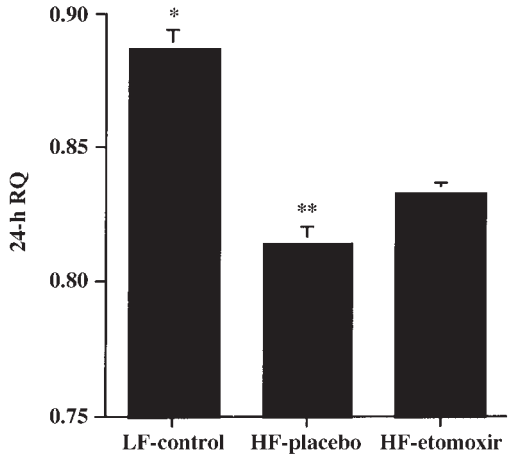

FIGURE 1. Comparison of mean (±SE) 24-h respiratory quotients (RQs) during the 3 treatments: low-fat (LF)-control, high-fat (HF)-placebo, and HF-etomoxir. *Significantly different from HF-etomoxir and HF-placebo, $P < 0.005$ (paired t test with Bonferroni correction; $n = 9$). **Significantly different from HF-etomoxir, $P < 0.05$ (paired t test with Bonferroni correction; $n = 10$).

RQs were significantly higher with the LF-control diet than with the HF-placebo ($P < 0.0005$) or the HF-etomoxir treatments ($P < 0.005$) (**Figure 1**).

There was a significant treatment effect and time × treatment interaction effect on sleeping RQs ($P < 0.05$). With the LF-control treatment, sleeping RQs were significantly higher than with the HF-placebo treatment during both nights ($P < 0.0005$), and the changes in sleeping RQ from night 1 to night 2 differed significantly between the HF-etomoxir and the LF-control treatments ($P < 0.05$). Sleeping RQs did not differ significantly between the HF-etomoxir and HF-placebo treatments during the first night, but during the second night, sleeping RQs were significantly higher with the HF-etomoxir treatment than with the HF-placebo treatment ($P < 0.05$) (**Figure 2**).

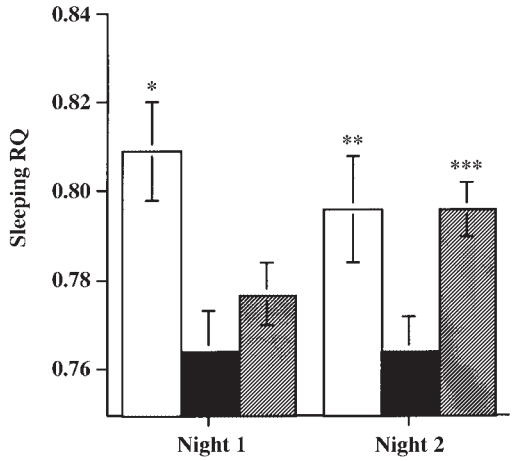

FIGURE 2. Comparison of mean (±SE) sleeping RQs during the 2 consecutive nights between the treatments [low-fat (LF)-control (□), high-fat (HF)-placebo (■), and HF-etomoxir (▨)]. There were significant treatment effects and time × treatment interaction effects ($P < 0.05$) by two-factor repeated-measures ANOVA. *Significantly different from HF-placebo on night 1, $P < 0.05$ ($n = 9$). **Significantly different from HF-placebo on night 2, $P < 0.005$ ($n = 9$). ***Significantly different from HF-placebo on night 2, $P < 0.005$, and from HF-etomoxir on night 1, $P < 0.05$ ($n = 9$).

Whole-body fat oxidation tended to be inhibited by etomoxir and was 13.7% less than with the HF-placebo treatment ($P = 0.06$), whereas carbohydrate oxidation tended to be concomitantly increased with etomoxir ($P = 0.08$). Protein oxidation was not significantly different between the HF-etomoxir and HF-placebo treatments. Fat oxidation was significantly lower with the LF-control treatment than with the HF-placebo ($P < 0.0005$) or HF-etomoxir treatments ($P < 0.001$). Carbohydrate oxidation, on the other hand, was higher with the LF-control treatment than with the HF-etomoxir ($P < 0.05$) or the HF-placebo treatments ($P < 0.001$). Protein oxidation was significantly higher with the LF-control treatment than with the HF-etomoxir ($P < 0.0005$) or HF-placebo treatments ($P < 0.0005$) (**Figure 3**). FQs were significantly different from RQs with the HF-etomoxir treatment (FQ = 0.803 ± 0.000, RQ = 0.833 ± 0.004, $P < 0.001$) and the LF-control treatment (FQ = 0.907 ± 0.001, RQ = 0.887 ± 0.006, $P < 0.05$) but not with the HF-placebo treatment (FQ = 0.803 ± 0.000, RQ = 0.814 ± 0.006, $P > 0.05$).

For all 3 treatments, the energy balance was not significantly different from zero. With the HF-etomoxir treatment, the carbohydrate balance was negative ($P < 0.001$), the fat balance was positive ($P < 0.0001$), and the protein balance was not significantly different from zero. With the HF-placebo treatment, none of the macronutrient balances was significantly different from zero. With the LF-control treatment, the fat balance was negative ($P < 0.01$), whereas there was a tendency toward a positive carbohydrate balance ($P = 0.07$) and the protein balance was positive ($P < 0.05$) (**Figure 4**).

Energy expenditure

Twenty-four-hour energy expenditures were not significantly different between the treatments (11.455 ± 0.27, 11.182 ± 0.30, and 11.007 ± 0.28 MJ/d for the LF-control, HF-placebo, and HF-etomoxir treatments, respectively; $P > 0.05$). There was no treatment effect, but there was a time × treatment interaction effect on SMR measured during the 2 nights. With the HF-etomoxir

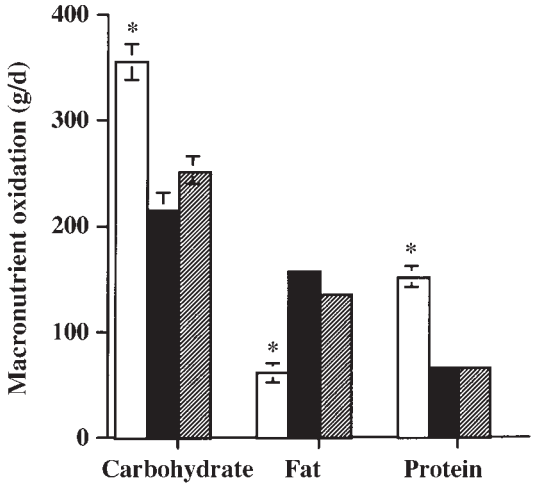

FIGURE 3. Comparison of mean (±SE) macronutrient oxidation between the 3 treatments [low-fat (LF)-control (□), high-fat (HF)-placebo (■), and HF-etomoxir (▨)]. *Significantly different from HF-etomoxir and HF-placebo, $P < 0.001$ (paired t test with Bonferroni correction; $n = 9$).

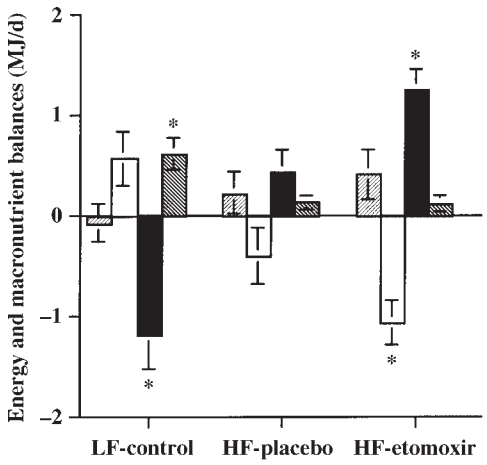

FIGURE 4. Comparison of mean (±SE) energy (▨), carbohydrate (□), fat (■), and protein (▧) balances during the 3 treatments [low-fat (LF)-control (n = 9), high-fat (HF)-placebo (n = 10), and HF-etomoxir (n = 10)]. *Significantly different from zero, $P < 0.05$.

treatment, SMRs were significantly lower than with the LF-control treatment during the second night ($P < 0.05$; night 1: 7.13 ± 0.16 compared with 7.19 ± 0.17 MJ/d; night 2: 6.99 ± 0.13 compared with 7.33 ± 0.13 MJ/d).

Blood parameters

There was a significant treatment effect for plasma glucose ($P < 0.05$), but no significant differences were observed between the HF-etomoxir and HF-placebo treatments. Plasma glucose was lower with the LF-control treatment than with the HF-placebo treatment.

There were no significant treatment effects or time × treatment interaction effects for plasma lactate, BHB, and glycerol concentrations. There was also no treatment effect for fatty acids, but there was a time × treatment effect: fatty acid concentrations increased significantly more with the HF-etomoxir treatment than with the HF-placebo treatment ($P < 0.05$). There was also a treatment effect for triacylglycerol concentration, with significantly lower concentrations with the HF-etomoxir treatment than with the HF-placebo treatment and lower concentrations with the HF-placebo treatment than with the LF-control treatment. ($P < 0.0001$). There was no significant time × treatment effect (**Table 1**). The individual differences in BHB between the HF-etomoxir and HF-placebo treatments on day 4 correlated with the differences in 24-h RQs between the 2 treatments ($r^2 = 0.72$, $P < 0.005$).

Satiety ratings

The 24-h satiety and hunger scores (area under the curve) were not significantly different between the 3 treatments (data not shown). The hunger and satiety ratings did not differ significantly at any time point during the day (**Table 2**).

DISCUSSION

In the present study, we found that the repeated administration of the CPT-I blocker etomoxir increased 24-h RQs and tended to decrease 24-h whole-body fat oxidation but did not affect hunger and satiety ratings in healthy men. Although it has been shown previously that etomoxir increases RQs in type II diabetic subjects (33), to our knowledge this is the first time that an increase in 24-h RQs because of etomoxir has been observed in healthy humans.

In our previous study, the increase in RQs did not reach significance over 200 min after a single dose of etomoxir (19). In the present study, the sleeping RQ during the second night of the HF-etomoxir treatment was significantly higher than that during the first night, whereas there was no difference between the 2 nights in sleeping RQs with the placebo treatment. This indicates that the repeated administration of etomoxir resulted in a gradual decrease of whole-body fat oxidation, which might explain why we did not observe an effect on the RQ after a single dose of etomoxir in our previous study (19).

In the present study, we fed subjects either a low- or high-fat diet for 3 d at home and for 1 d in the respiration chamber to reach a state of substrate balance. However, even after 4 d of adaptation to the low-fat diet, the RQ and the FQ still differed

TABLE 1
Fasting blood concentrations during the 3 treatments, measured in the morning on days 4 and 5[1]

	Day	LF-control	HF-placebo	HF-etomoxir
Glucose (mmol/L)[2,3]	4	4.59 ± 0.10	4.82 ± 0.07	4.89 ± 0.15
	5	4.61 ± 0.11	4.84 ± 0.13	4.60 ± 0.14
Lactate (mmol/L)	4	0.66 ± 0.08	0.76 ± 0.09	0.80 ± 0.09
	5	0.95 ± 0.12	0.98 ± 0.12	0.98 ± 0.08
BHB (μmol/L)	4	158.4 ± 12.1	191.5 ± 16.2	169.3 ± 20.3
	5	204.3 ± 16.7	205.2 ± 8.3	203.1 ± 20.0
Glycerol (μmol/L)	4	47.7 ± 4.9	64.0 ± 8.5	59.2 ± 12.9
	5	92.7 ± 14.6	100.8 ± 25.2	88.7 ± 10.7
Fatty acids (μmol/L)[4]	4	185.9 ± 21.5	284.5 ± 37.1	219.9 ± 39.2
	5	401.7 ± 55.6	381.5 ± 44.1	487.4 ± 75.6
Triacylglycerol (μmol/L)[2,5]	4	1200.6 ± 203.5	677.2 ± 142.7	651.5 ± 166.5
	5	1154.2 ± 166.7	733.3 ± 131.6	416.4 ± 96.5

[1] $\bar{x}$ ± SE; n = 9. LF, low-fat; HF, high-fat; BHB, β-hydroxybutyrate.
[2] Significant treatment effect, $P < 0.05$ (two-factor repeated-measures ANOVA).
[3] The LF-control treatment was significantly different from the HF-placebo treatment, $P < 0.05$ (Bonferroni-adjusted post hoc test).
[4] Significant time × treatment effect, $P < 0.05$ (two-factor repeated-measures ANOVA). There was a significantly larger increase with the HF-etomoxir treatment than with the HF-placebo treatment, $P < 0.05$ (Bonferroni-adjusted post hoc test).
[5] The HF-etomoxir treatment differed significantly from the HF-placebo treatment and the HF-placebo treatment differed significantly from the LF-control treatment, $P < 0.0001$ (Bonferroni-adjusted post hoc test).

TABLE 2
Hunger and satiety ratings on a 100-mm visual analogue scale[1]

	Hunger			Satiety		
	LF-control	HF-placebo	HF-etomoxir	LF-control	HF-placebo	HF-etomoxir
Before breakfast	63.5 ± 7.4	70.9 ± 4.3	75.9 ± 5.4	34.0 ± 9.5	25.4 ± 6.3	21.7 ± 5.4
After breakfast	18.2 ± 4.9	31.6 ± 7.6	32.8 ± 10.4	70.4 ± 7.8	53.4 ± 9.0	60.3 ± 11.2
During the morning	42.2 ± 6.7	60.5 ± 9.0[2]	38.1 ± 10.8	51.7 ± 7.5	21.4 ± 8.4[2]	41.2 ± 10.0
Before lunch	77.7 ± 4.7	72.6 ± 6.4	63.4 ± 7.4	25.2 ± 7.7	17.1 ± 3.8	26.4 ± 6.4
After lunch	19.9 ± 6.4	21.8 ± 6.7	22.3 ± 7.05	63.0 ± 11.8	72.8 ± 7.3	64.2 ± 8.7
During the afternoon	44.9 ± 9.0	44.8 ± 10.2	41.4 ± 10.2	40.4 ± 9.3	51.7 ± 9.2	49.0 ± 9.8
Before dinner	71.8 ± 5.5	84.3 ± 3.2	81.0 ± 7.1	21.0 ± 5.0	13.4 ± 2.7	18.8 ± 5.6
After dinner	22.6 ± 7.3	21.2 ± 5.8	18.4 ± 9.2	59.9 ± 11.2	72.2 ± 7.3	69.7 ± 12.4[3]
During the evening	35.1 ± 8.8[3]	44.7 ± 8.9	29.3 ± 9.4	55.3 ± 13.1[3]	39.7 ± 8.1	52.4 ± 10.4
Before going to bed	45.3 ± 12.2[3]	52.5 ± 9.8	31.2 ± 10.5	41.6 ± 13.6[3]	36.1 ± 7.7	46.3 ± 9.7

[1] $\bar{x}$ ± SE; $n = 9$ except where indicated otherwise. LF, low-fat; HF, high-fat. There were no significant differences at any time point (repeated-measures ANOVA).
[2] $n = 7$.
[3] $n = 8$.

significantly, indicating that the adaptation was not completely achieved. Earlier studies with carbohydrate diets reported similar results (3). Hill et al (34) found that even after 7 d of a high-carbohydrate diet adaptation was not completely achieved, whereas subjects on a high-fat diet showed a smaller difference between the RQ and the FQ. With the HF-placebo treatment, the subjects' RQ was not significantly different from their FQ, showing that fat oxidation had adapted to the high-fat diet within 4 d. This is in accordance with our previous results, indicating that adaptation of fat oxidation to a high-fat diet is accomplished within 3–7 d (35). With the HF-etomoxir treatment of the present study, the RQ was significantly higher than the FQ, revealing that etomoxir partly reversed the adaptive increase in fat oxidation.

With respect to triacylglycerol concentrations, clomoxir, which also inhibits CPT-I, has been shown to increase plasma triacylglycerols acutely and to have a triacylglycerol-lowering effect when administered chronically (36). Accordingly, we found an increase in plasma triacylglycerols after a single administration of etomoxir in our earlier study, whereas in the present study, after repeated administration, we observed a decrease in plasma triacylglycerol concentrations. Chronic administration of clomoxir has been shown to increase cardiac lipoprotein lipase activity and postheparin plasma lipoprotein lipase, which leads to an increased clearance of triacylglycerols from plasma (37). Such a mechanism may also be responsible for the triacylglycerol-lowering effect of etomoxir reported here.

The plasma concentration of BHB can be used as an indicator of hepatic fatty acid oxidation and is therefore especially interesting when studying the effect of hepatic fatty acid oxidation on satiety. However, in contrast to our previous observation after a single administration of etomoxir (19), plasma BHB was, on average, not significantly affected by repeated administration of etomoxir in the present study. However, individual differences in BHB between the etomoxir and placebo treatments on day 4 were strongly correlated with the difference in 24-h RQs between the 2 treatments. Therefore, our results are consistent with a suppressive effect of etomoxir on hepatic fatty acid oxidation, but there seems to be a large interindividual variability in this effect. It is also possible that the inhibition of hepatic fatty acid oxidation by etomoxir is more pronounced in the acute than in the chronic situation, where adaptational changes in response to inhibition of CPT-I might occur.

The absence of a general effect of repeated etomoxir administration on plasma BHB on days 4 and 5 is in agreement with the absence of an effect of etomoxir on hunger. Yet, it should also be considered in this context that hunger and satiety ratings do not always correspond exactly to actual food intake (15). To keep subjects in energy balance, we provided them with a fixed diet, which made it impossible to measure ad libitum food intake. Furthermore, in our earlier study subjects were selected who habitually ate a high-fat diet, reflecting a population who is adapted to a high-fat diet for a long time and who might be conditioned to rely on hepatic fatty acid oxidation as a satiety factor. Although the subjects in the present study were adapted in terms of whole-body substrate oxidation, other adaptations such as conditioning might only occur during a longer term.

Protein and carbohydrate have been shown to be oxidized before fat, and accordingly, a hierarchy in satiety has been described in which protein and carbohydrate are more satiating than is fat (38). We confirmed these findings in a previous study, showing that 24-h satiety ratings in healthy women were lower when a high-fat diet was consumed than when a high-protein–high-carbohydrate diet was consumed (20). In the present study, we used diets with the same macronutrient composition as in the previous study (20), but this time with a 3-d adaptation period at home. In this setting, the subjects were adapted to the high-fat diet and to the low-fat diet. Thus, it was possible to examine the effect of substrate oxidation in an adapted rather than in an acute situation, and under these conditions we could not detect any difference in 24-h satiety between the high-fat and low-fat diets.

In conclusion, we found a clear effect of repeated etomoxir administration on whole-body substrate oxidation, and our results suggest that hepatic fatty acid oxidation also may be inhibited by etomoxir. However, under the current conditions, we did not find an effect on hunger and satiety ratings. The results show that the acute effects of etomoxir cannot be completely extrapolated to the longer term and emphasize the importance of a period of long adaptation to dietary fat for effects on satiety.

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