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Role of glycogen-lowering exercise in the change of fat oxidation in response to a high-fat diet

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Schrauwen, Patrick, Wouter D. Van Marken Lichtenbelt, Wim H. M. Saris, and Klaas R. Westerterp. Role of glycogen-lowering exercise in the change of fat oxidation in response to a high-fat diet. *Am. J. Physiol.* 273 (*Endocrinol. Metab.* 36): E623–E629, 1997.—One of the candidate factors for determining the increase of fat oxidation after a switch from a reduced-fat diet to a high-fat diet is the size of the glycogen storage. Therefore, we studied the effect of low glycogen stores on fat oxidation after a switch from a reduced-fat diet to a high-fat diet. Twelve healthy, nonobese males and females (age: 22 ± 1 yr, body mass index: 21.0 ± 0.7, maximal power output: 254 ± 11 W) consumed a reduced-fat (RF) diet (30, 55, and 15% of energy from fat, carbohydrate, and protein, respectively) three times a day at home for 3 days (*days 1–3*). On two occasions subjects came to the laboratory on *day 3* at 1500 to perform an exhaustive glycogen-lowering exercise (EX), after which they went into the respiration chamber for a 36-h stay. On one occasion, subjects directly entered the respiration chamber at 1800 for a 36-h stay. In the respiration chamber they were given either a high-fat (HF) diet (60, 25, and 15% of energy from fat, carbohydrate, and protein, respectively) or a RF diet. In both cases they were fed at energy balance. All diets were consumed as breakfast, lunch, dinner, and two or more snacks per day. On the HF treatment, fat oxidation was below fat intake, indicating the slow change of oxidation to intake on a HF diet. After the HF+EX treatment, however, fat oxidation matched fat intake. In conclusion, lean subjects are capable of rapidly adjusting fat oxidation to fat intake when glycogen stores are lowered by exhaustive exercise.

respiration chamber; glycogen stores; obesity

THE INCIDENCE OF OBESITY is still increasing in affluent societies. Both increased fat content of the diet and decreased physical activity have been proposed to explain this phenomenon (4). Therefore, much attention has been paid to energy and substrate metabolism in obesity. It was found that weight maintenance requires, in addition to energy balance, balances of the separate macronutrients. For protein and carbohydrate, such balances are maintained within narrow ranges. Fat balance depends more on energy balance (1). Important work in this field is done by Flatt, who has postulated a two-compartment model (8). This model states that, in response to an increase in dietary fat content, fat oxidation can be increased by two mechanisms: *1*) glycogen stores can be maintained in a lower range, leading to lower glucose and insulin concentrations between meals, and hence higher fatty acid concentrations and higher rates of fat oxidation, and/or *2*) expansion of the adipose tissue mass, which leads to enhanced fat oxidation.

In humans, it has been shown that fat intake does not promote its own oxidation (26). In a previous study we showed that, when dietary fat content is increased, lean subjects are capable of adjusting fat oxidation to fat intake within 7 days (25). In this study, subjects spent 2 days on a reduced-fat diet (30% energy as fat) and, subsequently, 3 days on a high-fat diet (60% energy as fat) in the respiration chamber. After 7 days on the high-fat diet, subjects spent another day in the respiration chamber. Fat oxidation gradually increased on the high-fat diet and equaled fat intake 7 days after the dietary switch. During the first days on the high-fat diet, negative carbohydrate balances were found (on average −142 g). We therefore suggested that declining glycogen stores might be responsible for the gradual increase in fat oxidation. However, other mechanisms such as increased enzymatic capacity for fat oxidation might be responsible for the increase in fat oxidation.

In this study we investigated the role of glycogen stores in the change of fat oxidation in response to a high-fat diet. Glycogen was lowered by means of exercise bouts, and substrate oxidation was measured using a respiration chamber. We hypothesized that the change in fat oxidation in response to an increased fat intake can be accelerated when glycogen stores are low.

SUBJECTS AND METHODS

Subjects

The characteristics of the 12 volunteers (6 men, 6 women) participating in this study are shown in Table 1. All subjects were healthy, untrained (not active in any sport, no training history), and nonobese. Subjects' habitual energy intake was 11.0 ± 0.7 MJ/day, with 34 ± 1, 52 ± 2, and 13 ± 1% of energy from fat, carbohydrate, and protein, respectively. The study was approved by the Ethical Committee of the Maastricht University, and subjects gave their written informed consent.

Experimental Design

The experimental design is depicted in Fig. 1, *A-C*. Each subject followed three different treatments. Treatments were separated by ≥1 wk and were conducted in random order. Each treatment consisted of a 36-h stay in the respiration chamber. Before all three treatments, subjects were given a reduced-fat diet for consumption at home for 3 days (*days 1–3*). On two occasions subjects came to the laboratory on *day 3* at 1500 to perform an exhaustive glycogen-lowering exercise (EX) and then to enter the respiration chamber at 1800 for a 36-h stay. In the respiration chamber they were given either a high-fat diet (HF+EX) or a reduced-fat diet (RF+EX). The RF diet contained 30% energy as fat, as is often recommended in the prevention of obesity (4). On the third occasion no glycogen-lowering exercise was performed, but subjects directly entered the respiration chamber at 1800 for a 36-h

Table 1. *Subject characteristics*

	Age, yr	Height, m	Weight, kg	Percent Fat	BMI, kg/m²	W_{max}, W	W_{max}/kg FFM, W
Total	21.5 ± 0.7	1.79 ± 0.03	66.7 ± 2.2	19 ± 3	21.0 ± 0.7	254 ± 11	4.71 ± 0.14
Males	20.8 ± 1.1	1.85 ± 0.04[*]	68.0 ± 3.2	11 ± 3[†]	19.8 ± 0.4	279 ± 20[*]	4.66 ± 0.19
Females	22.2 ± 1.0	1.73 ± 0.02	65.5 ± 2.9	27 ± 1	22.2 ± 1.1	229 ± 11	4.77 ± 0.16

Values are means ± SE. BMI, body mass index; W_{max}, maximal power output; FFM, fat-free mass. [*] $P < 0.05$, [†] $P < 0.001$ vs. females.

stay, where they were given a high-fat diet (HF). On the morning of *day 5*, subjects left the respiration chamber at 0800.

Maximal power output. One week before the experimental treatment, each subject performed an incremental exhaustive exercise test on an electronically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands) to determine maximal heart rate and maximal power output (W_{max}). Exercise was performed until voluntary exhaustion or until the subject could no longer maintain a pedal rate of >60 rpm. Female subjects started at 50 W and male subjects at 75 W for 5 min. Thereafter, workload was increased by 50 W every 2.5 min. When subjects were approaching exhaustion, as indicated by heart rate and subjective scoring, the increment was reduced to 25 W. In practice, this meant that the last one to three load increments were 25 W. Heart rate was registered continuously using a Polar Sport tester (Kempele, Finland). In each individual W_{max} was calculated from

$$W_{max} = W_{out} + (t/150) \times \partial W$$

in which W_{out} is the highest workload completed by the subject, t is the time (in s) performed on the last workload, and ∂W is the final uncompleted load increment (15).

Glycogen-lowering exercise. After fasting for 2 h, the subjects came to the lab at 1500 to perform a glycogen-lowering exercise test. After a warm-up at 50% of their W_{max} for 5 min, subjects cycled for 2 min at 90% of W_{max} followed by 2 min at 50% of W_{max}. This was repeated until subjects were no longer able to perform the high-intensity exercise. The maximal intensity was then lowered to 80% of W_{max}. Again, when this intensity could no longer be maintained, the maximal intensity was decreased to 70% of W_{max}. The test was ended after exhaustion. Subjects were allowed to consume water during exercise. During the exercise heart rate was measured continuously with a Polar Sport tester. Energy expended during the exercise was calculated by assuming a mechanical efficiency of 20% (11).

Diets

Before the experiment, subjects filled in a 3-day food intake record to estimate habitual diet composition. Metabolizable energy intake and macronutrient composition of the diet were calculated using the Dutch food composition table (28). In the table metabolizable energy is calculated by multiplying the amount of protein, fat, and carbohydrate with the Atwater factors (16.74, 37.66, and 16.74 kJ/g for carbohydrate, fat, and protein, respectively) (19). The amount of protein, fat, and carbohydrate was multiplied by 0.909, 0.948, and 0.953, respectively, to correct for digestibility of macronutrients. All experimental diets were consumed as breakfast, lunch, dinner, and two or more snacks per day. The composition of experimental diets is given in Table 2. All snacks had the same macronutrient composition as the experimental diet.

On *days 1* and *2* and the first part of *day 3*, an RF diet for consumption at home was provided. Subjects were given a fixed amount of food (based on their food intake record) and

ad libitum access to snacks. On the evening of *day 3*, subjects consumed their dinner and evening snack (either RF or HF) in the respiration chamber. In the HF treatment, energy intake for dinner and evening snack was fixed at 35 and 15% of estimated daily energy expenditure [1.7 × basal metabolic rate (BMR) based on the Harris and Benedict equations; for women: BMR = 2.74 + 0.774 × H + 0.040 × BM − 0.020 × A, and for men: BMR = 0.28 + 2.093 × H + 0.058 × BM − 0.028 × A, in which BMR is in MJ/day, H is height in meters, BM is body mass in kilograms, and A is age in years] (12). In the RF+EX and HF+EX treatments, the evening snack had an energy content equal to energy expended during the exercise test. 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 it was shown that, with a comparable activity protocol used in the chamber, a physical activity index of 1.58 was reached (20).
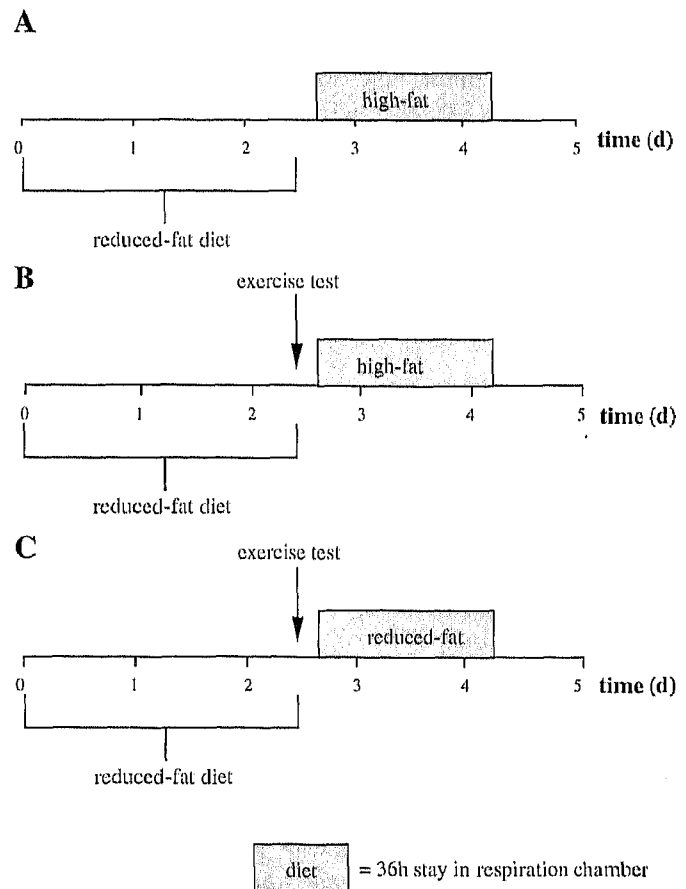
A

B

C

Fig. 1. Experimental design of the 3 different treatments. *A*: high-fat diet (HF) treatment; *B*: HF plus exercise (HF+EX) treatment; *C*: reduced-fat diet + EX (RF+EX) treatment. Time measured in days (d).

Table 2. *Composition of experimental diets*

	Reduced-Fat Diet	High-Fat Diet
Protein	15	15
Carbohydrate	55	25
Fat	30	60
Food quotient	0.88	0.80

Values of protein, carbohydrate, and fat are expressed as % of total energy.

Procedures

Body composition. Subjects weighed themselves in the respiration chamber on the morning of *days 4* and *5*, without clothing, after voiding, and before eating and drinking. Measurements were done on a digital balance (Seca delta, model 707) with an accuracy of 0.1 kg.

Whole body density was determined by underwater weighing in the morning in the fasted state. Body weight was measured with a digital balance with an accuracy of 0.01 kg (Sauter, type E1200). Lung volume was measured simultaneously with the helium dilution technique by use of a spirometer (Volugraph 2000, Mijnhardt). Percent body fat was calculated using the equations of Siri (27). Fat-free mass (in kg) was calculated by subtracting fat mass from total body mass.

Indirect calorimetry and physical activity. O_2 consumption and CO_2 production were measured in a whole-room indirect calorimeter (24). The respiration chamber is a 14-m^3 room furnished with a bed, chair, television, radio, telephone, intercom, wash bowl, and toilet. The room is ventilated with fresh air at a rate of 70–80 l/min. The ventilation rate is measured with a dry gas meter (Schlumberger, type G6, The Netherlands). The concentrations of O_2 and CO_2 are measured using a paramagnetic O_2 analyzer (type Magnos G6, Hartmann & Braun, Frankfurt, Germany) and an infrared CO_2 analyzer (type Uras 3G, Hartmann & Braun). Ingoing air is analyzed every 15 min and outgoing air once every 5 min. The gas sample to be measured is selected by a computer that also stores and processes the data. Energy expenditure is calculated from O_2 consumption and CO_2 production according to the method of Weir (32).

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 for 30 min at intervals of 5 min of exercise alternated with 5 min of rest, at a rate of 60 steps per min with a bench height of 33 cm, and was repeated three times a day. Thus subjects exercised for 45 min per day, at a relative low-to-medium intensity. At 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 stay in the respiration chamber urine was collected in two batches, the first from 2000 to 0800 and the second over the subsequent 24-h interval. Subjects were requested to empty the bladder 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; volume and nitrogen concentration were measured, the latter with a nitrogen analyzer (type CHN-O-Rapid, Heraeus).

Twenty-four-hour energy expenditure and substrate oxidation. Subjects stayed in the respiration chamber for 36 h. Data from 2000 on *day 3* to 0800 on *day 4* are presented to study short-term effects of treatments. For calculation of

balances, 24-h energy expenditure (EE) and 24-h respiratory quotient (RQ) were measured from 0800 on *day 4* to 0800 on *day 5*. SMR was defined as the lowest mean EE during three subsequent hours measured between 0000 and 0800 with a minimal activity level as indicated by the radar system.

Carbohydrate, fat, and protein oxidation were calculated using O_2 consumption, CO_2 production, and urinary nitrogen losses with the equations of Brouwer (5)

Protein oxidation (g/day) = 6.25 × N

Fat oxidation (g/day) = 1.718 × $\dot{V}O_2$ − 1.718 × $\dot{V}CO_2$

$$- 0.315 \times P$$

Carbohydrate oxidation (g/day)

$$= 4.17 \times \dot{V}CO_2 - 2.965 \times \dot{V}O_2 - 0.390 \times P$$

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

Statistical Analysis

All data are presented as means ± SE. Equality of RQ and food quotient (FQ), energy intake, and EE, as well as substrate intake and substrate oxidation, was determined by calculating the 95% confidence intervals for differences. A nonparametric Wilcoxon test, using a Bonferroni correction, was used to detect any differences in measured parameters between treatments.

RESULTS

Time until exhaustion during the exercise test was not significantly different between the RF+EX and HF+EX treatments: 71 ± 4 and 69 ± 4 min, respectively. Also, no difference in EE during the exercise tests was found: 3.4 ± 0.8 and 3.5 ± 0.3 MJ for RF+EX and HF+EX treatments, respectively.

Body weight, as measured in the respiration chamber, was not significantly different among any of the treatments (Table 3).

SMR measured during the first night was significantly increased in the RF+EX treatment compared with the HF treatment ($P < 0.05$). In the HF+EX treatment, SMR tended to be increased compared with the HF treatment ($P = 0.06$). However, SMR measured during the second night was not significantly different among any of the treatments (Table 3). Twenty-four-hour EE (Table 4) and physical activity index (= 24-h

Table 3. *SMR, PAI, and body weight measured in respiration chamber*

Treatment	kJ/min		PAI	Body Weight, kg
	Night 1	Night 2		
RF + EX	4.52 ± 0.14*	4.42 ± 0.15	1.60 ± 0.02	66.8 ± 2.3
HF	4.32 ± 0.14	4.42 ± 0.15	1.57 ± 0.03	66.6 ± 2.3
HF + EX	4.52 ± 0.17†	4.44 ± 0.15	1.59 ± 0.02	66.7 ± 2.3

Values are means ± SE. SMR, sleeping metabolic rate; PAI, physical activity index; RF and HF, reduced-fat and high-fat diet, respectively; EX, exercise. PAI: 24-h energy expenditure/24-h SMR. *$P < 0.05$, †$P = 0.056$ vs. HF.

Table 4. *Energy intake, expenditure, and balance of subjects on 3 different treatments as measured in respiration chamber*

Treatment	Intake	Expenditure	Balance
RF+EX	10.02 ± 0.38	10.19 ± 0.38	−0.17 ± 0.19
HF	10.00 ± 0.37	9.99 ± 0.32	+0.00 ± 0.21
HF+EX	10.04 ± 0.37	10.17 ± 0.38	−0.13 ± 0.16

Values are means ± SE expressed in MJ for a 24-h (8:00 AM–8:00 AM) measurement.

EE/SMR, Table 3) were not significantly different among treatments.

On the evening after the exercise test, subjects were given an amount of energy to compensate for the EE during the exercise bout. Therefore, of course, a positive energy balance was measured during the first 12 h in the chamber. However, this positive energy balance was not significantly different between the RF+EX and HF+EX treatments (2.89 ± 0.2 vs. 2.90 ± 0.24 MJ). RQ during the first 12 h in the respiration chamber was 0.843 ± 0.014, 0.846 ± 0.023, and 0.803 ± 0.015 for the RF+EX, HF, and HF+EX treatments, respectively. The RQ was significantly lower in the HF+EX than in the RF+EX and HF treatments ($P < 0.01$). In the HF treatment all energy of the positive energy balance (1.71 ± 0.19 MJ) was stored as fat. In the RF+EX treatment a positive carbohydrate balance of 136.6 ± 22.5 g and a positive fat balance of 9.7 ± 9.4 g were reached, whereas in the HF+EX treatment those values were +48.7 ± 21.8 and +49.3 ± 26.6 g for carbohydrate and fat, respectively. Thus glycogen was more repleted in the RF+EX than in the HF+EX treatment and, as a result, differences in glycogen store were obtained.

In all three tests, 24-h energy balance (*day 4*) was not significantly different from zero. Twenty-four-hour RQ was significantly different among all treatments ($P < 0.05$). RQ was lowest in the HF+EX treatment and highest in the RF+EX treatment (Fig. 2). RQ was significantly different from FQ in the HF and RF+EX treatment ($P < 0.05$). In the HF+EX treatment, RQ and FQ were not significantly different (Fig. 2).

Twenty-four-hour protein oxidation was not significantly different among treatments (Table 5). In the RF+EX treatment, protein intake was significantly higher than in the HF and HF+EX treatments (84 vs. 78 g/day, Table 5, $P < 0.05$), resulting in a significantly positive protein balance in the RF+EX treatment (Fig. 3, $P < 0.05$).

Twenty-four-hour carbohydrate oxidation was significantly different among all treatments, being highest in the RF+EX treatment and lowest in the HF+EX treatment ($P < 0.01$, Table 5), resulting in positive carbohydrate balance in the RF+EX treatment and negative carbohydrate balance in the HF treatment (Fig. 3).

Twenty-four-hour fat oxidation was significantly different among all treatments, being lowest in the RF+EX treatment and highest in the HF+EX treatment ($P < 0.05$, Table 5), resulting in negative fat balance in the

RF+EX treatment, positive fat balance in the HF treatment, and zero fat balance in the HF+EX treatment (Fig. 3).

DISCUSSION

The results of the present study demonstrate that, after a switch from an RF diet to an HF diet under conditions of energy balance, fat oxidation can be increased rapidly to levels that match fat intake when glycogen stores are lowered by exhaustive exercise. After glycogen-lowering exercise in combination with an HF diet, fat oxidation was equal to fat intake, whereas on the HF diet without glycogen-lowering exercise, fat oxidation was not increased sufficiently to match fat intake. After glycogen-lowering exercise in combination with the RF diet, glycogen was partly repleted and the increase in fat oxidation was less marked, providing another indication of the important role of glycogen content in the regulation of fat oxidation.

Flatt (8) has proposed a model in which he states that, on an HF diet, fat oxidation can be increased by two mechanisms. First, fat oxidation can be raised by maintaining glycogen stores in a lower range. Second, expansion of the fat mass will lead to increase in fat oxidation (2). In our experiment, subjects were fed at energy balance and therefore expansion of the fat mass was negligible. We found that, on the HF diet, fat oxidation was increased sufficiently to equal fat intake when glycogen stores were low. Therefore, our data are in agreement with the model of Flatt and demonstrate the importance of glycogen stores in increasing fat oxidation on an HF diet. In both the RF+EX and the HF+EX treatments, glycogen was lowered by means of exercise. During the first 12 h in the chamber, carbohydrate balance was more positive during the RF+EX
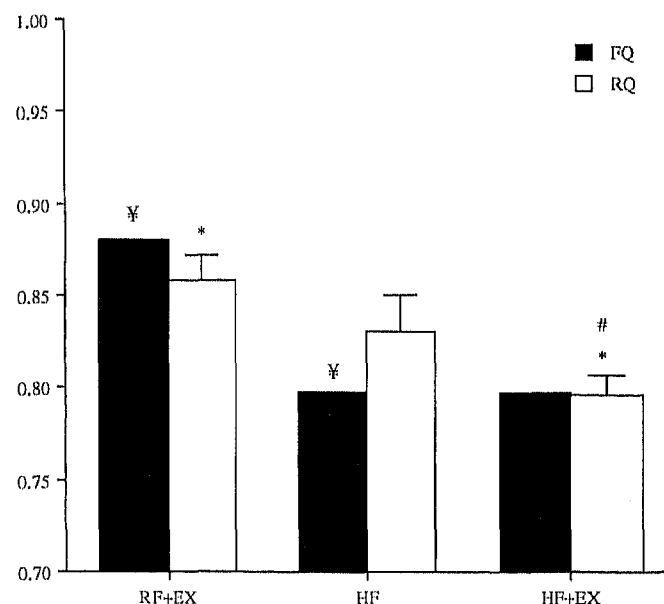

Fig. 2. Twenty-four-hour respiratory quotients (RQ) and food quotients (FQ) as measured in respiration chamber for *day 4* (means ± SE). Significant difference ($P < 0.05$) vs. *HF; #RF+EX; ¥RQ.

Table 5. *Carbohydrate, fat, and protein intake and oxidation of subjects on 3 different treatments as measured in the respiration chamber*

Treatment	CHO		Fat		Protein	
	Intake	Oxidation	Intake	Oxidation	Intake	Oxidation
RF + EX	334 ± 13*†	283 ± 13*†	80 ± 3*†	101 ± 6*†	85 ± 3*†	67 ± 4
HF	160 ± 6	219 ± 10†	160 ± 6	124 ± 8†	76 ± 5	68 ± 3
HF + EX	159 ± 6	149 ± 6	161 ± 6	156 ± 9	77 ± 4	75 ± 4

Values are means ± SE expressed in g/day (8:00 AM–8:00 AM). CHO, carbohydrate. *$P < 0.05$ vs. HF; †$P < 0.05$ vs. HF + EX.

treatment than during the HF+EX treatment (+137 g vs. +49 g). When the size of the positive carbohydrate balance and the prior exercise regimen are considered, most likely this positive carbohydrate balance is stored as glycogen. Therefore, glycogen was more repleted in the RF+EX treatment. The difference in fat oxidation between the RF+EX and HF+EX treatment can thus be explained by differences in both glycogen stores and exogenous carbohydrate availability. However, the higher fat oxidation in the HF+EX treatment compared with the HF treatment (same exogenous carbohydrate availability) indicates the important role glycogen stores play in the regulation of fat oxidation.

In this study, we assumed that subjects on the RF diet (which was almost similar to their habitual diet) would have been in substrate balance. This would mean that the glycogen-lowering exercise increased fat oxidation by 21 g on the RF diet, whereas on the HF diet, fat oxidation was increased by 32 g, the difference most likely due to lower glycogen stores in the HF+EX treatment compared with the RF+EX treatment.

Using indirect calorimetry for the assessment of substrate oxidation requires some precaution. The assumptions involved in using indirect calorimetry to determine substrate utilization have been reviewed by Livesey and Elia (17). They concluded that the accuracy of indirect calorimetry for assessing substrate oxida-

tion is at best within 5% of the true value. Stubbs et al. (29) reported an accuracy of ±20 g/day and 9.5 g/day for 24-h carbohydrate and fat oxidation, respectively, as measured in a respiration chamber. Thus changes in substrate oxidation of 64 and 23 g/day for carbohydrate and fat oxidation, respectively, reported in the present study (Table 5) are well detectable.

In our study we lowered the glycogen stores by means of exercise. It is well known that during exercise at high intensities the major substrate for oxidation is carbohydrate. It has repeatedly been shown in our laboratory by Kuipers et al. (16) and Wagenmakers et al. (31) and in other laboratories (6, 13, 18, 33) that glycogen stores in muscle are significantly decreased both in male and female subjects after exhaustive exercise protocols. The energy expended in both exercise tests was the same, so it can be assumed that glycogen was lowered to the same level. To prevent increases in fat oxidation due to an exercise-induced negative energy balance on the exercise day, energy intake on the first evening in the chamber was based on the energy expended during exercise. This resulted in a positive energy balance that was of the same magnitude in the HF+EX and RF+EX treatments. However, there was a difference in carbohydrate balance. Thus the protocol used must have resulted in differences in glycogen repletion and, therefore, differences in fat oxidation between the HF+EX and RF+EX treatments.

It is possible that the exercise bout itself resulted in other (hormonal) adaptations that accelerated fat oxidation. Bielinski et al. (3) found in their study that 2.5 h after cessation of exercise, free fatty acid (FFA), glycerol, and glucagon concentrations returned to their control values. This indicates that the (hormonal) disturbances induced by exercise are not long lasting. In our study the elevation in fat oxidation on the HF+EX treatment was long lasting. RQ during sleep, for example, was not significantly different between the 1st and 2nd nights in the respiration chamber, in either the RF+EX or HF+EX treatment (data not shown). If the elevation in fat oxidation was an exercise effect other than glycogen lowering, it would be expected that fat oxidation would have returned to lower levels after 36 h. We therefore conclude that the increase in fat oxidation was long lasting and cannot be explained by the exercise itself but can be explained by low glycogen stores.

The mechanism by which low glycogen levels may lead to increased fat oxidation is not clear. Randle et al. (21) introduced the "glucose-fatty acid cycle" in 1963 to
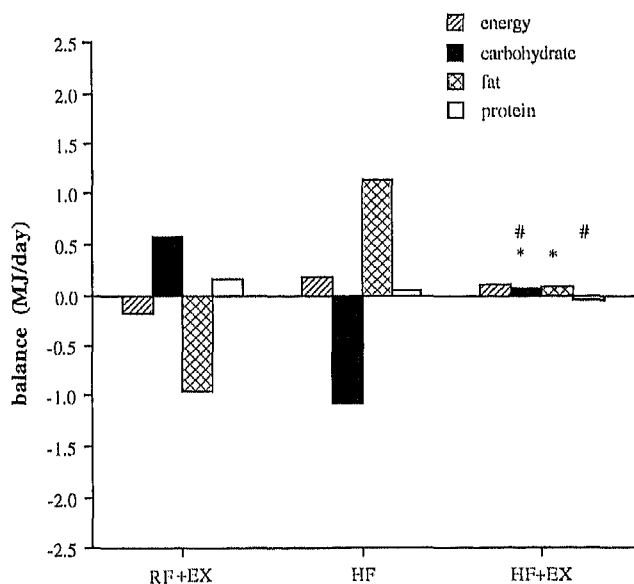

Fig. 3. Twenty-four-hour energy and substrate balances for *day 4* as measured in respiration chamber (means ± SE). Significant difference ($P < 0.05$ by Wilcoxon signed-rank test) vs. *HF; #RF+EX.

describe the interaction between fat and carbohydrate oxidation. They suggested that an increased availability of FFA leads to an increased fat oxidation. In our study the glycogen-lowering exercise, especially in combination with the HF diet, might have resulted in lower insulin levels and, thus, less inhibition of lipolysis (7), in turn leading to increased FFA availability and, according to the glucose-fatty acid cycle, increased fat oxidation. However, a recent study in rats showed that glycogen depletion per se does not result in increased FFA uptake and oxidation but increased blood glucose utilization, even at high FFA levels (30). In our experiment, fat oxidation was highest when total carbohydrate availability was low. It therefore seems that carbohydrate oxidation takes priority over fat oxidation and that total carbohydrate availability regulates fat oxidation, for example, through the conversion of acetyl-CoA into malonyl-CoA, which is an important inhibitor of carnitine palmitoyltransferase, necessary for FFA uptake and thus oxidation (22). This might explain why fat oxidation does not match fat intake on the short term as long as endogenous carbohydrate availability is high. Further studies are necessary to elicit the exact mechanism behind the increase in fat oxidation when glycogen levels are decreased.

Recently, Fleury et al. (10) reported the discovery of a gene that codes for the uncoupling protein-2 (UCP2), which might have a role in diabetes and obesity. UCP2, which is expressed in a number of adult human tissues, might be involved in the regulation of energy balance by influencing EE and thermogenesis. On an HF diet, UCP2 mRNA levels were significantly increased, and raised levels of FFA may very well activate UCP2 in white adipocytes. Future work must reveal whether this finding is important for the interpretation of our results.

This experiment was performed in healthy, nonobese subjects. It has to be examined whether the same results can be found in obese subjects. Obesity often has been linked to a high fat intake, and therefore much attention has been focused on decreasing the percentage of energy intake derived from fat (14). Because of the high energy density of high-fat foods, this strategy may lead to decreased energy intake and in this way be effective in the treatment of obesity. Another factor that might explain the increased prevalence of obesity in Western societies is a low level of physical activity (9, 23). Our results show that acute exercise (accompanied by decreasing glycogen concentration) is an effective method to handle a high fat intake. The same level of physical activity in combination with a RF diet resulted in a negative fat balance. Therefore, regular physical activity can be an effective strategy in the prevention of obesity. Apart from training effects that might stimulate fat oxidation, regular physical activity can prevent obesity by causing glycogen stores to be maintained in a lower range (9).

In conclusion, our results demonstrate that, after switching from a RF diet to a HF diet, lean subjects are capable of adjusting fat oxidation to fat intake when glycogen stores are lowered by exhaustive exercise.

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