Changes in fat oxidation in response to a high-fat diet.

Changes in fat oxidation in response to a high-fat diet[1,2]

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ABSTRACT Intervention studies have shown that the adaptation of fat oxidation to fat intake, when the dietary fat content is changed, is not abrupt. This study was conducted to measure the time course of adaptation of oxidation rates to increases in the fat content of the diet while subjects were fed at energy balance. Twelve healthy, nonobese males and females [age: 26 ± 2 y, body mass index (in kg/m^2): 21.4 ± 0.5; and habitual fat intake: 29 ± 1% of energy] consumed a low-fat diet for 6 d (days 1–6) followed by a high-fat diet for 7 d (days 7–13). Days 5–9 and 13 were spent in a respiration chamber. After adjustment for energy intake to 24-h energy expenditure on day 5, subjects were in energy balance (range: −0.15 to 0.23 kJ/d) on days 6–9 and 13. Fat balance was zero on day 6 but became positive after subjects changed to the high-fat diet (1.06 ± 0.15, 0.75 ± 0.15, and 0.55 ± 0.14 MJ/d for days 7, 8, and 9, respectively, $P < 0.05$), reaching a new balance on day 13, 7 d afterward. In conclusion, when in energy balance, lean subjects are capable of adjusting fat oxidation to fat intake within 7 d of when dietary fat content is increased. *Am J Clin Nutr* 1997;66:276–82.

KEY WORDS Respiration chamber, substrate oxidation, sex differences, high-fat diet, fat oxidation, humans, energy expenditure

INTRODUCTION

Weight maintenance requires that, in the long term, energy intake matches energy expenditure. Apart from energy balance, this also requires the oxidation rate to be equal to intake for separate nutrients. For both protein and carbohydrate it has been shown that the rate of oxidation is well adjusted to intake (1, 2). The body's storage capacity for fat is 100 times the storage capacity for carbohydrate. Therefore, fat stores are considered to be the main energy stores for humans and maintaining zero fat balance has little priority. Indeed, it has been shown that fat balance is poorly regulated and positive or negative energy balances (energy intake minus energy expenditure) are accommodated by gains or losses of fat (3, 4). These principles are postulated in the two-compartment model of Flatt (5). This model states that when the dietary fat content increases, fat oxidation can be raised by two mechanisms: *1*) glycogen concentration 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, or *2*) expansion of the adipose tissue mass, which leads to enhanced fatty acid oxidation.

In our Western society, with a surplus of food available, it is unlikely that the body would compensate for the lower carbohydrate content of a high-fat diet by working at low glycogen concentrations. It is more likely that food intake will be increased. As a result, high-fat diets will lead to a positive energy balance and therefore to gains of fat mass. Indeed, it has been shown that voluntary energy intake is higher with high-fat diets (6, 7) and that high-fat diets are fattening (8–10). Therefore, ad libitum consumption of a high-fat diet will lead to expansion of the body's fat stores, which will increase fat oxidation (11) until a new equilibrium is reached between fat intake and fat oxidation.

However, when energy intake is fixed, rapid shifts in substrate oxidation have been shown (12). In Hill's experiment, subjects were studied for 7 d on a diet with a food quotient of 0.770 and for 7 d on a diet with a food quotient of 0.917. Twenty-four–hour respiratory quotient (24-h RQ) was determined 3 and 7 d after the start of each diet by using a respiration chamber. 24-h RQ shifted in the direction of the food quotient on both diets. However, even after 7 d, no complete adjustment was observed. In the respiration chamber subjects were fed in energy balance. However, as suggested by the authors, they might have been in negative energy balance on days outside the respiration chamber. To our knowledge, no studies have been reported that measured both acute (within days) and long-term (≤ 1 wk) adaptation of substrate oxidation to alterations in diet composition.

The fat content of the diet may be of special relevance in the development and treatment of obesity. It has been suggested that in obese people, adaptation to a high-fat diet is diminished (13, 14). However, before overweight subjects are studied, it is important to know how long adaptation to an increase in dietary fat content takes in normal-weight subjects. We therefore measured substrate oxidation on a habitual (low-fat) diet and subsequently the adaptation to a high-fat diet during 7 d while feeding subjects in energy balance. We hypothesized that complete adaptation to the changed fat content of the diet can occur within a few days.

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SUBJECTS AND METHODS

Subjects

The characteristics of the 12 volunteers (6 men and 6 women) participating in this study are shown in **Table 1**. All subjects were healthy and had a habitual diet that could be considered low fat ($\bar{x} \pm$ SEM energy intake: 9.0 ± 0.7 MJ, of which 29 ± 1%, 54 ± 2%, and 16 ± 1% was provided as fat, carbohydrate, and protein, respectively, as determined with a 3-d food intake record). Subjects were nonobese and did not have a family history of obesity. The study was approved by the Ethical Committee of the Maastricht University and all subjects gave their written informed consent.

Experimental design

Subjects consumed a low-fat diet for 6 d, followed by a high-fat diet for 7 d. On days 1–4, subjects were instructed to consume a low-fat diet and to fill in a food intake record. On days 5 and 6 subjects were fed a low-fat diet in the respiration chamber. On days 7–13 subjects consumed a high-fat diet. On days 10–12 this high-fat diet was given to the subjects for consumption at home to guarantee that diet composition did not change. Subjects entered the respiration chamber on the evenings of days 4 and 12, and left the chamber in the morning (0800) of days 10 and 14.

Diets

All food was consumed as breakfast, lunch, dinner, and two or more snacks per day. The composition of the experimental diets is given in **Table 2**. All snacks had a fixed macronutrient composition. Metabolizable energy intake and macronutrient composition of the diets were calculated by using the Dutch food composition table (15). In the table, metabolizable energy is calculated by multiplying the amount of protein, fat, and carbohydrate by the Atwater factors (16) (16.74, 37.66, and 16.74 kJ/g for carbohydrate, fat, and protein, respectively). It was shown that the metabolizable energy of a diet decreases when the fiber content of the diet increases (17). Therefore, the diets were composed such that the fiber content was comparable (1.41 ± 0.04 compared with 1.13 ± 0.06 g/MJ for low and high-fat diets, respectively).

On days 1–4 and days 10–12, the days spent at home, subjects were free to eat as much as they wanted. On day 5, the first day in the respiration chamber, subjects were given an amount of energy equal to 1.5 times their 24-h sleeping metabolic rate (24-h SMR), as measured during the night. On days 6–10 and day 13 the amount of energy given was equal to the amount of energy expended on day 5. In this way, energy intake could be individually adjusted to energy expenditure, assuming no major changes in energy expenditure during the experiment.

TABLE 1
Subject characteristics measured at the end of the experimental period[1]

Characteristic	Males (n = 6)	Females (n = 6)	All subjects (n = 12)
Age (y)	28.5 ± 3.0	23.0 ± 1.0	25.8 ± 1.7
Height (m)	1.83 ± 0.03	1.71 ± 0.03	1.77 ± 0.03
Weight (kg)	72.0 ± 3.4	62.0 ± 1.9	67.0 ± 2.4
Percentage body fat (%)	15.1 ± 1.3	23.1 ± 2.4	19.1 ± 1.8
BMI (kg/m^2)	21.4 ± 0.7	21.3 ± 0.8	21.4 ± 0.5

[1] $\bar{x}$ ± SEM.

TABLE 2
Composition of experimental diets

	Low-fat diet	High-fat diet
Protein (% of energy)	15	15
Carbohydrate (% of energy)	55	25
Fat (% of energy)	30	60
Food quotient	0.878	0.798

Body composition

Subjects weighed themselves every morning during their stay in the respiration chamber, after voiding, and before eating and drinking. Measurements were made on a digital balance (Seca delta, model 707; Seca, Hamburg, Germany) accurate to 0.1 kg. On the morning of day 14, whole-body density was determined by underwater weighing in the fasted state directly after subjects left the respiration chamber. Body weight was measured with a digital balance accurate to 0.01 kg (Sauter, type E1200; August Sauter, Albstadtl-Ebingen, Germany). Lung volume was measured simultaneously with the helium dilution technique by using a spirometer (Volugraph 2000; Mijnhardt, Bunnik, Netherlands). Percentage body fat was calculated by using the equations of Siri (18). Fat-free mass (FFM; in kg) was calculated by subtracting fat mass from total body mass.

Indirect calorimetry and physical activity

Oxygen consumption and carbon dioxide production were measured in a whole-room indirect calorimeter, which was described previously (19). 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 was measured with a dry gas meter (type G6; Schlumberger, Dordrecht, Netherlands). The concentrations of oxygen and carbon dioxide were measured by using a paramagnetic oxygen analyzer (type Magnos G6; Hartmann & Braun, Frankfurt, Germany) and an infrared carbon dioxide analyzer (type Uras 3G; Hartmann & Braun). Ingoing air was analyzed 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 (20).

In the respiration chamber subjects followed an activity protocol with 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 exercise alternated with 5 min rest, at a rate of 60 steps/min with a bench height of 33 cm, and was repeated three times a day. Thus, subjects exercised for 45 min/d, at a low-to-medium intensity. During the day, no sleeping or other exercise was allowed during the stay in the respiration chamber. Spontaneous 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 24-h urine samples were collected from 0800 to 0800. Subjects had to empty their bladders at 0800 so that urine produced during the night could be included with the urine sample of the previous day. 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 by using a nitrogen analyzer (type CHN-O-Rapid; Heraeus, Hanau, Germany).

Twenty-four–hour energy expenditure and substrate oxidation

Twenty-four–hour energy expenditure (24-h EE) and 24-h RQ were calculated from 0800 to 0800. SMR was defined as the lowest mean energy expenditure during three subsequent hours measured between 0000 and 0800. For calculation of the thermic effect of food (TEF), energy expenditure from 0830 to 2330 was plotted against radar output over 30-min intervals. The intercept of the regression line at the lowest radar output (offset) represents the energy expenditure in the inactive state: SMR and TEF. TEF was determined by subtracting SMR from energy expenditure in the inactive state.

Carbohydrate, fat, and protein oxidation were calculated by using oxygen consumption, carbon dioxide production, and urinary nitrogen losses with the following equations of Brouwer (21):

$$\text{Protein oxidation (g/d)} = 6.25 \times \text{N} \quad (1)$$

Fat oxidation (g/d)

$$= 1.718 \times \dot{V}O_2 - 1.718 \times \dot{V}CO_2 - 0.315 \times P \quad (2)$$

Carbohydrate oxidation (g/d)

$$= 4.17 \times \dot{V}CO_2 - 2.965 \times \dot{V}O_2 - 0.390 \times P \quad (3)$$

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

Blood analysis

Ten milliliters of venous blood was sampled on the morning of days 4, 10, and 14 after an overnight fast. Blood was collected in tubes containing EDTA to prevent clotting and immediately centrifuged at 1000 × g (3000 rpm) and 4 °C for 10 min. Plasma was frozen in liquid nitrogen and stored at −80 °C until further analysis. Plasma substrates were determined by using the hexokinase method (LaRoche, Basel, Switzerland) for glucose, the Wako NEFA C test kit (Wako Chemicals, Neuss, Germany) for fatty acids, the glycerolkinase-lipase method (Boehringer, Mannheim, Germany) for glycerol and triacylglycerols, and the ultrasensitive human insulin radioimmunoassay kit (Linco Research, St Charles, MO) for insulin.

Statistical analysis

All data are presented as mean ± SEM. Equality of respiratory quotient and food quotient, as well as energy intake and energy expenditure, was determined by calculating the 95% CIs for respiratory quotient minus food quotient and energy intake minus energy expenditure. To test whether the decline in respiratory quotient observed between days 3 and 9 was statistically significant, individual slopes were calculated and tested by using the nonparametric t test (Wilcoxon). A repeated-measures one-way analysis of variance (ANOVA) was used to detect differences in any variables between days. When significant differences were found, a Tukey post hoc test was used to determine the exact location of this difference. Sex differences in any variable were analyzed by using repeated-measures two-factor ANOVA.

RESULTS

On days 1–4 subjects were instructed to consume a low-fat diet and to fill in a food intake record. This resulted in an energy intake of 8.4 ± 0.7 MJ—of which 25 ± 2%, 57 ± 2%, and 16 ± 1% were provided as fat, carbohydrate, and protein, respectively—and a food quotient of 0.890 ± 0.004.

There was a significant difference in body weight between days ($P < 0.05$). Post hoc testing revealed that body weight showed a slight but significant decline of 0.6 ± 0.2 kg between days 5 and 13 (**Table 3**). No significant differences in body weight between days 5 and 9 were observed.

There were no significant differences in SMR between days (Table 3). There was a significant difference in physical activity index (PAI = average daily metabolic rate/SMR) between days ($P < 0.05$). Post hoc testing revealed that PAI was somewhat lower during day 13 than during days 5, 6, and 7 (Table 3). No significant differences in PAI between days 5 and 9 were observed. The somewhat lower PAI on day 13 can be explained by a diminished energy expenditure for physical activity on this day because subjects reported feeling bored and spent their time watching television.

On day 5 (the first day in the respiration chamber) the average PAI was 1.60. Because the 24-h energy requirement was estimated with 1.5 × SMR, a significantly negative energy balance was found. After individual adjustment of energy intake to energy requirement, energy balance was not significantly different from zero on days 6–9 or 13 (**Table 4**).

24-h RQ and food quotient are presented in **Figure 1**. On days 5 and 6, respiratory quotient and food quotient were not significantly different. Between days 7 and 13, 24-h RQ declined significantly ($P < 0.005$), resulting in nearly equal respiratory quotient and food quotient values on day 13. Sleeping respiratory

TABLE 3
Sleeping metabolic rate (SMR), physical activity index (PAI), and body weight as measured in the respiration chamber[1]

Diet and day	SMR	PAI[2]	Body weight
	kJ/min		*kg*
Low fat			
Day 5	4.373 ± 0.185	1.60 ± 0.03[3]	67.5 ± 2.5[3]
Day 6	4.379 ± 0.165	1.60 ± 0.03[3]	67.2 ± 2.6
High fat			
Day 7	4.369 ± 0.169	1.60 ± 0.03[3]	67.4 ± 2.5
Day 8	4.386 ± 0.161	1.57 ± 0.03	67.5 ± 2.5[3]
Day 9	4.460 ± 0.179	1.59 ± 0.04	67.3 ± 2.5
Day 13	4.389 ± 0.180	1.55 ± 0.02	66.9 ± 2.4

[1] $\bar{x}$ ± SEM; n = 12.
[2] 24-h Energy expenditure/sleeping metabolic rate.
[3] Significantly different from day 13, $P < 0.05$.

TABLE 4
Twenty-four–hour energy intakes, expenditures, and balances[1]

Diet and day	Intake	Expenditure	Balance
		MJ/d	
Low fat			
Day 5	9.109 ± 0.383	10.012 ± 0.336	−0.903 ± 0.212[2]
Day 6	9.980 ± 0.325	10.083 ± 0.386	−0.103 ± 0.132
High fat			
Day 7	9.988 ± 0.327	10.025 ± 0.370	−0.037 ± 0.131
Day 8	9.982 ± 0.317	9.914 ± 0.363	0.068 ± 0.116
Day 9	9.990 ± 0.317	10.140 ± 0.405	−0.150 ± 0.176
Day 13	9.976 ± 0.319	9.750 ± 0.396	0.226 ± 0.134

[1] $\bar{x}$ ± SEM; n = 12.
[2] Significantly different from zero balance, $P < 0.05$.

quotient also declined significantly between days 7 and 13 (from 0.823 ± 0.009 to 0.798 ± 0.008, $P < 0.005$).

Protein balance was not significantly different from zero during the measurement days (**Figure 2**). Carbohydrate oxidation was significantly different between days ($P < 0.001$). Post hoc testing showed that carbohydrate oxidation was not significantly different between days 5 and 6. However, carbohydrate oxidation declined significantly between days 6 and 13. This resulted in a significantly negative carbohydrate balance on days 7, 8, and 9. On days 5 and 6, as well as on day 13, carbohydrate balance was not significantly different from zero (Figure 2). Fat oxidation was significantly different between days ($P < 0.001$). Post hoc testing revealed that fat oxidation was not significantly different between days 5 and 6. However, fat oxidation increased significantly between days 6 and 13. Fat balance was significantly negative on day 5, whereas on days 7, 8, and 9 a positive fat balance was reached. On day 6 as well as on day 13, fat balance was not significantly different from zero (Figure 2).

When energy and substrate balance were adjusted for the baseline day (day 6) for each individual, carbohydrate balance was significantly negative for days 7, 8, and 9 (−1.2 ± 0.1, −0.8 ± 0.1, and −0.6 ± 0.2 MJ/d, respectively), whereas on day 13 carbohydrate balance was not significantly different from zero (−0.0 ± 0.2 MJ/d). Fat balance was significantly positive on days 7, 8, and 9 (1.3 ± 0.1, 1.0 ± 0.1, and 0.8 ± 0.2 MJ/d, respectively) and was not significantly different from zero on day 13 (0.5 ± 0.2 MJ/d). On all days, subjects were in protein balance (0.0 ± 0.0, −0.0 ± 0.0, 0.2 ± 0.1, and 0.1 ± 0.0 MJ/d for days 7, 8, 9, and 13, respectively) and energy balance (0.1 ± 0.1, 0.2 ± 0.1, −0.0 ± 0.1, and 0.3 ± 0.1 MJ/d for days 7, 8, 9, and 13, respectively).

Blood variables

There were no significant differences in glycerol, glucose insulin, and fatty acid concentrations between days 4, 10, and 14. On days 10 and 14 triacylglycerol concentration was significantly lower than on day 4 ($P < 0.005$).

Sex differences

This study was performed in both men and women. Some sex differences were observed. As expected, women had significantly lower body weight ($P < 0.05$), greater percentage body fat ($P < 0.05$), and less FFM ($P < 0.001$). No differences in body mass index or fat mass were observed between men and women.

Respiratory quotient was significantly higher for women on day 13 than for men (0.816 compared with 0.786, $P < 0.001$). Also, respiratory quotient declined significantly more between days 9 and 13 for men than for women ($P < 0.005$). There were no significant differences between men and women in (decline in) respiratory quotient between days 5 and 9.

Carbohydrate oxidation as a percentage of energy expenditure was significantly lower for men than for women on day 13 ($P < 0.001$). This resulted in a significant difference in carbohydrate balance ($P < 0.001$), with men being in positive carbohydrate balance and women in negative balance. Fat oxidation as a percentage of energy expenditure on day 13 was significantly higher for men than for women ($P < 0.05$). The male subjects were in negative fat balance and the female subjects were in positive fat balance, the difference being significant ($P < 0.01$). No differences in substrate oxidation or any other variables (except for some blood variables, *see* below) measured during the stay in the respiration chamber were observed between men and women.

Fatty acid concentration was significantly higher for women on day 10 than for men ($P < 0.05$, **Table 5**). Glycerol concentration increased significantly between day 4 and day 10 in women, whereas no change was seen in men ($P < 0.05$, Table 5). In women, the insulin concentration was significantly lower on days 10 and 14 than on day 4 ($P < 0.05$). No other differences in blood variables on any days were found.

DISCUSSION

The results of the present study show that, in situations in which energy balance is reached, substrate oxidation can be adjusted to substrate intake. After 7 d on a high-fat diet, fat oxidation was, on average, equal to fat intake. However, some sex differences were found. The adjustment of fat oxidation occurred without any change in energy expenditure or body weight during the days in the respiration chamber.

High-fat diets are often considered to be fattening (6, 9). It has been suggested that, together with raising energy intake, high-fat diets result in a lower energy expenditure (22, 23). In our study we did not find any difference in 24-h EE between low- and high-fat diets except for the last day. On day 13, energy expenditure was significantly lower than on any other day spent in the respiration chamber. It is possible that this was a diet effect, because 7 d after the start of the high-fat diet (day 13), subjects were in substrate balance and could be considered completely adapted to the high-fat diet. However, it is more likely that most subjects became bored and therefore were more sedentary on their last day in the respiration chamber. This was indeed indicated by a lower PAI. If any difference in energy expenditure between low- and high-fat diets would have occurred it was expected to occur in the TEF. It has been reported that carbohydrate-rich diets result in a greater TEF than do diets with a high fat content. We estimated TEF, as reported by Schutz et al (24), and found no difference in TEF between high- and low-fat diets (data not shown). Our results, therefore, agree with the studies of Roust et al (25) and Hill et al (12) and a recent study of Stubbs et al (26) performed under free-living circumstances. Furthermore, no changes in body

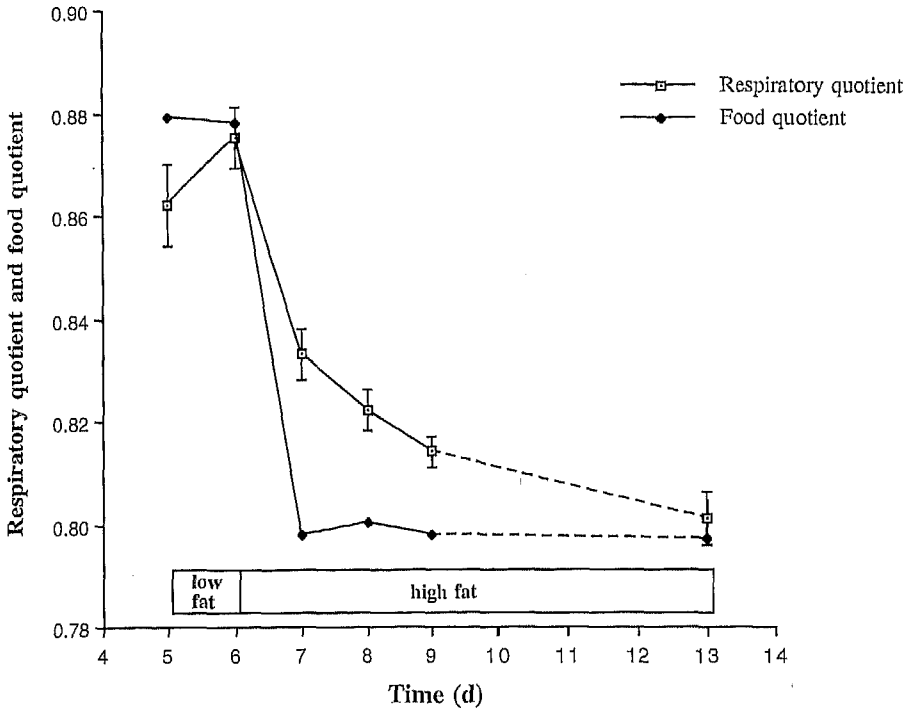

FIGURE 1. Twenty-four-hour respiratory quotients and 24-h food quotients as measured in the respiration chamber for days 5–9 and 13 ($\bar{x} \pm$ SEM; $n = 12$).

weight were seen in the first 3 d of the high-fat diet. If anything, body weight declined slightly with the high-fat diet (day 13). The decrease in body weight might be due to decreased glycogen stores. Thus, the present study does not support other studies like that of Prewitt et al (10), which show that consumption of high-fat diets is more fattening than consumption of isoenergetic low-fat diets.

This study shows that the body has a great capacity to adjust substrate oxidation to substrate intake over the long term ($\leq$ 1 wk) for carbohydrate and fat. Earlier studies could not show

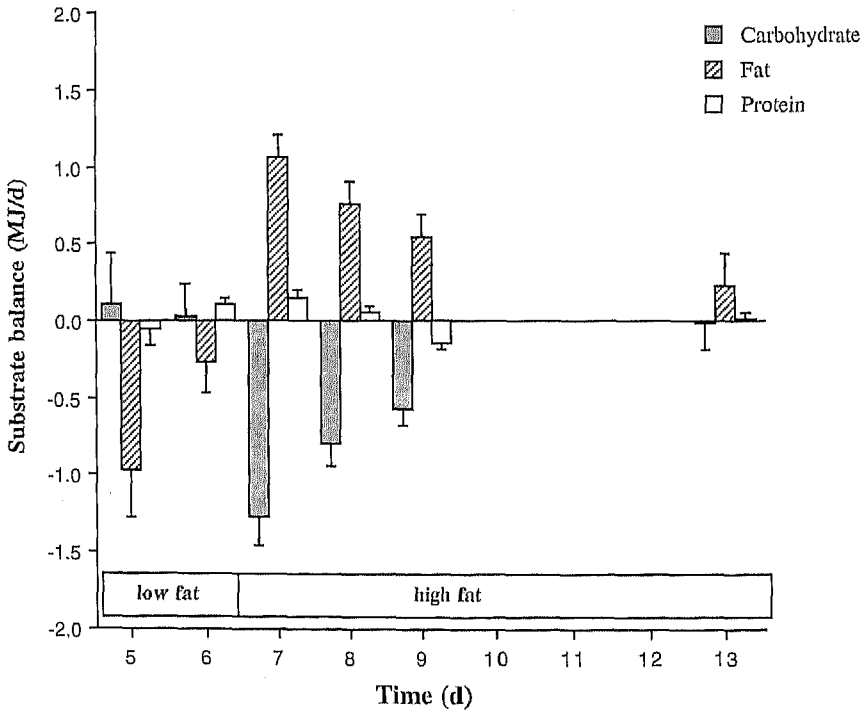

FIGURE 2. Substrate balances for days 5–9 and 13 as measured in the respiration chamber ($\bar{x} \pm$ SEM; $n = 12$).

TABLE 5
Blood indexes measured before, during, and after a high-fat diet[1]

Sex and day	Glucose	Triacylglycerols	Fatty acids	Glycerol	Insulin
	mmol/L	*μmol/L*	*μmol/L*	*μmol/L*	*pmol/L*
Females (n = 6)					
Day 4	4.73 ± 0.23	908 ± 217	289 ± 54	54 ± 5	72.15 ± 14.12
Day 10	4.57 ± 0.06	666 ± 91	386 ± 36[2]	86 ± 12[3]	48.93 ± 4.45[3]
Day 14	4.72 ± 0.10	703 ± 100	352 ± 31	73 ± 11	48.74 ± 6.55[3]
Males (n = 6)					
Day 4	5.13 ± 0.34	721 ± 93	253 ± 35	61 ± 8	39.5 ± 11.02
Day 10	4.80 ± 0.10	421 ± 40[3]	248 ± 26	59 ± 5	48.30 ± 6.34
Day 14	4.86 ± 0.10	482 ± 40	263 ± 45	64 ± 6	42.51 ± 3.65

[1] $\bar{x}$ ± SEM.
[2] Significantly different from males, $P < 0.05$.
[3] Significantly different from day 4, $P < 0.05$.

such an adaptation to a high-fat diet, probably because their subjects were not in energy balance. In contrast with other studies, in this experiment subjects were permanently in the chamber during the dietary switch and the subsequent days. During the stay in the respiration chamber, 24-h EE was virtually constant in the subjects. By prescribing activities throughout the day, we could limit daily fluctuations in energy expended in physical activity. This made it possible to adjust energy intake to energy expenditure after the first measurement day. With this protocol we were able to measure substrate oxidation of subjects in energy balance. The results show that humans have a capacity to adjust the respiratory quotient to food quotient in 7 d. Theoretically, the adaptation period under conditions of energy balance might be somewhat longer because our data on body weight suggest that subjects were in negative energy balance on the 3 d spent at home, which may have accelerated adaptation to the high-fat diet.

Fat oxidation gradually increased in this study. These results can be interpreted as an adaptation to a high fat intake. The consequence of an isoenergetic exchange of fat for carbohydrate is that the results can also be interpreted as being an adaptation to a low carbohydrate intake. However, the capacity of carbohydrate oxidation to adjust to carbohydrate intake has been shown to be rapid (1). Therefore, if the increase in fat oxidation was solely a consequence of the decrease in carbohydrate oxidation a more rapid adaptation would have been expected. In regulating substrate oxidation, the effect of glycogen content should be considered. According to Flatt's model (5), fat oxidation can be raised on high-fat diets by maintaining glycogen concentrations in a lower range. In our study, we found a positive fat balance and a negative carbohydrate balance on days 7–9. The negative carbohydrate balance (on average, −142 g between days 5 and 9) must have resulted in reduction of the glycogen stores, which was not, however, detectable in body weight. It therefore seems that the glycogen content of the body decreased until a new concentration was reached in which fat oxidation was sufficiently elevated to become in equilibrium with the elevated fat intake. However, it is also possible that fat oxidation was elevated because of increased enzymatic capacity for fat oxidation, which occurred because of the exposure to the high-fat diet. Further studies are necessary to investigate the role of glycogen content in the regulation of fat oxidation in a situation of energy balance.

It was assumed that changes in carbohydrate balance are reflected in changes in glycogen content. The fact that the sleeping respiratory quotient was also affected by the high-fat diet indicates that changes in the body's nutrient stores occurred. However, a part of the negative carbohydrate balance can be accounted for by enhanced gluconeogenesis from amino acid, lactate, or glycerol. We did not find any difference in urinary nitrogen excretion. It is therefore unlikely that protein breakdown was enhanced to make amino acids available for gluconeogenesis. Klein and Wolfe (27) showed that restriction of dietary carbohydrate is responsible for the metabolic responses to short-term fasting, such as enhanced lipolysis and gluconeogenesis. Carlson et al (28) found that fatty acid reesterification together with lipolysis are increased during fasting, in this way making fatty acids available for oxidation and maximizing glycerol release for gluconeogenesis. In our study we did not measure any endogenous lipid kinetics. Therefore, we cannot determine whether glycerol was released in favor of gluconeogenesis.

On the fourth day after the start of the high-fat diet (day 10) marked differences in plasma substrate concentrations were observed between men and women. Fasting fatty acid and glycerol concentrations were elevated in women compared with day 4, whereas in men no change in fatty acid and glycerol concentrations was seen. Also, a lower fasting insulin concentration was found on day 10 compared with day 4 in women but not in men. Those differences between men and women occurred without any accompanying differences in substrate oxidation or balances on days 5–9. It therefore seems that the elevation of fat oxidation was reached by different mechanisms in men and women. Jensen (29) showed recently that normal-weight women had greater suppression of fatty acid flux after consumption of a mixed meal than age-matched men, despite their greater percentage body fat, suggesting greater insulin sensitivity of adipose tissue lipolysis in women than in men. In our study, the lower insulin as well as the elevated fatty acid and glycerol concentration in women on day 10 compared with day 4 suggest that lipolysis increased to make fatty acids available for oxidation and glycerol available for gluconeogenesis. How fat oxidation is increased in men is not yet clear.

On the eighth day after the start of the high-fat diet (day 14) the differences in blood variables between men and women disappeared. However, on this day significant differences in substrate oxidation between men and women occurred. Women had higher carbohydrate oxidation (as a percentage of energy expenditure) and lower fat oxidation (as a percentage of energy

expenditure) than men. It is possible that women, despite their assumed increase in lipolysis, were not able to further increase fat oxidation. However, we did not control for activity on days 10–13 (the days they spent at home) and food intake on those days was ad libitum. It is therefore also possible that women were in positive energy balance and in this way partly repleted their glycogen stores. Further studies have to be conducted to examine possible sex differences in long-term adaptation to high-fat diets.

We showed that normal-weight persons were able to reach substrate balance on a high-fat diet after 1 wk. However, over the short term, substrate balances could not be maintained. We cannot conclude from these results whether obese or obesity-susceptible people would have the same capacity to maintain body weight on high-fat diets. Ample evidence exists that pre- and postobese people have a diminished capacity for fat oxidation (13, 30–32). It is also possible that in obese (or susceptible) persons the time course of adjusting fat oxidation to increased fat intake is delayed (14, 33). This would result in greater fat storage and glycogen mobilization. Therefore, obesity might result from cumulative positive fat balances due to day-to-day fluctuations in fat intake as will occur in affluent societies, where food is always available.

In conclusion, our results show that, when in energy balance, lean subjects are capable of adjusting fat oxidation to fat intake within 7 d of when dietary fat content is increased.

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