Stimulation of Oxygen Consumption Following Addition of Lipid Substrates in Liver and Skeletal Muscle From Rats Fed a High-Fat Diet

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We studied hepatic and skeletal muscle metabolic activity in rats fed a high-fat diet. Rats were fed a low-fat or high-fat diet for 15 days. At the end of the experimental period, full energy-balance determinations together with serum free triiodothyronine (FT_3), leptin, and free fatty acid (FFA) measurements were performed. In addition, we assessed fatty acid–stimulated oxygen consumption in perfused liver and in skeletal muscle homogenate. Rats fed a high-fat diet showed a significant increase in energy intake but no variation in body energy gain, due to a significant increase in energy expenditure. Serum FT_3 and FFA levels significantly increased in rats fed a high-fat diet versus rats fed a low-fat diet, while no variation was found in serum leptin levels. Perfused livers and skeletal muscle homogenates from rats fed a high-fat diet exhibited a significant increase in fatty acid–stimulated oxygen consumption. Our results suggest that the enhanced fatty acid oxidation rates in liver and skeletal muscle contribute to the maintenance of fat balance in response to increased fat intake, preventing excess fat deposition.

ENERGY STORES within the body are dependent on the relative proportions of energy intake and energy expenditure.[1] Energy intake is dependent on food consumption, while energy expenditure can be divided into three major components: the basal metabolic rate, the thermic effect of food (TEF), and the energy expended by physical activity.[2] In previous studies, we have shown that rats fed a high-fat diet overeat but resist becoming obese through a sympathetically mediated increase in energy expenditure.[3,4] The increase is due not only to a chronic increase in the fasting resting metabolic rate but also to an increase in the TEF.[5] In addition, we have shown that hepatocytes isolated from rats fed a high-fat diet exhibit increased hexanoate-supported respiration.[6] Thus, the increased energy expenditure found in rats fed a high-fat diet might be partly associated with an increase in hepatic lipid oxidation.[6]

Since fat, in general, is unable to promote an acute increase in its own oxidation,[7] it was of interest to further study fat oxidation in liver and skeletal muscle, which represent important contributors to fat utilization. To this end, we assessed oxygen consumption in perfused livers from rats fed a low-fat or high-fat diet using lipid substrates. We chose to use perfused liver because it represents a more physiological situation than isolated hepatocytes. In fact, it has been reported that energy metabolism in the liver is subject to zonation,[8] which is lost in isolated hepatocytes[9] and which may be of significance for the substrate oxidation rate in the intact organ. We also evaluated the fatty acid oxidation capacity in skeletal muscle homogenates from rats fed a low-fat or high-fat diet. Respiratory rates in liver and muscle were studied after addition of a medium-chain fatty acid, namely hexanoate, or supplementation with a long-chain fatty acid, namely palmitate.

In addition, measurements of energy balance and serum free fatty acid (FFA) levels were performed. Finally, since free triiodothyronine (FT_3) increases food intake and thermogenesis[10-11] and leptin is involved in the regulation of body weight,[12-13] we also measured serum FT_3 and leptin levels.

MATERIALS AND METHODS

Male Wistar rats aged about 30 days (bred in the animal house of the Heinrich-Heine University of Düsseldorf) were divided into two groups with the same mean body weight (about 74 g). Nine rats were fed a low-fat diet while the other nine rats were fed a high-fat diet. The composition of the two diets is listed in Table 1. The high-fat diet is characterized by a high fat content and the presence of a meat component, which is among the flavors most preferred by rats.[14-15] The experiment lasted 15 days.

All rats used in this study were individually housed in grid-bottomed cages at 24°C under an artificial circadian 12-hour light/12 h dark cycle, with ad libitum access to food and water. At the end of the experimental period, the rats were starved for 16 hours and liver perfusion was performed under pentobarbital anesthesia (12 mg/100 g body weight [BW]).

Other male Wistar rats were used for determination of serum FT_3, FFA, and leptin levels in fasted rats. Four rats were fed a low-fat diet and the other four a high-fat diet for 15 days, and thereafter they were starved for 16 hours. Rats were anesthetized with ethyl ether, and blood was collected via the inferior caval vein. Serum samples were stored at −20°C until the time of analysis.

Liver Perfusion

Livers were perfused "in situ" with a medium containing 120 mmol/L NaCl, 5 mmol/L KCl, 50 mmol/L HEPES, pH 7.4, 1 mmol/L KH_2PO_4, 2 mmol/L $CaCl_2$, and 1.2 mmol/L $MgSO_4$ in a nonrecirculating system.[16] The fluid was pumped through a temperature-regulated (37°C) membrane oxygenator supplemented with 100% O_2 to obtain about 90% O_2 saturation prior to entering the liver via a cannula inserted in the portal vein. The portal vein oxygen concentration, maintained constant by the oxygenator, was measured before and after each experiment. The effluent perfusate was collected via a cannula placed in the vena cava, and it passed by a platinum electrode for continuous monitoring of the venous oxygen concentration before being discarded. The flow rate was adjusted to the metabolic activity of the liver as judged from the venous oxygen concentration. It varied from experiment to experiment between 5 and 6 $mL \cdot min^{-1} \cdot g^{-1}$, but it was

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Submitted July 29, 1998; accepted April 26, 1999.

Supported by a grant from the Ministero dell' Università e della Ricerca Scientifica e Tecnologica (Italy).

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0026-0495/99/4810-0007$10.00/0

Table 1. Composition of Low- and High-Fat Diets (g/kg)

Component	Low-Fat	High-Fat
Standard chow*	1,000	280
Lyophilized meat†	0	395
Butter‡	0	178
Alphacel	0	120
AIN 76 mineral mix§	0	20
AIN 76 vitamin mix‖	0	7
Gross energy density (kJ/g)	15.88	19.85
Energy source (J/100 J)		
Protein	29	29
Lipid	10.6	50
Carbohydrate	60.4	21

*4RF21 (Mucedola, Settimo Milanese, Milan, Italy).

†Liomellin (STAR, Milan, Italy) containing (in 10 g) 5.8 g protein (N × 6.25), 1.2 g lipid, 2.57 g carbohydrate, 0.2 g minerals, and 0.2 g water.

‡Lurpak (Denmark), locally purchased, containing 10% water.

§American Institute of Nutrition (1977).

‖American Institute of Nutrition (1980).

constant in each individual experiment. Oxygen consumption rates were calculated from the arteriovenous oxygen concentration differences and the constant flow rate, and were referred to the wet weight of the liver. Substrates (hexanoate or palmitate) were added to the perfusion fluid before it entered the liver. The final concentration of substrates was hexanoate 0.5 mmol/L and palmitate 0.5 mmol/L. Palmitate was dissolved in fatty acid–free bovine serum albumin solution: 1 g palmitate was slowly added to 170 mL albumin solution (20 g/100 mL) under gentle stirring, which was continued for a further 2 hours at room temperature.

Livers were preperfused for 10 to 15 minutes with the perfusion medium until a steady state was reached, ie, the oxygen consumption rate remained constant. Then, the substrate was infused together with the same medium for a further 10 to 15 minutes until a new steady state was reached. The difference in oxygen consumption due to substrate addition was evaluated from the difference between the steady states before and after infusion of the substrate. The liver perfusion time was about 2 hours.

At the end of liver perfusion and after the removal of the gut contents, the carcasses were frozen until utilization for energy-balance measurements.

Skeletal Muscle and Liver Respiration

Eight male Wistar rats were fed a low-fat diet and another eight rats were fed a high-fat diet for 15 days for determination of oxidation rates in skeletal muscle and liver homogenates. At the end of the diet treatment and without any previous food deprivation, the animals were anesthetized by intraperitoneal injection of chloral hydrate (40 mg/100 g BW) and blood was collected via the inferior caval vein for determination of serum FT_3, FFA, and leptin levels. Serum samples were stored at −20°C until the time of analysis. Then, liver and hindleg muscles were removed for mitochondrial respiration rate measurements. Finally, after removal of the gut contents, the carcasses were frozen until utilization for energy-balance determinations. Corrections for the energy content and composition of liver and hindleg muscles were made in the energy-balance calculation.

The livers were finely minced and washed in a medium containing 220 mmol/L mannitol, 70 mmol/L sucrose, 20 mmol/L HEPES, pH 7.4, 1 mmol/L EDTA, and 0.1% (wt/vol) fatty acid–free bovine serum albumin. Tissue fragments were gently homogenized with the same medium (1:4 wt/vol) in a Potter Elvehjem homogenizer set at 500 rpm (4 strokes/min). Aliquots of the homogenate were then used for determination of respiratory activities.

Hepatic oxygen consumption was measured polarographically with a Clark-type electrode (Yellow Springs Instruments, Yellow Springs, OH) maintained in a water-jacketed chamber at 30°C in a medium containing 80 mmol/L KCl, 50 mmol/L HEPES, pH 7.0, 5 mmol/L KH_2PO_4, and 0.1% (wt/vol) fatty acid–free bovine serum albumin. The substrates were palmitoylcarnitine (40 μmol/L) + malate (2.5 mmol/L), palmitoyl coenzyme A (CoA) (40 μmol/L) + carnitine (2 mmol/L) + malate (2.5 mmol/L), and hexanoate (0.4 mmol/L) + malate (2.5 mmol/L). Measurements were performed in the presence of 0.6 mmol/L ADP.

Hindleg muscles were freed of excess fat and connective tissue, finely minced, and washed in a medium containing 100 mmol/L KCl, 50 mmol/L Tris, pH 7.5, and 0.1% (wt/vol) fatty acid–free bovine serum albumin. Tissue fragments were treated with protease nagarse (EC 3.4.21.62, 1 mg/g tissue) for 4 minutes and then homogenized with a medium (1:8 wt/vol) containing 100 mmol/L KCl, 50 mmol/L Tris, pH 7.5, 5 mmol/L $MgCl_2$, 1 mmol/L EDTA, 5 mmol/L EGTA, and 0.1% (wt/vol) fatty acid–free bovine serum albumin in a Potter Elvehjem homogenizer set at 500 rpm (4 strokes/min). Aliquots of the homogenate were then used for the determination of respiratory activities.

Skeletal muscle oxygen consumption was measured polarographically with a Clark-type electrode (Yellow Springs Instruments) maintained in a water-jacketed chamber at 30°C in a medium containing 30 mmol/L KCl, 6 mmol/L $MgCl_2$, 75 mmol/L sucrose, 1 mmol/L EDTA, 20 mmol/L KH_2PO_4, pH 7.0, and 0.5% (wt/vol) fatty acid–free bovine serum albumin. The substrates were palmitoylcarnitine (40 μmol/L) + malate (2.5 mmol/L), palmitoyl CoA (40 μmol/L) + carnitine (2 mmol/L) + malate (2.5 mmol/L), and hexanoate (0.4 mmol/L) + malate (2.5 mmol/L). Measurements were performed in the presence of 0.6 mmol/L ADP. State 4 respiration was measured in the presence of oligomycin (4 μg/mL) using palmitoyl coA + carnitine + malate as a substrate.

Energy-Balance Measurements

Body weight and food intake were monitored daily to allow calculations of body weight gain and gross energy intake. The feces and spilled food were also collected daily for energy content measurements. They were dried and ground to a powder before determining the energy content with a bomb calorimeter (Parr adiabatic calorimeter calibrated with dry benzoic acid standard; Pace Instruments, Moline, IL). The gross energy content of the low-fat and high-fat diets was also determined by the bomb calorimeter.

Digestible energy intake was obtained by subtracting the energy measured in the feces and spilled food (about 25% of gross energy intake for both groups of rats) from the gross energy intake measured from daily food consumption. Metabolizable energy (ME) intake was expressed as digestible energy intake × 0.96.[17] The gain in energy was obtained by subtracting the energy content of an initial group (eight rats killed at the beginning of the study) from that of each of the two experimental groups. The carcasses were autoclaved, chopped into small pieces, thoroughly mixed, and homogenized in water (final vol equal to twice the carcass weight) with a Polytron (Kinemica, Littau/Lucerne, Switzerland). Samples of homogenates were desiccated into a dry powder, from which small pellets (about 200 mg) were made. The energy content was measured with the bomb calorimeter, and the lipid content was measured according to the method of Folch et al.[18] Water content was determined by the difference in weight of the homogenate before and after drying at 70°C in a vacuum oven. Protein content was obtained by subtracting the energy as lipid from the body energy content previously measured with the bomb calorimeter, using the value of 23.5 kJ/g for the energy content of protein. Energy expenditure was calculated from the difference between ME intake and energy gain; gross efficiency was calculated as the percent of ME intake stored as body energy.

Serum FT_3, FFA, and Leptin Levels

The serum FT_3 level was measured using a radioimmunoassay kit (Coat-a-Count; Medical Systems, Genova, Italy). Serum FFA levels were measured using the acyl-CoA synthetase/acyl-CoA oxidase method with an enzymatic kit from Boehringer-Mannheim Biochemia (Milano, Italy). Serum leptin was determined using a radioimmunoassay kit from Mediagnost (Tübingen, Germany).

Statistical Analysis

The data are presented as the mean ± SE for different rats. Statistical significance between mean values was examined by a two-tailed Student's *t* test. Probability values less than .05 were considered to indicate a significant difference.

Materials

Hexanoate, palmitate, palmitoylcarnitine, palmitoyl CoA, malate, carnitine, ADP, and oligomycin were purchased from Sigma Chemical (St Louis, MO). All other reagents were from Merck (Darmstadt, Germany) and were of the highest purity commercially available.

RESULTS

Body Weight and Composition

There was no difference in the final body weight, the percent body fat, water, and protein, and body energy content in rats fed a high-fat diet compared with rats fed a low-fat diet (Table 2). The same results were obtained with rats that were not starved on the day before death.

Energy Balance

Table 3 lists the results for ME, protein, lipid, and carbohydrate intake and body energy, protein, and fat gain. A significant increase was found for ME, protein, and fat intake, while carbohydrate intake significantly decreased, in rats fed a high-fat diet compared with rats fed a low-fat diet. However, the body energy, lipid, and protein gain was not different between the two groups, whereas gross efficiency significantly declined in rats fed a high-fat diet versus rats fed a low-fat diet. Table 3 also shows that the percentage of ingested lipid that was stored as carcass energy significantly decreased while the percentage of lipid consumed significantly increased in rats fed a high-fat diet versus a low-fat diet. No difference was found for the percentage of ingested protein that was stored or consumed between the two experimental groups. The same results were obtained with rats that were not starved on the day before death.

Table 4 shows energy expenditure and partitioning of ME intake in rats fed a low-fat or high-fat diet. The total cost of storage was determined by taking into account that the energy loss in storing 1 kJ protein is 1.25 kJ,[19] while the corresponding energy cost for fat deposition is 0.36 kJ/kJ for diets with a high percentage of carbohydrates such as our low-fat diet and 0.16 kJ/kJ for diets with a high fat content such as our high-fat diet.[19] Energy expenditure and the values obtained for energy expenditure excluding the total cost of storage, ie, corrected energy expenditure (CEE), were significantly increased in rats fed a high-fat diet versus a low-fat diet. When CEE was expressed as a percentage of ME, a significant increase was found in rats fed a high-fat diet versus a low-fat diet. On the other hand, the percentage of ME intake used for storage of protein and lipid, as well as the percentage of ME intake stored as protein and lipid, significantly decreased in rats fed a high-fat diet compared with rats fed a low-fat diet. The same results were obtained with rats

Table 2. Body Weight, Composition, and Energy Content in Rats Fed a Low-Fat or High-Fat Diet

Parameter	Low-Fat	High-Fat	% Change*
Initial body weight (g)	74 ± 1	74 ± 1	—
Final body weight (g)	177 ± 2	175 ± 3	−1
Body water (%)	67.0 ± 0.5	66.7 ± 0.4	−0.4
Body lipid (%)	9.2 ± 0.4	9.5 ± 0.4	3.3
Body protein (%)	17.3 ± 0.3	17.4 ± 0.4	0.6
Body energy (kJ/g)	7.6 ± 0.2	7.8 ± 0.2	2.6

NOTE. Values are the mean ± SEM of 9 different rats after 15 days of diet treatment.

*Values refer to high-fat *v* low-fat.

Table 3. ME Intake and Body Energy Gain in Rats Fed a Low-Fat or High-Fat Diet

Parameter	Low-Fat	High-Fat	% Change
ME intake (kJ)	2,995 ± 25	3,876 ± 20†	29
Protein intake (kJ)	868 ± 30	1,124 ± 27†	29
Fat intake (kJ)	317 ± 10	1,938 ± 40†	511
Carbohydrate intake (kJ)	1,810 ± 40	814 ± 20†	−55
Body energy gain (kJ)	753 ± 20	763 ± 25	1.3
Protein gain (kJ)	421 ± 18	419 ± 20	−0.5
Fat gain (kJ)	332 ± 18	344 ± 16	3.6
Gross efficiency (%)	0.25 ± 0.01	0.20 ± 0.01*	−20
Protein oxidized/protein intake (%)	52 ± 2	63 ± 3*	21
Protein stored/protein intake (%)	48 ± 2	37 ± 3*	−23
Lipid oxidized/lipid intake (%)	0 ± 1	82 ± 2†	8,200
Lipid stored/lipid intake (%)	100 ± 1	18 ± 1†	−82

NOTE. Values are the mean ± SEM of 9 different rats and refer to the whole period of diet treatment. % Change values refer to high-fat *v* low-fat. Gross efficiency is energy gain/ME intake.

*$P < .01$ *v* low-fat.

†$P < .0001$ *v* low-fat.

Table 4. Energy Expenditure and Partitioning of ME Intake in Rats Fed a Low-Fat or High-Fat Diet

Parameter	Low-Fat	High-Fat	% Change
Energy expenditure (kJ)	2,242 ± 100	3,113 ± 100‡	39
Heat loss in storing protein (kJ)	526 ± 20	524 ± 18	−0.4
Heat loss in storing fat (kJ)	120 ± 8	55 ± 3‡	−54
Total cost of storage (kJ)	646 ± 30	579 ± 30	−10
CEE (kJ)	1,596 ± 100	2,534 ± 100‡	59
CEE/ME (%)	53 ± 2	65 ± 2†	23
Protein gain/ME (%)	14 ± 0.5	11 ± 0.5	−21
Fat gain/ME (%)	11.0 ± 0.5	9.0 ± 0.5*	−18
Cost of storage/ME (%)	22 ± 1	15 ± 1**	−32

NOTE. Values are the mean ± SEM of 9 different rats and refer to the whole period of diet treatment. % Change values refer to high-fat *v* low-fat. A value of 1.25 kJ/kJ was used to estimate the storage cost of protein. Values of 0.36 kJ/kJ (low-fat) and 0.16 kJ/kJ (high-fat) were used to estimate the storage cost of fat. Total cost of storage refers to heat loss associated with the storage of fat and protein.

*$P < .05$ *v* low-fat.

†$P < .001$ *v* low-fat.

‡$P < .0001$ *v* low-fat.

that were not starved on the day before death. Serum FT_3 and FFA levels significantly increased in rats fed a high-fat diet versus a low-fat diet, both in starved and fed rats (Table 5). No variation was found for serum leptin levels in response to a high-fat diet in fed and starved rats.

Table 6 shows the results for oxygen consumption measurements in perfused livers from rats fed a low-fat or high-fat diet. The liver in situ was perfused first without substrates and basal oxygen consumption was measured. Then, oxygen consumption was also measured following addition of hexanoate or palmitate to the perfusion medium. The effect of addition of substrates to the perfused liver in both groups of rats was a rapid increase in oxygen consumption. This increase (ΔO_2) was calculated as the difference between oxygen consumption measured after substrate infusion and oxygen consumption without substrate (Table 6). Perfused livers from rats fed a high-fat diet exhibited a significant increase in both basal oxygen consumption and ΔO_2, whatever the added substrate, compared with those from rats fed a low-fat diet.

Table 7 lists the results for fatty acid oxidation rates in liver and skeletal muscle homogenates from rats fed a low-fat or high-fat diet. Rats fed a high-fat diet exhibited a significant increase in skeletal muscle oxidation rates with all substrates used, while hepatic oxidation rates significantly increased with hexanoate or palmitoyl CoA as a substrate.

DISCUSSION

The energy-balance measurements in rats fed a low-fat or high-fat diet in the present study are in agreement with our previous results[20] obtained in rats of the same strain but from a different colony. Rothwell and Stock[21] found that rats from different colonies exhibit a different degree of lipid gain after a "cafeteria diet," and claimed that the genetic background influences the resistance to obesity. However, 30-day-old rats fed our high-fat diet showed no increase in lipid gain irrespective of the colony, since the excess of energy intake over the experimental period was completely balanced by an increased energy expenditure. The ME intake of rats fed a high-fat diet was 36% higher than that of rats fed a low-fat diet; however, body energy gain and lipid and protein content remained unchanged despite the increased protein and fat intake (Tables 2 and 3). In addition, in rats fed a high-fat diet, a higher percentage of the fat intake is oxidized (Table 3), probably because the oxidation of carbohydrates and proteins is not sufficient for the increased body energy requirements. Increased fat oxidation appears to be commensurate with fat intake, since fat does not tend to accumulate in adipose tissue of rats fed a high-fat diet. Therefore, in the young Wistar rats used in this study, an increased energy intake on a diet with a substantial fat content does not produce high levels of adiposity. The increase in fat consumption in the rats fed a high-fat diet is also in line with the increased capacity for fatty acid oxidation that occurs in the liver and skeletal muscle of these rats (Tables 6 and 7).

Table 6. Basal Oxygen Consumption and ΔO_2 in Perfused Livers From Rats Fed a Low-Fat or High-Fat Diet

Parameter	Low-Fat	High-Fat	% Change
Basal oxygen consumption	119.9 ± 3.2	157.9 ± 3.2*	32
ΔO_2 Hexanoate	38.6 ± 1.0	58.3 ± 2.0*	51
ΔO_2 Palmitate	16.2 ± 0.9	28.8 ± 1.5*	78

NOTE. Values are the mean ± SEM of 9 different rats and are expressed as μmol O_2/h · g wet weight of liver. % Change values refer to high-fat *v* low-fat.

*$P < .0001$ *v* low-fat.

Our results also show that energy expenditure and CEE significantly increased in rats fed a high-fat diet compared with those fed a low-fat diet, such that gross efficiency significantly declined (Tables 3 and 4). When CEE, which can represent the cost of body energy maintenance, was expressed as a percentage of ME, significantly higher values were obtained in rats fed a high-fat diet compared with rats fed a low-fat diet. This finding indicates that the CEE/ME ratio is not constant, whatever the energy intake, and suggests the presence of regulatory mechanisms controlled by ME intake, which are useful for limiting fat gain. The last result is in agreement with our previous results on a different rat colony[20] and is different from those obtained by LeBlanc et al,[22] who found no variation in the CEE/ME ratio in adult rats fed a cafeteria diet.

In an attempt to gain insight into the hormonal determinants of the increase in energy intake and expenditure found in rats fed a high-fat diet, we measured serum levels of FT_3 and leptin. It is well known that T_3 increases food intake and thermogenesis[10-11] and leptin is involved in the regulation of body weight; in fact, leptin acts by signaling the size of energy stores in adipose tissue.[12-13] Serum leptin levels were the same in both groups of rats (Table 5), in agreement with the finding that rats fed a low-fat or high-fat diet exhibited a similar body lipid content. On the other hand, a significant increase of 50% in serum FT_3 levels was found in rats fed a high-fat diet in the fed and the fasted state (Table 5), in agreement with similar results obtained by our group in another rat colony[3-4] and by Rothwell and Stock[21] in rats fed a cafeteria diet. Since all of these rats exhibited a lower efficiency in body energy gain in response to a high-fat diet, enhanced serum T_3 levels could thus be involved in the regulatory responses induced by high-fat feeding. In fact, it has been shown that T_3 stimulates the synthesis of three

Table 5. Serum FT_3, FFAs, and Leptin in Rats Fed a Low-Fat or High-Fat Diet

	Starved Rats			Fed Rats		
Parameter	Low-Fat	High-Fat	% Change	Low-Fat	High-Fat	% Change
FT_3 (ng/100 mL)	200 ± 18	300 ± 25*	50	350 ± 25	490 ± 33*	40
FFAs (mmol/L)	0.90 ± 0.10	1.10 ± 0.10*	22	0.50 ± 0.03	0.70 ± 0.03*	40
Leptin (pg/mL)	400 ± 38	400 ± 35	—	680 ± 48	690 ± 47	1

NOTE. Values are the mean ± SEM of 4 (starved) or 8 (fed) different rats after 15 days of diet treatment. % Change values refer to high-fat *v* low-fat.

*$P < .05$ *v* low-fat.

Table 7. Fatty Acid Oxidation Rates in Liver and Skeletal Muscle Homogenate From Rats Fed a Low-Fat or High-Fat Diet

Parameter	Liver			Skeletal Muscle		
	Low-Fat	High-Fat	% Change	Low-Fat	High-Fat	% Change
Hexanoate	2,881 ± 142	3,393 ± 137*	18	203 ± 24	336 ± 44†	66
Palmitoylcarnitine	4,258 ± 266	4,638 ± 270	9	2,280 ± 193	3,107 ± 195†	36
Palmitoyl CoA	3,846 ± 245	4,838 ± 378*	26	1,254 ± 100	1,770 ± 141†	41
Palmitoyl CoA + oligomycin	—	—		110 ± 17	170 ± 19*	55

NOTE. Values are the mean ± SEM of 8 different rats and are expressed as nmol O_2/min · g wet weight. % Change values refer to high-fat *v* low-fat.

*$P < .05$ *v* low-fat.

†$P < .01$ *v* low-fat.

proteins considered crucial for mammalian thermogenesis, namely uncoupling protein 1 (UCP1), UCP2, and UCP3 in brown adipose tissue, white adipose tissue, and skeletal muscle.[23-25] In addition, it is well known that futile cycling is increased in livers from hyperthyroid rats.[26] Support for the hypothesis is given by our previous result that hypothyroid rats fed a high-fat diet exhibited a higher lipid and energy gain and an increased efficiency of energy gain.[27]

Our present results also show that the increased body energy expenditure in rats fed a high-fat diet is coupled to a significant increase in oxygen consumption in perfused liver both in the absence and in the presence of added substrates (Table 6). The increase in basal respiration could reflect a higher oxidative capacity (1) associated with an improvement in substrate supply to the electron-transport chain due to the presence of high FFAs in the plasma (Table 5) and cells and (2) due to an enhancement of the capacity of respiratory-chain proteins by T_3.[28] This is in agreement with the enhanced serum FFA and T_3 levels found in rats fed a high-fat diet (Table 5).

The higher ΔO_2 following the addition of hexanoate in perfused livers from rats fed a high-fat diet is in agreement with our previous finding in hepatocytes from rats fed a high-fat diet.[6] It is indicative of an increase in mitochondrial medium-chain fatty acid oxidation, since hexanoate can be oxidized only within the mitochondrial matrix.[29] With palmitate, the percent increase in ΔO_2 that occurs in rats fed a high-fat diet is even higher than with hexanoate. We have previously found an increase in hepatocyte nonmitochondrial oxygen consumption in rats fed a high-fat diet.[30] Therefore, the increase in palmitate oxidation could be partly due to an enhanced peroxisomal fatty acid oxidation. Thus, an increased peroxisomal fatty acid oxidation capacity has been found in rats fed a high-fat diet[31] and in situations in which the delivery of fatty acids to the liver is increased.[32]

Respiratory data obtained on perfused liver are consistent with the findings found in liver homogenates from rats fed a low-fat or high-fat diet. In fact, a significant increase in hexanoate- and palmitoyl CoA-dependent oxygen consumption was found in rats fed a high-fat diet, while no variation was found when palmitoylcarnitine was used as a substrate (Table 7). It is known that palmitoyl CoA–supported respiration reflects the activity of carnitine palmitoyl transferase I (CPT I), CPT II, and the intramitochondrial β-oxidation pathway, while respiration with palmitoylcarnitine, which bypasses the step catalyzed by CPT I, represents an index of fatty acid oxidation per se.[33] Therefore, we can conclude that enhanced hepatic capacity for long-chain fatty acid oxidation is probably due to an increase in CPT I activity. In agreement, it has been shown that T_3[10] and a high-fat diet[31] stimulate the oxidation of fatty acids by increasing the activity of CPT I.

We also measured fatty acid oxidation rates in skeletal muscle, which is the largest organ in the body by mass, and is thus another important site of fatty acid oxidation. An even more significant increase in skeletal muscle oxygen consumption was found in rats fed a high-fat diet with all lipid substrates used (Table 7). This indicates that in skeletal muscle, an increased fatty acid oxidation capacity is achieved through an increase in the activity of CPT I and the other enzymes of β-oxidation. Our results are in agreement with previous findings that high-fat feeding influences the activity of skeletal muscle enzymes involved in the oxidation of fatty acids.[34-36] In addition, it has been recently reported that UCP3 is specifically expressed in brown adipose tissue and skeletal muscle[37-38] and is induced by high-fat feeding.[39] Thus, it can be suggested that at least part of the increased lipid-supported skeletal muscle respiration in rats fed a high-fat diet could be due to higher UCP3 levels and could therefore contribute to counteract the development of obesity. In agreement with this suggestion, we have also found increased palmitoyl CoA–supported respiration in the presence of oligomycin in rats fed a high-fat diet. In fact, the respiratory rate with oligomycin can be considered a rough index of "proton leak" and hence of UCPs.[40] However, further and more direct experiments are needed to verify whether increased UCP3 levels are responsible for the increased fatty acid oxidation capacity in the skeletal muscle of rats fed our high-fat diet.

Taken together, our results indicate an increase in hepatic and skeletal muscle fatty acid oxidation capacity in rats fed a high-fat diet. The increased hepatic fatty acid oxidation in rats fed a high-fat diet is of particular relevance in view of the fact that fatty acid oxidation has been shown to be partly independent of the ATP demand of cells in the liver.[41-44] This "uncoupling-like effect" of FFAs causes an increase in the hepatic capacity to metabolize excess dietary lipids, which may contribute to the resistance to obesity of rats fed a high-fat diet. Additionally, due to the important contribution of skeletal muscle to whole-body fat oxidation, the increase in the skeletal muscle fatty acid oxidation capacity is also of relevance in the maintenance of fat balance in rats fed a high-fat diet.

ACKNOWLEDGMENT

The authors wish to thank Professor Antonio Barletta for helpful discussions and Raffaella Crescenzo, Sonja Grundel, and Myung-Hee Oh for technical assistance.

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