Effect of high-fat feeding on metabolic efficiency and mitochondrial oxidative capacity in adult rats

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The changes in metabolic efficiency, body composition, and nutrient partitioning induced by high-fat feeding were evaluated in adult rats (90 d of age). The alterations in serum free triiodothyronine, insulin, and leptin levels, as well as in hepatic and skeletal muscle metabolism, were also assessed. Rats were fed either a low- or a high-fat diet for 2 weeks. Relative to the low-fat feeding, energy intake and expenditure, as well as body-energy gain, lipid gain, and energetic efficiency, were increased by the high-fat feeding. Increased serum leptin levels accompanied these variations. A positive correlation between serum leptin levels and percentage of body fat was found in the rats fed the low- or high-fat diet, with a significant divergence between the slope of the regression lines. Furthermore, a negative correlation between serum leptin level and energy intake was found in the rats fed the low-fat diet, while a positive correlation was found in the rats fed the high-fat diet. Finally, the high-fat feeding decreased the hepatic and skeletal muscle mitochondrial oxidative capacity. It is concluded that, in adult rats, a nutritional factor such as a high level of fat in the diet induces obesity, leptin resistance, and impairment of mitochondrial capacity, all phenomena typical of unrestrained aged rats.

Energy balance: Mitochondrial capacity: Triiodothyronine: Leptin

The prevalence of obesity is increasing in most countries around the world (World Health Organization 1998). To develop effective prevention strategies, it is important that the causes of weight gain are understood. Among the factors leading to the development of obesity, diet composition is certainly of importance (McCrory *et al.* 2000). In fact, it is known that diets rich in fat are generally associated with the development of obesity (Smith *et al.* 1998; Hill *et al.* 2000). However, it has been previously shown that post-weaning (about 30-d-old) and post-pubertal (about 50-d-old) rats fed a high-fat diet for 2 weeks exhibit increased energy intake associated with increased energy expenditure, which exceeds the obligatory cost of energy gain (Liverini *et al.* 1996; Iossa *et al.* 1999*a*). This regulatory increase in thermogenic capacity counteracts obesity development completely in post-weaning rats but only in part in post-pubertal rats, thus suggesting a weakness of regulatory responses to increased fat intake during growth. It has also been shown that, with increasing age, even rats fed a low-fat diet developed a spontaneous obesity (Barzilai & Rossetti, 1995). In agreement, it has previously been shown that in adult rats (aged between 90 and 180 d), which are characterised by the cessation of growth and development, ingested energy is higher

than expenditure and the excess is entirely stored as fat rather than burnt (Iossa *et al.* 1999*b*).

Taking into account these observations, it was considered of interest to evaluate the changes in energy balance following overfeeding on a high-fat diet for 2 weeks in adult rats (90 d of age), which are potentially prone to developing obesity.

The changes in energy expenditure found at the whole-body level reflect the changes in metabolic activity of single organs and tissues, especially those that are the main contributors to total metabolism, for example the liver and muscle (Rolfe & Brown, 1997). Since mitochondria are the main site of the oxidative process, changes in cellular metabolic activity could result from changes in mitochondrial mass and/or specific capacity. In fact, it has previously been found that decreased hepatic metabolic efficiency and increased skeletal muscle capacity to oxidise lipid substrates contributes to regulatory responses elicited by high-fat feeding in very young rats (Iossa *et al.* 1995, 2002). It was considered, therefore, of interest to determine the muscle and liver mitochondrial oxidative capacity in adult rats fed a high-fat diet.

Finally, to obtain information on the hormonal modifications induced by high-fat feeding in adult rats, leptin,

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Abbreviations: α-GPDH, α-glycerophosphate dehydrogenase; BSA, bovine serum albumin; HEPES, N'-(2-hydroxyethyl)piperazine-N-2-ethanesulfonic acid; ME, metabolisable energy; NEFA, non-esterified fatty acids; T$_3$, triiodothyronine.

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insulin, and free triiodothyronine (T_3) serum levels have been determined. In fact, it is well known that these hormones are important regulators of body weight, energy expenditure and food intake (Danforth & Burger, 1989; Freake & Oppenheimer, 1995; Friedman 2002; Leibel, 2002; Porte *et al.* 2002), and their serum levels are related to age (Iossa *et al.* 1999b, 2001; Kumar *et al.* 1999).

Methods and materials

Animals and diets

Male Wistar rats aged 90 d (Charles River Italia, Calco, Como, Italy) were caged singly in metabolism cages in a temperature-controlled room ($24 \pm 1°C$) with a 12 h light–dark cycle (07.00–19.00 hours). The rats were divided into three groups each composed of eight rats, with similar mean body weight (400 (SE 1) g) and with body weight normally distributed within each group. The treatment, housing, and killing of animals met the guidelines set by the Italian Health Ministry.

At the start of the study, one group of rats was killed to establish baseline measurements. The other two groups of rats were fed a low-fat diet or a high-fat diet for 2 weeks. The high-fat diet was formulated to differ from the low-fat diet only in the fat and carbohydrate contribution to the energy value but to be identical in terms of protein, vitamins, minerals, and fibre, as previously reported (Iossa *et al.* 2002). In the experimental period body weights and food intakes were monitored daily. Food spilled was carefully collected and accounted for in the food intake calculations. Faeces and urine were also collected daily, dried, and ground to a powder before the determination of their energy content with a bomb calorimeter calibrated with dry benzoic acid as a standard (Parr adiabatic calorimeter; Parr Instruments Co., Moline, IL, USA). Metabolisable energy (ME) intake was obtained by subtracting the energy measured in the faeces and urine from the gross energy intake, determined from daily food consumption and the gross energy density of the diets.

Determination of energy expenditure and energetic efficiency

At the end of the diet treatment, the animals, without any food deprivation, were anaesthetised by injection of chloral hydrate (40 mg/100 g body weight, intraperitoneally) and blood was taken via the inferior cava vein. Immediately after blood collection, the liver and hind-leg muscles were removed for preparation of homogenate and isolated mitochondria. Then, the rat carcasses were used for the determination of body energy content, as previously reported (Iossa *et al.* 1999a). Briefly, this parameter was measured with the bomb calorimeter on samples of carcass homogenised with a mass of water equal to twice the carcass weight in a Polytron homogeniser (Polytron Kinematica AG, Littau/Lucerne, Switzerland) and desiccated at 70°C in a vacuum oven. To take into account the energy content of the hind-leg skeletal muscle and the liver, samples of muscles and liver were dried and then the energy content measured with the bomb calorimeter.

Body energy gain was obtained as the difference between the final body energy content and the initial body energy content of the rats killed at the beginning of the experiment. Energy expenditure over the entire experimental period was calculated from the difference between ME intake and energy gain, while gross energetic efficiency was calculated as the percentage of body energy retained per ME intake. The cost of storage was determined by using the coefficient 1·25 kJ/kJ (Pullar & Webster, 1977) for protein and 0·36 kJ/kJ (low-fat diet; Pullar & Webster, 1977) or 0·16 kJ/kJ (high-fat diet; Rothwell *et al.* 1985) for fat. Net energy expenditure was obtained by subtracting the cost of fat and protein storage from total energy expenditure.

Body composition measurements

Water content was determined by the difference in weight of the carcass homogenate before and after drying at 70°C in a vacuum oven. Fat content was determined by the method of Folch *et al.* (1957) and converted to energy as fat using the coefficient of 39·2 kJ/g. Protein content was determined as described by Brooks *et al.* (1995) and converted to energy as protein by using the value of 23·5 kJ/g. Protein and fat gain was calculated as the difference between the protein and fat content at the end of the experimental period and the respective content of the rats killed at the beginning of the experiment.

Circulating substrate and hormone serum concentrations

The blood samples were centrifuged at $2000 \boldsymbol{g}_{av}$ for 10 min. The serum was removed and stored at $-20°C$ until used for the determination of free T_3, insulin, leptin, glucose, and non-esterified fatty acids (NEFA) concentration. Serum free T_3, insulin, and leptin concentrations were measured using radioimmunoassay kits (ICN Pharmaceuticals, Diagnostic Division, New York, NY, USA for free T_3, and Linco Research, Inc., St Charles, MO, USA for insulin and leptin). Each hormone was measured in a single assay to remove inter-assay variations. Serum NEFA and glucose were measured by the colorimetric enzymic method using commercial kits (Roche Diagnostics, Mannheim, Germany for NEFA, and Pokler Italia, Genova, Italy for glucose).

Preparation of homogenates and isolated mitochondria

After removal, the livers were finely minced and washed in a medium containing 220 mM-mannitol, 70 mM-sucrose, 20 mM-N'-(2-hydroxyethyl)piperazine-N-2-ethane-sulfonic acid (HEPES) (pH 7·4), 1 mM-EDTA, and 0·1 % (w/v) fatty-acid-free bovine serum albumin (BSA). Tissue fragments were homogenised with the above medium (1:4, w/v) in a Potter Elvehjem homogeniser (Heidolph, Kelheim, Germany) set at 500 rpm (4 strokes/min). Samples of homogenate were used for respiration measurements. The use of homogenate allows the linking of mitochondrial oxidative capacities with the effective mitochondrial protein mass. The remainder of the homogenate was centrifuged at $1000 \boldsymbol{g}_{av}$ for 10 min and the resulting supernatant fraction was again centrifuged

at $3000\,\boldsymbol{g}_{av}$ for 10 min. The mitochondrial pellet was washed twice and finally re-suspended in a medium containing 80 mM-KCl, 50 mM-HEPES (pH 7·0), 5 mM-KH_2PO_4, and 0·1 % (w/v) fatty-acid-free BSA. The protein content of the mitochondrial suspension was determined by the method of Hartree (1972) using BSA as the protein standard. Isolated mitochondria were then used for the determination of respiratory parameters and α-glycerophosphate dehydrogenase (α-GPDH; EC 1·1·1·8) activity by the method described by Lee & Lardy (1965).

Hind-leg muscles were freed of excess fat and connective tissue, finely minced and washed in a medium containing 100 mM-KCl, 50 mM-tri(hydroxymethyl)-aminomethane (pH 7·5), 5 mM-$MgCl_2$, 1 mM-EDTA, 5 mM-EGTA, and 0·1 % (w/v) fatty-acid-free BSA. Tissue fragments were treated with protease nagarse (EC 3.4.21.62; 1 mg/g tissue) for 5 min, washed, homogenised with the above medium (1:8, w/v) in a Potter Elvehjem homogeniser set at 500 rpm (4 strokes/min) and filtered. Samples of the homogenate were then used for the determination of mitochondrial respiration as well as for the preparation of isolated mitochondria. To this end, the homogenate was centrifuged at $500\,\boldsymbol{g}_{av}$ for 10 min and the supernatant fraction was centrifuged at $3000\,\boldsymbol{g}_{av}$ for 10 min. The resulting pellet containing mitochondria was washed twice, and finally re-suspended in a suspension medium containing 250 mM-sucrose, 50 mM-tri(hydroxymethyl)-aminomethane (pH 7·5), and 0·1 % fatty-acid-free BSA. The protein content of the mitochondrial suspension was determined by the method of Hartree (1972) using BSA as the protein standard. Isolated mitochondria were then used for the determination of respiratory parameters and α-GPDH activity.

Polarographic measurement of respiration

Mitochondrial O_2 consumption was estimated by a Clark-type electrode (Yellow Springs Instruments, Yellow Springs, OH, USA), maintained in a water-jacketed chamber at 30°C. Hepatic mitochondria (0·9 mg protein) or samples of the liver homogenate were incubated in a medium (3 ml) containing 80 mM-KCl, 50 mM-HEPES, 1 mM-EGTA, 5 mM-KH_2PO_4 (pH 7·0), and 0·1 % (w/v) fatty-acid-free BSA. Skeletal muscle mitochondria (0·3 mg protein) or samples of the muscle homogenate were incubated in a

medium (3 ml) containing 30 mM-KCl, 6 mM-$MgCl_2$, 75 mM-sucrose, 1 mM-EDTA, 20 mM-KH_2PO_4 (pH 7·0), and 0·1 % (w/v) fatty-acid-free BSA.

The substrates used for both liver and muscle respiration were 10 mM-succinate $+$ 3·75 μM-rotenone, 10 mM-glutamate $+$ 2·5 mM-malate or 40 μM-palmitoyl-carnitine $+$ 2·5 mM-malate. State 3 measurements were performed in the presence of 0·6 mM-ADP. State 4 respiration was measured in the presence of oligomycin (4 μg/ml) in the homogenates or in the absence of ADP in isolated mitochondria. The ratio between state 3 and 4, called the respiratory control ratio, was calculated according to Estabrook (1967).

Statistical analysis

Data are given as means with their standard errors for eight different rats per group. Statistical analysis was performed by the two-tailed Student's t test. Regression analyses were also performed. Probability values less than 0·05 were considered to indicate a significant difference. All analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA).

Materials

ADP, malate, glutamate, succinate, rotenone, palmitoylcarnitine, α-glycerophosphate, nagarse, iodonitrotetrazolium violet, phenazine methosulfate, and oligomycin were purchased from Sigma Chemical Co., St Louis, MO, USA. All other reagents were of the highest purity commercially available.

Results

The changes in body composition and energy content are given in Table 1. The percentage of body fat and body energy content significantly increased in the rats fed the high-fat diet compared with the rats fed the low-fat diet.

Table 2 provides the changes in energy balance, partitioning, and efficiency over the experimental period. The rats fed the high-fat diet exhibited significantly higher ME and fat intakes together with body-energy and fat gain. Both energy expenditure and net energy expenditure (the value obtained for energy expenditure excluding the

Table 1. Body weight and composition in rats at baseline and in rats fed a low-fat or a high-fat diet†

(Mean values with their standard errors for eight rats per group)

| | Baseline | | Diet | | | | |
| | | | Low-fat | | High-fat | | |
	Mean	SE	Mean	SE	Mean	SE	Difference (%)‡
Body weight (g)	400	1	444	4	454	5	2·2
Body water (%)	69·1	0·1	64·7	0·5	63·3	1·2	− 2·3
Body fat (%)	7·4	0·1	11·1	0·6	14·1*	1·1	27
Body protein (%)	17·5	0·2	17·9	0·4	16·8	0·6	− 6
Body energy (kJ/g)	7·0	0·1	8·5	0·2	9·5*	0·4	11·8

* Mean value was significantly different to that for rats fed the low-fat diet ($P < 0.05$).
† For details of diets and procedures, see Iossa et al. (2002) and p. 954.
‡ Percentage difference values refer to the high-fat diet compared with the low-fat diet.

Table 2. Energy balance, partitioning of metabolisable energy (ME) intake, and efficiency in rats fed a low-fat or a high-fat diet†

(Mean values with their standard errors for eight rats per group)

Diet...	Low-fat		High-fat		Difference (%)‡
	Mean	SE	Mean	SE	
ME intake (kJ)	4405	278	6037*	383	37
Fat intake (kJ)	467	29	3019*	191	546
Body-energy gain (kJ)	974	34	1513*	241	55
Protein gain (kJ)	193	35	139	15	−28
Fat gain (kJ)	789	61	1354*	140	72
Energy expenditure (kJ)	3431	255	4524*	259	32
Cost of storage (kJ)	526	55	382*	37	−27
NEE (kJ)	2905	190	4142*	169	42
NEE/ME × 100 (%)	66·0	0·5	69·5	3·6	4·5
Fat gain/ME × 100 (%)	18·5	0·6	22·4*	1·5	21
Fat gain/fat intake × 100 (%)	174	22	43·2*	8·3	−75
Energetic efficiency (%)	22·0	0·8	25·0*	1·1	14

* Mean value was significantly different to that for rats fed the low-fat diet ($P < 0.05$).

† Values refer to the whole period of the diet treatment (2 weeks). For details of diets and procedures, see Iossa *et al.* (2002) and p. 954.

‡ Percentage difference values refer to the high-fat diet compared with the low-fat diet.

NEE, net energy expenditure (the difference between energy expenditure and cost of storage).

Fig. 1. (A), Serum free triiodothyronine (T_3) and leptin levels in rats fed a low-fat (□) or a high-fat (▨) diet. Values are means, with their standard errors shown by vertical bars (five rats per group). * Mean value for leptin level was significantly different from that for the low-fat diet ($P < 0.05$). (B), Correlation between serum leptin levels and percentage of body fat in rats fed a low-fat (○) or a high-fat (●) diet. Each point represents an individual value. The association for the low-fat diet was r^2 0·871 ($P = 0.02$); that for the high-fat diet was r^2 0·987 ($P = 0.0006$). (C), Correlation between daily metabolisable energy (ME) intake and serum leptin levels in rats fed a low-fat (○) or a high-fat (●) diet. Each point represents an individual value. The association for the low-fat diet was r^2 0·934 ($P = 0.007$); that for the high-fat diet was r^2 0·855 ($P = 0.024$).

total cost of storage) significantly increased in the rats fed the high-fat diet compared with the rats fed the low-fat diet. When net energy expenditure was expressed as the percentage of ME intake, no significant variation was found in the rats fed the high-fat diet. As for fat partitioning, Table 2 also shows that the percentage of ME intake stored as fat was significantly higher in the rats fed the high-fat diet, while the percentage of fat intake stored as fat decreased in the rats fed the high-fat diet compared with the rats fed the low-fat diet. Finally, gross energetic efficiency significantly increased in the rats fed the high-fat diet compared with the rats fed the low-fat diet.

As for serum leptin and free T_3 levels, no significant variation was found in serum free T_3, while serum leptin levels significantly increased in the rats fed the high-fat diet compared with the rats fed the low-fat diet (Fig. 1 (A)). Fig. 1 (B) shows the correlation between leptin and body fat in the rats fed the low- or the high-fat diet. Serum leptin levels were positively correlated with the percentage of body fat in both groups of rats, but the slopes of the regression lines were significantly different. A significant inverse correlation was found between serum leptin levels and ME intake in the rats fed the low-fat diet, whereas a direct correlation occurred between these parameters in the rats fed the high-fat diet (Fig. 1 (C)). The serum levels of NEFA, glucose, and insulin as well as insulin:glucose are presented in Table 3. Serum NEFA, glucose, and insulin levels were slightly elevated in the rats fed the high-fat diet compared with the rats fed the low-fat
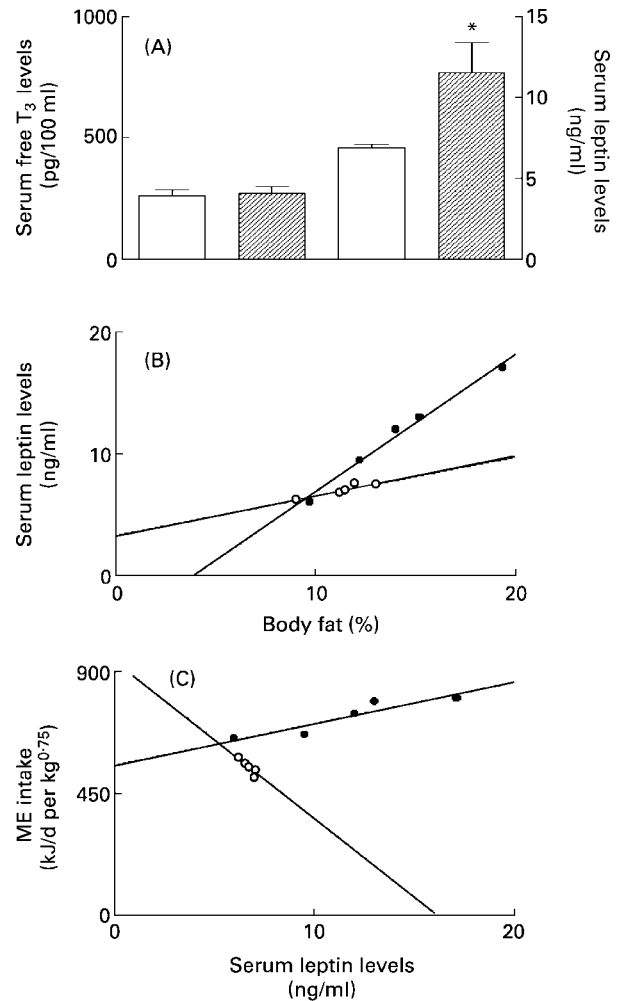
diet. However, statistical analysis indicated no significant difference between the two groups of rats. In addition, no change was found in insulin:glucose.

Mitochondrial state 3 and 4 respiratory capacities were measured in the liver and skeletal muscle homogenates to assess the metabolic activity of the whole tissue stimulated by NAD-and FAD-linked substrates. As for the liver, whatever was the substrate, mitochondrial capacities significantly increased in the liver homogenates from the rats fed the high-fat diet compared with the rats fed the low-fat diet (Table 4). In addition, a significant inverse correlation was found between the percentage of fat intake stored as body fat and hepatic palmitoyl-carnitine-supported

Table 3. Serum non-esterified fatty acids (NEFA), glucose and insulin levels and serum insulin:glucose in rats fed a low-fat or a high-fat diet*

(Mean values with their standard errors for eight rats per group)

Diet...	Low-fat		High-fat		Difference (%)†
	Mean	SE	Mean	SE	
NEFA (mmol/l)	0·215	0·017	0·240	0·019	12
Glucose (mg/100 ml)	168	9	180	9	7
Insulin (ng/ml)	2·89	0·1	3·11	0·2	8
Insulin:glucose ($\times 10^{-6}$)	1·72	0·1	1·73	0·1	0·6

* For details of diets and procedures, see Iossa et al. (2002) and p. 954.
† Percentage difference values refer to the high-fat diet compared with the low-fat diet.

respiration (Fig. 2). On the contrary, no variation was found in skeletal muscle homogenate respiration between the rats fed the low-fat diet and the rats fed the high-fat diet (Table 4).

State 3 and 4 respiratory capacities were also measured in the isolated liver and skeletal muscle mitochondria to take into account the changes in specific respiratory capacity due to high-fat feeding. The respiratory control ratio values indicated that isolated mitochondria were well coupled (Table 5). Whatever substrate was used, both state 3 and 4 respiratory capacities significantly decreased in the rats fed the high-fat diet compared with the rats fed the low-fat diet (Table 5). Liver and skeletal muscle mitochondrial α-GPDH specific activity was not affected by diet treatment (Table 5).

Discussion

The results of the present study show that high-fat feeding in adult rats induces not only an increase in fat storage but also impairment in liver and muscle mitochondrial respiratory capacities.

The evaluation of the changes in energy balance shows that adult rats are much more prone to become obese compared with young rats, when fed diets rich in fat. In fact, the adults rats fed the high-fat diet for 2 weeks exhibited an increase in ME intake and in the percentage of ME intake stored as fat (Table 2), as well as a higher body fat content (Table 1). The present results are different from those previously obtained in post-weaning rats fed a high-fat diet for 2 weeks. Mollica et al. (1999) found increased ME intake, but no change in body fat content between low-fat- or high-fat-fed rats, due to a decrease in the percentage of ME intake stored as fat. The difference can be attributed to the fact that in post-weaning rats, the energy ingested in excess is dissipated through an adaptive increase in energy expenditure, as indicated by the increase in the percentage of ME intake utilised for body energy maintenance and by the decrease in energetic efficiency (Iossa et al. 1997). Since the efficiency of nutrient utilisation is higher for fat than for carbohydrate or protein (Jéquier, 2002), high-fat feeding tends to increase rather than decrease energetic efficiency. It follows that only the presence of adaptive thermogenesis could justify a decrease in metabolic efficiency during a dietetic regimen rich in fat. The presence of diet-induced thermogenesis to limit excess energy storage in young rats overeating palatable food (rich in fat) has been previously suggested by the classic studies of Rothwell & Stock (1982). On the other hand, the present results show that when net energy expenditure, which represents the cost of body energy maintenance, was expressed as a percentage of ME intake, the values obtained did not significantly change between rats fed a low- or a high-fat diet. These results suggest that in adult rats regulatory mechanisms useful to counteract obesity development are absent and, therefore, the excess ingested energy will contribute to enlarge fat stores. In agreement, energetic efficiency increased in the adult rats fed the high-fat diet (Table 2). However, an interesting observation is that the ability to utilise fat as

Table 4. Mitochondrial respiration (ng atoms O/min × g wet tissue) in liver and skeletal muscle homogenates from rats fed a low-fat or a high-fat diet†

(Mean values with their standard errors for eight rats per group)

Diet...		Low-fat		High-fat		Difference (%)‡
		Mean	SE	Mean	SE	
Liver						
Glutamate	State 3	5207	241	6172*	360	18
	State 4	251	20	315*	21	25
Palmitoyl-carnitine	State 3	4650	133	6034*	260	30
	State 4	280	24	340*	14	21
Succinate	State 3	9218	393	11246*	327	22
	State 4	985	51	1179*	73	20
Skeletal muscle						
Glutamate	State 3	3446	79	3796	224	10
	State 4	171	37	168	35	−2
Palmitoyl-carnitine	State 3	2160	222	2304	211	7
	State 4	122	20	116	16	−5
Succinate	State 3	5729	460	5413	584	−6
	State 4	945	111	832	66	−12

* Mean value was significantly different to that for rats fed the low-fat diet ($P < 0.05$).
† For details of diets and procedures, see Iossa et al. (2002) and p. 955.
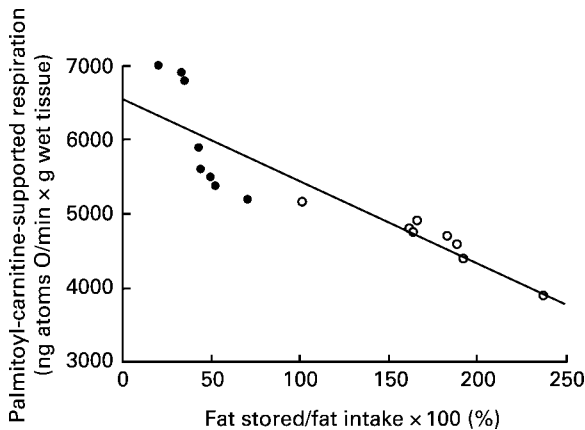‡ Percentage difference values refer to the high-fat diet compared with the low-fat diet.

Fig. 2. Correlation between hepatic palmitoyl-carnitine-supported respiration and percentage of fat intake stored as body fat in rats fed a low-fat (○) or a high-fat (●) diet. Each point represents an individual value (r^2 0.7828; $P < 0.0001$).

a metabolic fuel is elevated in adult rats fed a high-fat diet. In fact, the present results also show that when fat gain was expressed as a percentage of fat intake, significantly lower values were obtained for the rats fed the high-fat diet (Table 2). In addition, a negative correlation was found between the percentage of fat intake stored as body fat and palmitoyl-carnitine-supported respiration only in the liver (Fig. 2). It follows that, in adult rats, changes in fat storage seem to be related to changes in fat oxidation in the liver but not in skeletal muscle. On the other hand, when the dietary regimen was applied to 25-d-old rats (Iossa *et al.* 2002), a negative correlation between the

percentage of fat intake stored as body fat and skeletal muscle palmitoyl-carnitine-supported respiration was found. Therefore, the mechanisms of regulation of lipid oxidation in skeletal muscle in response to increased dietary intake are switched on in young rats, resistant to obesity, while they are not activated in adult rats, which develop obesity.

To gain insight into the hormonal modification induced by high-fat feeding in 90-d-old rats, the serum levels of free T_3, insulin, and leptin, all involved in the regulation on nutrient metabolism, were measured (Muller & Seitz, 1984; Friedman, 2002; Porte *et al.* 2002). High-fat feeding did not affect the serum free T_3 levels in adult rats (Fig. 1 (A)). A different finding was obtained in post-weaning rats (Iossa *et al.* 1995), where higher serum free T_3 levels were found in the rats fed the high-fat diet compared with the rats fed the low-fat diet, associated with increased hepatic mitochondrial α-GPDH activity, an enzyme that is induced by T_3 (Bobyleva *et al.* 2000). These results allowed Iossa *et al.* (1995) to suggest an involvement of T_3 in adaptive responses, which occur in young rats fed a high-fat diet. In fact, it is known that the α-GPDH enzyme is rate-limiting for the α-glycerophosphate shuttle (Tyzbir *et al.* 1981) and reportedly is involved in processes that could decrease metabolic efficiency (Lardy *et al.* 1995). In the present work, in agreement with the finding that T_3 levels did not change in the 90-d-old rats fed the high-fat diet, no changes were found in α-GPDH activities both in the liver- and skeletal-muscle-isolated mitochondria (Table 5).

Since serum insulin and glucose levels as well as insulin: glucose did not significantly change between the two

Table 5. Respiration (ng atoms O/min $\times$ mg protein) in liver and skeletal muscle mitochondria from rats fed a low-fat or a high-fat diet†

(Mean values with their standard errors for eight rats per group)

Diet...		Low-fat		High-fat		Difference (%)‡
		Mean	SE	Mean	SE	
Liver						
Glutamate	State 3	84	7	62*	4	−26
	State 4	8·4	0·4	6·7*	0·3	−20
	RCR	10·0	0·7	9·2	0·4	−8
Palmitoyl-carnitine	State 3	77	4	59*	2	−23
	State 4	11·5	0·5	9·0*	0·4	−22
	RCR	6·7	0·3	6·6	0·4	−0·01
Succinate	State 3	152	9	108*	3	−29
	State 4	23	0·7	18*	1	−22
	RCR	6·6	0·3	6·0	0·3	−9
α-GPDH activity (nmol/min × mg protein)		10	1	9	2	−0·1
Skeletal muscle						
Glutamate	State 3	1039	75	756*	76	−27
	State 4	64	4	48*	3	−25
	RCR	16·2	0·7	15·8	1·0	−2
Palmitoyl-carnitine	State 3	382	21	257*	19	−33
	State 4	60	5	44*	4	−27
	RCR	6·4	0·3	5·8	1·0	−9
Succinate	State 3	1037	36	842*	55	−19
	State 4	173	11	139*	9	−20
	RCR	6·0	1·0	6·1	0·3	2
α-GPDH activity (nmol/min × mg protein)		85	6	95	11	12

RCR, respiratory control ratio; α-GPDH, α-glycerophosphate dehydrogenase.
* Mean value was significantly different to that for rats fed the low-fat diet ($P < 0.05$).
† For details of diets and procedures, see Iossa *et al.* (2002) and p. 955.
‡ Percentage difference values refer to the high-fat diet compared with the low-fat diet.

groups of rats (Table 3), the feeding of a high-fat diet for 2 weeks is characterised by normal insulin sensitivity. This result is not in agreement with previous studies, where a high-fat diet induced insulin resistance (Spicer, 2002; Woods *et al.* 2003). However, in these studies, the high-fat-diet treatment lasted for more than 2 weeks. It seems that the length of the high-fat feeding is an important factor for the development of insulin resistance.

As for serum leptin levels, a significant increase was found in the adult rats fed the high-fat diet compared with the control rats (Fig. 1 (A)) in agreement with the greater body fat content exhibited by these rats. On the other hand, Mollica *et al.* (1999) found no variations in leptin levels and body fat content in post-weaning rats fed a high-fat diet. Also, Iossa *et al.* (1999b) showed increased serum leptin levels and body fat content in middle-aged rats (180 d of age) fed a low-fat diet. It can be suggested that high-fat feeding could anticipate the effect of ageing on body-fat gain and serum leptin levels. A significant positive correlation between serum leptin levels and body fat mass was always found (Fig. 1 (B)), in agreement with the observation that leptin circulates in proportion to body mass in rodents (Friedman, 2002). Interestingly the slope of the regression line obtained for the adult rats fed the high-fat diet was significantly higher than that found in the control rats. This result suggests that the increase in leptin levels found in the adult rats fed the high-fat diet is due to other factors beyond increased body fat stores. It has been shown that the sympathetic nervous system inhibits *ob* gene transcription in adipose tissue through β_3 adrenergic receptors (Li *et al.* 1997; Evans *et al.* 1999). In addition, it has been found that high-fat feeding adversely affects adipocyte adrenergic receptor expression and function (Collins *et al.* 1997). It can be speculated that the higher leptin levels, found in the adult rats fed the high-fat diet, could be due not only to higher body fat mass, but also to defective adrenergic control of adipocyte function. Consistent with the inhibitory action of leptin on food intake (Schwartz *et al.* 1996), a significant inverse correlation between serum leptin levels and ME intake was found in the rats fed the low-fat diet. On the other hand, a significant positive correlation was found between these two parameters in the rats fed the high-fat diet (Fig. 1 (C)). This finding suggests that high-fat feeding in 90-d-old rats eliminates the susceptibility to the anorexic effect of leptin. It should be noted that a reduction in the anorexic effect of leptin has been reported in old rats (Scarpace *et al.* 2000). In addition, in a previous study, it was found that from 90 d of age onwards a progressive reduction in the responsiveness of the rat to the leptin signal takes place (Iossa *et al.* 1999b). Therefore, it can be suggested that a nutritional factor, such as a high fat level in the diet, can induce the reduction in responsiveness to leptin, a phenomenon typical of ageing. This suggestion is in agreement with the observation that the chronic administration of a high-fat diet to rats results in a reduced uptake of leptin into the brain and an impaired intracellular signal transduction (El-Haschimi *et al.* 2000). Since it has been shown that the capacity to resist obesity development tends to decrease with age (Barzilai & Rossetti, 1995; Iossa *et al.* 1999b),

it is conceivable that high-fat feeding in adult rats anticipates the fall in physiological mechanisms that allow the maintenance of body weight.

The present results also showed that adult rats fed a high-fat diet exhibited a significant decrease in specific respiratory capacities whatever substrate was used for both liver- and skeletal-muscle-isolated mitochondria (Table 5). On the other hand, higher skeletal muscle mitochondrial-specific respiratory capacities have previously been found in post-weaning rats fed a high-fat diet compared with rats fed a low-fat diet (Iossa *et al.* 2002), but no diet-related difference was found in the liver-specific respiratory capacity of mitochondria isolated from post-pubertal rats (Iossa *et al.* 1999a). Taking into account that a general decline in mitochondrial oxidative capacities is associated with the ageing process (Ames *et al.* 1995), it can be suggested that high-fat feeding causes an early onset of mitochondrial decay in adult rats. However, in the present study the impairment in oxidative capacities in isolated mitochondria did not affect whole-tissue oxidative function. In fact, no variation was found in the mitochondrial oxidative capacity of skeletal muscle homogenates and a significant increase in liver homogenates was found. It can be speculated that an increase in the mass of muscle and liver mitochondria compensates for the reduced oxidative capacities found in isolated mitochondria. This suggestion is consistent with the recovered mitochondrial protein contents from the liver and skeletal muscle, which were significantly higher in the rats fed the high-fat diet (data not shown). In addition, Nemeth *et al.* (1992) have found increases in the oxidative enzymes of the tricarboxylic acid cycle and in β-hydroxyacyl-CoA dehydrogenase, an enzyme for fatty acid oxidation, measured in skeletal muscle homogenate from adult rats fed a high-fat diet.

Taken together, the present results demonstrate that adult rats have a lower capacity to resist diet-induced obesity, probably due to the failure of adaptive mechanisms, which counteract obesity in young rats. In addition, the present study found that a nutritional factor, such as a high level of fat in the diet, induces an impairment of mitochondrial respiratory capacities, even if, probably, compensated by an increase in mitochondrial protein mass. The overall conclusion of the present study is that high-fat feeding in 90-d-old rats induces an early onset of obesity and impairment in isolated mitochondrial oxidative capacity, both phenomena typical of ageing.

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