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Fat balance and hepatic mitochondrial function in response to fat feeding in mature rats

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OBJECTIVE: To study the effects of fat feeding on fat balance and hepatic mitochondrial function in postpubertal male rats.
DESIGN: Rats were fed low fat, medium fat or high fat diet for 15 days.
MEASUREMENTS: Energy balance, body composition, resting metabolic rate (RMR), mitochondrial state 3 and state 4 oxygen consumption rates, succinic dehydrogenase (EC 1.3.99.1) and mitochondrial α-glycerophosphate dehydrogenase (EC 1.1.1.8) activities.
RESULTS: Rats fed medium fat or high fat diet, in comparison with rats fed low fat diet, showed a significantly greater metabolisable energy intake and energy expenditure. In addition, body energy and lipid gains were significantly higher in rats fed medium fat or high fat diet than in rats fed low fat diet. Mitochondrial respiration and enzymatic activities were not affected by fat feeding.
CONCLUSION: These results indicate that in postpubertal rats fed high fat diets, the increase in energy expenditure counteracts only in part the excess fat deposition. This is probably due to the impairment in regulatory responses, and enhances thermogenesis.

Keywords: fat feeding; fat balance; hyperphagia; mitochondrial respiration

Introduction

When energy intake and expenditure are not in balance, the body may change its metabolic rate. In fact, under conditions of fasting[1,2] or food restriction,[3,4] metabolic rate decreases to partially compensate for the energy deficit. In contrast, when energy intake exceeds energy expenditure through overfeeding, metabolic rate may increase and some of the excess energy is released in the form of heat production. This is particularly true for young rats: in fact, several works have found that young rats fed a high-energy diet overeat but resist becoming obese through a regulatory increase in energy expenditure[5–7] both at the level of brown adipose tissue[8] and liver.[9,10] However, the regulatory nature of the increased energy expenditure has been a matter of debate.[11,12]

We have previously shown that Wistar rats fed a high fat diet between 30 and 45 d of age overate but failed to exhibit excess weight gain due to a concomitant increase in energy expenditure, compared to rats fed a low fat diet.[13–15] We also suggested that a contribution to the increased energy expenditure could be given by the liver, as we found an increase in flavin-adenine dinucleotide ($FADH_2$) oxidation and a decrease in nicotinamide-adenine dinucleotide (NADH) oxidation in hepatic mitochondria together with an increased hepatic fatty acid utilisation.[13,14,16] Taking into account that similar results were obtained by us in several experiments using rats of the same strain but from other colonies,[17–19] we concluded that the strain of rats we used is very resistant to obesity between 30 and 45 d of age. Since the capacity of the rats to resist the onset of obesity tends to decrease with age,[20] it appeared of interest to verify whether this obesity resistance peculiar to young Wistar rats is maintained with increasing age. To this end, 50 d old rats were fed diets enriched in fat for a 2-week period.

In these rats we carried out full energy balance, resting metabolic rate (RMR), and hepatic mitochondrial respiration measurements in order to know if they are able to develop responses similar to those found in young rats, such as hyperphagia, increased energy expenditure, decreased efficiency, and changed oxidative capacity in hepatic mitochondria.[14–16]

Methods and materials

Animals

The animals studied were 50 d old male Wistar rats (weighing about 200 g) obtained from Charles River Italia (Calco, Como, Italy) just after weaning. The animals were housed individually in grid-bottomed cages under controlled conditions (24°C and an artificial circadian 12-h light/dark cycle) and given food and water *ad libitum*. Treatment, housing, and killing of animals met the guidelines set by the Italian Health Ministry.

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Experimental design

On arrival, all rats were provided with a low fat diet (Mucedola 4RF21; Settimo Milanese, Milan, Italy) for a 4-week baseline period. The composition (% energy) of this diet was protein 29.0, lipid 10.6, and carbohydrate 60.4; 12.50 kJ metabolisable energy/g. After the baseline period the rats were divided into four groups of similar mean body weight, and with body weight normally distributed within each group. The first group was killed, as later reported, for the determination of energy content and body composition. The second group, used as the control group, continued on the low fat diet. The third group had free access to a medium fat diet (82% low fat diet, 10% lyophilised meat, 5% butter, 2.8% alphacel, 0.1% AIN 76 vitamin mix, 0.5% AIN, 76 mineral mix, g/g; composition (% energy): protein 29.0, lipid 25.0, and carbohydrate 46.0; 14.3 kJ metabolisable energy/g). The last group had free access to high fat diet (28% low fat diet, 39.5% lyophilised meat, 17.8% butter, 12% alphacel, 0.7% AIN 76 vitamin mix, 2% AIN 76 mineral mix, g/g). The composition (% energy) of this diet was protein 29.0, lipid 50.0, and carbohydrate 21.0; 15.80 kJ metabolisable energy/g. The medium fat and high fat diets, both with the same protein content as the low fat diet, are characterised by an increased fat content and by the presence of a meat component which is among the flavours most preferred by rats.[21,22] The diet treatment lasted 15 d. In this period body weights and food intakes were monitored daily to allow calculations of body weight gain and gross energy intake. The faeces and spilled food were also collected daily, dried, and ground to a powder before determining their energy content with a bomb calorimeter calibrated with dry benzoic acid standard (Parr adiabatic calorimeter of Parr Instruments Co., Moline, Il, USA). The gross energy content of the diets was also determined with the bomb calorimeter, and the values were 15.88 kJ/g for low fat diet, 18.1 kJ/g for medium fat diet, and 19.85 kJ/g for high fat diet.

At the end of the experimental period, before the rats were killed for energy balance measurements and for determination of respiration in isolated mitochondria, they were used for RMR determination

RMR determination

Oxygen consumption was measured to all the rats between 09.00 h to 11.00 h with an oxygen consumption monitor (Columbus Instruments, Columbus, Ohio, USA) in a chamber at 24°C. Although most of the rats became quiet after about 30 min in the chamber, all were allowed to adapt to the conditions for a minimum of 60 min before beginning the measurements. RMR was obtained over a period of at least 10 min during which the animals remained quiet.

Energy balance measurements

Digestible energy intake was obtained by substracting the energy measured in the faeces and spilled food from the gross energy intake as measured from daily food consumption. Metabolisable energy (ME) intake was expressed as digestible energy intake $\times$ 0.96.[23] The metabolisable energy content of the diets was calculated as ME intake/food intake ratio of the three groups of rats. At the end of the diet treatment, the animals were anaesthetised by the intraperitoneal injection of chloral hydrate (40 mg/100 g body weight), killed by decapitation, and the liver was removed. Then, after gut content removal, the carcasses were weighed, autoclaved for 90 min, chopped into small pieces, thoroughly mixed, and finally homogenised with water (volumes equal to twice the carcass weight) in a Polytron (Kinematica AG, Lucerne, Switzerland). Aliquots of the homogenates were dessicated at 70°C in a vacuum oven. Then, small pellets (about 200 mg) of the dried homogenate were made and the body energy content was measured with the bomb calorimeter. Corrections were made for the energy content of the liver. The gain in energy during the 15 d period of the diet treatment was obtained by subtracting the energy content of the rats killed at the beginning of the diet treatment from that of each of the three experimental groups. Energy expenditure was calculated from the difference between ME intake and energy gain.

Body composition measurements

Aliquots of the carcass homogenate were analysed for lipid, protein, and water content. Lipid content was determined gravimetrically after extraction in chloroform–methanol and evaporation to constant weight by a rotating evaporator (Heidolh, Germany) by the method of Folch *et al*.[24] The energy as lipid was calculated from the lipid content by using the coefficient of 39.2 kJ/g for the energy content of lipid. Protein content was determined by the Biuret method after extraction in SDS-NaOH as described by Brooks *et al*.[25] The energy as protein was calculated from the protein content by using the value of 23.5 kJ/g for the energy content of protein. Water content was determined by the difference in weight of the homogenate before and after drying at 70°C in a vacuum oven.

Preparation of isolated mitochondria

Livers were rapidly removed, finely minced and washed with a medium containing 220 mM mannitol, 70 mM sucrose, 20 mM Hepes, pH 7.4, 1 mM EDTA, and 0.1% (w/v) fatty acid free bovine serum albumin. Tissue fragments were gently homogenised with the same medium (1 : 10, w/v) in a Potter Elvehjem homogeniser set at 500 rpm (4 strokes/min). Liver mitochondria were isolated from the homogenate as previously reported.[26,27] Briefly, the homogenate was filtered through sterile gauze and freed of debris and nuclei by centrifugation at $1000 \times g_{av}$ for 10 min; the resulting supernatant was again centrifuged at $3000 \times g_{av}$ for 10 min. The mitochondrial pellet was

washed twice and finally resuspended in a medium containing 80 mM KC1, 50 mM Hepes, pH 7.0, 5 mM KH_2PO_4, 0.1% (w/v) fatty acid free bovine serum albumin. It should be noted that enzymatic and electron microscopy characterisation has shown that our isolation procedure (centrifugation at $3000 \times g_{av}$ for 10 min) results in a cellular fraction which is constituted essentially by mitochondria.[28,29] The protein content of the mitochondrial suspension was determined by the method of Hartree[30] using bovine serum albumin as the protein standard.

Mitochondrial respiration and enzyme activities

Mitochondrial oxygen consumption was measured polarographically with a Clark-type electrode (Yellow Springs Instruments, Yellow Springs, OH, USA), maintained in a water jacketed chamber at 30°C. Mitochondria (1 mg of protein) were incubated in 3 ml of the above suspension medium. Measurements were made within 2 h following the isolation of the mitochondria. The mitochondria were allowed to oxidise their endogenous substrates for a few minutes. Substrates were then added at the concentration reported in Table 6 to determine state 4 oxygen consumption rate. Six minutes later, adenosine diphosphate (ADP) (at a final concentration of 0.3 mM) was added and state 3 rate was measured. Succinic dehydrogenase (EC 1.3.99.1) and mitochondrial α-glycerophosphate dehydrogenase (EC 1.1.1.8) activities were measured by the method described by Lee and Lardy.[31]

Statistical analysis

Data are given as means ± s.e.m. Statistical analyses were performed by one-way analysis of variance (ANOVA). *Post-hoc* comparison between group pairs was made with the Newman-Keuls test after ANOVA had established significant differences among groups. *P*-values < 0.05 were considered to indicate a significant difference. All analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA).

Materials

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

Results

Body weight and composition

Figure 1 shows daily body weights of rats fed low fat, medium fat or high fat diet for 15 d. Starting from 5d of diet treatment, weight gain of rats consuming high fat diet was slightly higher than that of rats fed low fat or medium fat diet. This weight difference was maintained until the termination of the experimental feeding period, but the final body weights of the three groups reported in Table 1 did not significantly vary.

Changes in body composition and energy content are given in Table 1. Note that rats fed medium fat or

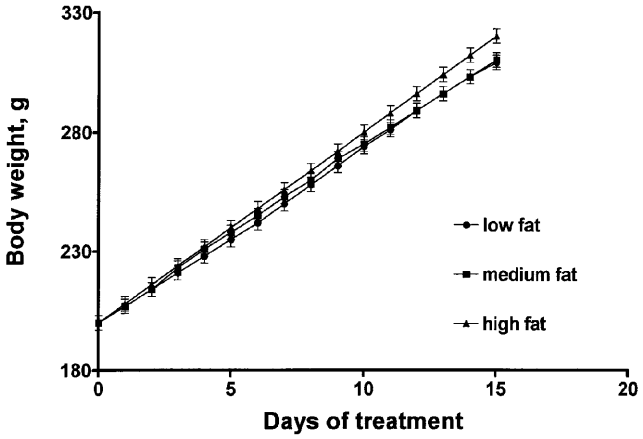

Figure 1 Body weight gain in rats fed low fat, medium fat or high fat diet. Each point represents mean weight ± s.e.m. of eight (medium fat) or sixteen (low fat and high fat) rats.

Table 1 Body weight, composition, and energy content in rats fed low fat, medium fat or high fat diet

	Low fat	*Medium fat*	*High fat*
Body weight, g	309 ± 5 (16)	310 ± 4 (8)	320 ± 5 (16)
% change		0.3	4
Body water, %	67.6 ± 0.2 (16)	65.1 ± 0.3 (8)**	63.8 ± 0.4 (16)**,§
% change		− 4	− 6
Body lipid, %	10.8 ± 0.3 (16)	13.5 ± 0.3 (8)*	14.2 ± 0.8 (16)**
% change		25	31
Body protein, %	15.1 ± 0.4 (8)	14.5 ± 0.4 (8)	13.0 ± 0.4 (8)*,§
% change		− 4	− 14
Body energy, kJ/g	8.1 ± 0.1 (16)	9.0 ± 0.1 (8)*	8.9 ± 0.2 (16)**
% change		11	10

Values are the means ± s.e.m. Numbers of rats shown in parentheses.
% Change values refer to high fat or medium fat compared to low fat.
Baseline values of eight rats at the beginning of the diet treatment were: body weight, g: 200 ± 2; body water, %: 68.1 ± 0.2; body lipid, %: 8.9 ± 0.2; body protein, %: 16.4 ± 0.3; body energy, kJ/g: 7.8 ± 0.1.
*$P < 0.01$, **($P < 0.001$ compared to rats fed low fat diet.
§$P < 0.05$ compared to rats fed medium fat diet.

high fat diet had a significantly increased percentage of body lipid and energy content compared to rats fed low fat diet. Percentage of body water significantly decreased in rats fed medium fat or high fat diet compared to rats fed low fat diet, while percentage of body protein significantly decreased only in rats fed high fat diet.

Energy balance

In Table 2 are reported ME intake as well as body energy, protein, and lipid gain. A significant increase was found in ME intake in rats fed medium fat or high fat diet compared to rats fed low fat diet. Taking into account the composition of the diet, rats fed medium fat diet ingested the same amount of carbohydrate, more protein (+31%) and fat (+221%), while rats fed high fat diet ingested more protein (+30%) and fat (+513%), but carbohydrate intake significantly decreased (−55%). Body energy and lipid gain were significantly higher in rats fed medium fat or high fat diet compared to rats fed low fat diet, while protein gain was significantly lower only in rats fed high fat diet. Table 2 also shows that the percentage of ingested lipid and protein which was stored as carcass energy significantly decreased in rats fed medium fat or high fat diet compared to rats fed low fat diet.

In Table 3 are reported energy expenditure and partitioning of ME intake in rats fed low fat, medium fat or high fat diet. The total cost of storage was

Table 2 Metabolisable energy intake and body energy gain in rats fed low fat, medium fat or high fat diet

	Low fat	*Medium fat*	*High fat*
ME intake, kJ	4254±161 (16)	5567±151 (8)***	5530±253 (16)***
% change		31	30
Body energy gain, kJ	936±47 (16)	1228±51 (8)***	1290±50 (16)***
% change		31	38
Protein gain, kJ	326±29 (8)	286±26 (8)	207±25 (8)*§
% change		−12	−37
Lipid gain, kJ	610±32 (16)	943±28 (8)***	1083±63 (16)***
% change		55	77
Protein gain/protein intake, %	26±2 (8)	18±1 (8)**	13±2 (8)***
% change		31	50
Lipid gain/fat intake, %	135±7 (16)	65±1 (8)***	39±5 (16)***§§
% change		−52	−71

Values are the means±s.e.m. The values refer to the whole period of the diet treatment. Numbers of rats are shown in parentheses.
% Change values refer to high fat or medium fat compared to low fat.
ME = metabolisable energy.
*$P<0.05$, **$P<0.01$, ***$P<0.001$, compared to rats fed low fat diet.
§$P<0.05$, §§$P<0.01$, compared to rats fed medium fat diet.

Table 3 Energy expenditure and partitioning of metabolisable energy intake in rats fed low fat, medium fat or high fat diet

	Low fat	*Medium fat*	*High fat*
Energy expenditure, kJ	3318±121 (16)	4339±125 (8)***	4240±150 (16)***
% change		31	28
Heat loss in storing protein, kJ	407±37 (8)	357±35 (8)	259±34 (8)*
% change		−22	−36
Heat loss in storing fat, kJ	220±12 (16)	151±4 (8)**	173±26 (16)*
% change		−31	−21
Total cost of storage, kJ	627±38 (8)	508±45 (8)	432±40 (8)**
% change		−19	−31
Corrected energy expenditure (CEE), kJ	2691±94 (8)	3831±92 (8)***	3808±89 (8)***
% change		42	42
CEE/ME, %	63.0±2.0 (8)	69.0±2.0 (8)	69.0±2.7 (8)
% change		10	10
Protein gain/ME, %	8.0±0.5 (8)	5.0±0.4 (8)**	4.0±0.8 (8)***
% change		−38	−50
Lipid gain/ME, %	14.0±0.7 (16)	17.0±0.6 (8)*	20.0±1.0 (16)***§
% change		21	43
Cost of storage/ME, %	15.0±0.5 (8)	9.0±0.4 (8)***	8.0±0.9 (8)***
% change		−40	−47

Values are the means±s.e.m. The values refer to the whole period of the diet treatment. Numbers of rats shown in parentheses. % Change values refer to high fat or medium fat compared to low fat.
Values of 1.25 kJ/kJ was used to estimate the storage cost of protein.
Values of 0.36 kJ/kJ (low fat diet) and 0.16 kJ/kJ (medium fat and high fat diet) were used to estimate the storage cost of fat. Total cost of storage refers to the heat loss associated with the storage of fat and protein.
CEE = Energy expenditure excluding the total cost of storage.
ME = Metabolisable energy.
*$P<0.05$, **$P<0.01$, ***$P<0.001$, compared to rats fed low fat diet.
§$P<0.05$, compared to rats fed medium fat diet.

determined taking into account that the energy loss in storing 1 kJ of protein is 1.25 kJ,[32] while the corresponding energy cost for fat deposition is 0.36 kJ/kJ for diets with a high percentage of carbohydrates,[32] such as our low fat diet, and 0.16 kJ/kJ for diets enriched in fat, such as our medium fat or high fat diet.[33] Energy expenditure and the values obtained for energy expenditure excluding the total cost of storage, called corrected energy expenditure (CEE), were significantly increased in rats fed medium fat or high fat diet compared to rats fed low fat diet. When CEE was expressed as a percentage of ME, no significant variation was found in rats fed medium fat or high fat diet. The percentage of ME intake used for storage of protein and lipid significantly decreased in rats fed medium fat or high fat diet compared to rats fed low fat diet. In addition, the percentage of ME intake stored as protein decreased in rats fed medium fat or high fat diet, while the percentage stored as lipid increased in rats fed medium fat or high fat diet compared to rats fed low fat diet.

Metabolic rate

Figure 2 shows that RMR of rats fed medium fat or high fat diet was significantly higher (+20% and +27% respectively) than that of rats fed low fat diet. Because most published values of metabolic rate are normalised to body weight$^{0.75}$, this procedure was followed for these data. Similar results were obtained if RMR was expressed in terms of body protein content. In fact, in this case RMR was significantly increased by 24% in rats fed medium fat diet and by 46% in rats fed high fat diet.

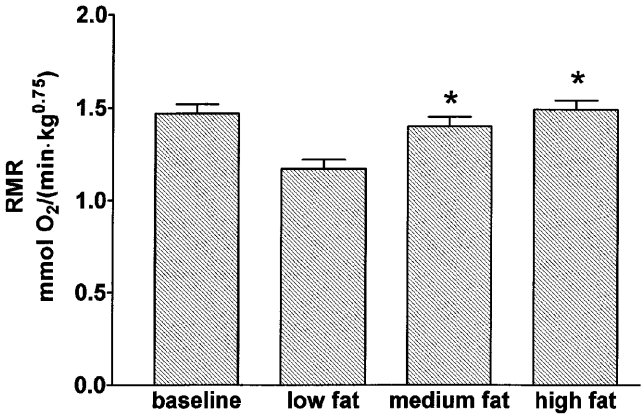

Figure 2 Resting metabolic rate (RMR) in rats fed low fat, medium fat or high fat diet.
Baseline values are the means ± s.e.m of forty different rats at the beginning of the diet treatment.
Low fat, medium fat, and high fat values are the means ± s.e.m of eight (medium fat) or sixteen (low fat and high fat) different rats after fifteen days of the diet treatment.
*$P < 0.05$ compared to rats fed low fat diet.

Hepatic mitochondrial enzymes and mitochondrial protein mass

Succinic dehydrogenase (SDH) specific activity was assayed as a mitochondrial marker enzyme. SDH activity measured both in the liver homogenate (9.5 ± 0.2 μmol/min × g liver in rats fed low fat diet, 9.8 ± 0.5 in rats fed medium fat diet, and 10.0 ± 0.9 in rats fed high fat diet) and in the mitochondria (0.27 ± 0.01 μmol/min × mg protein in rats fed low fat diet, 28 ± 0.02 in rats fed medium fat diet, and 0.26 ± 0.04 in rats fed high fat diet) was not affected by diet treatment. As a consequence, the mitochondrial protein mass per gram liver, which was calculated as previously reported[28,29] from SDH activity in the liver homogenate and in the mitochondria, was similar in the three experimental groups. As for the mitochondrial α-glycerophosphate dehydrogenase specific activity, it was not affected by diet treatment (29.3 ± 2.7 nmol/min × mg of protein in rats fed low fat diet, 30.2 ± 2.8 in rats fed medium fat diet, and 28.3 ± 3.2 in rats fed high fat diet).

Respiratory activities with NAD- and FAD-linked substrates

Table 4 shows that there was no difference in hepatic mitochondrial respiration using NAD- and FAD-linked substrates in rats fed medium or high fat diet compared to rats fed low fat diet.

Table 4 Hepatic mitochondrial respiratory activity in rats fed low fat, medium fat or high fat diet

		Low fat	*Medium fat*	*High fat*
Succinate +	State 3	152.3 ± 8.6	144.3 ± 5.9	148.5 ± 6.7
Rotenone	State 4	24.4 ± 1.1	23.9 ± 1.9	23.1 ± 2.1
Glutamate +	State 3	86.8 ± 7.0	88.1 ± 6.9	89.2 ± 7.1
Malate	State 4	9.2 ± 0.4	8.9 ± 0.7	9.0 ± 0.5
Pyruvate +	State 3	35.2 ± 1.5	34.8 ± 2.1	33.2 ± 1.8
Malate	State 4	6.7 ± 0.4	7.0 ± 0.5	6.5 ± 0.6
Palmitoyl-carnitine +	State 3	87.6 ± 4.6	82.9 ± 3.3	88.7 ± 4.2
Malate	State 4	14.0 ± 1.1	13.2 ± 0.9	13.8 ± 1.2
Palmitoyl-CoA +	State 3	85.1 ± 6.3	83.4 ± 5.6	79.5 ± 5.3
carnitine + Malate	State 4	14.5 ± 1.1	13.9 ± 1.0	14.9 ± 1.2

Values are the means ± s.e.m of sixteen (low fat and high fat) or eight (medium fat) different rats after fifteen days of the diet treatment.
States 3 and 4 respiratory rates are expressed as nmol O/min × mg protein. Substrate concentrations: succinate 10 mM, rotenone 3.75 μM, glutamate 10 mM, pyruvate 10 mM, palmitoylcarnitine 40 μM, palmitoyl-Coa 40 μM, malate 2.5 mM, carnitine 2 mM.

Discussion

The present investigation mainly focuses on the energy intake and utilisation in rats fed low fat, medium fat or high fat diet for 15 days during the postpubertal period.

Rats fed medium fat or high fat diet showed a significant increase in ME intake, compared to rats fed low fat diet (Table 2). Body lipid mass and lipid gain significantly increased in rats fed medium fat or high fat diet. On the other hand, lower protein gain was found only in rats fed high fat diet despite the increased protein intake (see Table 1 and 2), probably because in these rats, due to their lower carbohydrate intake, an increase in hepatic gluconeogenesis from aminoacids[34] takes place. We have previously found that 30 d old Wistar rats fed high fat diet showed the same level of hyperphagia (30%), but their body energy gain as well as protein and fat deposition were not significantly altered compared to control rats,[15] due to an increased capacity to utilise fat for metabolic needs. This increased capacity is also found in postpubertal rats fed medium fat or high fat diet, as indicated by the significant decrease in fat gain/fat intake ratio (Table 2), even if this increase does not balance increased fat intake, so that body fat stores increase. In addition, one possible explanation of the differences in protein gain found in rats of different age fed high fat diet could be that in younger animals, due to the higher capacity to utilise fat, a greater amount of glycerol coming from triglyceride hydrolysis can partly satisfy gluconeogenesis needs.

Our results also show that RMR as well as energy expenditure and energy expenditure excluding the costs of fuel storage (corrected energy expenditure, CEE) significantly increased in rats fed medium fat or high fat diet (Figure 2 and Table 3). The increased CEE here observed is in agreement with the enhanced thermogenic activity of brown adipose tissue,[35] white adipose tissue,[36] and skeletal muscle[36] found in rats fed high fat diet.

To clarify if the excess energy expenditure here observed is due not only to obligatory thermogenesis linked to storage of extra energy intake[11] but also to a regulatory process associated with consumption of fat-rich diets, percentage partitioning of metabolisable ingested energy into energy stored as fat and protein, cost of energy storage, and CEE has been determined (see Table 3). The results show that a greater percentage of ME intake is utilised to store fat, while a lower percentage is used to synthesise body protein in rats fed medium fat or high fat diet, compared to rats fed low fat diet. The percentage of ME intake associated with the cost of energy storage significantly decreased in rats fed medium fat or high fat diet because of their higher lipid gain and lower protein gain. In fact, the process of synthesising body protein is energetically more expensive than the replenishment of fat reserve. In addition, when CEE, which can represent the cost of the body energy maintenance, was expressed as a percentage of ME, the values obtained in the three experimental groups did not significantly change with an average of 67%. This result is similar to that obtained by LeBlanc *et al*, who found no variation in CEE/ME ratio in adult rats fed a cafeteria diet.[37] On the other hand, it is different from that found by us in younger rates, where CEE/ME ratio significantly increased in rats fed high fat diet compared to controls.[15] Therefore, it appears that regulatory mechanisms controlled by ME intake are weakened in older rats.

We have previously found that 30 d old rats fed high fat diet exhibited an increase in $FADH_2$ oxidation[13] and a decrease in NADH oxidation[14] in hepatic mitochondria together with an increased hepatic respiration of liver cells.[15] These results allowed us to suggest a fall in hepatic metabolic efficiency, which may represent one of those regulatory mechanisms useful to counteract obesity onset in very young rats.[14] On the other hand, present results indicate that in 50 d old rats fed medium fat or high fat diet hepatic mitochondrial capacity to oxidise various substrates was not affected. In addition, the increased fat oxidation found in rats fed medium fat or high fat diet is not coupled to an increase in hepatic fatty acid oxidation capacity. In fact, as shown in Table 4, there were no significant differences due to the diet in the hepatic mitochondrial rates of palmytoylcarnitine or palmitoyl-CoA oxidation. Respiration supported by the latter substrate reflects the activity of carnitine palmitoyltransferase (CPT) I, CPT II, and the intramitochondrial β-oxidation pathway, while respiration with palmitoylcarnitine, which bypasses the step catalysed by CPT I, represents an index of fatty acid oxidation per se.[38–41] However, *in vivo*, mitochondrial capacity usually is not reached.[42] Above-mentioned findings confirm previous data that nutritional states do not affect mitochondrial capacity to oxidise fatty acids.[42]

Taken together, previous[13–16] and present results demonstrate that in general high levels of energy intake are associated with increased energy expenditure which exceeds the obligatory cost of energy gain. It seems that in postpubertal rats the increase in energy expenditure counteracts only in part excess fat deposition, probably due to a decrease in regulatory responses. This impairment both in rats and humans may be the cause of the tendency to accumulate fat peculiar to the animals, which grow older.

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