International Journal of Obesity (2002) 26, 65–72

0307–0565/02 $25.00
www.nature.com/ijo

PAPER

Skeletal muscle oxidative capacity in rats fed high-fat diet

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OBJECTIVE: To investigate whether young rats respond to high-fat feeding through changes in energy efficiency and fuel partitioning at the level of skeletal muscle, to avoid obesity development. In addition, to establish whether the two mitochondrial subpopulations, which exist in skeletal muscle, ie subsarcolemmal and intermyofibrillar, are differently affected by high-fat feeding.
DESIGN: Weaning rats were fed a low-fat or a high-fat diet for 15 days.
MEASUREMENTS: Energy balance and lipid partitioning in the whole animal. State 3 and state 4 oxygen consumption rates in whole skeletal muscle homogenate. State 3 and state 4 oxygen consumption rates, membrane potential and uncoupling effect of palmitate in subsarcolemmal and intermyofibrillar mitochondria from skeletal muscle.
RESULTS: Rats fed a high-fat diet showed an increased whole body lipid utilization. Skeletal muscle NAD-linked and lipid oxidative capacity significantly increased at the whole-tissue level, due to an increase in lipid oxidative capacity in subsarcolemmal and intermyofibrillar mitochondria and in NAD-linked activity only in intermyofibrillar ones. In addition, rats fed a high-fat diet showed an increase in the uncoupling effect of palmitate in both the mitochondrial populations.
CONCLUSIONS: In young rats fed a high-fat diet, skeletal muscle contributes to enhanced whole body lipid oxidation through an increased mitochondrial capacity to use lipids as metabolic fuels, associated with a decrease in energy coupling.
International Journal of Obesity (2002) **26,** 65–72. DOI: 10.1038/sj/ijo/0801844

Keywords: skeletal muscle lipid oxidation; high-fat diet; lipid partitioning; intermyofibrillar and subsarcolemmal mitochondria

Introduction

The maintenance of a stable body weight depends on the ability to adapt energy expenditure to changes in energy intake. In addition, when changes in the composition of the diet take place, the body must adapt macronutrient oxidation to intake. This adaptation relies on the ability to partition dietary nutrients between oxidation and storage. As for protein and carbohydrate balance, it is accepted that changes in intakes are able to elicit changes in utilization of the above macronutrients.[1] On the other hand, an increase in lipid intake is not always followed by an increase in lipid utilization, so that diet-induced obesity may develop.[2–4] We have previously shown that young Wistar rats fed a high-fat diet are able to avoid obesity development through an increase in whole body energy expenditure and lipid oxidation.[5–7] These changes must be related to modification in metabolic activity of organs and tissues, especially those that are the main contributors to total metabolism, eg liver and muscle.[8] Since mitochondria are the main site of oxidative processes, changes in cellular metabolic activity could result from changes in mitochondrial mass and/or specific activity. As for liver contribution to the response to high-fat feeding, we have found an increase in hepatic fatty acid oxidation rates in rats fed high-fat diet.[5,6] Concerning skeletal muscle, several studies have found changes in mitochondrial enzyme activities in rats fed a high-fat diet.[9–12] However, to our knowledge none of these studies has dealt with changes in the whole tissue oxygen consumption, which is a more direct index of muscle metabolic activity. In fact, measurements of mitochondrial oxygen consumption in homogenates take into account variations not only in mitochondrial specific activity but also in mitochondrial protein mass, and therefore avoid extrapolations to whole tissue of oxidative capacities measured in isolated organelles.[13] In the light of this observation, we examined the effect of high-fat feeding on skeletal muscle oxidative capacity. To this end,

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Received 7 February 2001; revised 1 May 2001;
accepted 6 July 2001

together with full energy balance measurements, we assessed mitochondrial oxidative capacity in tissue homogenates, by using lipid and non-lipid substrates, to obtain information on the various mitochondrial oxidative systems. In addition, it is well known that two distinct populations of mitochondria exist within muscle cells,[14–16] and that these two populations adapt differently to experimental conditions (ie exercise, immobilization).[15,17,18] However, no studies so far have examined the effect of high-fat feeding on the activity of these two mitochondrial populations. We have therefore also measured oxidative capacity in isolated subsarcolemmal and intermyofibrillar organelles.

Methods and materials

Animals and diets

Male Wistar rats of about 25 days of age (Charles River Italia, Calco, Como, Italy) were housed individually in metabolic cages with free access to food and water in a temperature-controlled room ($24 \pm 1^{\circ}$C) under a 12 h light–dark cycle (07:00–19:00 h). Treatment, housing and killing met the guidelines of the Italian Health Ministry.

At the start of the study, rats were divided into three groups, each composed of eight rats, with similar mean body weight (77 ± 2 g) and with body weight normally distributed within each group. One group of rats was killed for the determination of initial body energy content and composition. The other two groups of rats were fed a low-fat diet or a high-fat diet for 15 days (Table 1).

Energy balance

Metabolizable energy (ME) intake was obtained by subtracting the energy measured in faeces and urine (with a bomb calorimeter) from the gross energy intake, determined from daily food consumption and gross energy content of the diets (measured with bomb calorimeter). Body energy content was measured with the bomb calorimeter (Parr adiabatic calorimeter of Parr Instruments Co., Moline, IL, USA) on aliquots of carcass homogenized with water (final volume equal to twice the carcass weight) in a Polytron homogenizer (Polytron Kinematica AG, Littau/Lucerne, Switzerland) and desiccated at 70°C in a vacuum oven. Corrections were made for skeletal muscle energy content. Body energy gain was calculated as the difference between the final and the initial body energy content. Energy expenditure was calculated from the difference between ME intake and energy gain, while gross efficiency was obtained from the ratio between energy gain and ME intake. Cost of storage was calculated by using the coefficient 1.25 kJ/kJ[19] for protein and 0.36 (low-fat)[19] or 0.16 (high-fat)[20]) kJ/kJ for lipid. Net energy expenditure (NEE) was obtained by subtracting the cost of lipid and protein storage from total energy expenditure.

Table 1 Composition of low- and high-fat diets (g/kg)

Component	*Low-fat diet*	*High-fat diet*
Standard chow[a]	1000	280
Lyophilized meat[b]	0	395
Butter[c]	0	178
Alphacel	0	120
AIN 76 mineral mix[d]	0	20
AIN 76 vitamin mix[e]	0	7
Metabolizable energy density (kJ/g)[f]	12.50	15.80
Gross energy density (kJ/g)	15.88	19.85
Energy (J/100 J) from		
Protein	29	29
Lipid	10.6	50
Carbohydrate	60.4	21

[a]4RF21, Mucedola, Settimo Milanese, Milano, Italy.
[b]Liomellin, STAR s.p.a., 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, 0.2 g water.
[c]Lurpak, Denmark, locally purchased, containing 10% water.
[d]American Institute of Nutrition (1977).
[e]American Institute of Nutrition (1980).
[f]Metabolizable energy/g of the two experimental diets was calculated using the coefficient (kJ/g) 15.67, 16.72, 37.62 and 0 for carbohydrate, protein, fat, fibre, respectively.

Body composition measurements

Water content was determined by the difference in weight of the homogenate before and after drying at 70°C in a vacuum oven. Lipid content was determined by the method of Folch *et al*[21] and converted to energy as lipid by using the coefficient of 39.2 kJ/g. Protein content was determined as described by Brooks *et al*[22] and converted to energy as protein by using the value of 23.5 kJ/g.

Preparation of skeletal muscle homogenate

Hind leg muscles were freed of excess fat and connective tissue, finely minced and washed in a medium containing 100 mM KCl, 50 mM TRIS, pH 7.5, 5 mM $MgCl_2$, 1 mM EDTA, 5 mM EGTA, 0.1% (w/v) fatty acid free bovine serum albumin (BSA). Tissue fragments were treated with protease nagarse (E.C. 3.4.21.62; 1 mg/g tissue) for 5 min, washed, homogenized with the above medium (1 : 8, w/v) in a Potter Elvehjem homogenizer (Heidolph, Kelheim, Germany) set at 500 rpm (4 strokes/min) and filtered through sterile gauze. Aliquots of the homogenate were then used for the determination of respiratory activities.

Preparation of skeletal muscle intermyofibrillar and subsarcolemmal mitochondria

Tissue fragments obtained as described above were homogenized with the above medium (1 : 8, w/v) at 500 rpm (4 strokes/min). Homogenate was then centrifuged at $500\,g_{av}$ for 10 min and the resulting precipitate was subsequently used for the preparation of the intermyofibrillar mitochondria. The supernatant was centrifuged at $3000\,g_{av}$ for 10 min and the pellet containing subsarcolemmal mitochondria was washed twice and resuspended in suspension medium (250 mM sucrose, 50 mM Tris, pH 7.5, 0.1% fatty

acid-free BSA). The pellet from the 500 g_{av} centrifugation was resuspended in a small amount of homogenization solution and treated with protease nagarse (1 mg/g tissue) for 5 min. The suspension was then homogenized, filtered through sterile gauze and centrifuged at 3000 g_{av} for 10 min. The resulting supernatant was rapidly discarded and the pellet was resuspended and centrifuged at 500 g_{av} for 10 min. The supernatant containing the intermyofibrillar mitochondria was centrifuged at 3000 g_{av} for 10 min, the pellet was washed once and resuspended in suspension medium.

Measurement of mitochondrial respiration and membrane potential

Oxygen consumption was measured polarographically with a Clark-type electrode (Yellow Springs Instruments, Yellow Springs, OH, USA) at 30°C, in a medium containing 30 mM KCl, 6 mM $MgCl_2$, 75 mM sucrose, 1 mM EDTA, 20 mM KH_2PO_4, pH 7.0, 0.1% (w/v) fatty acid-free BSA. Substrates used were succinate 10 mM + rotenone 3.75 μM, glutamate 10 mM + malate 2.5 mM or palmitoylCoA 40 μM + carnitine 2 mM + malate 2.5 mM. 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 skeletal muscle homogenates and in the absence of ADP in mitochondria. Addition of oligomycin (4 μg/ml) to state 4 respiration of mitochondria was without effect.

Mitochondrial membrane potential was measured with safranin O using a JASCO dual-wavelength spectrophotometer (511 – 533 nm).[23] Measurements were made at 30°C, in a medium containing 30 mM LiCl, 6 mM $MgCl_2$, 75 mM sucrose, 1 mM EDTA, 20 mM Tris – PO_4, pH 7.0, 0.1% (w/v) fatty acid-free BSA, succinate 10 mM, rotenone 3.75 μM, oligomycin 4 μg/ml, before and after the addition of 45 μM palmitate. The absorbance readings were converted to mV membrane potential using the Nernst equation: $\Delta\psi = 61$ mV log ($[K^+]_{in}/[K^+]_{out}$).[23] Calibration curves for each preparation were obtained by altering the extramitochondrial K^+ level ($[K^+]_{out}$) in the 0.1 – 20 mM range. The change in absorbance caused by the addition of 3 μm valinomycin was plotted against $[K^+]_{out}$. $[K^+]_{in}$ was then estimated by extrapolation of the line to the zero uptake point.

Enzyme activities and protein content

Published procedures were used for the determination of protein content,[24] succinic dehydrogenase (SDH),[25] citrate synthase[26] and ATPase[15] specific activity.

Statistical analysis

Data are summarized using means with their standard errors for eight rats. Statistical analyses were performed using two-tailed unpaired Student's *t*-test or two-way analysis of variance for main effects and interactions. Correlation between selected parameters was performed by linear regression analysis. All analyses were performed using GraphPad Prism (Graphpad Software Inc., San Diego, CA, USA).

Materials

ADP, malate, glutamate, succinate, rotenone, palmitoylCoA, carnitine, nagarse, iodonitrotetrazolium violet, phenazine methosulphate, dithiobis-nitrobenzoic acid, acetyl CoA, oxalacetic acid, phosphoenolpyruvate, pyruvate kinase/lactic dehydrogenase, ATP, NADH, cytochrome c, oligomycin, safranin O were purchased from Sigma Chemical Co., St Louis, MO, USA. All other reagents were of the highest purity commercially available.

Results

Figure 1 shows results of energy balance measurements in rats fed a low-fat or a high-fat diet. Rats fed a high-fat diet significantly increased ME intake and energy expenditure, while body energy gain was similar to that of rats fed a low fat diet (Figure 1A). Accordingly, no difference in final body weight (206 ± 4 and 207 ± 4 g for rats fed a low-fat or a high-fat diet, respectively) and body growth curves (Figure 2) was found between the two groups. Parameters of metabolic efficiency, ie lipid partitioning, gross efficiency, and NEE/ME intake ratio all showed a decreased efficiency in rats fed a high-fat diet. In fact, gross efficiency (Figure 1C) and the percentage of lipid intake stored as body lipid (Figure 1B) significantly decreased, while NEE/ME intake ratio (Figure 1C) significantly increased in rats fed a high-fat diet compared to those fed a low-fat diet.

Skeletal muscle mitochondrial state 3 and 4 respiration was measured in homogenates to take into account changes in mass and activity of mitochondria of the whole tissue due to high fat feeding. Table 2 shows a significant increase in state 3 respiration using NAD-linked substrate glutamate + malate, as well as a significant increase in state 3 and 4 respiration with lipid substrate palmitoylCoA + carnitine + malate in rats fed a high-fat diet. Figure 3A shows a significant inverse correlation between lipid storage and lipid-supported respiration in skeletal muscle homogenates from rats fed a low-fat or a high-fat diet. A significant inverse correlation was also found between lipid-supported respiration/citrate synthase ratio and lipid storage (Figure 3B).

State 3 and 4 respiration was measured in isolated subsarcolemmal and intermyofibrillar mitochondria from rats fed a low-fat or a high-fat diet to take into account changes in specific activity due to high fat feeding (Tables 3 and 4). Firstly, we detected the intactness and purity of the two mitochondrial populations. In agreement with others,[15–17] we found that the contamination of subsarcolemmal and intermyofibrillar mitochondria by other ATPase-containing membranes was lower than 10% in both groups of rats, with no variation due to high-fat feeding. Our RCR values indicate that the mitochondria were well coupled. Then we assured that the differences in functionality of the two

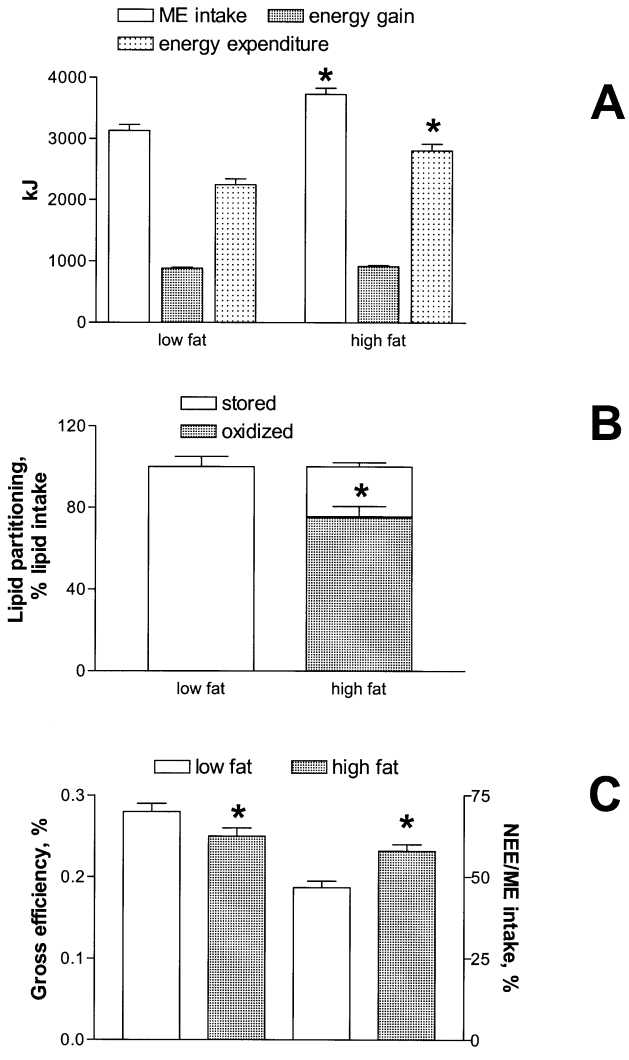

Figure 1 Metabolizable energy (ME) intake, energy gain and energy expenditure (A), lipid partitioning (B), together with gross efficiency and net energy expenditure (NEE)/ME intake ratio (C) in rats fed a low-fat or a high-fat diet. Values are the means ± s.e.m. of eight different rats. *$P < 0.05$ compared to low fat (unpaired two-tailed Student's *t*-test).

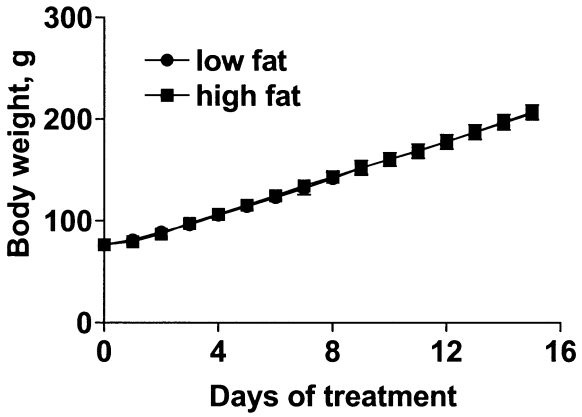

Figure 2 Body growth curve in rats fed a low-fat or a high-fat diet for 15 days. Each point represents the mean ± s.e.m. of eight different rats.

Table 2 Mitochondrial respiration and enzyme activities in skeletal muscle homogenates from rats fed a low-fat or a high-fat diet

		Low-fat	*High-fat*
Glutamate (10 mM) + malate (2.5 mM)			
	State 3	3506 ± 229	4153 ± 200*
	State 4	149 ± 11	153 ± 13
	RCR	24 ± 2	29 ± 3
PalmitoylCoA (40 μM) + carnitine (2 mM) + malate (2.5 mM)			
	State 3	2393 ± 107	3032 ± 113*
	State 4	122 ± 8	146 ± 6*
	RCR	20 ± 1	22 ± 2
Succinate (10 mM) + rotenone (3.75 μM)			
	State 3	5259 ± 288	5296 ± 142
	State 4	972 ± 35	1064 ± 50
	RCR	5.5 ± 0.4	5.0 ± 0.4
SDH activity		6.1 ± 0.3	6.4 ± 0.3
CS activity		28.9 ± 1.0	29.2 ± 0.9

Values are the means ± s.e.m. of eight different rats. State 3 and state 4 mitochondrial respiration rates are expressed as natoms O/ min × g wet tissue. Succinic dehydrogenase (SDH) and citrate synthase (CS) activities are expressed as μmol/min × g wet tissue.
*$P < 0.05$ compared to low-fat (unpaired two-tailed Student's *t* test).

mitochondrial populations are not due to differences in isolation procedures. Nagarse treatment of subsarcolemmal mitochondria had no effect on state 3 and 4 respiratory activities and addition of cytochrome c (3 nmol/mg protein) only enhanced state 3 respiration by approximately 30 and 10% in subsarcolemmal and intermyofibrillar mitochondria, respectively, in agreement with others.[16] Protein yield was 2.65 ± 0.09 (low-fat) or 2.83 ± 0.9 (high-fat) mg/g tissue for intermyofibrillar mitochondria, in agreement with others.[15,16] For subsarcolemmal mitochondria, protein yield was 0.50 ± 0.04 (low-fat) or 0.7 ± 0.04 (high-fat) mg/g tissue, lower than those of others,[15,16] due to the lower gravitational field used by us (3000*g* instead of 9000*g* as in Krieger *et al*[15] and Cogswell *et al*[16]). However, we thought it would be essential, for comparing the two populations of mitochondria, to use the same gravitational field for their isolation, to be sure that the differences found were not due to differences in preparation. Rats fed high-fat diet exhibited significantly higher state 3 respiration with glutamate + malate and state 3 and 4 respiration with palmitoylCoA + carnitine + malate in intermyofibrillar mitochondria (Table 3). A significant increase in state 3 and 4 respiration with palmitoyl-CoA + carnitine + malate was found in subsarcolemmal mitochondria from rats fed a high-fat diet (Table 4). Table 5 shows that addition of a natural uncoupler, such as palmitate, induced a decrease in mitochondrial membrane

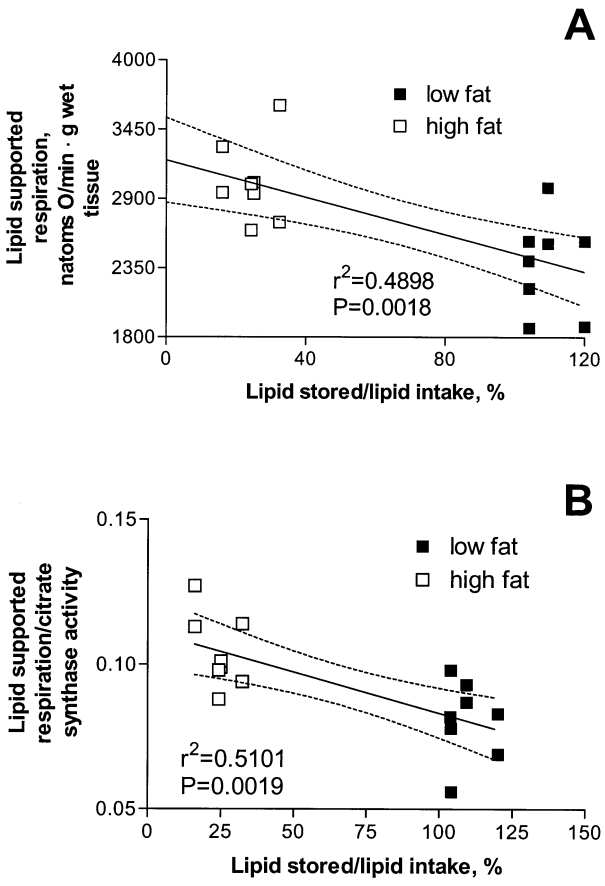

Figure 3 Correlation with 95% confidence interval between skeletal muscle lipid-supported respiration and percentage of lipid intake stored as body lipid (A); correlation with 95% confidence interval between the ratio of lipid-supported respiration to citrate synthase activity and the percentage of lipid intake stored as body lipid (B).

Table 3 Respiration and enzyme activities in skeletal muscle intermyofibrillar mitochondria from rats fed a low-fat or a high-fat diet

		Low-fat	*High-fat*
Glutamate (10 mM) + malate (2.5 mM)			
	State 3	819 ± 40	948 ± 40*
	State 4	46 ± 2	46 ± 3
	RCR	17.7 ± 0.4	21.2 ± 1.3
PalmitoylCoA (40 μM) + carnitine (2 mM) + malate (2.5 mM)			
	State 3	350 ± 18	421 ± 29*
	State 4	41 ± 2	48 ± 2*
	RCR	8.5 ± 0.4	8.8 ± 0.7
Succinate (10 mM) + rotenone (3.75 μM)			
	State 3	866 ± 57	976 ± 50
	State 4	167 ± 8	182 ± 17
	RCR	5.2 ± 0.3	5.6 ± 0.3
SDH activity		1.3 ± 0.2	1.4 ± 0.1
CS activity		4.7 ± 0.3	4.9 ± 0.2

Values are the means ± s.e.m. of eight different rats. State 3 and state 4 mitochondrial respiration rates are expressed as natoms O/min × mg protein. Succinic dehydrogenase (SDH) and citrate synthase (CS) activities are expressed as μmol/min × mg protein.
*$P < 0.05$ compared to low-fat (unpaired two-tailed Student's *t* test).

Table 4 Respiration and enzyme activities in skeletal muscle subsarcolemmal mitochondria from rats fed a low-fat or a high-fat diet

		Low-fat	*High-fat*
Glutamate (10 mM) + malate (2.5 mM)			
	State 3	388 ± 35	414 ± 34
	State 4	32 ± 3	32 ± 2
	RCR	12.7 ± 1.0	12.9 ± 0.9
PalmitoylCoA (40μ M) + carnitine (2 mM) + malate (2.5 mM)			
	State 3	135 ± 9	183 ± 7*
	State 4	28 ± 1	32 ± 1*
	RCR	4.9 ± 0.4	5.7 ± 0.2
Succinate (10 mM) + rotenone (3.75 μ M)			
	State 3	362 ± 25	408 ± 33
	State 4	77 ± 5	79 ± 4
	RCR	4.8 ± 0.3	5.2 ± 0.4
SDH activity		1.1 ± 0.1	1.1 ± 0.1
CS activity		4.5 ± 0.5	4.2 ± 0.2

Values are the means ± s.e.m. of eight different rats. State 3 and state 4 mitochondrial respiration rates are expressed as natoms O/min × mg protein. Succinic dehydrogenase (SDH) and citrate synthase (CS) activities are expressed as μmol/min × mg protein.
*$P < 0.05$ compared to a low-fat (unpaired two-tailed Student's *t* test).

Table 5 Mitochondrial membrane potential in state 4 and after the addition of palmitate in subsarcolemmal and intermyofibrillar mitochondria in rats fed a low-fat or a high-fat diet

			ANOVA: P		
	Low-fat	*High-fat*	*D*	P	*D*×P
Intermyofibrillar					
State 4, mV	162 ± 6	200 ± 6			
			< 0.05	< 0.05	< 0.05
+ palmitate 45 μM, mV	129 ± 8	148 ± 7			
Subsarcolemmal					
State 4, mV	193 ± 15	194 ± 8			
			< 0.05	< 0.05	< 0.05
+ palmitate 45 μM, mV	137 ± 7	119 ± 6			
Percentage decrease after palmitate			*D*	*M*	*D*×*M*
Intermyofibrillar	20 ± 1	26 ± 1			
			< 0.05	< 0.05	NS
Subsarcolemmal	29 ± 1	39 ± 1			

Values are reported as means ± s.e.m. of eight different rats. For membrane potential values: D, main effect of diet; P, main effect of palmitate; D×P, interaction between diet and palmitate (two-way analysis of variance). For percentage decrease after palmitate: D, main effect of diet; M, main effect of mitochondrial type; D×M, interaction between diet and mitochondrial type (two-way analysis of variance).

potential, both in subsarcolemmal and intermyofibrillar mitochondria. The uncoupling effect of palmitate was significantly higher in rats fed a high-fat diet compared to controls, and was higher in subsarcolemmal mitochondria compared to intermyofibrillar ones, whatever the diet.

Discussion

The results of the present study clearly show that in skeletal muscle from young rats fed a high-fat diet there is an increase in mitochondrial lipid oxidative capacity, which contributes to avoid obesity development.

As for energy balance, our present data confirm previous findings,[5,7,27] showing that when young Wistar rats are fed high-fat diet, adaptative increase in whole body lipid utilization takes place, together with a decrease in metabolic efficiency, which avoids excess lipid deposition.

Measurements of skeletal muscle mitochondrial respiratory activity were first performed in skeletal muscle homogenate. Rats fed a high-fat diet exhibited a significant increase in state 3 respiration with NAD-linked substrate, but not with FAD-linked substrate. This result implies that an increase in rate-limiting steps upstream of the system involved in succinate oxidation occurs after high-fat feeding. A significant increase not only in state 3 but also in state 4 lipid-supported respiration, and therefore in lipid oxidative capacity, was also found in rats fed a high-fat diet compared to rats fed a low-fat diet. In agreement, other studies have shown that high-fat diets selectively increase the activity of enzymes of the β-oxidation pathway.[9–12,28] The different variations in mitochondrial respiratory activity with the various substrates used here suggest that no variation in mitochondrial protein mass occurs in rats fed a high-fat diet, since an increase in the mass of mitochondria should have the same effect for all the substrates. In addition, no variation was found in the activity of two mitochondrial marker enzymes, succinic dehydrogenase and citrate synthase. The lack of effect of a high-fat diet on succinic dehydrogenase and citrate synthase activity is in agreement with others[10] and indicates that the tricarboxylic acid cycle maintains a constant level of activity, while changes take place at an upstream enzymatic step.

We have also measured mitochondrial specific respiratory activities in two specialized mitochondrial populations which exist in skeletal muscle cell, one located beneath the sarcolemma (subsarcolemmal mitochondria) and the other associated with the myofibrils (intermyofibrillar mitochondria).[14–16] Our results show lower state 3 and 4 respiratory rates whatever the substrate in subsarcolemmal mitochondria compared to intermyofibrillar ones, in agreement with others,[15–17] while no difference in succinic dehydrogenase and citrate synthase could be detected between the two populations. As for succinic dehydrogenase, the literature gives conflicting results, which show either higher[15] or lower[16] activity in intermyofibrillar mitochondria compared to subsarcolemmal ones. On the other hand, our result showing similar citrate synthase activity in the two mitochondrial populations is in agreement with others.[16] Subsarcolemmal and intermyofibrillar mitochondrial respiratory activities are differentially regulated in response to high-fat feeding. In fact, we found an increase in lipid-supported respiration in both populations of mitochondria and an increase in NAD-linked respiration in intermyofibrillar mitochondria. Differential regulation of mitochondrial activity in the two subpopulations has been previously found in response to other environmental stimuli,[15,17,18] and could be due to differences in protein synthesis capacity,[15] rates of import of nuclear-coded mitochondrial proteins[29] and/or rates of protein degradation.[15,29]

Our results also suggest that increased skeletal muscle lipid oxidative capacity is only due to an increase in mitochondrial specific activity. Our previous works have shown that high-fat feeding also induces an increase in hepatic lipid oxidative capacity.[5–6] Therefore, our results, as a whole, point to liver and skeletal muscle as important contributors to obesity resistance of rats fed a high-fat diet. The concerted action of high-fat feeding on lipid oxidation in various tissues could be exerted though various mechanisms. In fact, rats fed a high-fat diet have enhanced serum free triiodothyronine (T_3) levels,[5] and it is well known that T_3 stimulates the oxidation of fatty acids.[30] In addition, in rats fed a high-fat diet serum levels of free fatty acids (FFA) are higher than in controls,[6] and FFA are natural ligands of peroxisome proliferator-activated receptors (PPARs).[31] Since PPARα, which is expressed both in liver and skeletal muscle, regulates the transcription of genes coding for enzyme of mitochondrial fatty acid oxidation pathway, ie carnitine palmitoyltransferase I,[32] it can be speculated that increased FFA could directly stimulate their own oxidation via PPARs, both at liver and muscular level.

We also tested the effect of a natural energy uncoupler, palmitate, on membrane potential of the two mitochondrial populations. The results show that both intermyofibrillar and subsarcolemmal mitochondria from rats fed a high-fat diet were more sensitive to the uncoupling effect of palmitate. In addition, subsarcolemmal mitochondria were more sensitive than intermyofibrillar ones, whatever the diet, thus suggesting a specific role of subsarcolemmal mitochondria in the regulation of cellular energy coupling. It has been proposed that the uncoupling effect of fatty acids depends on the activity of mitochondrial carriers, such as the ATP/ADP antiporter, the dicarboxylate carrier, the aspartate/glutamate antiporter[33] and uncoupling proteins (UCP) 2 and 3.[34] Therefore, the higher sensitivity to palmitate of skeletal muscle mitochondrial populations from rats fed a high-fat diet could be due to an increase in the above mitochondrial carriers. Accordingly, an increase in UCP3 mRNA levels has been found in skeletal muscle from rats fed a high-fat diet,[35] and our preliminary study shows an increase in ATP/ADP antiporter in skeletal muscle mitochondria from rats fed a high-fat diet.

We have also found a strong negative correlation between skeletal muscle lipid supported respiration and the percentage of lipid intake stored as body lipid (Figure 1A), which underline the importance of the increased skeletal muscle lipid oxidative capacity in obesity resistance of rats fed a high-fat diet. In addition, we calculated the ratio of lipid-supported respiration to citrate synthase activity, to obtain an estimate of the relative importance of lipid oxidation

pathway *vs* total oxidative capacity.[11] This ratio was then related to the percentage of lipid intake stored as body lipid and a significant negative correlation was found (Figure 1B). Previous studies have demonstrated a relationship between muscle lipid utilization preference and obesity susceptibility.[11] Our data confirm this relationship, since rats resistant to obesity when fed a high-fat diet exhibit a higher preference for lipid metabolism in skeletal muscle, a tissue that contributes significantly to whole body lipid utilization. The high capacity of young rats to resist obesity when fed a high-fat diet could be due to the fact that high-fat feeding started soon after weaning. It has been shown that skeletal muscle has a high lipid utilization capacity during suckling and at weaning, which then declines to adult levels.[36] Therefore, high-fat feeding soon after weaning might prolong the duration of the high muscle lipid oxidative capacity and therefore elicit adaptative mechanisms of weight maintenance. In agreement, we have found that when a high-fat diet was given to adult rats, an increase in body lipid content was found.[37] In conclusion, our present results indicate that in rats fed a high-fat diet skeletal muscle contributes to the increased whole body lipid utilization, through an increase in lipid oxidative capacity associated with a decrease in energy coupling.

Acknowledgements

We wish to thank Professor Antonio Barletta for helpful discussion. This work was supported by University 'Federico II' of Naples.

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