Acetyl-L-Carnitine Supplementation Differently Influences Nutrient Partitioning, Serum Leptin Concentration and Skeletal Muscle Mitochondrial Respiration in Young and Old Rats[1]

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ABSTRACT Variations in energy balance, body composition, and nutrient partitioning induced by acetyl-L-carnitine (ALCAR) supplementation were studied in young (2 mo) and old (24 mo) Wistar rats. Changes in skeletal muscle metabolism as well as in serum free triiodothyronine and leptin levels were also evaluated. Rats were administered 0 (control) or 15 g/L ALCAR in their drinking water for 1 mo. ALCAR treatment significantly decreased body lipid percentage in young rats and significantly increased body protein percentage in old rats. The percentage of metabolizable energy (ME) intake stored as lipid was lower in ALCAR-treated young rats, whereas the percentage of ME intake stored as protein was greater in ALCAR-treated old rats compared with their age-matched controls. In addition, ALCAR supplementation significantly decreased serum leptin levels in old rats. Elevated skeletal muscle respiration was found in old rats treated with ALCAR, due to an increase in mitochondrial protein mass. In conclusion, ALCAR supplementation decreases efficiency of lipid deposition in young rats and increases efficiency of protein deposition in old rats. In addition, ALCAR supplementation partly reduces the leptin resistance that occurs in old rats, and improves ATP production in skeletal muscle mitochondria through an increase in mitochondrial protein content. J. Nutr. 132: 636–642, 2002.

KEY WORDS: • *acetyl-L-carnitine* • *aging* • *energy balance* • *skeletal muscle* • *leptin* • *rats*

Both humans and rodents tend to gain weight as they age (1–3); therefore, rats are considered the preferred animal model for studying changes in energy partitioning with age. In previous work, we found that the partitioning of energy from incoming nutrients is differently regulated with age (4). In fact, young rats were characterized by storage of lipid and protein, whereas in middle-aged rats, protein deposition approached zero and the excess of the ingested energy over energy expenditure was stored entirely as fat, so that age-associated obesity began to develop (4). Interestingly, a number of studies in a variety of species have shown that L-carnitine supplementation influences nutrient partitioning and thus body composition (5–9). Because carnitine, a cosubstrate of carnitine acyl-transferase, is essential for translocation of fatty acids into the mitochondrial matrix (10), it is conceivable that carnitine level influences fatty acid utilization. In addition, we have shown that dietary supplementation with a carnitine derivative, acetyl-L-carnitine (ALCAR),[3] slowed some hepatic metabolic dysfunction due to age, probably through maintenance of mitochondrial function (11).

Because to our knowledge a complete study on energy utilization in young and old rats supplemented with carnitine or carnitine derivatives has not been done, we carried out measurements of energy balance, fuel partitioning and resting metabolic rate in young (2 mo) and old (24 mo) rats, whose diet was supplemented with ALCAR. In addition, it was of interest to evaluate the effect of ALCAR on serum leptin and triiodothyronine (T_3) levels in these rats because these hormones are important regulators of body weight, energy expenditure and food intake (12–14). Finally, because it has been suggested that ALCAR supplementation improves muscle exercise capacity (15), we also measured mass and function of skeletal muscle mitochondria in young and old rats treated with ALCAR.

MATERIALS AND METHODS

Materials. ADP, malate, glutamate, succinate, rotenone, palmitoylCoA, carnitine, nagarse, iodonitrotetrazolium violet, phenazine methosulfate, phosphoenolpyruvate, pyruvate kinase/lactic dehydrogenase, ATP, NADH, oligomycin, and ALCAR were purchased from Sigma Chemical (St. Louis, MO). All other reagents were of the highest purity commercially available.

Animals. Male Wistar rats were purchased from the animal house of Heinrich-Heine University of Düsseldorf, Germany. At the start of the study, rats were divided into six groups, three groups aged 2 mo (young rats), and the remaining groups aged 24 mo (old rats). In each group, body weight was normally distributed and had similar means (338 ± 12 g for young rats and 600 ± 26 g for old rats). One group of young and one of old rats were immediately killed for the determination of initial body energy content and composition. All

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[3] Abbreviations used: ALCAR, acetyl-L-carnitine; BSA, bovine serum albumin, ME, metabolizable energy; RCR, respiratory control ratio; RMR, resting metabolic rate; SDH, succinic dehydrogenase; T_3, triiodothyronine.

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other rats were housed individually in metabolic cages at 24°C under an artificial circadian 12-h light:dark cycle, with free access to a standard stock diet (Mucedola 4RF21; Settimo Milanese, Milan, Italy) and water. This diet contained (g/100 g) 3 fat, 18.5 protein, 18.5 fiber, 7 mineral, 41 carbohydrate and adequate vitamin content. Its gross energy density was 15.88 kJ/g wet food (determined by bomb calorimetry), and its metabolizable energy (ME) was 12.50 kJ/g. Energy composition (kJ/100 kJ) was 29.0 protein, 10.6 lipid and 60.4 carbohydrate. One group of young and old rats served as control, whereas the other two groups received water supplemented with a 15 g/L (pH adjusted to 6.0) solution of ALCAR. Young and old rats drank about the same amount of water per day (data not shown), which would provide a daily ALCAR dose of ~1.0 g/(kg body · d). The treatment period lasted 1 mo. Treatment, housing and killing met the guidelines of the Italian Health Ministry.

Resting metabolic rate. At the end of the experimental period, resting metabolic rate (RMR) was measured in all rats in the morning in an open-circuit oxygen consumption system (O_2-ECO, Columbus Instruments, Columbus, OH), designed to monitor oxygen consumption in small animals. The instrument was calibrated with room air before and after each measurement. Although most rats quieted down after ~30 min in the chamber, all rats were allowed to adapt to the experimental environment for a minimum of 60 min before beginning the measurements. RMR was measured in the fed state and the day after in food-deprived rats in a chamber at 24°C over a period of at least 10 min during which the rat remained quiet.

Energy balance. ME intake was obtained by subtracting the energy measured in feces and urine using bomb calorimetry calibrated with dry benzoic acid standard (Parr adiabatic calorimetry of Parr Instruments, Moline, IL) from the gross energy intake. This was determined from daily food consumption and the gross energy content of the diet.

After RMR measurements, the food-deprived rats were anesthetized by the intraperitoneal injection of chloral hydrate (40 mg/100 g body), and blood was taken as well as hind leg muscles. After evacuation of intestinal fecal contents, the carcasses, including gut, were weighed, autoclaved for 90 min, chopped into small pieces, thoroughly mixed and finally homogenized with water (volume equal to twice the carcass weight) in a Polytron homogenizer (Polytron Kinematica AG, Littau/Lucerne, Switzerland). Aliquots of the homogenates were desiccated at 70°C in a vacuum oven. Then, small pellets (~200 mg) of the dried homogenate were made and the body energy content was measured with bomb calorimetry. To take into account the energy content of hind leg skeletal muscle, used for determination of "in vitro" respiration rate, aliquots of muscles were dried and their energy content measured with bomb calorimetry. Body energy, protein and fat gain were calculated as the difference between the body energy, 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. Energy expenditure was calculated from the difference between ME intake and energy gain. The total cost of storage was determined taking into account that the energy loss in storing 1 kJ of protein is 1.25 kJ (16). The corresponding energy cost for fat deposition is 0.36 kJ/kJ for diets with a high percentage of carbohydrates such as the stock diet used here (17).

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. Fat content was determined by the method of Folch et al. (18), and converted to energy as fat by using the coefficient of 39.2 kJ/g. Protein content was determined as described by Brooks et al. (19), and converted to energy as protein by using the value of 23.5 kJ/g.

Free triiodothyronine and leptin serum concentrations. The blood samples, collected via the inferior cava vein, were centrifuged at 2000 × *g* for 10 min. Serum was removed and stored at −20°C until used for determination of free T_3 and leptin concentrations. Serum-free T_3 and leptin concentrations were measured using RIA kits (ICN Pharmaceuticals, Diagnostic Division, New York, NY for free T_3, and Linco Research, St Charles, MO, for leptin). Each hormone was measured in a single assay to remove interassay variations.

Preparation of skeletal muscle homogenate and isolated mitochondria. Skeletal muscle was freed of excess fat and connective tissue, finely minced and washed in a medium containing 100 mmol/L KCl, 50 mmol/L Tris, pH 7.5, 5 mmol/L $MgCl_2$, 1 mmol/L EDTA, 5 mmol/L EGTA, 1 g/L fatty acid–free bovine serum albumin (BSA). Tissue fragments were treated with protease nagarse (EC 3.4.21.62, 1 mg/g tissue) for 5 min, washed, homogenized for 1 min with the above medium (1:8, wt/v) in a Potter Elvehjem homogenizer (Heidolph, Kelheim, Germany) set at 500 rpm (4 strokes/min) and filtered. Aliquots of the homogenate were then used for the determination of mitochondrial respiration and succinic dehydrogenase activity (SDH) using the method of Lee and Lardy (20), as well as for preparation of isolated mitochondria. To this end, the homogenate was centrifuged at 500 × *g* for 10 min and the supernatant was centrifuged at 3000 × *g* for 10 min. The resulting pellet containing mitochondria was washed twice and finally resuspended in suspension medium containing 250 mmol/L sucrose, 50 mmol/L Tris, pH 7.5, 1 g/L fatty acid–free BSA. The protein content of the mitochondrial suspension was determined by the method of Hartree (21) using BSA as the protein standard. Isolated mitochondria were then used for the determination of respiratory parameters and SDH activity.

Measurement of mitochondrial respiration. Oxygen consumption was measured polarographically with a Clark-type electrode (Yellow Springs Instruments, Yellow Springs, OH). Isolated mitochondria (0.3 mg protein) were incubated in 3 mL of a medium containing 30 mmol/L KCl, 6 mmol/L $MgCl_2$, 75 mmol/L sucrose, 1 mmol/L EDTA, 20 mmol/L KH_2PO_4, pH 7.0, 1 g/L fatty acid–free BSA, at 30°C. Substrates used were succinate (10 mmol/L) + rotenone (3.75 μmol/L), glutamate (10 mmol/L) + malate (2.5 mmol/L) or palmitoylCoA (40 μmol/L) + carnitine (2 mmol/L) + malate (2.5 mmol/L). State 3 measurements were performed in the presence of 0.6 mmol/L ADP. State 4 respiration was measured in the presence of oligomycin (4 mg/L) and in the absence of ADP. The ratio between state 3 and state 4 (RCR) was calculated according to Estabrook (16).

Statistical analysis. Data are summarized as means ± SEM, $n = 6$. Statistical analyses were performed by two-way ANOVA for the main effects of age and ALCAR treatment as well as for their interaction, coupled with the Bonferroni post-test. Regression analyses were also performed. Probability values < 0.05 were considered to indicate a significant difference. All analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA).

RESULTS

Final body weight and the body water percentage did not differ in either young or old rats due to ALCAR treatment (**Table 1**). Body lipid percentage increased significantly with age and was lower in ALCAR-treated young rats compared with their controls (Table 1). Body protein percentage was lower in old rats than in young rats and was greater due to ALCAR only in old rats (Table 1). Body energy content was greater in old than in young rats and ALCAR treatment increased it only in old rats (Table 1).

ME intake, energy expenditure and net energy expenditure (energy expenditure excluding the total cost of storage) decreased with age, and ALCAR supplementation decreased energy expenditure and net energy expenditure only in old rats (**Table 2**). Body energy, protein and fat gain decreased with age. ALCAR treatment increased body protein gain in old rats and decreased fat gain in young rats, so that body energy gain increased in old rats and decreased in young rats (Table 2).

The percentage of ME intake stored as fat decreased with age and was lower than controls in ALCAR-treated young rats (**Fig. 1***A*). The percentage of ME intake stored as protein decreased with age in controls but was greater in ALCAR-treated old rats than in the other three groups (Fig. 1*B*).

The percentage of ME intake utilized for energy maintenance (net energy expenditure/ME intake) was greatest in control old rats and least in old rats treated with ALCAR (**Fig. 2***A*). The percentage of ME intake stored as body energy, an

TABLE 1

Body weight and composition in young and old rats with or without acetyl-L-carnitine (ALCAR) supplementation for 1 mo[1]

					ANOVA		
	Young	Young + Alcar	Old	Old + Alcar	Main effect of age	Main effect of ALCAR	Interaction
Final body weight, *g*	399 ± 9[b]	386 ± 9[b]	606 ± 26[a]	590 ± 24[a]	S	N	N
Body water, *g/100 g*	61.5 ± 1.0	63.7 ± 1.0	63.0 ± 4.0	57.0 ± 1.0	N	N	N
Body fat, *g/100 g*	15.3 ± 0.2[b]	12.8 ± 0.2[c]	22.0 ± 0.8[a]	22.0 ± 0.8[a]	S	S	S
Body protein, *g/100 g*	15.9 ± 0.8[a]	16.9 ± 0.8[a]	10.0 ± 0.5[b]	14.0 ± 0.7[a]	S	S	S
Body energy, *kJ/g*	9.7 ± 0.3[c]	9.0 ± 0.2[c]	11.0 ± 0.3[b]	13.0 ± 0.4[a]	S	S	S

[1] Values are means ± SEM, $n = 6$. S, significant $P < 0.05$; N, not significant $P \geq 0.05$. Means in a row without a common letter differ, $P < 0.05$.

index of metabolic efficiency, was lower in ALCAR-treated young rats and greater in ALCAR-treated old rats than in their respective controls (Fig. 2*B*). The percentage of ME intake utilized for lipid and protein storage was lower in old than in young control rats and it significantly increased due to ALCAR treatment only in old rats (Fig. 2*C*).

Serum free T_3 levels significantly decreased with age and ALCAR treatment had no effect (**Fig. 3***A*). Serum leptin levels were greater in old than in young control rats and were lower due to ALCAR supplementation only in old rats (Fig. 3*A*). RMR decreased significantly with age whether rats were fed or food-deprived. ALCAR-treatment had no effect in either condition (Fig. 3*B*).

In skeletal muscle homogenates, no effect of age was found on state 3 respiration, and ALCAR supplementation increased it only in old rats (**Table 3**). On the other hand, ALCAR supplementation did not influence state 4 respiration, which increased significantly with age (Table 3). In addition, we measured state 3 and state 4 respiration in isolated muscle mitochondria to take into account changes in respiratory specific activity due to ALCAR supplementation (**Table 4**). Isolated mitochondria were pure and intact. In agreement with others (22,23), we found that the contamination of isolated mitochondria by other ATPase-containing membranes was <10% in all groups. The RCR indicated the high quality of the mitochondrial preparations (Table 4). There were no effects of age or ALCAR supplementation on state 3 respiration. State 4 respiration, in contrast, increased with age and was lower in ALCAR-treated old rats than in controls. We calculated the mitochondrial protein mass (mg/g wet tissue) by dividing the SDH activity in muscle homogenates (Table 3) by the SDH activity in isolated muscle mitochondria (Table 4). This calculation could be done because our mitochondrial preparations were minimally contaminated as discussed above. Mitochondrial protein mass was greater in ALCAR-treated old rats than in controls (**Fig. 4**).

Significant inverse correlations were found between serum leptin levels and ME intake in each group of rats (**Fig. 5**). In addition, slopes of the regression lines for the correlation between leptin and ME intake were different in old rats compared with young rats and in old rats after ALCAR supplementation compared with control old rats (Fig. 5).

DISCUSSION

We studied the effect of ALCAR supplementation on body composition, nutrient partitioning, and skeletal muscle metabolism in young and old Wistar rats. ALCAR is used largely as a nutritional supplement because of its importance in cellular energy production. Its use could be equivalent to that of L-carnitine because ALCAR is deacetylated during or immediately after uptake into intestinal cells and a portion of the newly formed intracellular free carnitine is apparently reacetylated (24). However, at the cellular level, ALCAR seems to be the active molecule in some metabolic pathways (25,26). Carnitine and ALCAR are both involved in the transport of

TABLE 2

Energy balance in young and old rats with or without acetyl-L-carnitine (ALCAR) supplementation for 1 mo[1]

					ANOVA		
	Young	Young + Alcar	Old	Old + Alcar	Main effect of age	Main effect of ALCAR	Interaction
			kJ				
ME intake[2]	8408 ± 350[a]	8101 ± 350[a]	6467 ± 250[b]	5894 ± 300[b]	S	N	N
Body energy gain	1141 ± 50[a]	825 ± 20[b]	613 ± 30[c]	1104 ± 35[a]	S	S	S
Energy expenditure	7268 ± 228[a]	7276 ± 174[a]	5854 ± 200[b]	4790 ± 200[c]	S	S	S
Total cost of storage	688 ± 40[b]	563 ± 29[b]	308 ± 50[c]	895 ± 55[a]	N	S	S
Net energy expenditure	6580 ± 279[a]	6713 ± 241[a]	5546 ± 320[b]	3896 ± 345[c]	S	S	S
Protein gain	206 ± 15[b]	223 ± 16[b]	96 ± 4.5[c]	557 ± 50[a]	S	S	S
Fat gain	1000 ± 99[a]	610 ± 78[b]	523 ± 65[c]	553 ± 30[c]	S	S	S

[1] Values are means ± SEM, $n = 6$. S, significant $P < 0.05$; N, not significant $P \geq 0.05$. Means in a row without a common letter differ, $P < 0.05$.
[2] ME, metabolizable energy; energy expenditure is the difference between ME intake and body energy gain; net energy expenditure is the difference between energy expenditure and total cost of storage.

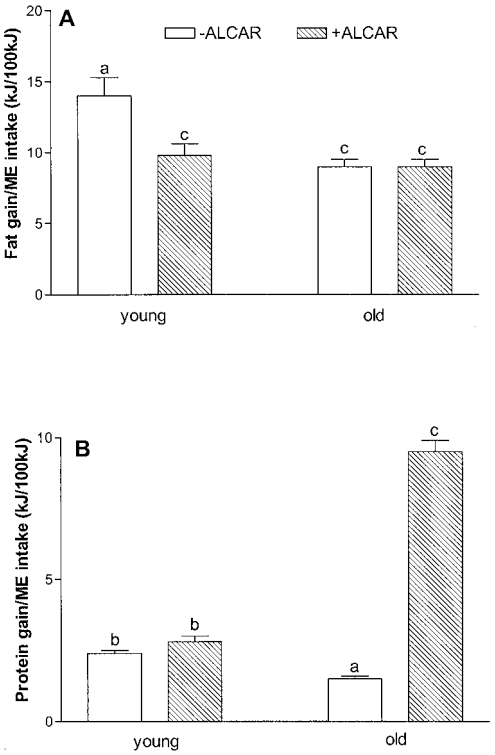

FIGURE 1 Fat (*A*) and protein gain (*B*), expressed as percentage of metabolizable energy (ME) intake, in young and old rats with or without acetyl-L-carnitine (ALCAR) supplementation for 1 mo. Values are means ± SEM, $n = 6$. Main effect of age, $P < 0.05$; main effect of ALCAR treatment, $P < 0.05$; interaction, $P < 0.05$. Means with a different superscript letter differ significantly ($P < 0.05$).

fatty acids and acetyl groups into the mitochondria. ALCAR is also an intracellular energy reservoir of acetyl groups (10).

Old rats had greater body energy and fat content, but lower body protein content than young rats (Table 1). These changes could be due to a decrease in the ability to use fat as a metabolic fuel with age, so that more amino acids would be utilized for energy production rather than for body protein synthesis. This suggestion is in agreement with our previous work that showed an age-related decline in fatty acid oxygen consumption in perfused liver (11). Taking into account the lower tissue carnitine levels found in old rats by Kalaiselvi and Panneerselvam (27), the impairment in fat utilization could be due in part to low levels of carnitine.

The present results also showed that long-term ALCAR dietary treatment differently influenced body composition and nutrient partitioning in young and old rats. In fact, we found that ALCAR supplementation significantly decreased fat gain and body fat content in young rats and significantly increased protein gain and body protein content in old rats (Tables 1,2).

Decreased fat gain in young rats supplemented with ALCAR is consistent with other studies conducted in a variety of species. In fact, Ji et al. (7) found that carnitine-fed salmon had lower lipid concentrations in white muscle and visceral organs. Rabie and Szilagyi (9) showed that in broilers supplemented with carnitine, the quantity and percentage of abdominal fat were reduced. Finally, in rats treated with caffeine, carnitine and choline, there was a significant loss of adipose fat mass (6). Lower fat gain of young rats treated with ALCAR (Table 2) could be due to increased fat utilization as metabolic fuel. This suggestion is in agreement with our previous work that showed increased fatty acid oxygen consumption in perfused liver from rats treated with ALCAR (11) and with the results of Penn et al. (8), who showed a decrease in fatty acid oxidative rates in liver homogenates from carnitine-deprived piglets. However, the greater fat utilization found in young rats treated with ALCAR was not associated with increased energy expenditure (Table 2). A possible explanation could be the trend for a lower ME intake ($P = 0.17$) found in these rats (Table 2), so that carbohydrate and protein oxidation was insufficient to meet body energy needs and a greater amount of fat would be oxidized. Interestingly, after ALCAR treatment, a substantial decrease in body fat without changes in protein balance could be induced through only minor changes in ME intake.

ALCAR supplementation increased protein deposition (Table 2) and hence metabolic efficiency (Fig. 2*B*) only in old rats. This increase can be the result of enhanced protein synthesis and/or reduced protein catabolism. The increased metabolic efficiency found in old rats after ALCAR treatment means a higher efficiency of oxidative phosphorylation at the cellular level (see also below). Consequently, more ATP could be produced during fatty acid oxidation and more amino acids could be channeled through protein synthesis. The information regarding carnitine's effects on protein metabolism in the literature is controversial. Owen et al. (28) found that carcass proteins were unaffected by L-carnitine supplementation in weaning pigs. In contrast, Tao et al. (29) found an increased nitrogen balance in intravenously fed growing rats supple-

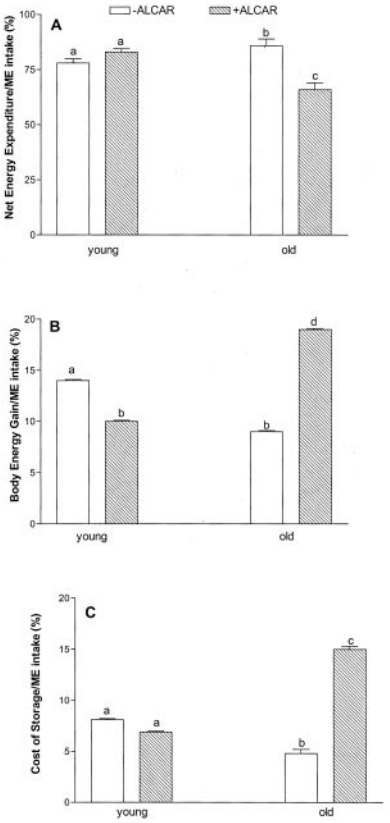

FIGURE 2 Net energy expenditure (*A*), body energy gain (*B*), and cost of storage (*C*), expressed as a percentage of metabolizable energy (ME) intake, in young and old rats with or without acetyl-L-carnitine (ALCAR) supplementation for 1 mo. Values are means ± SEM, $n = 6$. Main effect of age, $P < 0.05$; main effect of ALCAR treatment, $P < 0.05$; interaction, $P < 0.05$. Means with a different superscript letter differ significantly ($P < 0.05$).

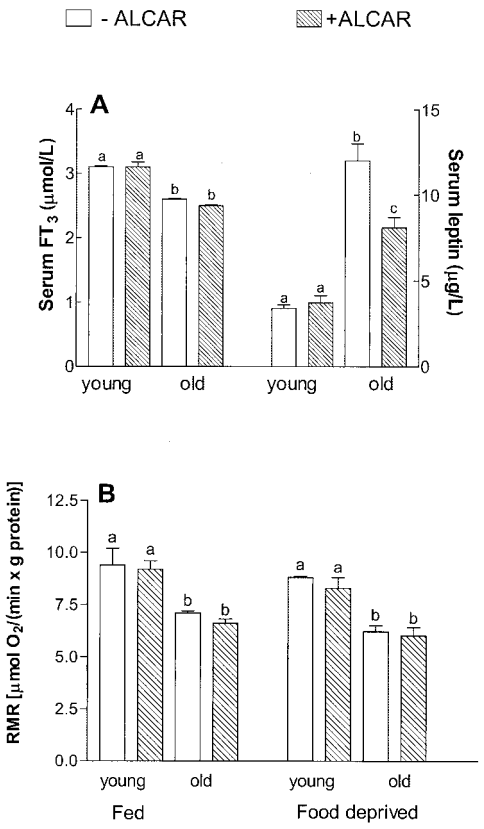

FIGURE 3 Serum concentration of free triiodothyronine (FT_3) and leptin (*A*), and resting metabolic rate (RMR) (*B*), in young and old rats with or without acetyl-L-carnitine (ALCAR) supplementation for 1 mo. Values are means ± SEM, $n = 6$. For serum concentration of FT_3 (*A*), main effect of age, $P < 0.05$; main effect of ALCAR treatment, $P \geq 0.05$; interaction, $P \geq 0.05$. For serum concentration of leptin (*A*), main effect of age, $P < 0.05$; main effect of ALCAR treatment, $P < 0.05$; interaction, $P < 0.05$. For RMR (*B*), main effect of age, $P < 0.05$; main effect of ALCAR treatment, $P \geq 0.05$; interaction, $P \geq 0.05$. Means with a different superscript letter differ significantly ($P < 0.05$).

mented with carnitine. In addition, Heo et al. (5) found that carnitine reduced urinary nitrogen excretion and increased protein accretion in growing pigs fed a low energy diet. The above different effects of ALCAR administration could be explained by the species studied, dietary treatment as well as by the age of the animals used in the various studies.

Our results also showed that RMR, measured in fed and food-deprived rats (Fig. 3*B*), as well as energy expenditure and net energy expenditure (energy expenditure excluding the cost of fuel storage) (Table 2), decreased significantly with age. Therefore, the reduction in energy expenditure found in old animals is due in part to a decrease in RMR. ALCAR supplementation did not restore the age-dependent decline in RMR and energy expenditure.

To gain insight into the hormonal modifications induced by ALCAR supplementation, we measured serum levels of free T_3 and leptin, which are involved in the regulation of nutrient metabolism (12–14). ALCAR treatment neither affected serum free T_3 levels in young rats nor restored the age-dependent decline in old rats (Fig. 3*A*). A similar finding was obtained by Janssens et al. (30) in exercising pigeons in which decreased thyroid hormone levels were not influenced by orally supplemented carnitine. Our serum leptin results (Fig. 3*A*) confirm previous observations that serum leptin levels increase with age (4,31). Interestingly, ALCAR treatment decreased serum leptin levels in old rats, but not in young rats. The latter result is different from that of Hongu and Sachan (6), who supplemented a diet with caffeine, choline and carnitine. A significant positive correlation ($r^2 = 0.97$, $P < 0.0001$) between serum leptin levels and body fat mass was always found, in agreement with the observation that leptin circulates in proportion to body fat mass in rodents (32). In addition, we found significant inverse correlations between serum leptin levels and ME intakes (Fig. 5). This result is consistent with the inhibitory action of leptin on food intake (33). Interestingly, the slope of the regression line obtained for old rats (−5.3 ± 0.8) was significantly higher than that obtained for young rats (−43.2 ± 6.3) and for old rats treated with ALCAR (−40.4 ± 1.8). This finding suggests that the responsiveness to the anorexic effect of leptin is reduced in old

TABLE 3

Mitochondrial respiration and succinic dehydrogenase (SDH) activity in skeletal muscle homogenates from young and old rats with or without acetyl-L-carnitine (ALCAR) supplementation for 1 mo[1]

					ANOVA		
	Young	Young + Alcar	Old	Old + Alcar	Main effect of age	Main effect of ALCAR	Interaction
			natoms O/(min × wet tissue)				
Glutamate + malate							
State 3	4379 ± 202[b]	4352 ± 200[b]	3823 ± 109[c]	4639 ± 170[a]	N	S	S
State 4	316 ± 18[b]	338 ± 22[b]	415 ± 24[a]	421 ± 22[a]	S	N	N
PalmitoylCoA + carnitine + malate							
State 3	3231 ± 135[b]	3311 ± 120[b]	2830 ± 40[c]	3320 ± 50[a]	N	S	S
State 4	283 ± 22[b]	285 ± 14[b]	327 ± 26[a]	351 ± 28[a]	S	N	N
Succinate + rotenone							
State 3	4399 ± 180[b]	4500 ± 137[b]	3845 ± 75[c]	4508 ± 120[a]	N	S	S
State 4	855 ± 48[b]	793 ± 45[b]	1089 ± 50[a]	1004 ± 50[a]	S	N	N
			μmol/(min × g wet tissue)				
SDH activity	3.56 ± 0.25[b]	3.47 ± 0.26[b]	3.52 ± 0.40[b]	5.04 ± 0.40[a]	S	S	S

[1] Values are means ± SEM, $n = 6$. S, significant $P < 0.05$; N, not significant $P \geq 0.05$. Means in a row without a common letter differ, $P < 0.05$.

TABLE 4

Respiration and succinic dehydrogenase (SDH) activity in skeletal muscle isolated mitochondria from young and old rats with or without acetyl-L-carnitine (ALCAR) supplementation for 1 mo[1]

					ANOVA		
	Young	Young + Alcar	Old	Old + Alcar	Main effect of age	Main effect of ALCAR	Interaction
			natoms O/(min × mg protein)				
Glutamate + malate							
State 3	616.8 ± 40.1	604.0 ± 42.3	517.2 ± 49.3	522.6 ± 51.9	N	N	N
State 4	30.1 ± 1.7[c]	38.0 ± 2.0[c]	67.7 ± 2.7[a]	49.2 ± 2.7[b]	S	S	S
RCR	21.1 ± 0.5[a]	16.6 ± 0.5[b]	8.9 ± 0.3[d]	11.4 ± 0.5[c]	S	S	S
PalmitoylCoA + carnitine + malate							
State 3	246.0 ± 14.5	223.8 ± 18.0	247.2 ± 22.5	259.4 ± 22.4	N	N	N
State 4	26.3 ± 1.5[c]	27.6 ± 1.5[c]	46.2 ± 1.0[a]	39.2 ± 1.0[b]	S	S	S
RCR	9.5 ± 0.1[a]	7.7 ± 0.1[b]	5.6 ± 0.2[d]	6.6 ± 0.2[c]	S	S	S
Succinate + rotenone							
State 3	746.1 ± 70.2	708.4 ± 55.0	713.3 ± 43.0	710.0 ± 56.1	N	N	N
State 4	126.0 ± 2.0[c]	114.9 ± 2.0[c]	149.0 ± 2.0[a]	128.8 ± 2.0[b]	S	S	S
RCR	6.0 ± 0.4	6.3 ± 0.5	5.3 ± 0.4	5.7 ± 0.5	N	N	N
			μmol/(min × mg protein)				
SDH activity	0.50 ± 0.06	0.48 ± 0.02	0.50 ± 0.05	0.54 ± 0.05	N	N	N

[1] Values are means ± SEM, $n = 6$. S, significant $P < 0.05$; N, not significant $P \geq 0.05$. Means in a row without a common letter differ, $P < 0.05$. RCR, respiratory control ratio.

rats but can be restored by ALCAR supplementation. The reduced sensibility to the anorexic effect of leptin in old rats is in agreement with previous studies showing reduced response to systemic (34) or intracerebroventricular injection of leptin in old rats (35).

In the present work, mitochondrial oxidative capacities together with mitochondrial protein content were assessed in skeletal muscle, a tissue that contributes greatly to whole-body energy expenditure and lipid utilization. Measurements of mitochondrial respiratory activities were performed on both homogenates and isolated mitochondria. ALCAR treatment increased maximal oxidative capacity (state 3 respiration) in homogenates (Table 3) but not in isolated mitochondria (Table 4) in old rats, due to increased mitochondrial protein content, which occurred in old rats supplemented with ALCAR. Moreover, we observed a significant increase in state 4 respiration with age, in both homogenates and isolated mitochondria. With the limitation that state 4 respiratory rate in the presence of saturating amounts of oligomycin can give only a rough indication of the mitochondrial proton leak, the increase in state 4 respiratory rate may indicate that the proton leak pathway increases with age. In agreement with this suggestion, Lal et al. (36) found that the proton leak was lower in skeletal muscle mitochondria from young than from old rats. An implication of this finding could be that the inefficiency of the oxidative phosphorylation system is greater in old than in young rats, so that mitochondria from old rats could not completely satisfy cellular energy requirements, with a possible impairment in the functional ability of the muscle cell. Inter-

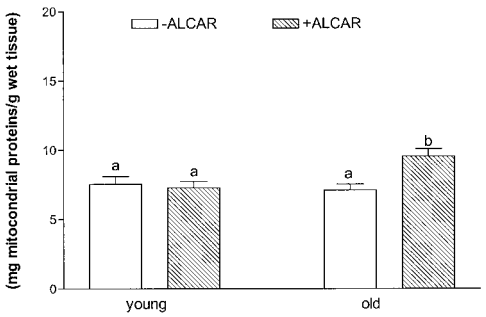

FIGURE 4 Skeletal muscle mitochondrial proteins, expressed as mg/g wet tissue, in young and old rats with or without acetyl-L-carnitine (ALCAR) supplementation for 1 mo. Values are means ± SEM, $n = 6$. Main effect of age $P \geq 0.05$; main effect of ALCAR treatment, $P < 0.05$; interaction, $P < 0.05$. Means with a different superscript letter differ significantly ($P < 0.05$).

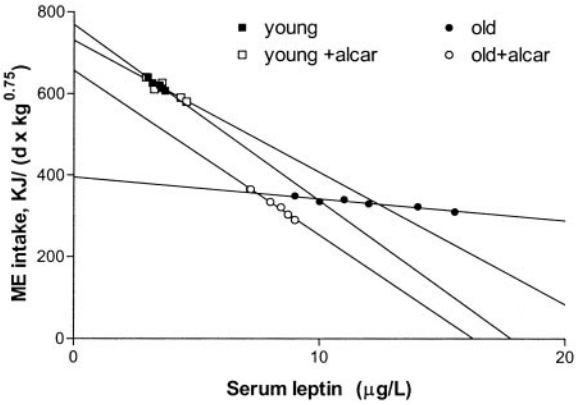

FIGURE 5 Correlation between metabolizable energy (ME) intake and serum leptin, in young and old rats with or without acetyl-L-carnitine (ALCAR) supplementation for 1 mo. Each point represents an individual value. young ($r^2 = 0.9406$, $P < 0.01$); young + ALCAR ($r^2 = 0.8565$, $P < 0.01$); old ($r^2 = 0.9191$, $P < 0.01$); old + ALCAR ($r^2 = 0.9921$, $P < 0.0001$).

estingly, ALCAR treatment decreased state 4 respiratory rate only in old rats and therefore could restore the capacity of muscle cells to produce energy.

In conclusion, the results of this work indicate that ALCAR supplementation differently influences nutrient partitioning in young and old rats. In fact, lipid deposition is lower in young rats, whereas protein deposition is enhanced in old rats. In addition, ALCAR supplementation in old rats can reduce leptin resistance and improve ATP production in skeletal muscle mitochondria through an increase in mitochondrial protein content and, perhaps, in the efficiency of the oxidative phosphorylation system.

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