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Research Article

Acetyl-L-carnitine treatment stimulates oxygen consumption and biosynthetic function in perfused liver of young and old rats

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Abstract. The effect of treatment with acetyl-L-carnitine on hepatic mitochondrial respiration and biosynthetic function in perfused liver from young (90 days) and old (22–24 months) rats was studied. Rats were given a 1.5% (w/v) solution of acetyl-L-carnitine in their drinking water for 1 month and oxygen consumption together with the rate of gluconeogenesis, urea synthesis, and ketogenesis with and without added substrates were measured in perfused liver. Mitochondrial oxygen consumption was also assessed in liver homogenate and isolated mitochondria to determine the maximal capacity for oxidative phosphorylation. Acetyl-L-carnitine treatment almost completely restored the age-dependent decline in oxygen consumption, gluconeogenesis, urea synthesis, and ketogenesis found in perfused liver of old rats to the levels found in young rats. In addition, acetyl-L-carnitine treatment increased oxygen consumption and biosynthetic function in perfused liver from young rats. After acetyl-L-carnitine treatment, we found detectable 3-oxoacyl-CoA-transferase activity associated with a consumption of ketone bodies in young and old rats. Finally, oxygen consumption measured in homogenate and isolated mitochondria did not change with age and acetyl-L-carnitine treatment. Our results show that in perfused liver, acetyl-L-carnitine treatment slows the age-associated decline in mitochondrial respiration and biosynthetic function. In addition, treatment of young rats with acetyl-L-carnitine has a stimulating effect on liver metabolism, probably through an increase in ATP production.

Key words. Gluconeogenesis; urea synthesis; ketogenesis; isolated mitochondria; ageing.

Ageing is known to be associated with alterations in various aspects of cell function. At the hepatic level, ageing causes a decline in various metabolic pathways [1]. The molecular basis of this decline is still not well understood, but it seems that mitochondrial decay plays an important role in the ageing process [2, 3]. Studies using hepatocytes have shown an age-related decline in mitochondrial membrane potential, respiratory control ratios, and oxygen consumption [4]. In addition, we have previously shown age-related changes in mitochondrial performance in perfused liver from rats at different stages of development: weaning (24 days), adolescence (60 days), adulthood (180 days) and middle age (365 days). In fact, we found that oxygen consumption, urea synthesis, gluconeogenesis from lactate and ketone body production decline with increasing age [5]. These impairments occur at a relatively early life stage, and the decrease in oxygen consumption could contribute to enhancement of the rate of free radical production. The rate of mitochondrial free radical generation seems to be

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dependent on the activity of the electron transport chain, and it increases as complete reduction of oxygen is impaired [5].

Recent studies have shown that acetyl-L-carnitine (ALCAR), a normal biomolecule [6] can slow the age-related deficit in mitochondrial function in various tissues [7, 8]. At the hepatic level, ALCAR was able to slow the age-related decline in mitochondrial membrane potential and cellular oxygen consumption found in hepatocytes from old rats [9, 10].

In an attempt to gain insight into the ALCAR effects, we decided to investigate whether the stimulatory effects of ALCAR on hepatic mitochondrial respiration found in old rats [10] also occur for other hepatic functional parameters. To this end, in the present study we assessed perfused liver oxygen consumption, stimulated by various substrates, together with the rate of gluconeogenesis, urea synthesis and ketogenesis in young (90 days) and old (22–24 months) rats, whose diet was supplemented by ALCAR. Mitochondrial respiration was also assessed in liver homogenate and isolated mitochondria of these above rats to determine if changes in this parameter due to ageing and ALCAR treatment found in perfused liver are associated with variation in maximal capacity for oxidative phosphorylation.

Methods and Materials

Animals. Male Wistar rats bred in the animal house of Heinrich-Heine-University of Düsseldorf, were used for the experiments. They were housed individually in grid-bottomed cages at 24 °C under an artificial circadian 12 h light/12 h dark cycle. At the start of the study, 24 rats were divided into four groups each composed of 6 rats, two groups aged 3 months (young rats) and the remaining groups aged about 24 months (old rats). One group of young and one of old rats, used as controls, received ad libitum standard stock diet and water, while the other two groups received ad libitum standard stock diet and water supplemented with a 1.5% (w/v, pH adjusted to 6) solution of ALCAR. The treatment period lasted 1 month. At the end of the experimental period, the rats were used for measurement of oxygen consumption, gluconeogenesis, urea synthesis and ketogenesis in perfused liver.

Another 32 male Wistar rats were divided into four groups each composed of 8 rats. The age and the treatment of these groups were the same as above. These rats were used to determine oxygen consumption in liver homogenates and isolated mitochondria, as well as for measurement of 3-oxoacyl-CoA-transferase activity in liver extracts. Animal care, housing and killing met the guidelines of the European Community.

Liver perfusion. Perfusions were performed on livers from 24-h-fasted rats since during 24-h fasting, liver glycogen is reduced to a level that has been shown previously to minimise glucose production via glycogenolysis [11].

Rats were anaesthetised with sodium pentobarbital (Nembutal; Sanofi, Bad Segeberg, Germany) by intraperitoneal injection of 12 mg/100 g body weight in isotonic solution. Before opening the abdomen, heparin (250 I.E./100 g) was injected into the femoral vein. Livers were perfused in situ with Krebs-Henseleit bicarbonate buffer [12], pH 7.4, in a non-recirculating system [13]. The fluid was pumped through a temperature-regulated (37 °C) membrane oxygenator supplemented with 95% O_2/5% CO_2, v/v, before entering the liver via a cannula inserted into the portal vein. The arterial oxygen concentration, maintained constant by the oxygenator, was measured before and after each experiment. The effluent perfusate was collected via a cannula placed in the vena cava. It passed by a platinum electrode for continuous monitoring of the venous oxygen concentration, and perfusate samples were collected every 1–2 min for metabolite analyses. The flow rate was adjusted to the metabolic activity of the liver as judged from the venous oxygen concentration. From experiment to experiment it varied between 5 and 6 ml min^{-1} g^{-1}, but was constant in each individual experiment. Substrates were added to the perfusion fluid before entering the liver. The final concentrations of substrates were: β-hydroxybutyrate 0.5 mM + acetoacetate 0.5 mM, lactate + pyruvate 2 mM at a ratio lactate/pyruvate of 7, glycerol 12 mM, NH_4HCO_3 1.2 mM, hexanoate 0.5 mM, palmitate 0.5 mM. Palmitate was dissolved in a bovine serum albumin solution: 1 g palmitate was slowly added to 170 ml albumin solution (20 g/100 ml) under gentle stirring which was continued for a further 2 h at room temperature.

Before addition of the substrates, the liver was perfused for 10–15 min until a steady state was reached, i.e. the oxygen consumption rate remained constant. Following the addition of each substrate, the liver was allowed to reach steady state before further additions (ca 10–15 min).

After termination of the perfusion, the wet weight of the liver was determined.

Metabolic rates. Glucose, ammonia, urea, lactate, pyruvate, β-hydroxybutyrate and acetoacetate concentrations were measured in perchloric acid extracts of the perfusate samples using enzymatic analysis by spectrophotometric procedures coupled to the appearance or disappearance of NADH [14]. The steady-state metabolic rates were calculated from the arteriovenous concentration differences of oxygen, glucose, urea, β-hydroxybutyrate and acetoacetate, the flow rate, and the liver wet weight.

Preparation of liver homogenates and isolated mitochondria. After 24 h fasting, rats were anaesthetised by an intraperitoneal injection of choral hydrate (400 mg/kg body weight) and livers were collected. After removal of the liver, small samples (2 g) were taken for preparation of tissue extracts and immediately frozen in liquid nitrogen. The remainder of the tissue was finely minced and washed with a medium containing 220 mM mannitol, 70 mM sucrose, 20 mM Tris, 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 (1:4, w/v) in a Potter Elvehjem homogeniser set at 500 rpm (4 strokes/min) and filtered through sterile gauze. Aliquots of the homogenate were then used to determine respiratory activities, while the remaining homogenate was further processed to prepare isolated mitochondria. Homogenate was freed of nuclei by centrifugation at 1000 g_{av} for 10 min; the resulting supernatant was centrifuged at 3000 g_{av} for 10 min. The mitochondrial pellet was washed twice and finally resuspended in a suspension medium containing 80 mM KCl, 50 mM Hepes, pH 7.0, 5 mM KH_2PO_4, 1 mM EGTA, 0.1% (w/v) fatty-acid-free bovine serum albumin. The protein content of the mitochondrial suspension was determined by the method of Hartree [15] using bovine serum albumin as the protein standard.

Measurement of respiration in liver homogenates and mitochondria. Maximal oxygen consumption rates were measured in homogenates and isolated mitochondria polarographically with a Clark-type electrode (Yellow Springs Instruments, Yellow Springs, Ohio), maintained in a water-jacketed chamber at 30 °C, as previously reported [16]. Briefly, aliquots of homogenates and isolated mitochondria were incubated in 3 ml of the above suspension medium and were allowed to oxidise their endogenous substrates for a few minutes. Substrates were then added at the following concentration: 40 µM palmitoylcarnitine + 2.5 mM malate, 40 µM palmitoylcoenzyme A + 2 mM carnitine + 2.5mM malate. Measurements were performed in the presence of 0.6 mM ADP.

Determination of 3-oxo acyl CoA-transferase activity. The determination of the rate of acetoacetyl-CoA formation from succinyl-CoA and acetoacetate was made in liver extracts according to Williamson et al. [17]. Briefly, samples of frozen liver were finely minced and homogenised (1:4 w/v) with a medium containing 0.25 M sucrose, 1 mM 2-mercaptoethanol, 10 mM Tris, pH 7.4, in a Potter-Elvehjem homogeniser. The homogenate was immediately exposed to ultrasonic vibration for exactly 30 s at 15 kHz (100 W). The ultrasonically treated homogenate was then centrifuged for 20 min at 30000 g_{av}. The supernatant fluid was considered to contain the total soluble protein of the cell (i.e. cytoplasm and mitochon-

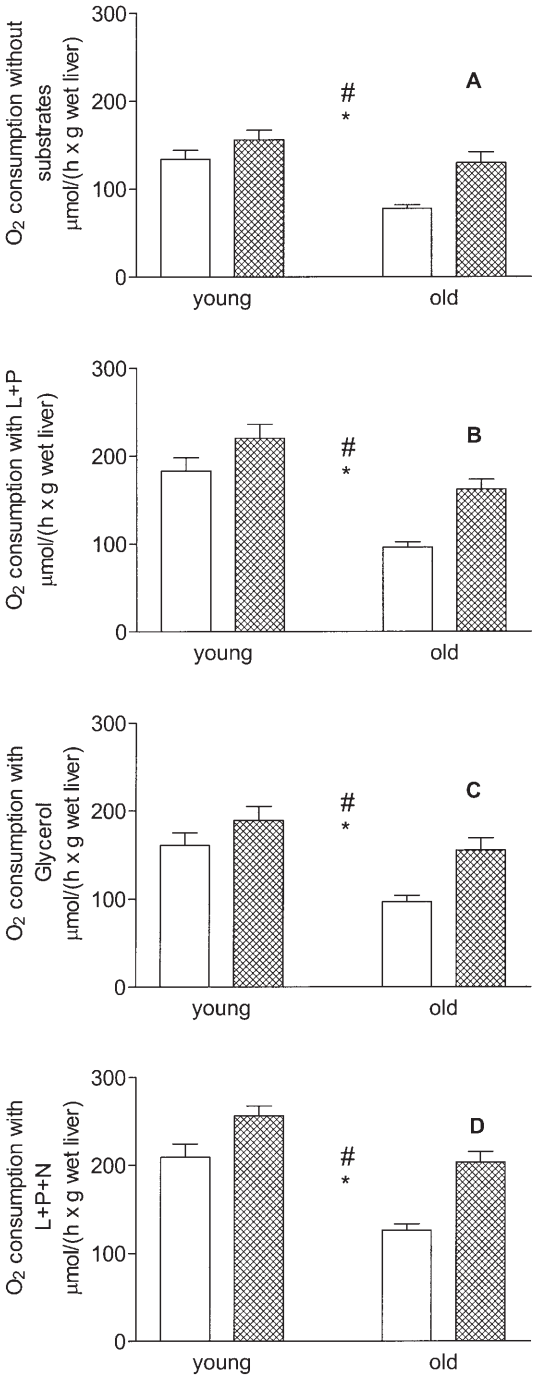

Figure 1. Effect of ALCAR treatment on oxygen consumption measured in perfused liver of young and old rats in the absence of substrates (*A*), in the presence of lactate + pyruvate (L + P) as substrates (*B*), in the presence of glycerol as substrate (*C*); or in the presence of lactate + pyruvate + NH_4HCO_3 (L + P + N) as substrates (*D*). Statistical difference by ANOVA ($P < 0.05$): # effect of age, * effect of ALCAR treatment.

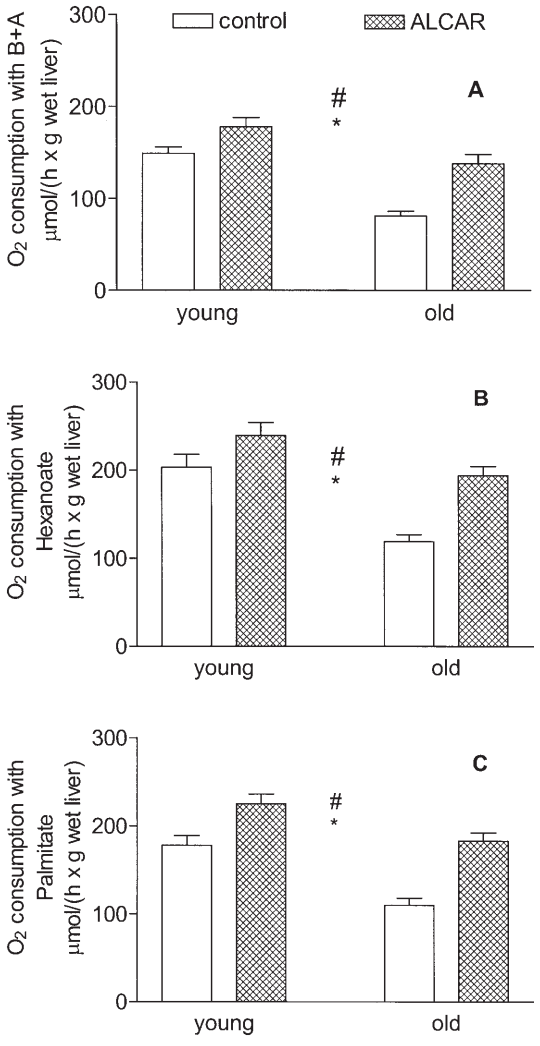

Figure 2. Effect of ALCAR treatment on oxygen consumption measured in perfused liver of young and old rats in the presence of β-hydroxybutyrate + acetoacetate (B + A) as substrates (*A*), in the presence of hexanoate as substrate (*B*), or in the presence of palmitate as substrate (*C*). Statistical difference by ANOVA ($P < 0.05$): # effect of age, * effect of ALCAR treatment.

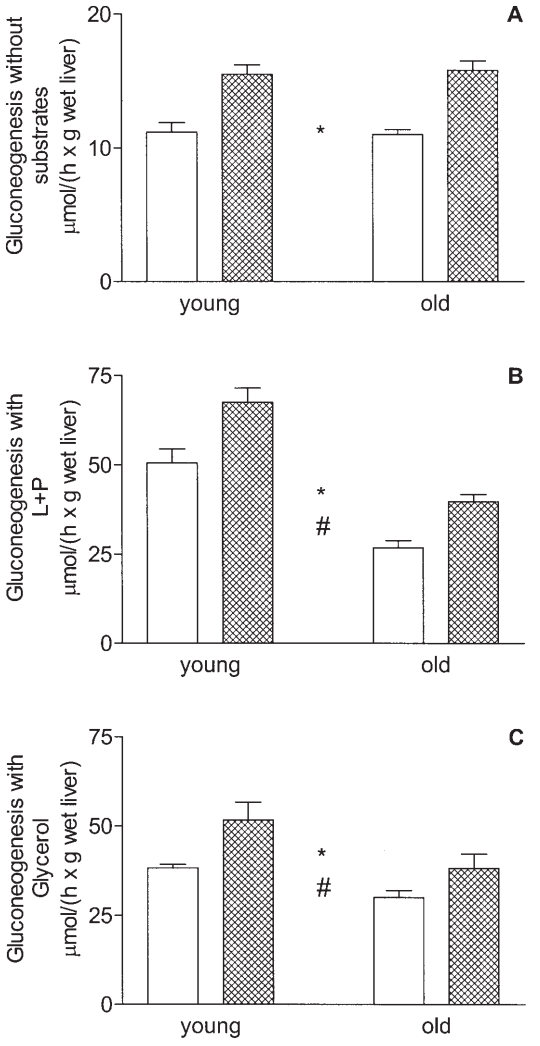

Figure 3. Effect of ALCAR treatment on gluconeogenesis measured in perfused liver of young and old rats in the absence of substrates (*A*), in the presence of Lactate + Pyruvate (L + P) as substrates (*B*), or in the presence of glycerol as substrate (*C*). Statistical difference by ANOVA ($P < 0.05$): # effect of age, * effect of ALCAR treatment.

drial matrix) and its enzyme activity is referred to in the text as the tissue activity. Enzyme activity was measured at 25 °C in a medium containing 50 mM Tris, pH 8.5, 5 mM MgCl, 5 mM Iodoacetamide, 0.1 mM succinyl-CoA, and 1 mM acetoacetate.

Statistical analysis. Data are given as means ± SEM of different rats. Statistical analyses were performed by two-tailed unpaired Student's t test or two-way analysis of variance (ANOVA) for the main effects of age and ALCAR treatment as well as for the interaction effect between age and ALCAR treatment. Probability values less than 0.05 were considered to indicate a significant difference. All analyses were performed using GraphPad Prism (GraphPad Software, San Diego, Calif.).

Materials. β-Hydroxybutyrate, acetoacetate, lactate, pyruvate, NH_4HCO_3, hexanoate, palmitate, glycerol, β-hydroxybutyrate dehydrogenase, NAD, NADH, ATP, ADP, NADP, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, hexokinase, glutamate dehydrogenase, urease, iodoacetamide, succinyl-CoA ADP, malate, palmitoylcarnitine, carnitine, palmitoylCoA, and fatty-acid-free bovine serum albumin were from Boehringer (Mannheim, Germany) or Sigma (Munich, Germany). Other chemicals were from Merck (Darmstadt, Germany). All reagents were of the highest commercially available purity.

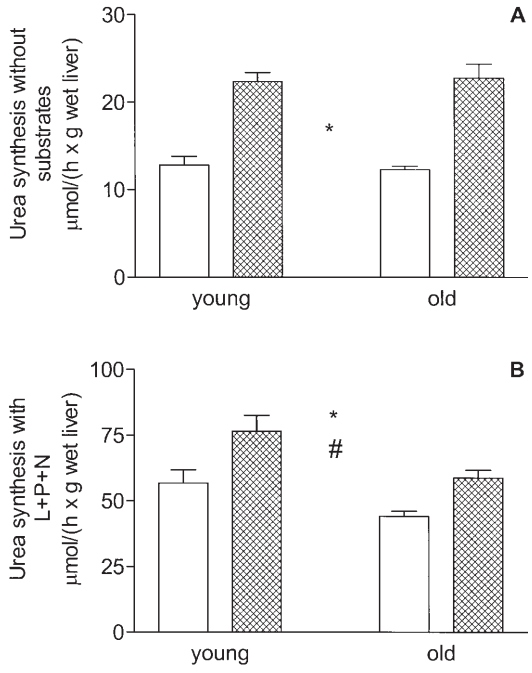

Figure 4. Effect of ALCAR treatment on urea synthesis measured in perfused liver of young and old rats in the absence of substrates (*A*), or in the presence of lactate + pyruvate + NH_4HCO_3 (L + P + N) as substrates (*B*). Statistical difference by ANOVA ($P<0.05$): # effect of age, * effect of ALCAR treatment.

Results

Livers were perfused in situ in the absence of exogenous substrates and basal oxygen consumption was measured (fig. 1A). The results show a significant decline in hepatic oxygen consumption with age, while treatment with ALCAR significantly increased basal oxygen consumption in both young and old rats. We also measured oxygen consumption in the presence of various substrates. The addition of a substrate to perfused liver caused a rapid (less than 1 min) increase in oxygen consumption (figs 1B–D, 2). Whatever the substrate, oxygen consumption was significantly lower in old compared to young rats, while it significantly increased in young and old ALCAR-treated rats compared to their respective controls.

Gluconeogenesis was measured both without substrates and following addition of lactate + pyruvate or glycerol, in the presence of 1 mM hydroxybutyrate + acetoacetate to saturate respiration (fig. 3A–C). Hydroxybutyrate + acetoacetate did not interfere with gluconeogenesis; in fact, the addition of hydroxybutyrate + acetoacetate to Krebs-Henseleit buffer was without effect on gluconeogenesis (not shown). Gluconeogenesis with lactate + pyruvate or glycerol significantly decreased with age and significantly increased in ALCAR-treated rats compared to their respective controls, whereas basal gluconeogenesis did not significantly change with age and significantly increased in ALCAR-treated rats compared to their respective controls.

The rate of urea synthesis in the absence of exogenous substrates is shown in figure 4A. It did not change with age, while treatment with ALCAR significantly increased the rate of urea synthesis in both young and old rats. For a reasonably high rate of urea synthesis, a nitrogen source must be added together with a precursor for oxalacetate. Therefore, ammonia and lactate + pyruvate were added to prevent a possible shortage of intermediates and energy. In this condition, the production of urea decreased with age and significantly increased in ALCAR-treated rats compared to their respective controls (fig. 4B).

The rate of ketogenesis and the hydroxybutyrate/acetoacetate ratio with and without added substrate are reported in table 1. The rate of ketogenesis in the absence of substrate did not change with age or ALCAR treatment. The hydroxybutyrate/acetoacetate ratio significantly increased in old compared to young rats and significantly

Table 1. Effect of ALCAR treatment on ketogenesis and the β-hydroxybutyrate/acetoacetate (B/A) ratio measured in perfused livers of young and old rats.

Addition	Young	Young + ALCAR	Old	Old + ALCAR	ANOVA		
					main effect of age	main effect of ALCAR treatment	interaction
None							
A + B	26.0 ± 1.1	30.0 ± 1.9	29.0 ± 0.7	31.0 ± 2.0	NS	NS	NS
B/A	0.24 ± 0.02	0.25 ± 0.02	0.34 ± 0.01	0.26 ± 0.012	<0.05	<0.05	<0.05
Hexanoate							
A + B	139.0 ± 0.8	163.0 ± 0.7	108.0 ± 4.3	140.0 ± 1.0	<0.0001	<0.0001	NS
B/A	0.99 ± 0.01	2.0 ± 0.1	2.0 ± 0.1	2.95 ± 0.034	<0.0001	<0.0001	NS
Palmitate							
A+B	55.0 ± 0.8	96.0 ± 3.6	68.0 ± 2.8	93.0 ± 6.4	NS	<0.0001	NS
B/A	0.49 ± 0.01	2.28 ± 0.12	2.25 ± 0.13	3.8 ± 0.1	<0.0001	<0.0001	NS

Values are reported as mean ± SE of six experiments [µmol/h × g wet liver)]. NS, not significant.

Table 2. Effect of ALCAR treatment on ketone body consumption rate and 3-oxoacyl-CoA-transferase activity measured in livers of young and old rats.

	Young	Young + ALCAR	Old	Old + ALCAR
Ketone body consumption [µmol/(h × g wet liver)]	–	28.0 ± 1	–	15.0 ± 1*
3-Oxoacyl-CoA-transferase nmol/[(min × g wet liver)]	–	62.7 ± 3.2	–	29.8 ± 5.0*

Values are reported as mean ± SE of six experiments (– undetectable). * $P < 0.05$ compared to young + ALCAR (two-tailed Student's t-test).

decreased only in old rats after ALCAR treatment. With hexanoate added, ketone body production declined with age and increased after ALCAR treatment. With palmitate, ketone body production increased after ALCAR treatment, while no variation due to age was observed. With both fatty acids, the redox hydroxybutyrate/acetoacetate ratio significantly increased in old compared to young rats and after ALCAR treatment.

Table 2 reports hepatic ketone body consumption rate and 3-oxoacyl-CoA-transferase activity, the enzyme which catalyses the rate-limiting step of ketone body degradation. The results show the presence of ketone body consumption and 3-oxoacyl-CoA-transferase activity only after ALCAR treatment, with significantly lower values in old compared to young rats.

Table 3 shows no variation in maximal respiratory rates of homogenate and isolated mitochondria using palmitoylCoA + malate or palmitoylcarnitine + malate as the substrate.

Discussion

In this work we studied the effect of ALCAR administration on liver metabolism in young and old rats. This natural biomolecule, when administered orally, is absorbed in the jejunum by simple diffusion and Na^+-dependent active transport [18]. In addition, ALCAR is deacetylated during or immediately after uptake into intestinal cells and a portion of the newly formed intracellular free carnitine is apparently reacetylated [19]. Therefore, use of ALCAR as a dietary supplement could be equivalent to that of L-carnitine. However, at least in some metabolic pathways [20–22], ALCAR seems to be the metabolically active molecule.

Our present results show that ALCAR treatment in both young and old rats significantly increases oxygen consumption, urea synthesis, gluconeogenesis and ketone body production, all assessed in perfused liver. In addition, since oxygen consumption and the above-mentioned biosynthetic functions are reduced in old compared to young rats, treatment of old rats with ALCAR almost completely restores them to the levels found in young rats.

The decrease in oxygen consumption without added substrates found in old rats suggests a decline in NADH oxidation. In fact, there is a concomitant increase in the hydroxybutyrate/acetoacetate ratio, measured in the effluent perfusate as an index of the mitochondrial NADH/NAD ratio [23]. The reduced NADH oxidation is probably due to lower energy requirements and not impairment of respiratory chain activity; in fact, maximal oxidative capacity measured in homogenate and isolated mitochondria did not vary. ALCAR treatment significantly increased oxygen consumption in young and old rats. This finding is in agreement with the results obtained by Hagen et al. [10], who found in isolated hepato-

Table 3. Effect of ALCAR treatment on oxygen consumption measured in liver homogenate and isolated mitochondria of young and old rats.

	Young	Young + ALCAR	Old	Old + ALCAR	ANOVA		
					main effect of age	main effect of ALCAR treatment	interaction
Isolated mitochondria [nmol O/(min × mg protein)]							
Palmitoyl CoA + malate	95.4 ± 6.0	91 ± 7	102 ± 7	107 ± 8	NS	NS	NS
Palmitoylcarnitine + malate	92.8 ± 8	86 ± 7	99.7 ± 7	110 ± 10	NS	NS	NS
Homogenate nmol O/(min × g wet liver)							
Palmitoyl CoA + malate	4601 ± 96	4157 ± 295	4897 ± 200	4776 ± 446	NS	NS	NS
Palmitoylcarnitine + malate	4459 ± 107	4898 ± 110	5427 ± 277	4761 ± 402	NS	NS	NS

Values are reported as mean ± SEM of eight experiments. NS, not significant.

cytes an increase in oxygen consumption after ALCAR treatment in old rats. These authors, however, did not report data on the effect of ALCAR treatment on young rats. Moreover, the increase in the hydroxybutyrate/acetoacetate ratio found in old compared to young rats is almost completely suppressed by ALCAR treatment, suggesting an increase in NADH oxidation.

We also measured oxygen consumption and the rate of ketogenesis in perfused livers in the presence of a lipid substrate, namely palmitate or hexanoate. Palmitate oxidation involves acylcarnitine formation and translocation, while that of hexanoate does not. Ketogenesis from hexanoate and oxygen consumption with hexanoate or palmitate was significantly decreased in old compared to young rats. In addition, ALCAR treatment increased ketogenesis and oxygen consumption whatever the substrate, both in young and old rats. As for ketogenesis, the greater effect of ALCAR treatment was on ketogenesis from palmitate in young rats (+74%). Part of this increase is conceivably due to an increased activity of carnitine-acylcarnitine exchange across the inner mitochondrial membrane, which is of great importance for oxidation of long-chain fatty acids. In addition, since the activity of the carnitine acyltransferase system controls the rate of fatty acid oxidation [6], it may be responsible for the age-related decrease in oxygen consumption that we observed. In agreement, studies on mitochondria indicate that the activity of this exchange reaction decreases significantly with age [24]. The lower intramitochondrial pool of carnitine has been suggested to be in part responsible for this age effect [25], and ALCAR treatment slows the age-related decrease in the level of the intramitochondrial pool of carnitine [25]. To gain further insight into the mechanism of increased oxygen consumption found after ALCAR treatment, we measured oxygen consumption using as substrate palmitoylcarnitine or palmitoyl-CoA in isolated mitochondria, to obtain direct information on maximal oxidative capacity. To link this parameter with the effective mitochondrial protein mass, oxygen consumption was also measured in the homogenate. The results indicate that there was no variation in oxidative capacity in either mitochondria or homogenate. Therefore, the increased oxygen consumption found in perfused liver after ALCAR treatment is possibly due to an increase in the activity of acyl-CoA-synthetase, the enzyme which catalyses the activation of fatty acids to acyl-CoA esters, rather than to an increase in maximal oxidative capacity. In agreement, an increase in the hydroxybutyrate/acetoacetate ratio was found after ALCAR treatment. Thus, ALCAR could stimulate liver metabolic activity, increasing liver ATP production, which is consistent with the increase in hepatic gluconeogenesis and urea synthesis that we found.

An interesting result was obtained when the livers were perfused with hydroxybutyrate + acetoacetate. The observed increase in oxygen consumption obtained with these substrates should only be due to an increase in NADH production coming from hydroxybutyrate oxidation to acetoacetate, since the liver is generally accepted as unable to utilise ketone bodies as substrates [17, 26]. However, we found consumption of ketone bodies in young and old rats after ALCAR treatment, indicating that in these rats, ketone bodies are converted back acetyl-CoA. This would suggest that, in young and old rats after ALCAR treatment, the enzyme which catalyses the rate-limiting step of ketone body degradation, 3-oxoacyl-CoA-transferase, is present. This result is quite unexpected: only foetal [27] and weaning liver [5] are known to be able to oxidise ketones, while 3-oxoacyl-CoA-transferase is not present in the liver of adult rats [17, 26]. The absence of 3-oxoacyl-CoA-transferase in liver mitochondria has been taken as evidence for many years that the liver cannot utilise ketone bodies. This concept should be revised in the light of our results demonstrating this enzyme in young and old rats after ALCAR treatment. In these rats, the role of 3-oxoacyl-CoA-transferase may be to produce a substrate cycle between acetoacetyl-CoA and acetoacetate with a consequent wastage of energy. Another implication of the presence of 3-oxoacyl-CoA-transferase in rat liver after ALCAR supplementation is to reduce the high rate of ketogenesis by removal of acetoacetate and by inhibition of flux through the HMG-CoA-synthase reaction [28]. In fact, the rate of ketogenesis in isolated mitochondria is very sensitive to the physiological concentration of acetoacetyl-CoA [29].

Using liver perfusion, we measured the rate of biochemical pathways like gluconeogenesis and urea synthesis and their respective oxygen consumptions. Gluconeogenesis was measured in presence of lactate + pyruvate or glycerol. Gluconeogenesis from lactate + pyruvate involves mitochondria, whereas that from glycerol does not. Despite the utilisation of different metabolic pathways by the two substrates, gluconeogenesis and oxygen consumption fell in old compared to young rats. The last result is partially in agreement with Sastre et al. [1] who found, in isolated hepatocytes, a decrease with age only in gluconeogenesis from lactate + pyruvate. Age-related declines in the activity of phosphoenolpyruvate carboxykinase [30, 31], considered the rate-limiting enzyme of gluconeogenesis from lactate [1], are consistent with our results. Moreover, we found that ALCAR treatment increased gluconeogenesis in both old and young rats. The effect of ALCAR on gluconeogenesis from lactate + pyruvate could be explained as follows. The lower rate of gluconeogenesis from lactate + pyruvate has been reported as due to an impaired transport of malate across the mitochondrial membrane using the dicarboxylate carrier [1] and this impairment is associated with a concomitant decrease in mitochondrial cardiolipin content [25]. Therefore, ALCAR treatment, by increasing the

cardiolipin concentration [25], would positively influence the activity of protein carriers.

Urea synthesis, an exclusive biosynthetic function of the liver, decreased with age; ALCAR treatment reversed this effect and increased the urea synthesis rate in young rats, probably due to its stimulating effect on ATP production.

The results of this work clearly demonstrate that in perfused liver, ALCAR treatment has a stimulating effect on metabolism. ALCAR treatment can slow some cellular metabolic dysfunctions due to age, probably through maintenance of mitochondrial function.

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