Clinical and Experimental Pharmacology and Physiology (2013) **40**, 205–211 doi: 10.1111/1440-1681.12050

Changes in hepatic lipogenic and oxidative enzymes and glucose homeostasis induced by an acetyl-L-carnitine and nicotinamide treatment in dyslipidaemic insulin-resistant rats

Maria R Ferreira,* Maria del C Camberos,† Dante Selenscig,* Lucía C Martucci,† Adriana Chicco,* Yolanda B Lombardo* and Juan C Cresto†

**Department of Biochemistry, School of Biochemistry, Litoral University, Santa Fe, and †Endocrinology Research Centre (CEDIE), Ricardo Gutierrez Hospital, Buenos Aires, Argentina*

SUMMARY

1. Normal rats fed a sucrose-rich diet (SRD) develop dyslipidaemia and insulin resistance. The present study examined whether administration of the mitochondrial nutrients nicotinamide and acetyl-L-carnitine reversed or improved these metabolic abnormalities.

2. Male Wistar rats were fed an SRD for 90 days. Half the rats then received daily injections of nicotinamide (25 mg/kg, i.p.) and acetyl- L-carnitine (50 mg/kg, i.p.) for a further 90 days. The remaining rats in the SRD-fed group and those in a normal chow-fed control group were injected with an equal volume of saline solution for the same period. The following parameters were determined in all groups: (i) liver activity of fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC) and carnitine-palmitoyl transferase-1 (CPT-1); (ii) hepatic and skeletal muscle triacylglycerol content, plasma glucose, insulin, free fatty acid (FFA) and triacylglycerol levels and pancreatic insulin content; and (iii) glucose tolerance.

3. Administration of nicotinamide and acetyl-L-carnitine to the SRD-fed rats reduced dyslipidaemia, liver steatosis, muscle triacylglycerol content and hepatic FAS and ACC activities and increased CPT-1 activity. In addition nicotinamide and acetyl-L-carnitine improved the glucose disappearance rate (K_g), normalized plasma glucose levels and moderately increased insulinaemia without altering pancreatic insulin content. Finally, nicotinamide and acetyl-L-carnitine administration reduced bodyweight gain and visceral adiposity.

4. The results of the present study suggest that altering key hepatic lipogenic and fatty acid oxidative enzymatic activity could improve dyslipidaemia, liver steatosis and visceral adiposity. Indeed, administration of nicotinamide and acetyl-L-carnitine improved glucose intolerance and normalized plasma glucose levels.

Correspondence: Yolanda B Lombardo, Department of Biochemistry, School of Biochemistry, Litoral University, CC 242 (3000) Santa Fe, Argentina. Email: ylombard@fbcb.unl.edu.ar

Received 17 February 2012; revision 11 December 2012; accepted 18 December 2012.

© 2012 The Authors
Clinical and Experimental Pharmacology and Physiology
© 2012 Wiley Publishing Asia Pty Ltd

Key words: acetyl-L-carnitine, dyslipidaemia, glucose homeostasis, insulin resistance, lipogenesis inhibition, nicotinamide, sucrose-rich diet.

INTRODUCTION

Several studies have identified worldwide increases in diabetes and its long-term complications, with subsequent increases in social and economic costs.[1,2] Approximately 90% of patients with diabetes have Type 2 diabetes, which is characterized by insulin resistance. Type 2 diabetes develops with dyslipidaemia, hyperinsulinaemia, lipotoxicity and hypertension. There is abundant evidence that increased plasma triacylglycerol (TAG) and free fatty acid (FFA) levels and excessive lipid accumulation in insulin target tissues, such as muscle and liver, are important factors that contribute to whole-body insulin resistance.[3–5] Moreover, insulin resistance is reportedly associated with impaired skeletal muscle oxidative capacity and reduced mitochondrial number and function.[6,7] Therefore, mitochondria also participate in insulin resistance.

Petersen *et al.*[8] demonstrated that neither lipolysis nor plasma levels of tumor necrosis factor (TNF)-α, interleukin (IL)-6, resistin or adiponectin are significantly increased in healthy offspring of patients with type 2 diabetes. However, these patients had significantly increased skeletal muscle intracellular lipid content, which was accompanied by reduced mitochondrial oxidative phosphorylation and ATP production.

When an oral glucose tolerance test was administered to both healthy, lean elderly people and young participants who had been matched for lean body mass and fat mass, Petersen *et al.*[6] observed that the elderly participants were markedly insulin resistant compared with the young controls. This resistance was attributed to reduced insulin-stimulated muscle glucose metabolism. These changes were associated with increased fat accumulation in the muscle and liver and with a 40% reduction in mitochondrial oxidative and phosphorylation activity. These results support the hypothesis that mitochondrial oxidative phosphorylation has a central role in insulin resistance and that reduced activity induces tissue insulin resistance regardless of the origin of the mitochondrial damage.

Several studies have evaluated the effects of mitochondrial nutrients, including L-carnitine, nicotinamide and folic acid among others, on glucose metabolism and insulin sensitivity in

animal models of obesity, Type 2 diabetes and dyslipidaemia.[9–12] Unger[13] demonstrated that nicotinamide treatment prevents β-cell abnormalities, loss of glucose stimulated insulin secretion (GSIS) and the glucose transporter GLUT-2 and accumulation of TAG in obese Zucker diabetic fatty rats. There is evidence that L-carnitine, which transports fatty acids into the mitochondria, plays a central role in fatty acid and carbohydrate metabolism by modulating the intramitochondrial acetyl-coenzyme A (CoA) : CoA ratio and pyruvate dehydrogenase complex (PDHc) flux. Overall, mitochondrial nutrients are suggested to increase fatty acid oxidation, decrease both plasma and liver TAG content, improve glucose homeostasis and insulin sensitivity, prevent reduced skeletal muscle mitochondrial biogenesis and reduce bodyweight and adiposity.

Cresto *et al.*[14] recently reported on the effects of acetyl-L-carnitine and nicotinamide treatment in a Type 1 diabetes model. These authors demonstrated that combined treatment with acetyl-L-carnitine and nicotinamide recovered insulin secretion and normalized plasma insulin levels in Type 1 diabetic mice that had hyperplastic and hypertrophic β-cells, suggesting that the nicotinamide-mediated increase in cytosolic NAD^+ and the acetyl-L-carnitine-mediated increase in acetyl-CoA augment mitochondrial NADH, citric acid flux and ATP production.

Conversely, chronic (6–8 months) administration of a sucrose-rich diet (SRD) to normal rats induces insulin resistance. This model resembles some of the biochemical and hormonal aspects present in human metabolic syndrome, such as dyslipidaemia, moderate hyperglycaemia, severe glucose intolerance in response to i.v. glucose challenge, hepatic and muscle TAG accumulation and visceral adiposity, which is accompanied by decreased skeletal muscle PDHc activity.[15] Moreover, perfusion of isolated islets indicates an absence of the first insulin release peak and an increase in the second phase, with altered glucose oxidation and reduced PDHc activity.[16,17]

To the best of our knowledge, no studies have investigated whether administration of the mitochondrial nutrients acetyl-L-carnitine and nicotinamide in combination has beneficial effects on dyslipidaemia and impaired glucose homeostasis in the SRD-fed animal model. Therefore, the aim of the present study was to investigate whether daily i.p. injections of acetyl-L-carnitine and nicotinamide improved or reversed liver steatosis, dyslipidaemia and altered glucose homeostasis in chronically (180 days) SRD-fed rats. To assess this we analysed the effects of nutrient treatment on: (i) liver TAG content, hepatic fatty acid synthase (FAS), acetyl CoA carboxylase (ACC) and carnitine-palmitoyl transferase-1 (CPT-1); (ii) plasma TAG, FFA, glucose and insulin levels, as well as pancreatic insulin content; and (iii) glucose utilization in response to an i.v. glucose challenge. Moreover, changes in bodyweight and energy intake were determined.

METHODS

Animal model and diets

Male Wistar rats (2.5 months old; 180–190 g) were purchased from the National Institute of Pharmacology (Buenos Aires, Argentina) and housed in a colony room under a 12 h light–dark cycle (lights on 1900–0700 h) at constant temperature (22°C) and humidity.

After a 1 week acclimation period, rats were randomly divided into control and experimental groups. The experimental group (n = 28) was fed a purified SRD containing (by weight) 62.5 g/100 g sucrose, 17 g/100 g casein-free vitamins, 8 g/100 g corn oil, 7.5 g/100 g cellulose, 3.5 g/100 g salt mixture (AIN-93 M-MX), 1 g/100 g vitamin mixture (AIN-93 M-VX), 0.2 g/100 g choline chloride and 0.3 g/100 g DL-methionine. The control group (n = 14) received the same semisynthetic diet, except that the sucrose was replaced by corn starch (62.5%; high-starch diet; CD). The SRD group was fed the diet for 90 days, after which the rats were randomly divided into two groups. The rats in the first group continued on the SRD for a further 90 days, whereas rats in the second group were given daily i.p. injections of 25 mg/kg nicotinamide dissolved in saline solution and 50 mg/kg acetyl-L-carnitine (Neurex®) in addition to the SRD for a further 90 days (SRD + T). In addition, rats in CD group and those continuing on the SRD only were injected with an equal volume of saline solution i.p. daily for a further 90 days.

After 70 days on the extended treatment regimen, six rats in each group were killed and plasma metabolite (TAG, glucose and insulin) levels and liver TAG content were determined. The remaining animals in each of the three groups continued on until the end of the experimental period (90 days).

The weight of each rat was recorded twice weekly for the entire 180 days of the study. Furthermore, energy intake and weight gain were assessed twice weekly in eight animals in each group. Rats were housed in individual cages and energy intake was estimated on the basis of the amount of food consumed.

At the end of the experimental period, food was removed at 0700 h and the experiments were performed between 0700 and 1000 h, unless stated otherwise. At least six rats from each dietary group were used in each experiment. Rats were anaesthetized with sodium pentobarbital (60 mg/kg, i.p.). In one group of animals, *in vivo* experiments were conducted as described below. In another group, blood samples were collected from the jugular vein and centrifuged at 2700 *g* for 10 min at room temperature; the plasma obtained was either used immediately or stored at −20°C until use. The liver and skeletal muscle (gastrocnemius) were removed, weighed, frozen immediately and stored at −80°C until use. Epididymal and retroperitoneal fat pads were also removed and weighed. The pancreas was removed immediately and insulin content was assessed as described below. The Human and Animal Research Committee of the School of Biochemistry, University of Litoral, Santa Fe, Argentina approved the study protocol. Adequate measures were taken to minimize rat pain and discomfort.

Analytical methods

Plasma TAG, FFA and glucose levels were determined by spectrophotometric methods, whereas insulin was determined using an immunoassay, as described previously.[15] The insulin immunoassay was calibrated against a rat insulin standard (Novo Nordisk, Copenhagen, Denmark) and the sensitivity of the assay was 0.5 μU/mL. Intra-assay coefficients of variation (CV) for insulin concentrations in the range 1–5, 5–10 and 10–50 μU/mL were 8.7%, 6.2% and 5.1%, respectively; the corresponding interassay CV for the same concentration ranges were 6.6%, 5.0% and 5.2%, respectively.[18] Frozen liver homogenates and skeletal mus-

© 2012 The Authors
Clinical and Experimental Pharmacology and Physiology © 2012 Wiley Publishing Asia Pty Ltd

cle powder were used for TAG determination with standard spectrophotometric methods, as described previously.[15] The pancreas was minced into small pieces (2–3 mm) in 27 mL acid–ethanol extraction fluid at 4°C. Insulin was extracted from the pancreas using the procedure described by Davoren.[19] The resulting crude insulin precipitate was collected by centrifugation at room temperature for 20 min at 10 000 *g*, resuspended in approximately 0.3 mL of 0.1 mol/L HCl, measured by radioimmunoassay and expressed as mU/total pancreatic wet weight.

Glucose tolerance test

An i.v. glucose tolerance test (IVGTT) was performed by administering 500 mg/kg glucose solution to anaesthetized rats that had fasted for 16–18 h. The methodology of the IVGTT has been described in detail elsewhere.[20] The IVGTT blood glucose removal constant (kg) and area under the curve (AUC) were calculated as described previously.[21]

Liver enzymatic activity assays

The ACC activity was determined using the methods of Zimmermann *et al.*[22] Briefly, 1 g frozen liver was homogenized with 3 volumes of phosphate bicarbonate buffer (composition (in mmol/L): $KHCO_3$ 70; K_2HPO_4 85; KH_2PO_4 9; dithiothreitol 1, pH 7.0). The cytosolic fraction was obtained after centrifuging the supernatant at 100 000 *g* for 1 h at 4°C. The ACC activity was measured using an NADH-linked assay.[22] The assay media (56 mmol/L Tris-HCl, pH 8.0, 10 mmol/L $MgCl_2$, 11 mmol/L EDTA, 4 mmol/L ATP, 52 mmol/L $KHCO_3$, 0.75 mg/mL bovine serum albumin (BSA), 0.5 mmol/L NADH and 1.4 mmol/L phosphoenolpyruvate) was mixed with 5.6 U/mL pyruvate kinase and 5.6 U/mL lactate dehydrogenase. The baseline was followed at 30°C until a constant slope was reached. For every 2.3 volumes of medium, 1 volume of activated homogenate was added and the reaction was started with acetyl-CoA (0.125 mmol/L final concentration). For enzymatic activation, 1 volume of homogenate was incubated with 1 volume of activation buffer (20 mmol/L citrate, 100 mmol/L Tris-HCl, pH 8.0, 1.5 mg/mL BSA, 20 mmol/L $MgCl_2$ and 20 mmol/L reduced glutathione (GSH, pH 7.5) for 15 min at 37°C. The FAS activity was assessed in cytosolic liver tissue fractions by measuring malonyl CoA-dependent NADPH oxidation at 37°C as described by Halestrap *et al.*[23] Activity of CPT-1 was determined spectrophotometrically using the method described by Karlic *et al.*[24]

Statistical analysis

Sample size was calculated using measurements that had been made previously with rats fed either a CD or SRD[15–17] with 80% power. Results are expressed as the mean ± SEM. Statistical analyses were performed using SPSS version 17.0 (SPSS, Chicago, IL, USA). Statistical comparisons were performed across the different dietary groups. The statistical significance among groups was determined by single-factor one-way analysis of variance (ANOVA). The IVGTT results were also analysed by ANOVA at each time point. When significant effects were identified, Tukey's post hoc test was used for comparisons. Significance was set at two-tailed $P < 0.05$.[25]

RESULTS

Bodyweight gain, energy intake and liver, epididymal and retroperitoneal fat pad weights

Bodyweight gain and energy intake were carefully monitored in the three dietary groups throughout the experiment. The weight gain and energy intake recorded in the CD- or SRD-fed rats during the first 90 day feeding period were comparable. The SRD-fed rats had significantly increased ($P < 0.05$) bodyweight compared with those in the CD-fed rats at the end of the 180 day experimental period, in accordance with previous studies.[15,16] However, bodyweight gain in the SRD + T group was similar to that in the CD-fed group. Energy intake was significantly increased in the SRD group, whereas energy intake in the SRD + T group was similar to that in the CD-fed group (Table 1). The SRD-fed rats had significantly increased epididymal and retroperitoneal fat pad weights, whether expressed as total weight or relative to total bodyweight, compared with the CD-fed rats. There was a significant reduction in both fat pad weights in the SRD + T group. However, fat pad weights in the SRD + T rats remained increased compared with those in age-matched CD-fed rats. No significant changes were found in liver weights normalized against bodyweight in the three dietary groups (Table 1).

Plasma FFA, glucose and insulin concentrations and pancreatic insulin content

As described previously,[15] after 180 days, SRD-fed rats had significantly increased plasma FFA and glucose levels without changes

Table 1 Total bodyweight, bodyweight gain, energy intake, epididymal adipose, retroperitoneal adipose and liver tissue weights in rats fed a control diet, a sucrose-rich diet (SRD) or an SRD supplemented by the administration of nicotinamide (25 mg/kg, i.p.) and acetyl-L-carnitine (50 mg/kg, i.p.)

	CD	SRD	SRD + T
Bodyweight (g)			
Initial bodyweight	176 ± 5	180 ± 4	–
90 days	365 ± 13	393 ± 10	398 ± 12
180 days	415 ± 12	498 ± 14*†	443 ± 16
Bodyweight gain (g)			
90–180 days	51.0 ± 5.6	105 ± 9*†	44.4 ± 7.1
Energy intake (kJ/day)			
Day 0–Day 90	273 ± 12	284 ± 13	280 ± 11
Day 90–Day 180	290 ± 13	365 ± 12*†	314 ± 13
Epididymal adipose tissue weight			
Total (g)	7.3 ± 0.1	15.0 ± 0.7*†	10.4 ± 0.2*
Relative (g/100 g bodyweight)	1.7 ± 0.1	3.0 ± 0.1*†	2.3 ± 0.1*
Retroperitoneal adipose tissue weight			
Total (g)	6.3 ± 0.7	12.4 ± 1.1*†	9.0 ± 0.9*
Relative (g/100 g bodyweight)	1.5 ± 0.1	2.6 ± 0.1*†	2.1 ± 0.1*
Relative liver weight (g/100 g bodyweight)	3.2 ± 0.1	3.1 ± 0.1	3.1 ± 0.1

Data are the mean ± SEM ($n = 8$ per group). *$P < 0.05$ compared with the control diet (CD)-fed group; †$P < 0.05$ compared with the nutrient-supplemented SRD (SRD + T) group (Tukey's test).

Note, SRD + T rats were fed the SRD from Day 0 to Day 180 and received nutrient supplementation from Day 90 to Day 180.

© 2012 The Authors
Clinical and Experimental Pharmacology and Physiology © 2012 Wiley Publishing Asia Pty Ltd

in insulin concentrations compared with age-matched CD-fed rats. A similar pattern was observed in rats that were SRD-fed for 90 days (data not shown). However, treating the SRD group with acetyl-L-carnitine and nicotinamide for the final 90 days of feeding reduced plasma glucose levels to values that were similar to those in the CD-fed rats. The SRD + T group also had significantly reduced plasma FFA levels and significantly increased plasma insulin levels. Interestingly, after 70 days treatment, SRD rats had significantly higher plasma glucose levels than the CD-fed rats, whereas there were no changes in insulinaemia (Table 2). The insulin : glucose ratio was also increased in the SRD + T rats at the end of the study. Conversely, SRD-fed rats had a decreased insulin : glucose ratio. At the end of the experiment, no significant differences in pancreatic insulin content were found among the dietary groups when expressed either as mU/total pancreatic wet weight or mU/100 g bodyweight (Table 2). Moreover, weight of the pancreas was similar in all dietary groups (data not shown).

Glucose tolerance

To assess glucose handling *in vivo*, a bolus of glucose was injected i.v. The IVGTT blood glucose levels are shown in Fig. 1. As indicated by the table at the bottom of Fig. 1, the glucose disappearance rates (K_g values) were significantly decreased ($P < 0.05$) in SRD- compared with CD-fed rats at the end of the experiment. In the SRD + T group, K_g values increased significantly ($P < 0.05$) compared with those in the SRD-fed group, but were lower than those for the CD-fed rats. The incremental glucose values integrated over a 60 min period after glucose injection ($\Delta G_{0–60}$; Fig. 1) differed significantly ($P < 0.05$) between the SRD and both the CD and SRD + T groups.

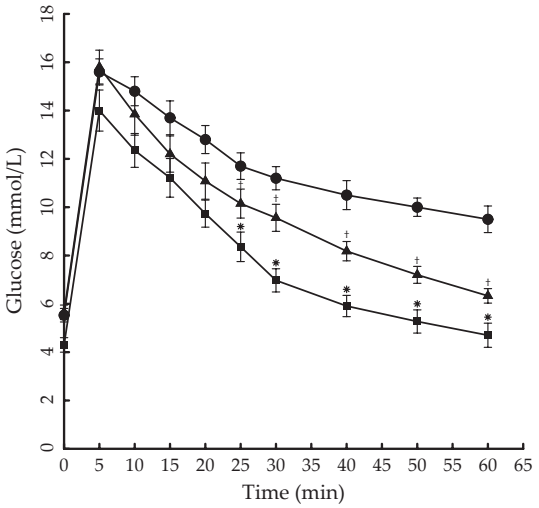
Table 2 Plasma free fatty acids, glucose and insulin concentrations, as well as pancreatic insulin content, in rats fed a control diet, a sucrose-rich diet (SRD) or an SRD supplemented by the administration of nicotinamide (25 mg/kg, i.p.) and acetyl-L-carnitine (50 mg/kg, i.p.)

	CD	SRD	SRD + T
Plasma			
FFA (μmol/L)	333 ± 19	648 ± 37*†	461 ± 37*
Glucose (mmol/L)			
Day 70 of treatment	6.7 ± 0.1	7.9 ± 0.1*†	7.4 ± 0.2*
Day 90 of treatment	7.0 ± 0.1	8.5 ± 0.3*†	7.1 ± 0.1
Insulin (μU/mL)			
Day 70 of treatment	63.5 ± 7.3	65.2 ± 8.3	65.2 ± 14.4
Day 90 of treatment	69.8 ± 8.0	67.8 ± 11.6†	116 ± 17*
Insulin: glucose ratio (at 90 days) (μU/μmol)	9.6 ± 0.5	7.5 ± 0.6*†	13.6 ± 1.4*
Pancreatic insulin			
Total (mU/total pancreatic wet weight)	211 ± 56	194 ± 28	195 ± 29
Relative (mU/100 g bodyweight)	47.1 ± 12.6	39.8 ± 5.6	38.4 ± 4.2

Data are the mean ± SEM ($n = 6$ per group). *$P < 0.05$ compared with the control diet (CD)-fed group; †$P < 0.05$ compared with the nutrient-supplemented SRD (SRD + T) group (Tukey's test).

Note, SRD + T rats were fed the SRD from Day 0 to Day 180 and received nutrient supplementation from Day 90 to Day 180.

FFA, free fatty acids.



Group	K_g (× 10^{-2}/min)	$\Delta G_{0–60}$ (mmol/min)
CD	2.39 ± 0.24*	7.73 ± 0.36
SRD	0.93 ± 0.21	11.51 ± 1.00‡
SRD+T	1.56 ± 0.31†	9.46 ± 0.77

Fig. 1 Intravenous glucose tolerance test at the end of the experimental period in rats fed either a control diet (CD; ■), a sucrose rich diet (SRD; ●) or an SRD supplemented by the administration of nicotinamide (25 mg/kg, i.p.) and acetyl-L-carnitine (50 mg/kg, i.p.; SRD + T; ▲). Glucose disappearance rates (K_g) were calculated from the slopes of the regression lines that were obtained with log-transformed glucose values after glucose administration. Glucose responses during the acute glucose challenge were used to calculate the incremental blood glucose values integrated over the 60 min period after the injection of glucose ($\Delta G_{0–60}$). Values are the mean ± SEM ($n = 6$ per group). *$P < 0.05$ compared with the SRD and SRD + T groups at particular time points. **$P < 0.05$ compared with the SRD group. Table insert: †$P < 0.05$ compared with SRD and SRD + T. ‡$P < 0.05$ compared with the SRD group. §Compared with CD and SRD + T groups.

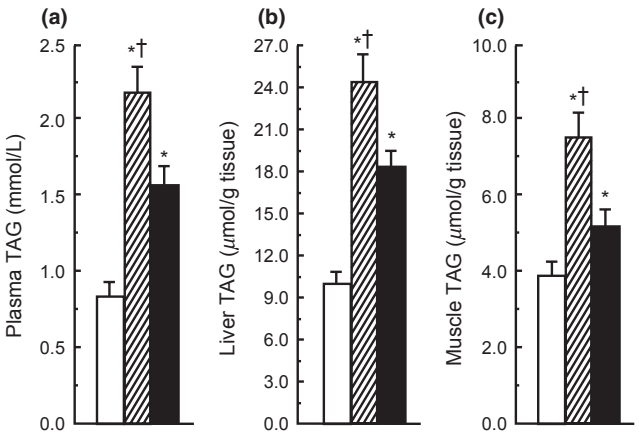


Fig. 2 (a) Plasma, (b) liver and (c) skeletal muscle triacylglycerol (TAG) content in rats fed a control diet (CD; □), a sucrose-rich diet (SRD; ▨) or an SRD supplemented by the administration of nicotinamide (25 mg/kg, i.p.) and acetyl-L-carnitine (50 mg/kg, i.p.; SRD + T; ■). Data are the mean ± SEM ($n = 6$ per group). *$P < 0.05$ compared with the CD group; †$P < 0.05$ compared with the SRD + T group.

Plasma, liver and skeletal muscle TAG content

Figure 2 shows TAG content in the plasma, liver and skeletal muscle in the different groups at the end of the experimental per-

© 2012 The Authors
Clinical and Experimental Pharmacology and Physiology © 2012 Wiley Publishing Asia Pty Ltd

Table 3 Liver acetyl-CoA carboxylase, fatty acid synthase and carnitine-palmitoyl transferase-1 activity in rats fed a control diet, a sucrose-rich diet (SRD) or an SRD supplemented by the administration of nicotinamide (25 mg/kg, i.p.) and acetyl-L-carnitine (50 mg/kg, i.p.)

	CD	SRD	SRD + T
ACC (pkat/mg protein)	783 ± 44	1433 ± 77*†	1032 ± 50*
FAS (pkat/mg protein)	106 ± 7	277 ± 16*†	219 ± 14*
CPT-1 (pkat/mg protein)	20.0 ± 0.6	9.3 ± 0.9*†	20.7 ± 1.1

Data are the mean ± SEM ($n = 6$ per group). *$P < 0.05$ compared with the control diet (CD)-fed group; †$P < 0.05$ compared with the nutrient-supplemented SRD (SRD + T) group (Tukey's test).

Note, SRD + T rats were fed the SRD from Day 0 to Day 180 and received nutrient supplementation from Day 90 to Day 180.

ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; CPT-1, carnitine-palmitoyl transferase-1.

iod. The SRD group had significantly increased plasma TAG concentrations compared with the age-matched CD-fed group. A significant reduction in plasma TAG levels was observed in the SRD + T group, although the values in this group remained greater than those in the CD-fed group. Liver and skeletal muscle TAG content exhibited a similar pattern to that described for plasma TAG levels. Furthermore, when acetyl-L-carnitine and nicotinamide were administered for 70 days, a similar pattern was observed for plasma and liver TAG levels (data not shown).

Liver ACC, FAS and CPT-1 enzyme activities

As indicated in Table 3, *de novo* lipogenesis enzyme activities were significantly increased in SRD- compared with CD-fed rats. The ACC and FAS activity was significantly reduced in the SRD + T compared with SRD group, but were still higher than activity in CD-fed rats. Conversely, mitochondrial CPT-1 activity, which is related to fatty acid oxidation, was significantly reduced in the SRD-fed group compared with activity in the age-matched CD-fed group. The activity of CPT-1 in the SRD + T group was similar to that in the CD group.

DISCUSSION

Feeding normal rats an SRD is a well-established model of dyslipidaemia, insulin resistance and visceral adiposity. In the present study, we investigated whether mitochondrial nutrient administration could reverse or improve these metabolic abnormalities caused by feeding of the SRD. There were several new findings observed in SRD-fed rats after 90 day nicotinamide and acetyl-L-carnitine administration. First, dyslipidaemia and liver steatosis were significantly reduced by nutrient administration. The increased hepatic *de novo* lipogenic enzyme activities (ACC and FAS) observed in SRD-fed rats was significantly reduced by nutrient treatment, although the values remained higher than those in CD-fed rats; however, CPT-1 activity, a key enzyme involved in mitochondrial fatty acid oxidation, was similar to that in control animals. Furthermore, nicotinamide and acetyl-L-carnitine administration significantly decreased skeletal muscle TAG content. Second, nutrient treatment improved glucose intolerance in response to a glucose challenge, which was accompanied by plasma glucose level normalization without changes in pancreatic insulin content. Moreover, after nicotinamide and acetyl-L-carnitine treatment, visceral adiposity and bodyweight gain were significantly reduced in SRD-fed rats.

Studies assessing mitochondrial nutrient administration to animals alone or in combination suggest that these nutrients improve lipid and glucose metabolism and decrease bodyweight gain. In this regard, Mingorance *et al.*[26] reported that dietary supplementation of propionyl-L-carnitine for 20 weeks partially corrected hyperinsulinaemia, liver TAG content and insulin resistance in obese, insulin-resistant Zucker rats. Power *et al.*[27] reported that supplementing obese mice with L-carnitine reduced lipid overload and glucose intolerance by enhancing the mitochondrial efflux of excess acyl groups in insulin-responsive tissues. Moreover, Shen *et al.*[28] demonstrated that R-α lipoic acid, acetyl-L-carnitine, nicotinamide and biotin treatment in combination improved glucose tolerance, decreased basal insulin secretion and plasma FFA levels, did not increase bodyweight and helped maintain mitochondrial biogenesis in Type 2 diabetic Goto-Kakizaki rat skeletal muscle. The results of the present study add to the information obtained in these previous studies by demonstrating that treating SRD-fed rats with acetyl-L-carnitine and nicotinamide in combination decreases hepatic *de novo* lipogenic enzyme FAS and ACC activity and enhances mitochondrial fatty acid oxidation CPT-1 activity, which significantly reduces liver steatosis and dyslipidaemia in this animal model. Furthermore, the reduced lipogenesis and increased fatty acid oxidation in the SRD + T group suggest that nicotinamide and acetyl-L-carnitine play a putative role in regulating mitochondrial biogenesis.

Several studies in rats and humans[5,15,29] have demonstrated that insulin resistance is strongly correlated with local TAG and long-chain acyl-CoA accumulation within skeletal muscle. In this regard, we have reported impaired glucose oxidation and insulin sensitivity, as well as increased intracellular TAG and long-chain acyl-CoA content, in the skeletal muscle of rats fed an SRD for 6–8 months.[15,29] In the present study, acetyl-L-carnitine and nicotinamide administration significantly decreased the TAG content of skeletal muscle from SRD-fed rats. The reduced plasma lipid availability because of decreased plasma TAG and FFA levels induced by these nutrients, among other possible mechanisms, could contribute to these outcomes. Recent evidence suggests that mitochondrial fatty acid overload causes incomplete β-oxidation and intramuscular accumulation of lipotoxic metabolites, which may contribute to mitochondrial dysfunction and insulin resistance. Therefore, carnitine-mediated lipid sequestration may have promoted mitochondrial performance as well as insulin signalling.[30,31] Moreover, Zhang *et al.*[32] reported enhanced glucose uptake in TNF-α-treated rat L6 myoblasts cell culture after the addition of acetyl-L-carnitine, suggesting that acetyl-L-carnitine inhibits TNF-α-induced insulin resistance through the AMP-activated protein kinase pathway in skeletal myocytes. Furthermore, the impaired glucose homeostasis in SRD-fed rats was completely normalized and was accompanied by a significant improvement in dyslipidaemia. Interestingly, the low plasma insulin : glucose ratio observed in SRD-fed rats in the present study was significantly increased in the SRD + T group.

The improvement in glucose intolerance in response to an i.v. glucose challenge observed in the SRD + T group, despite significantly lower K_g values compared with the CD-fed group, suggests that the administration of these nutrients ameliorated the

© 2012 The Authors
Clinical and Experimental Pharmacology and Physiology © 2012 Wiley Publishing Asia Pty Ltd

impaired insulin sensitivity in chronic SRD-fed rats. However, pancreatic insulin storage was similar in the three dietary groups. The results of the present study do not address possible mechanisms underlying the mitochondrial nutrient treatment-mediated normalization of basal plasma glucose levels and improved i.v. glucose tolerance in this experimental model. However, plasma insulin levels were similar after 70 days of nicotinamide and acetyl-L-carnitine administration and only a moderate increase in insulin levels was observed after 90 days of treatment, suggesting that these rats were still insulin resistant. However, recent publications that analysed the effects of carnitine and acetyl-L-carnitine alone or in combination with nicotinamide may provide mechanistic clues. For example, Yoshikawa *et al.*[33] reported that oral administration of zinc–carnitine reduced glucose levels and improved glucose tolerance in Type 2 diabetic KK-A^y mice. Carnitine supplementation also promoted glucose oxidation in diabetic mice.[27] Cresto *et al.*[14] recently demonstrated that acetyl-L-carnitine and nicotinamide administration improved bodyweight, glucose and plasma insulin levels and insulin release from perfused pancreatic slices in multiple low-dose streptozotocin (STZ)-induced diabetic mice. Furthermore, treatment normalized the index of insulin-immunopositive β-cells as well as β-cell size. In Sprague-Dawley rats, nicotinamide treatment prevented STZ-induced diabetes. Fasting blood glucose, serum insulin and C-peptide levels were all within the normal range in the nicotinamide-treated group.[34] The nicotinamide protection of β-cells may be facilitated by inhibition of apoptosis and nitric oxide generation.[34] Shima *et al.*[35] demonstrated an improvement of fed hyperglycaemia after nicotinamide treatment, which was accompanied by increased β-cell mass in pancreatectomized Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Mingrone *et al.*[9] reported that both L-carnitine and acetyl-L-carnitine improve insulin-mediated glucose disposal in healthy subjects and patients with type 2 diabetes.

In the present study, acetyl-L-carnitine and nicotinamide treatment decreased weight gain and visceral adiposity in SRD-fed rats. Similarly, Amin *et al.*[36] observed that L-carnitine treatment improved obesity in high-fat-fed white male albino rats, whereas Mingorance *et al.*[26] reported reduced bodyweight and adiposity in obese Zucker rats after oral propionyl-L-carnitine supplementation.

In conclusion, the effects of combined acetyl-L-carnitine and nicotinamide administration in a dyslipidaemic, insulin-resistant rat model suggest that reducing hepatic lipogenesis and enhancing fatty acid oxidation may improve dyslipidaemia, liver steatosis, muscle lipotoxicity and visceral adiposity. Furthermore, these nutrients improved whole-body peripheral glucose utilization in response to glucose challenge and normalized plasma glucose levels.

ACKNOWLEDGEMENTS

This work was supported financially by grants Proyecto de Investigación Ciencia y Tecnología (PICT) PICT 05-38157 and Banco Interamericano de Desarrollo (BID) BID 1728 OC/AR from the Agencia Nacional de Promoción Científica Tecnológica, Fundación Alberto J Roemmers and Laboratory Beta SA. The authors thank Drs JJ Anderson and R Gabach Laboratorio Beta SA, Buenos Aires, Argentina for providing the Neurex and Mr Walter Da Ru Department of Biochemistry, School of Biochemistry, Litoral University, Santa Fe, Argentina, for technical assistance.

REFERENCES

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; **27**: 1047–53.
2. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995–2025: Prevalence, numerical estimates, and projections. *Diabetes Care* 1998; **21**: 1414–31.
3. Kraegen EW, Cooney GJ. The role of free fatty acids in muscle insulin resistance. In: Marshall SM, Home PD, Rizza RA (eds). *The Diabetes Annual/12*. Elsevier Science BV, Amsterdam, 1999; pp. 141–50.
4. Lewis GF, Carpentier A, Adeli K, Giacca A. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr. Rev.* 2002; **23**: 201–29.
5. Hegarty BD, Furler SM, Ye J, Cooney GJ, Kraegen EW. The role of intramuscular lipid in insulin resistance. *Acta Physiol. Scand.* 2003; **178**: 373–83.
6. Petersen KF, Befroy D, Dufour S *et al.* Mitochondrial dysfunction in the elderly: Possible role in insulin resistance. *Science* 2003; **300**: 1140–2.
7. Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes* 2002; **51**: 2944–50.
8. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N. Engl. J. Med.* 2004; **350**: 664–71.
9. Mingrone G. Carnitine in type 2 diabetes. *Ann. N.Y. Acad. Sci.* 2004; **1033**: 99–107.
10. Giancaterini AGA, De Gaetano A, Mingrone G *et al.* Acetyl-L-carnitine infusion increases glucose disposal in type 2 diabetic patients. *Metabolism* 2000; **49**: 704–8.
11. Lee WJ, Song KH, Koh EH *et al.* Alpha-lipoic acid increases insulin sensitivity by activating AMPK in skeletal muscle. *Biochem. Biophys. Res. Commun.* 2005; **332**: 885–91.
12. Velykyi MM, Burda VA, Bironi NV, Oliiarnyk OD, Velykyi AM. The effect of nicotinamide on the enzymatic activity of the antioxidant defense in experimental diabetes. *Ukr. Biokhim. Zh.* 1996; **68**: 109–14.
13. Unger RH. Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes* 1995; **44**: 863–70.
14. Cresto JC, Fabiano de Bruno LE, Cao GF *et al.* The association of acetyl-L-carnitine and nicotinamide remits the experimental diabetes in mice by multiple low-dose streptozotocin. *Pancreas* 2006; **33**: 403–11.
15. Chicco A, D'Alessandro ME, Karabatas L, Pastorale C, Basabe JC, Lombardo YB. Muscle lipid metabolism and insulin secretion are altered in insulin-resistant rats fed a high sucrose diet. *J. Nutr.* 2003; **133**: 127–33.
16. Pighin D, Karabatas L, Rossi A, Chicco A, Basabe JC, Lombardo YB. Fish oil affects pancreatic fat storage, pyruvate dehydrogenase complex activity and insulin secretion in rats fed a sucrose-rich diet. *J. Nutr.* 2003; **133**: 4095–101.
17. Ferreira MR, Lombardo YB, Chicco A. β-Cell adaptation/dysfunction in an animal model of dyslipidemia and insulin resistance induced by the chronic administration of a sucrose-rich diet. *Islets* 2010; **2**: 367–73.
18. Chicco A, Basabe JC, Karabatas L, Ferraris N, Fortino A, Lombardo YB. Troglitazone (CS-045) normalizes hypertriglyceridemia and restores the altered patterns of glucose-stimulated insulin secretion in dyslipidemic rats. *Metabolism* 2000; **49**: 1346–51.
19. Davoren PR. The isolation of insulin from a single cat pancreas. *Biochim. Biophys. Acta* 1962; **63**: 150–3.

© 2012 The Authors
Clinical and Experimental Pharmacology and Physiology © 2012 Wiley Publishing Asia Pty Ltd

20. Lombardo YB, Chicco A, D'Alessandro ME, Martinelli M, Soria A, Gutman R. Dietary fish oil normalize dyslipidemia and glucose intolerance with unchanged insulin levels in rats fed a high sucrose diet. *Biochim. Biophys. Acta* 1996; **1299**: 175–82.
21. Gutman RA, Basilico MZ, Bernal C, Chicco A, Lombardo YB. Long-term hypertriglyceridemia and glucose intolerance in rats fed chronically an isocaloric sucrose-rich diet. *Metabolism* 1987; **36**: 1013–20.
22. Zimmermann R, Haemmerle G, Wagner EM, Strauss JG, Kratky D, Zechner R. Decreased fatty acid esterification compensates for the reduced lipolytic activity in hormone-sensitive lipase-deficient white adipose tissue. *J. Lipid Res.* 2003; **44**: 2089–99.
23. Halestrap AP, Denton RM. Insulin and the regulation of adipose tissue acetyl-coenzyme A carboxylase. *Biochem. J.* 1973; **132**: 509–17.
24. Karlic H, Lohninger S, Koeck T, Lohninger A. Dietary L-carnitine stimulates carnitine acyltransferases in the liver of aged rats. *J. Histochem. Cytochem.* 2002; **50**: 205–12.
25. Snedecor GWP, Cochran WG. *Statistical Methods*. Iowa State University Press, Ames. 1967.
26. Mingorance C, del Gonzalez PM, Dolores HM, Alvarez de SM. Oral supplementation of propionyl-L-carnitine reduces body weight and hyperinsulinaemia in obese Zucker rats. *Br. J. Nutr.* 2009; **102**: 1145–53.
27. Power RA, Hulver MW, Zhang JY *et al.* Carnitine revisited: Potential use as adjunctive treatment in diabetes. *Diabetologia* 2007; **50**: 824–32.
28. Shen W, Hao J, Tian C *et al.* A combination of nutriments improves mitochondrial biogenesis and function in skeletal muscle of type 2 diabetic Goto-Kakizaki rats. *PLoS ONE* 2008; **3**: e2328.
29. D'Alessandro ME, Chicco A, Lombardo YB. A long-term sucrose-rich diet increases diacylglycerol content and membrane nPKCθ expression and alters glucose metabolism in skeletal muscle of rats. *Nutr. Res.* 2006; **26**: 289–96.
30. Kelley DE, Mandarino LJ. Fuel selection in human skeletal muscle in insulin resistance: A reexamination. *Diabetes* 2000; **49**: 677–83.
31. Koves TR, Li P, An J *et al.* Peroxisome proliferator-activated receptor-gamma co-activator 1 alpha-mediated metabolic remodeling of skeletal myocytes mimics exercise training and reverses lipid-induced mitochondrial inefficiency. *J. Biol. Chem.* 2005; **280**: 33588–98.
32. Zhang Z, Zhao M, Li Q, Zhao H, Wang J, Li Y. Acetyl-L-carnitine inhibits TNF-alpha-induced insulin resistance via AMPK pathway in rat skeletal muscle cells. *FEBS Lett.* 2009; **583**: 470–4.
33. Yoshikawa Y, Ueda E, Sakurai H, Kojima Y. Anti-diabetes effect of Zn(II)/carnitine complex by oral administration. *Chem. Pharm. Bull.* 2003; **51**: 230–1.
34. Alenzi FQ. Effect of nicotinamide on experimental induced diabetes. *Iran. J. Allergy Asthma Immunol.* 2009; **8**: 11–18.
35. Shima K, Zhu M, Kuwajima M. A role of nicotinamide-induced increase in pancreatic beta-cell mass on blood glucose control after discontinuation of the treatment in partially pancreatectomized OLETF rats. *Diabetes Res. Clin. Pract.* 1998; **41**: 1–8.
36. Amin KA, Nagy MA. Effect of carnitine and herbal mixture extract on obesity induced by high fat diet in rats. *Diabetol. Metab. Syndr.* 2009; **1**: 17.

© 2012 The Authors
Clinical and Experimental Pharmacology and Physiology © 2012 Wiley Publishing Asia Pty Ltd