Acetyl-L-Carnitine Infusion Increases Glucose Disposal in Type 2 Diabetic Patients

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Little information is available in the literature on the effect of L-carnitine to improve glucose disposal in healthy control subjects and type 2 diabetic patients. No data are reported on the pharmacological properties of acetyl-L-carnitine (ALC) in type 2 diabetes mellitus. The present study evaluates glucose uptake and oxidation rates with either ALC or placebo administration in 18 type 2 diabetic patients. On different days, each patient received both a primed-constant infusion of ALC (5 mg/kg body weight [BW] priming bolus and either 0.025, 0.1, or 1.0 mg/kg BW/min constant infusion) and a comparable placebo formulation. During the infusion period, continuous indirect calorimetric monitoring and a euglycemic-hyperinsulinemic clamp (EHC) study were performed. The total end-clamp glucose tissue uptake (M value) was significantly increased by the administration of ALC (from 3.8 to 5.2 mg/kg/min, $P = .006$), and the dose dependence of this effect reached borderline statistical significance ($P = .037$). The increase in the M/I ratio was also highly significant after ALC administration (from 3.9 to 5.8×10^{-2} mg/kg/min/(μUI/mL, $P < .001$), while no statistically significant effect was attributable to the different dosages. The increase in the M value was related to increased glucose storage (highly significant effect of ALC) rather than increased glucose oxidation (no statistical significance). In conclusion, the effect of ALC on glucose disposal has no relationship to the amount administered. This could be due to an effect of ALC on the enzymes involved in both the glycolytic and gluconeogenetic pathways, and a possible reversibility of glycogen synthase inhibition in diabetic subjects.

IN THE EARLY 1960s, Randle et al[1] described a negative relationship between the free fatty acid (FFA) concentration and glucose oxidation in the isolated rat heart, which later gave rise to the concept of the FFA-glucose cycle, or Randle's cycle. Although the original hypothesis concerned the competition of these two energy substrates for access to their common oxidative pathway, ie, the tricarboxylic acid cycle, Felber et al[2] later extended this concept, showing that enhanced FFA oxidation inhibited the nonoxidative glucose metabolism in skeletal muscle.

The two major sources of intramitochondrial acetylcoenzyme A (acetyl-CoA) are pyruvate, produced from glycolysis in the cytosol and transported through the mitochondrial membranes, and FFA oxidation, which occurs within the mitochondria. Pyruvate decarboxylation is the irreversible key step in carbohydrate oxidation and is catalyzed by pyruvate dehydrogenase (PDH). In diabetic patients, a decrease in PDH activity leading to a decrease in the rate of glucose uptake and oxidation was found.

The major site at which fatty acids inhibit glucose oxidation is at the level of PDH. This enzyme is regulated by a phosphorylation-dephosphorylation cycle, with a PDH complex (PDC) kinase inhibiting PDC and a PDC phosphatase activating it.[3] The PDC kinase is activated by an increase in the intramitochondrial acetyl-CoA/CoA and NADH/NAD ratios. Consequently, increasing fatty acid levels will decrease the activity of PDC, resulting in a dramatic decrease in glucose oxidation.

Fatty acids are activated in the cytosol to long-chain acyl-CoA by acyl-CoA synthase. Acyl-CoA is transferred into the mitochondria by a complex of enzymes involving carnitine palmitoyltransferase-I ([CPT-I] the key regulator in the oxidation of long-chain fatty acids[4]), carnitine translocase, and CPT-II. Malonyl-CoA, which is produced in the cytosol by acetyl-CoA carboxylase, is a potent inhibitor of CPT-I.

Carnitine also participates in reversible *trans*-esterification reactions, forming acetylcarnitine via the carnitine acetyltransferase pathway.[5] By decreasing the intramitochondrial acetyl-CoA/CoA ratio through the trapping of acetyl groups, carnitine stimulates PDH activity and increases pyruvate oxidation and hence glucose oxidation.[6-9] The change in the acetyl-CoA/CoA ratio correlates with the efflux of acetylcarnitine through the mitochondria,[10,11] with a consequent increase in the activity of carnitine acyltransferase present on mitochondrial membranes.[9]

Therefore, acetyl-L-carnitine (ALC) might be regarded as a modulator of fuel substrate utilization in cells due to the role of carnitine in the metabolism of both lipids and carbohydrates.

Few studies[12,13] have evaluated the effect of carnitine on insulin sensitivity in type 2 diabetic patients, and show that whole-body glucose uptake and storage are enhanced by carnitine administration. Conversely, no information is available in the literature for such patients on the glucose fate during intravenous infusion of ALC, a naturally occurring derivative of carnitine. Since the possibility of therapeutically improving insulin resistance is currently minimal, it is important to quantify the effect of ALC on glucose metabolism in diseases such as type 2 diabetes mellitus in which an insulin resistance condition is frequently reported.

In the present study, we investigated the effect of a primed-constant infusion of different doses of ALC or placebo on glucose disposal as evaluated with the euglycemic-hyperinsulinemic clamp (EHC) and indirect calorimetric measurements.

SUBJECTS AND METHODS

Subjects

The study sample consisted of 18 type 2 diabetic patients. They were randomly assigned to 3 active-drug doses by means of 18 shuffled pairs

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of drug vials marked by a progressive number and day 1/day 2 in a double-blind fashion both for dosage and for order, ie, active/placebo. Anthropometric characteristics of the subjects are reported in Table 1. In all subjects, body composition was determined by the Hume and Weyers formula[14] to calculate total body water (TBW) in liters as follows: TBW in males = (0.2968 × weight in kg) + (0.1948 × height in cm) − 14.0129 and TBW in females = (0.1838 × weight in kg) + (0.3446 × height in cm) − 35.2701. Fat-free mass (FFM) was then computed by dividing TBW by 0.73. Fat mass (FM) was calculated as the difference between body weight (BW) and FFM.

The subjects were clinically euthyroid, had no stigmata of renal, cardiac, or hepatic dysfunction, and were not treated with agents (other than those for diabetes) able to affect carbohydrate or insulin metabolism. Diabetes was treated in the subjects with a therapeutic scheme consisting of 3 tablets per day of oral hypoglycemic agents ([OHAS] glibenclamide 2.5 mg + metformin 400 mg) plus a bedtime dose (15 to 20 UI) of human intermediate-acting insulin (Protaphane, Novo Nordisk, Gentofte, Denmark) over a 2- to 4-month period. One week before the experimental sessions, OHAs were suspended and blood glucose was controlled with rapid-acting insulin (Actrapid; Novo Nordisk) before the principal meals, maintaining the bedtime dose of intermediate-acting insulin as specified.

The subjects were studied in the postabsorptive state after a 12- to 14-hour overnight fast. In all patients, a blood sample was collected prior to the EHC study for determination of insulin by a radioimmunoassay method testing the ability of unlabeled insulin to displace the insulin tracer (3-^{125}Iodotyrosyl^{A14}Insulin; Pharmacia Biotech, Uppsala, Sweden) to exclude the presence of insulin antibodies, according to the method described by Savola et al.[15] Glycemia in the patients was maintained less than 100 mg/dL by small bolus doses of short-acting human insulin (Actrapid; Novo Nordisk) until the beginning of the study. All subjects consumed a weight-maintenance diet consisting of at least 250 g carbohydrate per day for 1 week before the study.

The study was performed according to the Declaration of Helsinki and the guidelines of the Institutional Review Board of the Catholic University in Rome. Written informed consent was obtained from all subjects before enrollment.

Experimental Protocol

All subjects were admitted to the Department of Metabolic Diseases of the Catholic University in Rome at 7 PM of the day before the study. At about 7 AM on the following morning, indirect calorimetric monitoring was started (time −30 minutes): the infusion catheter was inserted into an antecubital vein, the sampling catheter was introduced in the contralateral dorsal hand vein, and this hand was kept in a heated box (60°C) to obtain arterialized blood. Thirty minutes later (time 0 minutes), the primed-constant infusion of either ALC or placebo was started and continued over 180 minutes. After 1 hour (time 60 minutes), the EHC, according to De Fronzo et al,[16] has begun. A priming dose of short-acting human insulin was given during the initial 10 minutes in a logarithmically decreasing amount, to acutely increase serum insulin to the desired concentration. Insulinemia was then maintained constant with a continuous infusion of insulin at a rate of 40 mU/m^2/min for 110 minutes.

Table 1. Baseline Anthropometric and Metabolic Characteristics of the Subjects

Characteristic	Mean ± SD
No. of subjects	18
Sex ratio (male/female)	8/10
Age (yr)	52.3 ± 12
Weight (kg)	78.28 ± 15.8
Height (cm)	168 ± 12
BMI (kg · m^{-2})	27.46 ± 4.89
FFM (kg)	57.63 ± 11.08
FM (kg)	20 ± 6.75
EE (kJ/24 h)	
Saline infusion	6,794.6 ± 1,298
ALC infusion	6,810.8 ± 767
Fasting RQ	
Saline infusion	0.83 ± 0.04
ALC infusion	0.83 ± 0.02

Arterialized blood samples were collected every 20 minutes during the EHC study to measure the insulin concentration. During the EHC, the glucose level was monitored every 5 minutes and the infusion rate of a 20% glucose solution was adjusted following the algorithm detailed by De Fronzo et al.[16] Because serum potassium levels tend to decrease during this procedure, potassium chloride was administered during each study at a rate of 15 to 20 mEq/h to maintain serum potassium between 3.5 and 4.5 mEq/L.

ALC (Sigma Tau, Pomezia, Italy; as a preparation for intravenous administration) was continuously infused after a priming bolus (5 mg/kg BW) at a rate of 1 mg/kg/min (group A), 0.1 mg/kg/min (group B), and 0.025 mg/kg/min (group C). The subjects were restudied with the same scheme on a different day when saline solution was infused instead of ALC. The order of saline and ALC experiments was randomized.

The subjects voided before starting the study. Urine was collected during the infusion period and after the EHC for 24 hours to measure urinary nitrogen loss, which was used for calorimetric computations.

Respiratory gas exchange was measured by an open-circuit ventilated-hood system (monitor MBM-100, Deltatrac; Datex Instrumentarium, Helsinki, Finland). The energy expenditure (EE), respiratory quotient (RQ), and substrate oxidation rate were calculated from oxygen consumption, carbon dioxide production, and urinary nitrogen excretion according to the method of Ferrannini.[17]

Analytical Methods

The serum glucose level was measured by the glucose oxidase method using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Plasma insulin was analyzed by radioimmunoassay (RADIM, Pomezia, Italy). ALC levels were measured in plasma samples according to the method of Longo et al.[18]

Statistical Analysis

ANOVAs for repeated measures were performed on total tissue glucose uptake (M) at end-clamp, end-clamp insulinemia (I), their ratio (M/I), and end-clamp glucose storage (M-end-clamp glucose oxidation) using sex (male or female) and administered dosage (0.025, 0.1, and 1.0 mg/kg/min) as between-subject factors, ALC versus placebo as a within-subject factor, and anthropometric indices (body mass index [BMI], FFM, and age) as covariates. Nonsignificant covariates and factors were progressively eliminated to obtain final analyses for each dependent variable that include only simultaneously significant predictors.

A second type of repeated-measures design was used to investigate the effect of ALC administration on the calorimetrically obtained metabolic descriptors (EE, RQ, glucose oxidation [GOX], and lipid oxidation [LOX]). In this case, the averaged values of each metabolic descriptor during the half-hour before ALC or placebo administration (−30 through 0 minutes, T0), during the last half-hour of ALC or placebo administration before the clamp experiment (30 through 60 minutes, T1), and during the last hour of the clamp (120 through 180

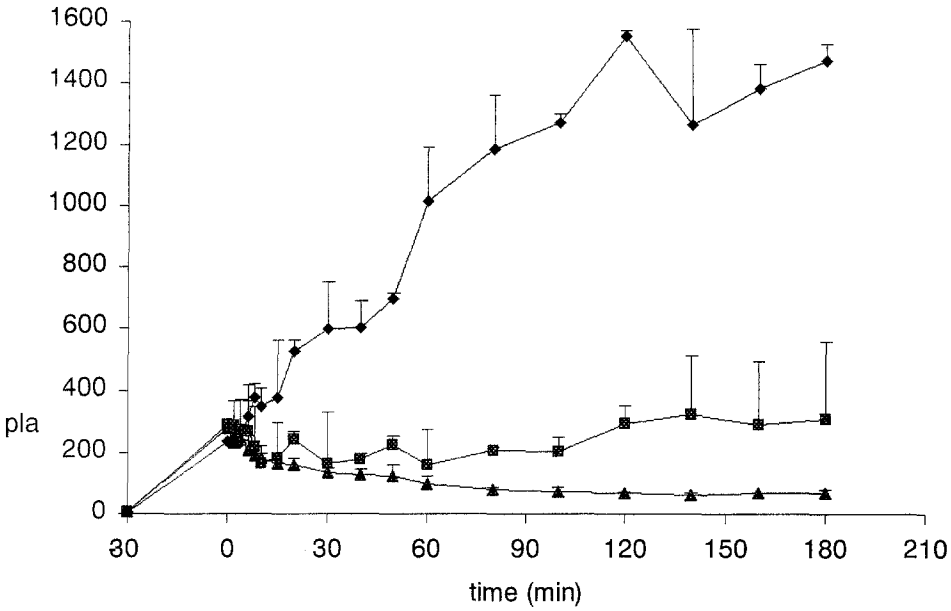

Fig 1. Time course of group-averaged ALC plasma concentrations after primed-constant infusion of 0.025 (▲), 0.1 (■), and 1.0 (◆) mg/min/kg BW.

minutes, T2) were obtained for each subject under both placebo (C0) and ALC (C1) administration. Nested within-subject factors were examined, nesting time (T0, T1, and T3) within ALC administration (C0 and C1). In this way, the effect of ALC on the change in these metabolic variables with ALC infusion at baseline and ALC infusion in the hyperinsulinemic state was assessed.

RESULTS

The time course of group-averaged ALC plasma concentrations during ALC primed-constant infusion at different rates in the 3 groups of patients (treated with 0.025, 0.1, and 1.0 mg/kg BW/min ALC infusion, respectively) is shown in Fig 1. Immediately before the primed infusions, the plasma ALC concentration was low and similar in all 3 groups (8.2 ± 2.71 nmol/mL, overall mean ± SD irrespective of subsequently administered dosage). Immediately after the priming bolus (equal to 5 mg/kg BW for all infusion rates), plasma ALC increased again to similar values in the 3 groups (267 ± 27.97 nmol/mL overall mean ± SD). With the infusion of ALC at different rates, the plasma concentration curves progressively stabilized to near–steady-state values, with a mean during the final hour of 65.84 ± 1.53 nmol/mL (0.025 mg/kg BW/min), 303.52 ± 13.57 nmol/mL (0.1 mg/kg BW/min), and 1,416.67 ± 125.76 nmol/mL (1.0 mg/kg BW/min).

During the EHC, steady-state plasma glucose was maintained close to the initial value, with a coefficient of variation of 3.0% to 3.8%.

Table 2 shows data obtained at steady state with both the ALC and placebo infusions separately by dosage for each subgroup of patients. The glucose uptake rate (M) was significantly different between experiments with and without ALC (overall difference, 3.8 to 5.2 mg/kg BW/min, $P = .006$); its dependency on the administered dose of ALC also showed statistical significance ($P = .037$). Similarly, there was a very highly significant increase in the M/I ratio due to ALC infusion (overall, from 3.8 to 5.9×10^{-2} mg/kg BW/min/(mUI/mL), $P < .001$), corresponding, on average, to an increase of about 55% over the corresponding placebo value. No statistically significant effect could be attributed to the different dosages with regard to the M/I ratio.

ALC infusion exerted a statistically highly significant effect on glucose storage (overall, from 1.80 to 3.05 mg/kg BW/min, $P = .005$). The dosage also showed a statistically significant effect ($P = .034$; see Table 2 for group averages).

A significant effect of ALC administration was not detected for either glucose or lipid oxidation. A small but significant reduction of plasma insulin levels could be attributed to ALC administration irrespective of dosage (from 112 to 97 μU/mL, $P = .004$). Data are reported separately for each patient subgroup in Table 3. No significant difference was found between placebo or ALC administration in each subgroup.

The repeated-measures ANOVA for the calorimetric data (EE, RQ, GOX, and LOX) did not show any significant effect of

Table 2. Metabolic Variables Measured Over the Last 40 Minutes of the EHC Study (mean ± SE)

Variable	Placebo (n = 18)	ALC Dose (mg/kg BW/min) 0.025 (n = 6)	0.1 (n = 6)	1.0 (n = 6)
M value (mg/kg BW/min)	3.81 ± 0.41	4.19 ± 0.84 (3.64 ± 0.60)	4.44 ± 0.56 (3.98 ± 0.90)	6.90 ± 1.34 (3.80 ± 0.74)
M/I ratio (mg/kg BW/min)/(μUI/mL)	0.0382 ± 0.0054	0.0460 ± 0.013 (0.0345 ± 0.012)	0.0632 ± 0.011 (0.0469 ± 0.011)	0.0684 ± 0.011 (0.0333 ± 0.004)
Glucose storage (mg/kg BW/min)	1.81 ± 0.36	2.47 ± 0.81 (1.87 ± 0.47)	2.08 ± 0.62 (1.76 ± 0.86)	4.61 ± 0.41 (1.79 ± 0.58)

NOTE. Data obtained with placebo infusion corresponding to each ALC dose are reported in parentheses.

Table 3. Plasma Insulin Measured Over the Last 40 Minutes of the EHC Study (mean ± SE)

	Plasma Insulin (μUI/mL)			
Treatment	Overall Population (N = 18)	0.025 mg/kg BW/min (n = 6)	0.1 mg/kg BW/min (n = 6)	1.0 mg/kg BW/min (n = 6)
Placebo	112 ± 35	129 ± 48	96 ± 24	109 ± 26
ALC	97 ± 34*	104 ± 49	85 ± 23	104 ± 28
Difference				
Absolute	−14 ± 18	−25 ± 25	−12 ± 12	−6 ± 11
Percent	−12 ± 14	−19 ± 19	−12 ± 12	−5 ± 11

NOTE. Data are provided as the absolute value and as the difference (absolute and percentage) between placebo and ALC administration. The overall mean plus the mean for each of 3 dosage groups are provided.

*$P = .004$ *v* placebo.

different ALC doses in changing the pattern of substrate utilization either under basal conditions or in the hyperinsulinemic state.

DISCUSSION

The present study reports the modifications of carbohydrate and lipid metabolism in type 2 diabetic patients induced by an artificial increase in the ALC plasma concentration. The main result of this study is that a higher plasma ALC level translates to a higher glucose uptake rate, while no significant effect could be demonstrated on glucose oxidation or lipid oxidation even with high-dose ALC.

The effect of ALC on some metabolic variables (such as insulin and the M/I ratio) was not uniformly larger with increasing dosage in the subjects examined. For other metabolic variables (such as the M value and glucose storage), the effect, although statistically significant, does not increase linearly with the dose. It is likely that this could partially depend on the current availability of the administered substance. A 40-fold increase in the ALC administration rate translates to only a 20-fold increase in its plasma concentration at steady state (Fig 1). Therefore, a 20-fold increase in the ALC plasma concentration corresponds to only a 4-fold increase in the effect on the metabolic variables.

Another point that should be taken into account is the current availability of ALC at the intracellular effector level. Two pools of acetyl-CoA molecules coexist in the cell: the larger one is located in the mitochondria and the smaller one in the cytosol. The mitochondrial acetyl-CoA pool derives directly from the β-oxidation of long-chain acyl-CoA and indirectly from pyruvate decarboxylation catalyzed by the PDC. ALC infusion very likely produces an increase in the acetyl-CoA cytosolic pool and simultaneously increases the ALC cellular concentration, forcing the passage of acetyl moieties across the mitochondrial membrane. Therefore, a back-inhibition of intramitochondrial acyl-CoA β-oxidation would occur with a subsequent reduction of acetyl-CoA formation. As observed in animals, the natural consequence of this process should be an inhibition of FFA oxidation and a simultaneous increase of glucose storage.[13]

The positive effect of ALC on glucose uptake independently of the amount administered suggests that this effect does not derive from the bulk delivery of acetyls to cells. Previous studies investigated the role of ALC in regulating enzyme action in mouse liver and muscle cells and chick embryo hepatocytes.[19,20] Hotta et al[21] showed that in mice with juvenile visceral steatosis, carnitine deficiency may affect the expression of gluconeogenic enzymes by increasing the level of glucokinase and L-type pyruvate kinase mRNA. Thus, an effect of carnitine on the expression of both glycolytic and gluconeogenic enzymes might be hypothesized.

Although we have not provided any data to show a direct correlation between ALC action and gene modulation, the ability of ALC to stimulate glucose uptake in type 2 diabetic patients in a dose-independent fashion seems to indirectly suggest a reversibility of the glycogen synthase inhibition found in diabetic subjects.[22-24] It has been hypothesized that there is a shift in substrate utilization from carbohydrates to lipids in type 2 diabetes, with a large proportion of the increase in lipid oxidation accounted for by an increase in intramuscular triglyceride mobilization.[25] Impaired PDH activity and increased β-oxidation have been described in these patients,[26] and low-dose ALC administration could therefore represent a feasible way to physiologically correct this enzymatic impairment of substrate utilization.

In conclusion, ALC definitely increases glucose utilization in type 2 diabetic patients as assessed by the EHC technique, without a dose-dependent effect. Further studies are needed to better clarify the mechanisms of ALC action on the enzymatic pathways in type 2 diabetic patients.

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