Ameliorating Hypertension and Insulin Resistance in Subjects at Increased Cardiovascular Risk

Effects of Acetyl-L-Carnitine Therapy

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Abstract—Insulin resistance, a key component of the metabolic syndrome, is a risk factor for diabetes mellitus and cardiovascular disease. Acetyl-L-carnitine infusion acutely ameliorated insulin sensitivity in type 2 diabetics with insulin resistance. In this sequential off-on-off pilot study, we prospectively evaluated the effects of 24-week oral acetyl-L-carnitine (1 g twice daily) therapy on the glucose disposal rate (GDR), assessed by hyperinsulinemic euglycemic clamps, and components of the metabolic syndrome in nondiabetic subjects at increased cardiovascular risk a priori segregated into 2 groups with GDR ≤7.9 (n=16) or >7.9 (n=16) mg/kg per minute, respectively. Baseline GDR and systolic blood pressure were negatively correlated (n=32; $P=0.001$; $r=-0.545$), and patients with GDR ≤7.9 mg/kg per minute had higher systolic/diastolic blood pressure than those with higher GDR. Acetyl-L-carnitine increased GDR from 4.89±1.47 to 6.72±3.12 mg/kg per minute ($P=0.003$, Bonferroni-adjusted) and improved glucose tolerance in patients with GDR ≤7.9 mg/kg per minute, whereas it had no effects in those with higher GDRs. Changes in GDR were significantly different between groups ($P=0.017$, ANCOVA). Systolic blood pressure decreased from 144.0±13.6 to 135.1±8.4 mm Hg and from 130.8±12.4 to 123.8±10.8 mm Hg in the lower and higher GDR groups, respectively ($P<0.05$ for both; $P<0.001$ overall) and progressively recovered toward baseline over 8 weeks posttreatment. Total and high molecular weight adiponectin levels followed specular trends. Diastolic blood pressure significantly decreased only in those with higher GDRs. Treatment was well tolerated in all of the patients. Acetyl-L-carnitine safely ameliorated arterial hypertension, insulin resistance, impaired glucose tolerance, and hypoadiponectinemia in subjects at increased cardiovascular risk. Whether these effects may translate into long-term cardioprotection is worth investigating. **(*Hypertension*. 2009;54:567-574.)**

Key Words: acetyl-L-carnitine ■ hypertension ■ metabolic syndrome ■ insulin resistance

Decreased insulin sensitivity (or insulin resistance) is a major risk factor for type 2 diabetes mellitus[1] and renal[2] and cardiovascular diseases.[3] Insulin resistance is a key component and, possibly, a pathogenic factor[4] of the metabolic syndrome, a clustering of hypertension, diabetes mellitus, dyslipidemia, and chronic inflammation, observed frequently in obese subjects and in subjects with a family history of diabetes mellitus and renal or cardiovascular disease. The syndrome is estimated to affect ≈30% of the adult population in Europe and ≤50% of subjects >60 years old in the United Kingdom.[5] The prevalence may vary from 10% to 50% according to different series and definitions[5] and increases with age and in urban areas.[6] Thus, with progressive population aging and urbanization, the syndrome is affecting a rapidly increasing proportion of subjects worldwide, which, in particular in developing countries, is expected to translate in a few years into an epidemic of chronic, noncommunicable diseases, such as type 2 diabetes mellitus and renal and cardiovascular diseases.[6]

In addition to genetic factors,[7] acquired and potentially treatable factors, such as decreased physical activity and obesity,[6,8] may play a central role in the pathogenesis of

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This trial has been registered at www.clinicaltrials.gov (identifier NCT00393770).

P.R., D.C., and G.R. participated in all stages of the study, made the initial interpretation of the study findings, and prepared the first draft of the report and the final article. G.L. participated to the study protocol finalization and, in cooperation with F.L., was in charge of patient care and monitoring. N.M. performed the statistical analyses. G.G. prepared the case record form and the database and participated in data handling. S.O. performed the leptin, resistin, and adiponectin measurements. All of the authors revised and approved the final version of the article.

This was a fully academic, independent study. The study was ideated, conducted, and internally monitored by the investigators of the Clinical Research Center for Rare Diseases "Aldo and Cele Daccò" of the Mario Negri Institute for Pharmacological Research. The sponsor had no involvement in data recording, analysis, and reporting.

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insulin resistance. Studies in the elderly,[9] in subjects with type 2 diabetes mellitus or obesity,[10] and in offspring of subjects with type 2 diabetes mellitus[11] consistently found that decreased insulin sensitivity is associated with a defect in mitochondrial oxidative phosphorylation secondary to accumulation of triglycerides and related lipids in muscle.[6] Conceivably, different biochemical changes in insulin-mediated signaling pathways may contribute to an impaired insulin-mediated glucose transport and metabolism that eventually results in insulin resistance and the clinical features of the metabolic syndrome.[6]

In the early 1990s, Capaldo et al[12] observed that whole body glucose use was acutely increased by L-carnitine intravenous infusion in insulin resistant subjects with type 2 diabetes mellitus. Subsequent studies showed that this effect is mediated by increased glucose storage and oxidative glucose use,[13] possibly through improved carnitine-mediated lipid metabolism. Indeed, carnitine acts as an obligatory cofactor for β-oxidation of fatty acids by facilitating the transport of long-chain fatty acids across the mitochondrial membrane as acylcarnitine esters. Additional mechanisms include a control action of carnitine on key enzymes involved in glucolysis and gluconeogenesis.[14,15] Similar effects have also been observed with acetyl-L-carnitine, a derivative product of carnitine available for oral administration[16] that, after ingestion, is in large part hydrolyzed to L-carnitine (half-life: 10 to 45 hours). The acetyl moiety is either rapidly used or stored as acyl-esters of varying chain length, in particular, in skeletal and cardiac muscles.[16] Thus, we hypothesized that chronic acetyl-L-carnitine therapy might help in achieving a sustained improvement of insulin-dependent glucose disposal and, consequently, of the cluster of functional and metabolic abnormalities associated with the insulin-resistant status. To address this issue we evaluated the effects of chronic supplementation of acetyl-L-carnitine on insulin sensitivity and several components of the metabolic syndrome in a cohort of subjects with insulin resistance. Subjects with normal or near-normal insulin sensitivity, but with clinical components of the metabolic syndrome, served as controls.

Methods

Participants

We included 21- to 58-year–old subjects expected to have a decreased insulin sensitivity because of the presence of ≥3 of the following risk factors: (1) first-degree relatives with type 2 diabetes mellitus (World Health Organization criteria); (2) body mass index ≥25 kg/m^2; (3) systolic or diastolic blood pressure (BP) ≥140 or ≥90 mm Hg, respectively, or concomitant antihypertensive therapy (see definitions); or (4) serum triglycerides ≥150 mg/dL.[5] Subjects with fasting morning blood glucose ≥125 mg/dL; serum creatinine >1.5 mg/dL; urinary protein excretion rate ≥0.5 g/24 hours or on concomitant treatment with steroids, nonsteroidal anti-inflammatory drugs, immunosuppressive agents, carnitine or carnitine derivative, or any drug that might directly affect insulin sensitivity and/or insulin secretion over the 6 months before the beginning of the study; and subjects unable to fully understand the purpose/risk of the study were excluded. All of the included subjects provided a written informed consent.

Study Design

This was an open, blind end point, longitudinal, sequential study primarily aimed at investigating the effect of acetyl-L-carnitine on insulin sensitivity in nondiabetic subjects at increased cardiovascular risk. Subjects and their doctors were aware of the study treatment, whereas nurses recording the BP values, technicians involved in laboratory tests, and statisticians performing final analyses were blinded to therapy.

After an initial screening evaluation, potentially eligible patients entered a 4-week run-in period, and those who fulfilled the selection criteria had a baseline physical evaluation and an ECG recording. A blood sample was collected for routine hematochemistry and to measure leptin, total and low-molecular-weight adiponectin, and resistin. Glucose disposal rate (GDR) was measured by an euglycemic hyperinsulinemic clamp and glucose tolerance by a standard oral glucose load. Then subjects entered a 24-week treatment period with 2 g/d of oral acetyl-L-carnitine (Nicetile, Sigma-Tau) given in 2 doses in the morning and in the evening, followed by a 16-week recovery period after active treatment withdrawal. BP was measured at screening evaluation (start run-in); at baseline (end run-in); at 1, 4, and 8 weeks after baseline; and every 8 weeks up to the study end. All of the other baseline evaluations were repeated at the end of the treatment period and 8 weeks after treatment withdrawal. No changes in diet or concomitant treatments were introduced throughout the whole study period. The study protocol was approved by the ethical committee of the Clinical Research Center and was conducted according to the Declaration of Helsinki Guidelines.

Assessments and Definitions

Blood Pressure

Trough systolic and diastolic (Korotkoff phase I/V) BPs were measured in the morning before treatment administration by use of an appropriate cuff with a sphygmomanometer and with the patient in a sitting position after ≥5 minutes of rest. Three measurements to the nearest 2 mm Hg were obtained, 2 minutes apart at each time point, and the average of the 3 measurements was recorded for statistical analyses. Each subject had BP measured on each occasion by the same operator with the same sphygmomanometer. Mean and pulse pressures were calculated by standard formulas.

Glucose Disposal Rate

The peripheral GDR was assessed by a hyperinsulinemic-euglycemic clamp, as described previously.[2] Briefly, insulin was infused at a constant rate of 2 mU·kg^{-1}·min^{-1}. Blood glucose concentration was allowed to decrease during the insulin infusion to 90±5 mg/dL, at which level it was maintained for 2 hours by a variable glucose infusion through an IVAC pump (IVAC 560). Whole-blood glucose concentration was assayed by the glucose-oxidized method every 5 minutes. During the last 30 minutes of the clamp, 3 blood samples were taken every 10th minute for insulin measurements to confirm a steady-state plasma insulin concentration. Because at the achieved plasma insulin concentration, the hepatic glucose production is totally suppressed, the amount of glucose required to maintain steady-state euglycemia was assumed to equal the total-body glucose disposal. Thus, total-body GDR was calculated as the mean of the glucose infusion rate during the last 30 minutes of the clamp and expressed as milligrams per minute per kilogram of body weight. We secondarily evaluated the effect of acetyl-L-carnitine on the GDR adjusted per kilogram of lean body mass (estimated by the equation proposed by James et al[17]).

Oral Glucose Tolerance Test

After an overnight fasting and a fasting morning blood glucose evaluation, patients were invited to drink a glucose solution containing 1.75 g of glucose per kilogram of body weight (up to a maximum dose of 75 g) within 5 minutes, and blood glucose was measured every 30 minutes up to 120 minutes after glucose administration. The area under the curve of blood glucose levels during the 120 minutes of observation was calculated and recorded for the analyses.

Laboratory Tests

Total adiponectin, leptin, and resistin plasma levels were measured by ELISA kit and high-molecular-weight adiponectin levels by enzyme immunoassay assay (Alpco Diagnostics), an assay validated

previously versus Western blot analysis.[18] Serum creatinine, potassium, and lipid concentrations and other routine laboratory parameters were measured by automatic analyzer Beckman Synchron CX9. Glycosylated hemoglobin was measured by high-performance liquid chromatography (normal laboratory range: 3.53% to 5.21%; Beckman System Gold Chromatograph).

Metabolic Parameters

The diagnosis of metabolic syndrome was based on World Health Organization criteria.[5] Impaired fasting glucose was defined as a fasting morning blood glucose concentration >100 mg/dL and <126 mg/dL[19] and impaired glucose tolerance as a blood glucose concentration >140 mg/dL and <200 mg/dL 2 hours after a standard oral glucose load.[19]

Sample Size Estimation

The primary aim of the study was to evaluate the treatment effect on GDR in patients with a baseline GDR ≤7.9 mg/kg per minute. The rationale for this approach rested on the evidence that, in a preliminary, explorative phase of the study in 10 consecutive subjects, 6-month treatment increased GDR by 25% from 4.9±1.6 to 6.1±1.6 mg/kg per minute in 4 subjects with baseline GDR ≤7.9 mg/kg per minute, whereas it had no appreciable effects in those with higher GDR at baseline. On the basis of these preliminary observations, we hypothesized that acetyl-L-carnitine therapy might be electively effective in ameliorating insulin sensitivity in subjects with more insulin resistance to start with. Thus, we aimed at testing the treatment effect in patients with a GDR <7.9 mg/kg per minute. To this purpose, we estimated that 16 subjects with baseline GDR ≤7.9 mg/kg per minute would have given the study an 80% power to detect as statistically significant (α=0.05, 2-tailed test) an expected 25% posttreatment increase in GDR. Assuming a 10% dropout rate, 17 subjects had to be included to have 16 subjects available for the analyses. Thus, we planned to include all of the consecutive eligible subjects until the target of 17 subjects with baseline GDR ≤7.9 mg/kg per minute was achieved. Subjects with baseline GDR >7.9 mg/kg per minute who were identified during the screening period were also included and served as controls for comparative analyses.

Statistical Analyses

Clinical characteristics of study subjects were described using mean±SD and percentages for continuous and categorical variables, respectively. Baseline characteristics of subjects with basal GDR ≤7.9 mg/kg per minute versus those with basal GDR >7.9 mg/kg per minute were compared using the χ^2 test, Fisher's exact test, unpaired *t* test, or Wilcoxon rank-sum test as appropriate.

Between-groups comparisons were carried out by means of ANCOVA, adjusting for baseline measurement. Within-group comparisons were assessed by means of repeated-measures ANOVA to examine treatment effects at different follow-up visits and baseline on BP, GDR, glucose tolerance, and laboratory tests. A $P<0.05$ was considered statistically significant. A Bonferroni correction was applied to adjust for multiple comparisons. All of the statistical analyses were performed using SAS version 9.1 (SAS Institute Inc).

Results

Study Subjects

Of 43 screened subjects, 36 fulfilled the selection criteria and entered the study; of them, 18 had a GDR ≤7.9 mg/kg per minute. After baseline evaluation, 4 subjects withdrew the consent to repeat the euglycemic hyperinsulinemic clamp and left the study. All of the remaining 32 patients completed all phases of the study (Table S1, please see http://hyper.ahajournals.org). Sixteen of them had basal GDR ≤7.9 mg/kg per minute. Most of subjects were adult men with a high prevalence of risk factors for cardiovascular disease and diabetes mellitus (Table 1).Baseline characteristics of withdrawn subjects were comparable to those of subjects completing the study (data not shown). At inclusion, 12 subjects (6 in each GDR group) were on antihypertensive therapy with an angiotensin-converting enzyme inhibitor (n=4), a β-blocker (n=3), a diuretic (n=2), an angiotensin receptor blocker (n=1),an α-blocker (n=1), and a fixed combination of a β-blocker with a diuretic (n=1). One subject was on lipid-lowering therapy with a statin. No change in these concomitant medications was introduced throughout the whole study period.

Subjects with GDR ≤7.9 mg/kg per minute compared with those with higher GDR more frequently satisfied the World Health Organization criteria for the metabolic syndrome and tended to be more frequently hypertensive and to have an higher prevalence of impaired fasting glucose or impaired glucose tolerance at inclusion. They also had higher systolic BP, serum uric acid, and triglycerides levels with lower high-density lipoprotein cholesterol levels. Consequently, they also had a significantly higher triglycerides/high-density lipoprotein cholesterol index (Table 1). At baseline evaluation, in the study group considered as a whole, there was a significant correlation between GDR and systolic BP (P=0.001; r=−0.545) and between GDR and high-molecular-weight adiponectin concentrations (P=0.001; r=−0.458).

Glucose Disposal Rate

In subjects with baseline GDR ≤7.9 mg/kg per minute, 6-month treatment with acetyl-L-carnitine significantly increased GDR by 37% from 4.89±1.47 to 6.72±3.12 mg/kg per minute (Bonferroni adjusted P=0.003 versus baseline). At the end of the recovery period, the GDR decreased to 6.31±2.53 mg/kg per minute and was still significantly higher than at baseline (Bonferroni adjusted P=0.021). In subjects with baseline GDR >7.9 mg/kg per minute, treatment had no appreciable effects (Table 2). Changes in GDR were significantly different between the 2 study groups at the end of the treatment period (P=0.017) but not at the end of the recovery period (P=0.352). Similar findings were found also when adjusting GDR values per kilogram of lean body mass (Table 2).

Glucose Tolerance and Other Metabolic Parameters

Consistent with the changes in GDR, in subjects with baseline GDR ≤7.9 mg/kg per minute, 6-month treatment with acetyl-L-carnitine significantly decreased blood glucose concentration at 60 and 90 minutes, as well as the area under the curve of blood glucose levels from 0 to 120 minutes after a standard glucose oral load, an effect that persisted even at the end of the recovery period (Figure 1). In subjects with baseline GDR >7.9 mg/kg per minute, treatment had no appreciable effects (Figure 1).

Four of the 13 subjects who satisfied the World Health Organization criteria for the metabolic syndrome at inclusion in the lower GDR group at the end of the study did not satisfy these criteria any longer. Consistently, all 4 of the patients who had an impaired glucose tolerance at inclusion in the lower GDR group recovered a normal glucose tolerance at the end of the treatment period. Three of these patients again showed an impaired glucose tolerance at the end of the

Table 1. Baseline Characteristics of the Study Subjects

Characteristic	Overall (n=32)	Basal GDR ≤7.9 mg/kg per min (n=16)	Basal GDR >7.9 mg/kg per min (n=16)	*P**
Age, y	44.3±9.3	45.1±9.1	43.4±9.8	0.6124
Male gender	22 (68.7)	12 (75.0)	10 (62.5)	0.4456
Current/former smoking	13 (40.6)	9 (56.2)	4 (25.0)	0.0719
Family history				
Type 1 diabetes mellitus	7 (21.9)	2 (12.5)	5 (31.2)	0.3944
Type 2 diabetes mellitus	32 (100)	16 (100)	16 (100)	…
Hypertension	28 (87.5)	13 (81.2)	15 (93.7)	0.5996
CVD+cerebrovascular disease	19 (59.4)	9 (56.2)	10 (62.5)	0.7189
Chronic kidney disease	8 (25.0)	6 (37.5)	2 (12.5)	0.2200
Clinical features				
Impaired fasting glucose	13 (40.6)	9 (56.3)	4 (25.0)	0.0719
Impaired glucose tolerance	4 (12.5)	4 (25.0)	0	0.1012
Hypertension	17 (53.1)	11 (68.8)	6 (37.5)	0.0765
Systolic BP, mm Hg	137.4±14.4	144.0±13.6	130.8±12.4	0.0072
Diastolic BP, mm Hg	83.9±10.1	86.6±9.8	81.1±10.0	0.1255
Body mass index, kg/m^2	31.5±3.9	32.7±4.9	30.3±2.0	0.0861
Metabolic syndrome	18 (56.3)	13 (81.3)	5 (31.3)	0.0044
Laboratory parameters				
Fasting blood glucose, mg/dL	95.8±14.4	96.8±16.8	94.8±12.1	0.7103
Total cholesterol, mg/dL	214.9±32.1	216.6±33.6	213.1±31.7	0.7735
HDL cholesterol, mg/dL	50.1±10.2	46.3±8.7	54.0±10.4	0.0359
LDL cholesterol, mg/dL	148.2±26.1	148.6±27.7	147.8±25.5	0.9327
Triglycerides, mg/dL	136.3±123.3	185.7±152.1	86.8±56.0	0.0297
Triglycerides/HDL	3.0±3.2	4.4±4.1	1.7±1.1	0.0229
Creatinine, mg/dL	0.88±0.13	0.92±0.14	0.84±0.12	0.0703
Uric acid, mg/dL	5.4±1.3	6.3±1.0	4.5±0.9	<0.001
ESR, mm/h	5.0±4.3	6.6±5.3	3.5±2.6	0.0742
CRP, mg/dL	0.2±0.4	0.1±0.1	0.2±0.4	0.4027

Data are presented as mean±SD or n (%). HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; CVD, cardiovascular diseases; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

*Data are from χ^2 test, Fisher's exact test, unpaired *t* test, or Wilcoxon rank-sum test, as appropriate.

recovery period. No appreciable changes in any of the above parameters were observed in the higher GDR group.

Other Laboratory Parameters

In subjects with baseline GDR >7.9 mg/kg per minute and in the study group as a whole, total and high-molecular-weight adiponectin plasma levels significantly increased at the end of the treatment period compared with basal evaluations and decreased toward baseline values at the end of the recovery period. A similar but nonsignificant trend was observed in subjects with GDR ≤7.9 mg/kg per minute (Table 2). In the lower GDR group, serum triglyceride levels nonsignificantly decreased by 15% at the end of the treatment period compared with baseline and recovered toward baseline at the end of the recovery period. Changes in the triglyceride/high-density lipoprotein cholesterol index followed a similar trend. None of the other considered laboratory parameters changed appreciably at different time points compared with baseline in the 2 groups considered separately as well as in the study group considered as a whole (data not shown).

Arterial BP

In both groups with baseline GDRs ≤7.9 or >7.9 mg/kg per minute, systolic BP progressively and significantly decreased during the treatment period compared with basal evaluations and progressively increased toward baseline values during the recovery period (Figure S1 and Figure 2). At the same time points, diastolic BP decreased significantly in the higher GDR group, whereas the decrease failed to achieve the statistical significance in the lower GDR group. The mean BP showed a trend to decrease in both groups, whereas the pulse pressure significantly decreased only in the lower GDR group. All of the changes progressively waned during the recovery period (Figure S1 and Figure 2).

In the study group considered as a whole there was a progressive and consistent reduction in systolic BP that, at the end of the treatment period, averaged 8 mm Hg compared with baseline. Diastolic BP also tended to decrease during the treatment period, but the reduction failed to achieve the statistical significance. Both mean and pulse pressure progressively and significantly decreased compared with base-

Table 2. Main Clinical Parameters Before, at the End of Treatment With Acetyl-L-Carnitine, and at the End of the Recovery Period in 32 Subjects at High Risk to Develop Diabetes Mellitus, Either Divided According to Baseline GDR Values or Overall

	Basal GDR ≤7.9 mg/kg per min			Basal GDR >7.9 mg/kg per min			Overall		
Variable	Baseline	Treatment	Recovery	Baseline	Treatment	Recovery	Baseline	Treatment	Recovery
Week	0	24	32	0	24	32	0	24	32
BMI, kg/m^2	32.7±4.9	32.2±5.0*	32.2±5.0*	30.3±2.0	30.2±2.3	30.5±2.3	31.5±3.9	31.2±4.0	31.4±4.0
OGTT AUC, mg*min/dL	17 690±1884	15 339±2422†	14 902±2393†	14 696±3223	15 388±2901	15 244±3523	16 242±2990	15 363±2619	15 067±2947
BW, kg	95.5±16.6	94.1±16.8	93.9±16.9	83.7±10.7	84.1±10.5	84.9±10.8	89.6±15.0	89.3±14.7	89.6±14.8
GDR, mg/kg of BW per min	4.89±1.47	6.72±3.12†	6.31±2.53*	10.24±2.29	9.94±2.53	10.42±2.84	5.56±3.31	8.33±3.24	8.37±3.37
LBM, kg	62.3±9.0	62.0±9.1	62.0±9.1	57.0±9.4	57.7±9.1	57.9±9.1	59.7±9.5	59.9±9.2	60.0±9.2
GDR, mg/kg of LBM per min	3.21±0.99	4.47±2.11*	4.23±1.84*	6.93±1.47	6.82±1.74	6.95±1.82	4.90±2.31	5.47±2.27	5.39±2.30
Total adiponectin, meq/L	4.3±2.2	5.2±3.0	4.0±2.2	5.2±1.9	6.8±2.2	5.6±2.0	4.7±2.1	6.0±2.7*	4.8±2.3
Hmw adiponectin, meq/L	1.8±1.7	2.5±2.3	1.9±2.0	2.6±1.6	3.7±1.8*	2.7±1.3	2.2±1.6	3.0±2.1*	2.3±1.7
Plasma resistin, meq/L	4.2±1.2	4.0±1.4	4.5±2.0	3.6±1.4	4.3±1.4	3.9±1.0	3.9±1.3	4.1±1.3	4.2±1.6
Plasma leptin, IU/L	23.3±11.9	23.6±15.4	23.5±22.0	29.4±23.2	26.7±23.2	22.7±18.3	26.0±17.8	25.0±19.0	23.1±19.9

BMI indicates body mass index; OGTT, oral glucose tolerance test; AUC, area under the curve; Hmw, high molecular weight; BW, body weight; LBM, lean body mass; meq, milliequivalent.

*$P<0.05$ and †$P<0.01$ vs baseline (by repeated-measures ANOVA with Bonferroni adjustment for multiple comparisons).

line, a trend that was largely sustained by the progressive reduction in systolic BP. All of the changes marginally recovered at 8 weeks and fully recovered toward baseline at 16 weeks after treatment withdrawal (Figure S1).

Safety Profile

Treatment was well tolerated in all of the subjects. A first-degree atrioventricular block combined to a sinus bradycardia was observed in 1 subject during the treatment period. This abnormality persisted after treatment withdrawal and was considered to be unrelated to the study treatment.

Discussion

Here we found that, in subjects with a clustering of risk factors for diabetes mellitus and cardiovascular disease, 6-month oral supplementation with acetyl-L-carnitine significantly reduced the arterial BP, increased plasma adiponectin levels, and improved the overall cardiovascular risk profile: effects that progressively waned after treatment withdrawal. In those who were more insulin resistant at inclusion, treatment also ameliorated insulin sensitivity and glucose tolerance, an effect that was associated with a reduced prevalence of the metabolic syndrome. The above findings could not be explained by changes in diet, physical activity, or pharmacological therapy that were not modified throughout the whole study period, or in body weight and body mass index that were stable over time. Consistent with previous reports in other clinical settings,[20,21] treatment with acetyl-L-carnitine was remarkably well tolerated in all of the patients.

The present study was designed to formally test whether oral therapy with acetyl-L-carnitine ameliorates insulin sensitivity and, more in general, the overall cardiovascular risk profile in subjects at increased cardiovascular risk because of obesity, hypertension, hyper-triglyceridemia, and family history of type 2 diabetes mellitus. Data showed that treatment significantly increased insulin sensitivity in those with baseline GDR <7.9 mg/kg per minute but had no appreciable effects in those with normal or near-normal insulin sensitivity to start with. Consistently, glucose tolerance improved in those with more severe insulin resistance but did not change appreciably in those with better insulin sensitivity. These data are consistent with previous observations that intravenous acetyl-L-carnitine infusion acutely ameliorated insulin sensitivity in patients with type 2 diabetes mellitus and severe insulin resistance.[12,20] Here we provide the evidence that this effect can be achieved by chronic oral supplementation even in the absence of overt type 2 diabetes mellitus. The impaired glucose disposal and tolerance that we observed in our subjects with more insulin resistance at inclusion may reflect a shift in substrate use from carbohydrates to lipids.[22] This is common in patients with type 2 diabetes mellitus and is most likely explained by impaired pyruvate dehydrogenase activity and increased β-oxidation.[23] Chronic acetyl-L-carnitine supplementation might correct this unbalance by modulating the expression of glycolytic and gluconeogenic enzymes.[14,15,24] It would also ameliorate mitochondrial glucose oxidation, acting as a transport molecule for free fatty acids and as an important acetyl-group donor in high-energy metabolism and free fatty acid β-oxidation.[13,25]

Treatment with acetyl-L-carnitine was also associated with a significant BP-lowering effect that ensued progressively during 24-week treatment and slowly waned over 16 weeks after treatment withdrawal. Treatment was particularly effective on systolic BP that uniformly decreased in both study groups regardless of baseline GDR, whereas the diastolic BP significantly decreased only in subjects with less severe insulin resistance to start with. These findings extend to humans experimental evidence that chronic L-carnitine treatment significantly decreased systolic BP with less effect on diastolic BP in spontaneously hypertensive rats.[26] In harmony with our present findings, posthoc analyses of a recent study of combined acetyl-L-carnitine and α-lipoic acid therapy in subjects with coronary artery disease found a significant reduction in systolic (but not diastolic) BP in the subgroup with the features of the metabolic syndrome at inclusion.[27]

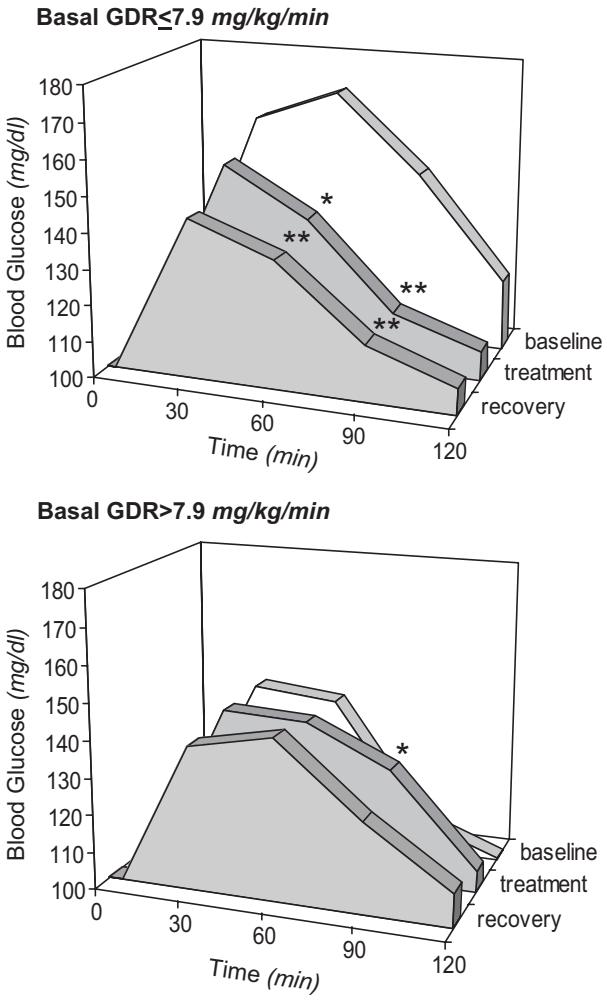

Figure 1. Blood glucose profiles over 120 minutes after a standard oral glucose load (oral glucose tolerance test) at baseline and at the end of the treatment and of the recovery periods in subjects with baseline GDR ≤7.9 mg/kg per minute (top) or >7.9 mg/kg per minute (bottom). *$P<0.05$ and **$P<0.01$ vs corresponding time points at baseline. Analyses were by repeated-measures ANOVA with Bonferroni correction for multiple comparisons.

However, it was impossible to establish whether BP reduction was explained by an effect of acetyl-L-carnitine, α-lipoic acid, or both. Thus, to the best of our knowledge, this is the first evidence that acetyl-L-carnitine therapy may have a clinically relevant antihypertensive effect in humans.

Of note, subjects with more insulin resistance at inclusion had remarkably higher BPs compared with those with normal or near-normal insulin sensitivity. Moreover, systolic BP and GDR were significantly correlated, and the highest BP levels were observed in subjects with more severe insulin resistance. This is consistent with epidemiological evidence that essential hypertension is strongly associated with insulin resistance[28] and with recent data that insulin resistance may precede and probably predispose to arterial hypertension.[29] Thus, amelioration of insulin sensitivity might explain, at least in part, the reduction in arterial BP achieved by acetyl-L-carnitine therapy in our study population. Other mechanisms, however, must be involved, because amelioration of insulin sensitivity was appreciable only in subjects who were more insulin resistant, whereas the BP was consistently reduced in all of the subjects, regardless of their GDR at inclusion. Of note, BP reduction was mirrored by a trend toward an increase in total and high-molecular-weight adiponectin levels that reached statistical significance in the study population as a whole and in subjects with higher GDR at inclusion and that waned after treatment withdrawal. We speculate that enhanced adiponectin bioavailability, possibly associated with enhanced NO production,[30,31] might play a role in acetyl-L-carnitine–induced BP reduction.[32,33] Regardless of the above, increasing adiponectin bioavailability might have clinical implications, because low adiponectin concentrations have been linked to myocardial infarction[34] and to the progression of subclinical coronary artery disease.[35] However, mechanisms of acetyl-L-carnitine–mediated increase in plasma adiponectin levels are unknown. A plausible speculation is that, as during physical exercise,[36] an improved oxidation of free fatty acids in skeletal muscle with a secondary increase in adiponectin expression[37] might be involved.

Limitations

First, this was a sequential off-on-off study without a placebo control arm. However, within each patient, data observed during the treatment period were compared with data recorded at baseline and after treatment withdrawal. Thus, each patient served as his own control, which allowed us to reasonably exclude that the study findings reflected an effect of chance. Second, the lack of a placebo treatment during the run-in and recovery periods does not allow us to definitely exclude some "trial effect." This effect, however, should result into a reduction in both systolic and diastolic BPs. Finding that acetyl-L-carnitine was associated with a remarkable reduction in systolic with only marginal changes in diastolic BP suggests that changes observed during the treatment period largely reflected a genuine effect of acetyl-L-carnitine. Third, patients were segregated a priori into 2 groups according to their GDR at inclusion. Thus, the increase in GDR observed in those with lower GDR at inclusion might partially reflect, at least in theory, a regression-to-the-mean phenomenon. The same phenomenon, however, should have resulted into a specular trend for GDR to decrease toward the mean in those with higher GDR to start with. Finding that the GDR was stable in these patients confirmed that different outcomes observed in the 2 groups reflected a different treatment effect and could not be explained by a regression to the mean. Fourth, in theory, inclusion of the 10 patients used for sample size estimation in final analyses might have increased the chances to detect a different treatment effect on the GDR between the 2 subgroups with different levels of insulin resistance at inclusion. This, however, could not affect the other findings, in particular, changes in BP and other variables that were observed in the study group as a whole, as well as in the 2 groups considered separately. Finally, this was a pilot, explorative study with a relatively small sample size. Study findings, however, may provide a sounding rationale for ad hoc studies to investigate the mechanisms involved in the BP-lowering effect of acetyl-L-carnitine and for adequately powered trials to

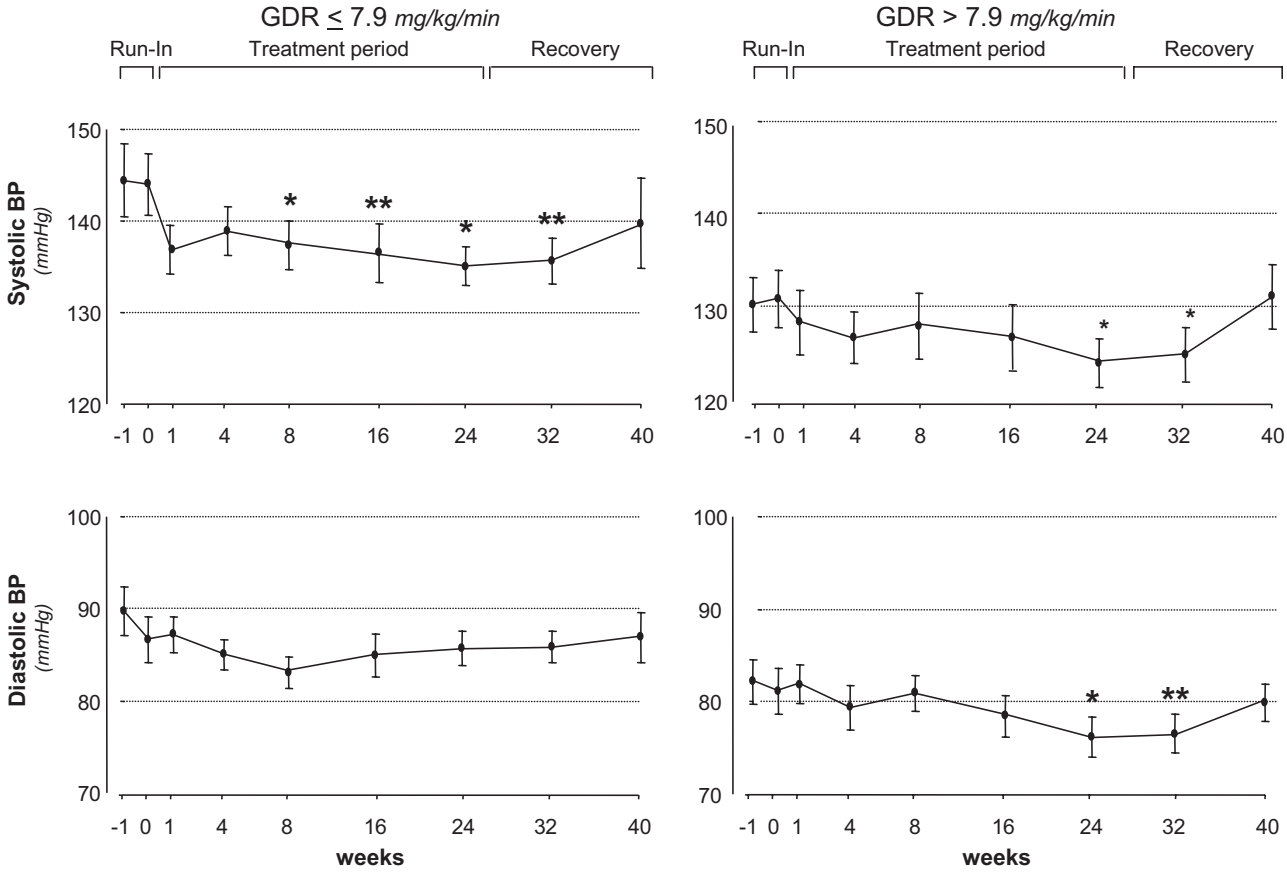

Figure 2. Systolic and diastolic BPs at different visits throughout the whole study period in subjects with baseline GDR ≤7.9 mg/kg per minute (left) or >7.9 mg/kg/min (right). *$P<0.05$ and **$P<0.01$ vs baseline. Analyses were by repeated-measures ANOVA.

assess the protective effects of chronic acetyl-L-carnitine therapy on target organs of hypertension and/or diabetes mellitus.

Perspectives

Chronic oral acetyl-L-carnitine supplementation ameliorated cardiovascular risk factors, such as arterial hypertension, insulin resistance, impaired glucose tolerance, and hypoadiponectinemia, in a cohort of subjects at increased cardiovascular risk and was well tolerated. Treatment with acetyl-L-carnitine appears to be particularly suitable for patients with obesity and/or type 2 diabetes mellitus, who often are either hypertensive and insulin resistant.[1,6,38] With the available armamentarium of antihypertensive medications, achieving recommended systolic BP targets is often difficult, in particular, in patients with type 2 diabetes mellitus and nephropathy.[39–41] Whether the availability of a novel therapeutic option that appears to be particularly effective in reducing the systolic BP may help limiting cardiovascular disease in this population is worth investigating.

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Disclosures

None.

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