ISSN 2472-1972

Blood Pressure and Metabolic Effects of Acetyl-L-Carnitine in Type 2 Diabetes: DIABASI Randomized Controlled Trial

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Context: Acetyl-L-carnitine (ALC), a mitochondrial carrier involved in lipid oxidation and glucose metabolism, decreased systolic blood pressure (SBP), and ameliorated insulin sensitivity in hypertensive nondiabetic subjects at high cardiovascular risk.

Objective: To assess the effects of ALC on SBP and glycemic and lipid control in patients with hypertension, type 2 diabetes mellitus (T2D), and dyslipidemia on background statin therapy.

Design: After 4-week run-in period and stratification according to previous statin therapy, patients were randomized to 6-month, double-blind treatment with ALC or placebo added-on simvastatin.

Setting: Five diabetology units and one clinical research center in Italy.

Patients: Two hundred twenty-nine patients with hypertension and dyslipidemic T2D >40 years with stable background antihypertensive, hypoglycemic, and statin therapy and serum creatinine <1.5 mg/dL.

Interventions: Oral ALC 1000 mg or placebo twice daily on top of stable simvastatin therapy.

Outcome and Measures: Primary outcome was SBP. Secondary outcomes included lipid and glycemic profiles. Total-body glucose disposal rate and glomerular filtration rate were measured in subgroups by hyperinsulinemic–euglycemic clamp and iohexol plasma clearance, respectively.

Abbreviations: ALC, acetyl-L-carnitine; BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; GDR, glucose disposal rate; GFR, glomerular filtration rate; HbA_{1c}, glycosylated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; IQR, interquartile range; LDL, low-density lipoprotein; SD, standard deviation; SBP, systolic blood pressure; T2D, type 2 diabetes.

Received 18 December 2017
Accepted 19 March 2018
First Published Online 22 March 2018

May 2018 | Vol. 2, Iss. 5
doi: 10.1210/js.2017-00426 | Journal of the Endocrine Society | 420–436

Results: SBP did not significantly change after 6-month treatment with ALC compared with placebo (−2.09 mm Hg *vs* −3.57 mm Hg, $P = 0.9539$). Serum cholesterol, triglycerides, and lipoprotein(a), as well as blood glucose, glycated hemoglobin, fasting insulin levels, homeostatic model assessment of insulin resistance index, glucose disposal rate, and glomerular filtration rate did not significantly differ between treatments. Adverse events were comparable between groups.

Conclusions: Six-month oral ALC supplementation did not affect blood pressure, lipid and glycemic control, insulin sensitivity and kidney function in hypertensive normoalbuminuric and microalbuminuric T2D patients on background statin therapy.

Freeform/Key Words: acetyl-L-carnitine, blood pressure, dyslipidemia, insulin-resistance, statin, type 2 diabetes mellitus

Up to 75% of patients with type 2 diabetes mellitus (T2D) worldwide are hypertensive [1]. Arterial hypertension constitutes a major risk factor for cardiovascular disease and a predictor of microvascular and macrovascular complications in patients with T2D [2]. Blood pressure (BP) control effectively decreases cardiovascular complications of T2D; however, the reduction of systolic BP (SBP) to normal range is difficult to achieve despite multidrug treatments [3, 4]. Additionally, the concomitant diastolic BP (DBP) reduction observed in patients on antihypertensive medications represents a risk factor for coronary events [5].

Dyslipidemia, another major cardiovascular risk factor, is also frequently observed in patients with T2D. Statins improve hypercholesterolemia by essentially reducing total and low-density lipoprotein (LDL) cholesterol fraction levels but they marginally affect triglycerides and lipoprotein(a), both associated with worse cardiovascular outcomes [2, 6]. Thus, novel treatment options to improve the control of hypertension and dyslipidemia in patients with T2D are needed.

L-Carnitine and its ester acetyl-L-carnitine (ALC) are mitochondrial carriers of acyl and acetyl groups, both of which are involved in lipid oxidation and glucose metabolism. These compounds stimulate the activity of the pyruvate dehydrogenase complex and activate the glycolytic pathway [7]. Additionally, they facilitate mitochondrial uptake of long-chain fatty acids, leading to improved β-oxidation and thus overcoming the mitochondrial oxidative phosphorylation defect observed in T2D [8, 9]. Such actions may in turn lead to more efficient oxidative glucose utilization and storage and counteract the shift in substrate use from carbohydrates to lipids commonly observed with insulin resistance [10, 11].

Previous studies reported an improvement in insulin sensitivity among patients with T2D treated with intravenous L-carnitine [12, 13]. In a pilot study, our group found that along with an improvement in insulin sensitivity, oral ALC also decreased SBP without affecting DBP in nondiabetic subjects at high cardiovascular risk [14]. The significant correlation between SBP and insulin resistance suggested that BP reduction could be explained, at least in part, by insulin sensitivity amelioration. This was likely not the only driver of SBP reduction, because the enhancement of insulin sensitivity was appreciable only in more severely insulin-resistant subjects, whereas BP was reduced in all subjects regardless of their glucose disposal rate (GDR) at inclusion.

Consistently, evidence from animal models of hypertension suggests that increased carnitine activity may be linked to systemic oxidative stress reduction and higher nitric oxide availability, along with a downregulation of renin–angiotensin–aldosterone system components [15, 16]; these data suggest that carnitine could exert a direct effect on the vascular tone and thus play a role in BP regulation.

Additionally, whereas small pilot studies in patients with T2D showed controversial results regarding the effects of oral L-carnitine on serum triglycerides and lipoprotein(a) [17–19], a recent meta-analysis showed that oral L-carnitine had no significant effect on these

serum lipid components, but produced a significant reduction in total and LDL cholesterol [20]. Moreover, data from the trials exploring the effect of the combined therapy with L-carnitine and simvastatin on lipid profile in T2D are encouraging, although not conclusive [21–23].

Taken together, the evidence suggests that oral ALC might reduce BP and drive positive effects on the lipid profile in patients with T2D. However, no prospective randomized and controlled study has tested the efficacy of oral ALC on BP and lipid metabolism when added on top of statins in this population. Therefore, we aimed to assess the effects of a 6-month therapy with oral ALC on SBP and metabolic profile in patients with hypertensive normoalbuminuric and microalbuminuric T2D with dyslipidemia on stable antihypertensive, hypoglycemic, and statin therapy.

1. Materials and Methods

A. Patients

In this prospective, randomized, phase III, double-blind, placebo-controlled trial we screened for eligibility patients from five outpatient clinics of northern Italy (DIABASI Study Organization, see Appendix 1). Inclusion criteria were T2D (World Health Organization criteria), age >40 years, arterial hypertension (defined according to the Seventh Report of the Joint National Committee of Prevention, Detection, Evaluation and Treatment of High Blood Pressure [24]: SBP ≥ 140 mm Hg or DBP ≥ 90 mm Hg and/or concomitant use of antihypertensive medications), dyslipidemia (based on National Cholesterol Education Program criteria [25] and/or patients who were already treated with lipid-lowering drugs), and serum creatinine concentrations <1.5 mg/dL on stable background antihypertensive, hypoglycemic, and lipid-lowering therapy. Patients with uncontrolled T2D (glycosylated hemoglobin > 110 mmol/mol or 12.2%), acute cardiovascular events during the last 3 months, history of hypersensitivity to the study drug, evidence of immunologically mediated renal disease, major systemic diseases, cancer, drug or alcohol abuse, as well as pregnant, lactating, and potentially childbearing women without adequate contraception or subjects unable to provide informed consent were excluded from the study. Every patient provided written informed consent before enrolment in the study.

B. Study Design and Intervention

After the screening evaluation, potentially eligible patients entered a 4-week run-in period (Fig. 1). Patients fulfilling selection criteria were also stratified according to previous therapy with statins (*i.e.*, previous statin therapy YES or NO). Patients already receiving statin therapy were maintained on an equivalent dose of simvastatin (10 to 20 mg/d) up to the study end, whereas patients who were not on statin therapy started simvastatin 10 or 20 mg/d as deemed clinically appropriate. A safety visit was performed 10 days after the screening visit. At the end of the 4-week run-in period, baseline evaluation of demographic characteristics, vital parameters, and laboratory analyses were performed. Within each stratum (*i.e.*, previous statin therapy YES or NO), patients were randomly allocated on a 1:1 basis to 6-month treatment with oral ALC (1000 mg twice each day) or placebo.

Computer-generated randomization was centralized at the Laboratory of Biostatistics of the coordinating center under the responsibility of an independent investigator. Randomization was stratified in blocks by center, with block size randomly varying to increase the unpredictability of the sequence. Patients received treatment boxes with a unique tag representing the randomly allocated study sequence. Placebo capsules were identical to ALC capsules in shape, smell, and taste. Patients, investigators, and all of the personnel involved in the study were blinded to treatment allocation whereas information regarding previous statin therapy and simvastatin dose during the run-in and the study treatment periods

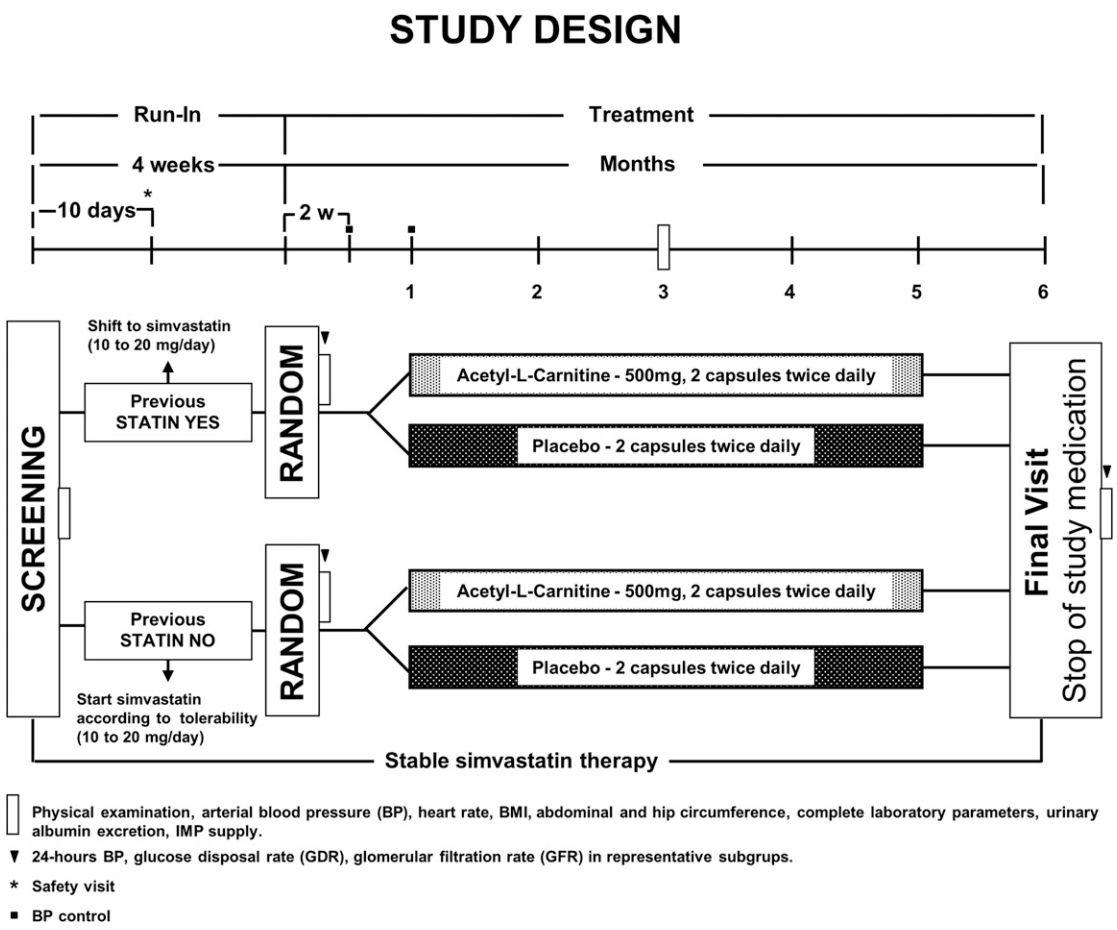

Figure 1. Study design.

remained unmasked. Study drug supply was performed at baseline visit and after 3 months of treatment. Clinical assessments and laboratory analyses were performed 3 and 6 months after randomization along with assessment of patient compliance to study drug and recommendations concerning physical activity and diet. At baseline and at final visits GDR and glomerular filtration rate (GFR) were evaluated in subgroups including the first 50 and 74 consenting patients, respectively. All patients were recommended to adhere to Italian Association of Diabetologists–Italian Society of Diabetology guidelines on diet and physical activity [26]. However, no substantial changes in diet, physical activity, or concomitant treatments were allowed throughout the study period to prevent confounding the study findings.

The study was carried out in accordance with the Declaration of Helsinki, and the clinical trial protocol was approved by the Ethics Committee of every participating center. The Clinical Research Center for Rare Diseases "Aldo e Cele Daccò" of the IRCCS Mario Negri Institute for Pharmacological Research (Ranica, Italy) coordinated and monitored the study and processed all laboratory samples. This study is registered on ClinicalTrial.gov (NCT00984750) and ClinicalTrialsRegister.eu (EUDRACT 2007-005925-31).

C. Outcomes

The primary outcome was the change in SBP after 6-month treatment with ALC compared with placebo. Secondary outcomes included changes in DBP, lipid and glycemic profile, insulin sensitivity, urinary albumin excretion, and GFR after ALC treatment compared with placebo.

D. Measurements

Office SBP and DBP were measured in the morning before treatment by means of an appropriate cuff, with the same sphygmomanometer (Omron 705IT; Omron, Hoofddorp, Netherlands) and with the patient in a sitting position after ≥5 minutes of rest. The average of three measurements taken 2 minutes apart was recorded for statistical analyses. Twenty-four–hour ambulatory BP was monitored and recorded in a representative subgroup of consenting patients by TM-2430 equipment (A&D Company, Tokyo, Japan) that was set to obtain measurements at 15-minute intervals during daytime (6:00 AM to 10:00 PM) and 30-minute intervals during nighttime (10:00 PM to 6:00 AM).

Blood was sampled the morning after overnight fasting for laboratory assessments. The patients were advised to maintain usual habits and to avoid physically intense/vigorous exercise, smoking, or substantial changes in regular diet the day before the examination. For the night before testing they were instructed to consume a low-carbohydrate meal and to avoid alcohol consumption. Eating after midnight was forbidden. The patients were allowed to drink water in the morning.

Insulin sensitivity was assessed by total GDR measured during the hyperinsulinemic–euglycemic clamp in a subgroup of 50 patients [27] and by homeostatic model assessment in all patients [28].

For the hyperinsulinemic–euglycemic clamp, insulin was infused at a constant rate of 4 mU/kg/min for 10 minutes and then decreased to 2 mU/kg/min. This infusion rate was maintained throughout the duration of the procedure. As soon as the blood glucose concentration decreased to 90 ± 5 mg/dL, the hyperinsulinemic–euglycemic clamp was started (time 0). From time 0, the blood glucose concentration was assayed with the glucose-oxidized method every 5 minutes and it was maintained at this level (90 ± 5 mg/dL) for 2 hours by a variable rate of 20% glucose solution infusion through an automated pump. During the last 30 minutes of the clamp, three blood samples were collected every 10 minutes for insulin measurements to confirm a steady-state plasma insulin concentration. Because at the achieved plasma insulin concentration the hepatic glucose production should be suppressed, the amount of glucose required to maintain steady-state euglycemia was assumed to be equal to 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 kilogram per minute.

The homeostatic model assessment of insulin resistance (HOMA-IR) index was calculated through the formula: HOMA-IR = [fasting serum glucose (mg/dL) × fasting insulin (μIU/mL)]/405. Lipoprotein(a) was measured by nephelometry (Immage; Beckman Coulter). Serum creatinine, lipid concentrations, and other routine laboratory parameters were assessed by a Beckman Coulter Synchron CX9 automatic analyzer whereas glycosylated hemoglobin (HbA_{1c}) was evaluated by high-performance liquid chromatography [normal laboratory range, 25.0 mmol/mol to 38.9 mmol/mol (International Federation of Clinical Chemistry and Laboratory Medicine) or 4.4% to 5.7% (Diabetes Control and Complications Trial); Beckman Coulter System Gold chromatograph].

Albuminuria was measured in three consecutive overnight urine collections by rate nephelometry (Array 360 system; Beckman Coulter, Milan, Italy). The sensitivity of the assay was 2 mg/L.

Glomerular filtration rate was measured by the plasma clearance of unlabeled iohexol [29]. Briefly, on the morning of renal function evaluation, 5 mL of iohexol solution (Omnipaque 300; GE Health Care, Milan, Italy) was injected intravenously during 2 minutes. Blood samples were then taken before iohexol injection (predose blank sample) and at different time points after completion of iohexol administration.

E. Sample Size Estimation and Statistical Analysis

On the basis of preliminary data in nondiabetic subjects with insulin resistance [14], SBP in eligible patients was expected to average 142.2 ± 16.3 mm Hg at baseline and decrease by

10 mm Hg at study end in those randomized to ALC. Assuming a similar SBP at baseline and a 3 mm Hg reduction at study end in those randomized to placebo, 104 patients per group would have given the trial an 80% power to detect a statistically significant difference ($P < 0.05$ two-tailed test) at 6 months. Assuming a 10% dropout rate, 114 patients per group had to be included to ensure at least 104 patients were available for the final analysis. Accordingly, a total of 229 patients were included and randomized. In a subgroup of consenting patients GFR and GDR were also measured. These were secondary, explorative outcome variables, and the number of patients to evaluate by the iohexol plasma clearance technique and hyperinsulinemic–euglycemic clamp was not calculated *a priori* on the basis of an expected treatment effect, but was dictated by feasibility, considering that both procedures are time- and cost-consuming and demanding for both patients and investigators.

Continuous variables were reported as mean [standard deviation (SD)] or median [interquartile range (IQR)], whereas categorical parameters were described by counts and percentages. Baseline characteristics of the patients were compared using the χ^2 test, Fisher's exact test, unpaired *t* test, or Wilcoxon rank-sum test, as appropriate. Statistical analyses were performed by modified intention to treat, which included all randomized patients who had received at least one dose of study drug. Within-group treatment effects were assessed by a paired *t* test or Wilcoxon signed rank test as appropriate. Between-group comparisons were carried out by means of analysis of covariance, adjusting for the measurements at randomization. All the statistical analyses were performed using SAS version 9.1 (SAS Institute) and STATA version 12. A *P* value of <0.05 was considered statistically significant.

2. Results

A. Patients' Characteristics

Of 247 participants screened from 26 June 2008 to 31 May 2011, 229 fulfilled the selection criteria and were randomized (116 patients to ALC and 113 to placebo). Ten of these patients, seven on ALC and three on placebo, did not complete the study because of consent withdrawal (n = 5), loss to follow-up (n = 3), an adverse event (n = 1), and protocol violation (n = 1) (Fig. 2).

All patients were white and 72% were males. According to the diagnostic criteria described in the Seventh Report of the Joint National Committee of Prevention, Detection, Evaluation and Treatment of High Blood Pressure [24], all of them were hypertensive. Overall, 95% of patients from both groups were on treatment with one or more antihypertensive medications, whereas the others were managed by dietary modifications alone according to guidelines [26]. SBP as well as other anthropometric, clinical, and laboratory parameters were comparable between groups at baseline. Although the prevalence of current smokers was higher in patients randomized to ALC compared with those on placebo, the difference between groups did not achieve statistical significance. Baseline characteristics of patients according to study treatment and stratification based on previous statin therapy are shown in Table 1. Of note, at the beginning of the study, baseline triglycerides and total and LDL cholesterol levels were higher in subjects who were on long-term statin therapy compared with those on short-term statin ($P < 0.001$ for all specified parameters). Baseline distribution of antihypertensive, hypoglycemic, and lipid-lowering agents was balanced between considered groups and strata (Table 1). Adherence to study drug was assessed by pill count scheduled at every visit. Compliance rates in the ALC and the placebo groups were similar and averaged 87%.

B. Effects of ALC vs *Placebo*

B-1. Blood pressure

SBP was not significantly different after 6 months of treatment with ALC or placebo, even after adjustment for baseline values ($P = 0.9539$). However, when compared with baseline,

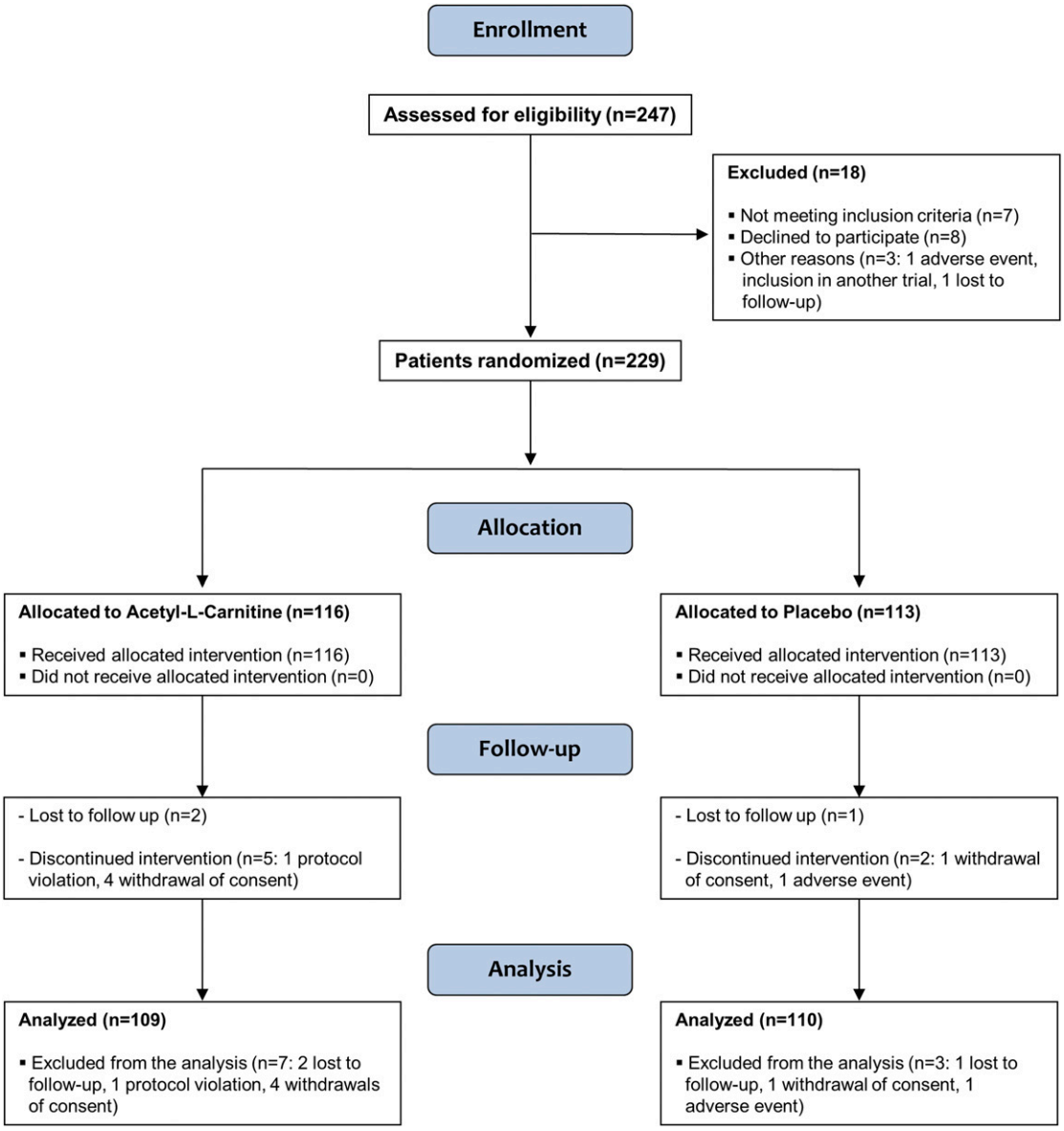

Figure 2. Flow diagram representing the disposition of subjects.

SBP did not appreciably change after treatment with ALC whereas it significantly decreased in patients allocated to placebo (from 138.1 ± 18.3 mm Hg to 134.5 ± 15.1 mm Hg; $P = 0.0187$) (Table 2).

Similarly, when strata were considered separately (previous statin YES or statin NO, *i.e.*, long- and short-term statin), the difference in SBP between ALC and placebo groups was not statistically significant. However, in the short-term statin therapy, SBP significantly decreased only when patients took placebo (137.6 ± 17.4 mm Hg to 133.2 ± 13.6 mm Hg; $P = 0.015$) (Table 3).

Overall, the effect of ALC on DBP was not significantly different from that of placebo ($P = 0.8994$) (Table 2), whereas DBP was significantly reduced after 6 months of ALC (from 79.9 ± 6.7 to 78.4 ± 7.5; $P = 0.0387$). Irrespective of the stratum considered, ALC and placebo had no appreciable effects on DBP compared with baseline.

Thirty-eight patients had changes in antihypertensive medications throughout the study period, of which 20 were on ALC (12 in the long-term statin and 8 in the short-term statin

Table 1. Baseline Characteristics of Patients Randomized to ALC or Placebo Therapy in the Study Group Considered as Whole (Overall) or According to Previous Treatment With Statins (YES or NO)

	Overall (n = 229)		Previous Statin Therapy YES (n = 117)		Previous Statin Therapy NO (n = 112)	
	ALC (n = 116)	Placebo (n = 113)	ALC (n = 61)	Placebo (n = 56)	ALC (n = 55)	Placebo (n = 57)
Demographic characteristics						
Age, y	64.9 ± 7.7	64.6 ± 7.5	65.4 ± 6.9	64.2 ± 73	64.4 ± 8.6	65.0 ± 7.7
Sex, male/female	81/35	84/29	41/20	41/15	40/15	43/14
Smoker, current/former	26/44	15/52	17/22	10/23	9/22	5/29
Weight, kg	83.3 ± 14.4	84.4 ± 15.3	84.0 ± 14.1	87.2 ± 17.0	82.6 ± 14.8	81.8 ± 13.2
BMI, kg/m^2	30.0 + 4.7	30.0 + 5.0	30.3 ± 4.3	30.9 ± 21.1	29.8 ± 5.2	29.2 ± 4.5
Clinical characteristics						
SBP, mm Hg	136.5 ± 14.3	138.1 ± 18.1	137.4 ± 13.6	138.4 ± 19.2	135.5 ± 15.0	137.8 ± 17.2
DBP, mm Hg	79.8 ± 6.7	79.0 ± 10.2	79.9 ± 6.6	78.5 ± 10.6	80.7 ± 6.8	79.6 ± 9.9
Mean BP, mm Hg	98.7 ± 7.5	98.7 ± 11.1	98.4 ± 7.2	98.4 ± 11.5	99.0 ± 7.9	99.0 ± 10.9
Laboratory parameters						
Serum glucose, mg/dL	148.2 ± 42.0	155.2 ± 42.1	150.9 ± 45.4	155.1 ± 44.6	145.2 ± 38.0	155.3 ± 40.1
HbA_{1c}, mmol/mol	51.0 ± 13.6	51.0 ± 13.2	52.7 ± 12.6	53.6 ± 14.6	49.1 ± 14.6	48.4 ± 11.3
HbA_{1c}, %	6.8 ± 1.24	6.8 ± 1.21	7.0 ± 1.15	7.1 ± 1.34	6.6 ± 1.34	6.6 ± 1.03
Insulin, μIU/mL	9.0 [6.4–15.1]	9.2 [6.1–14.9]	8.9 [7.3–15.8]	10.5 [6.3–16.9]	9.1 [5.7–14.5]	9.2 [6.1–14.9]
HOMA-IR	3.2 [2.0–5.9]	3.3 [2.1–5.6]	3.3 [2.1–6.1]	4.1 [2.0–6.0]	3.0 [1.8–5.8]	3.0 [2.1–4.7]
GDR, mg/kg/min[a]	5.6 [4.3–8.4]	5.8 [4.6–7.8]	5.6 [3.9–7.8]	5.6 [4.8–6.3]	5.7 [4.3–8.4]	6.3 [4.2–9.2]
Total cholesterol, mg/dL	158.2 ± 30.9	155.2 ± 33.7	167.0 ± 31.7	170.6 ± 35.1	148.5 ± 27.1	140.1 ± 24.3
HDL cholesterol, mg/dL	44.9 ± 13.3	44.8 ± 11.9	42.8 ± 10.8	45.3 ± 12.1	47.2 ± 15.4	44.2 ± 11.7
LDL cholesterol, mg/dL	93.5 ± 26.8	90.9 ± 26.6	100.9 ± 26.6	103.2 ± 27.2	85.4 ± 24.8	78.9 ± 19.9
Triglycerides, mg/dL	107.0 [83.0–144.5]	107.0 [74.0–148.0]	126.0 [85.0–173.0]	113.0 [84.0–182.0]	98.0 [64.0–124.0]	100.0 [70.0–126.0]
Lipoprotein(a), μIU/mL	9.0 [3.4–34.7]	11.5 [3.6–30.5]	11.3 [4.2–36.0]	16.8 [3.7–34.8]	6.1 [3.1–34.4]	9.3 [3.5–28.4]
Kidney function parameters						
Serum creatinine, mg/dL	0.93 ± 0.2	0.95 ± 0.2	0.92 ± 0.2	0.96 ± 0.2	0.94 ± 0.2	0.93 ± 0.2
GFR, mL/min/1.73 m^2[b]	97.1 ± 24.4	99.7 ± 25.5	99.2 ± 19.2	97.8 ± 27.8	94.5 ± 29.8	101.2 ± 24.0
Albuminuria, μg/min	11.9 [3.8–46.4]	10.6 [4.2–44.6]	14.4 [39–43.0]	18.6 [4.4–56.5]	11.7 [3.7–78.8]	7.6 [3.7–31.2]
Patients on pharmacological medications, n (%)						
Antihypertensive agents						
Any	110 (95)	107 (95)	58 (95)	54 (96)	52 (95)	53 (93)
Diuretics	73 (63)	60 (53)	41 (67)	28 (50)	32 (58)	32 (56)
Angiotensin-converting enzyme inhibitors	65 (56)	53 (47)	37 (61)	26 (46)	28 (51)	27 (47)
Angiotensin receptor blockers	35 (30)	36 (32)	16 (26)	18 (32)	19 (35)	18 (32)
Calcium channel blockers	37 (32)	32 (28)	21 (34)	16 (29)	16 (29)	16 (28)
Beta-blockers	26 (22)	40 (35)	19 (31)	22 (39)	7 (13)	18 (32)
Sympatholytic agents	34 (29)	32 (28)	16 (26)	18 (32)	18 (38)	14 (25)
Lipid-lowering agents						
Simvastatin	116 (100)	113 (100)	61 (100)	56 (100)	55 (100)	57 (100)
Simvastatin monotherapy	106 (91)	98 (87)	53 (87)	47 (84)	53 (96)	51 (89)
Any lipid-lowering drug additional to simvastatin:	10 (9)	15 (13)	8 (13)	9 (16)	2 (4)	6 (11)
Omega-3 fatty acid	9 (8)	13 (12)	8 (13)	9 (16)	1 (2)	4 (7)
Fibrates	1 (1)	2 (2)	0 (0)	0 (0)	1 (2)	2 (4)
Ezetimibe and omega-3 fatty acid	1 (1)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Hypoglycemic therapies						
Only diet	15 (13)	7 (6)	6 (10)	2 (4)	9 (16)	5 (9)
Any	101 (87)	106 (94)	55 (90)	54 (96)	46 (84)	52 (91)
Biguanides	87 (75)	86 (76)	46 (75)	41 (73)	41 (75)	45 (79)
Sulfonylureas	59 (51)	64 (57)	29 (48)	27 (48)	30 (54)	37 (54)
Thiazolidinediones	8 (7)	10 (9)	2 (3)	6 (11)	6 (11)	4 (7)
Meglitinides	2 (2)	2 (2)	1 (2)	2 (4)	1 (2)	0 (0)
Dipeptidyl peptidase-4 inhibitors	1 (1)	2 (2)	1 (2)	2 (4)	0 (0)	0 (0)
α-Glucosidase inhibitor	1 (1)	1 (1)	0 (0)	1 (2)	1 (2)	0 (0)
Oral hypoglycemic agents alone	70 (60)	82 (73)	32 (52)	41 (73)	38 (68)	41 (72)

Table 1. Continued

	Overall (n = 229)		Previous Statin Therapy YES (n = 117)		Previous Statin Therapy NO (n = 112)	
	ALC (n = 116)	Placebo (n = 113)	ALC (n = 61)	Placebo (n = 56)	ALC (n = 55)	Placebo (n = 57)
Insulin and other hypoglycemic agents	22 (19)	17 (15)	16 (26)	7 (13)	6 (11)	10 (18)
Insulin alone	9 (8)	7 (6)	7 (11)	6 (11)	2 (4)	1 (2)

Data are mean (SD) or median (IQR) for continuous variables and numbers (percentages) for dichotomous variables.
[a]Data from a subgroup of trial participants: 26, 12, and 14 patients allocated to ALC and 28, 9, and 19 patients allocated to placebo when considered as a whole (overall) and over statin YES and statin NO stratifications, respectively.
[b]Data from a subgroup of trial participants: 44, 24, and 20 patients allocated to ALC and 46, 20, and 26 patients allocated to placebo when considered as a whole (overall) and over statin YES and statin NO stratifications, respectively.

stratum) and 18 were on placebo (11 in the long-term statin and 7 in the short-term statin stratum). Differences between treatment groups considered as a whole and according to statin stratum were nonsignificant ($P = 0.8598$, $P = 1.0000$, and $P = 0.7864$ for overall, long-term, and short-term statin, respectively). Similar findings were observed in the subgroup of patients consenting to 24-hour BP monitoring (data not shown).

B-2. Laboratory, insulin resistance, and renal parameters

Overall, GDR, HbA_{1c}, and HOMA-IR did not significantly differ between the two treatments (Table 2). However, in ALC and placebo treatments, HbA_{1c} significantly increased [from 51.1 ± 14.0 mmol/mol to 54.4 ± 15.2 mmol/mol ($P = 0.0007$) and from 50.5 ± 12.2 mmol/mol to 53.0 ± 14.0 mmol/mol ($P = 0.0230$), respectively] whereas HOMA-IR decreased [from 3.1 (2.0 to 5.7) to 2.6 (1.7 to 4.8) ($P = 0.0251$) and from 3.3 (2.1 to 5.1) to 2.9 (1.7 to 5.2) ($P = 0.0267$), respectively] after 6 months (Table 2].

The effect of ALC on triglycerides, lipoprotein(a), LDL, HDL, and total cholesterol was not significantly different from that of placebo. After 6-month treatment with ALC all lipid parameters remained unchanged, whereas during placebo total and HDL cholesterol significantly increased and decreased from 155.7 ± 33.7 mg/dL to 161.3 ± 31.6 mg/dL ($P = 0.0164$) and from 44.9 ± 12.0 mg/dL to 43.3 ± 12.4 mg/dL ($P = 0.0174$), respectively. Throughout the study neither ALC nor placebo significantly affected kidney function (including directly measured GFR) and albuminuria (Table 2). When each stratum (long- and short-term statin) was considered separately, metabolic and renal parameters did not significantly differ between the ALC and placebo (Table 3). Most of the glycemic, lipid, and renal parameters remained stable during both treatments, but HOMA-IR significantly decreased after 6-month ALC and placebo in the short-term and in the long-term statin stratum, respectively. In the short-term statin stratum HbA_{1c} increased in ALC and placebo whereas insulin decreased only during ALC ($P < 0.01$ for all parameters). Also, in the same stratum, LDL ($P < 0.05$) and total cholesterol ($P < 0.01$) increased only when patients took placebo (Table 3).

B-3. Safety

Overall, 6-month therapy with ALC was well tolerated. Indeed, none of the patients discontinued the study medication because of treatment-related side effects. Adverse events were more frequent in the placebo than in the ALC group and most of them were mild to moderate in nature. Serious adverse events were slightly higher in patients randomized to

Table 2. Effects of 6 Months of Treatment with ALC or Placebo

	ALC (N = 109)		Placebo (N = 110)	
	Baseline	**6 Months**	**Baseline**	**6 Months**
Demographic characteristics				
Weight, kg	83.0 ± 14.4	83.1 ± 14.8	84.6 ± 15.5	84.4 ± 15.6
BMI, kg/m^2	30.0 ± 4.8	30.1 ± 5.0	30.0 ± 5.1	30.0 ± 5.2
Clinical characteristics				
SBP, mm Hg	135.4 ± 13.9	133.3 ± 13.7	138.1 ± 18.3	134.5 ± 15.1[a]
DBP, mm Hg	79.9 ± 6.7	78.4 ± 7.5[a]	79.4 ± 10.0	78.2 ± 9.1
Mean BP, mm Hg	98.4 ± 7.5	96.7 ± 7.7[a]	98.9 ± 11.1	97.0 ± 9.8[a]
Laboratory parameters				
Serum glucose	147.2 ± 41.7	144.0 ± 42.1	155.0 ± 42.1	146.5 ± 46.2
HbA_{1c}, mmol/mol	51.1 ± 14.0	54.4 ± 15.2[b]	50.5 ± 12.2	53.0 ± 14.0[a]
HbA_{1c}, %	6.8 ± 1.28	7.1 ± 1.39[b]	6.8 ± 1.12	7.0 ± 1.28[a]
Insulin, μIU/mL	8.7 [6.4–14.5]	7.8 [5.4–12.4]	8.9 [6.0–14.6]	8.5 [5.4–14.2]
HOMA-IR	3.1 [2.0–5.7]	2.6 [1.7–4.8][a]	3.3 [2.1–5.1]	2.9 [1.7–5.2][a]
GDR, mg/kg/min[c]	5.7 [4.2–8.4]	5.5 [4.9–7.2]	5.7 [4.6–7.2]	6.0 [4.9–7.6]
Total cholesterol, mg/dL	158.6 ± 31.7	160.7 ± 31.2	155.7 ± 33.7	161.3 ± 31.6[a]
HDL cholesterol, mg/dL	45.0 ± 13.5	43.5 ± 14.0	44.9 ± 12.0	43.3 ± 12.4[a]
LDL cholesterol, mg/dL	93.6 ± 27.6	94.3 ± 25.9	91.4 ± 26.4	94.8 ± 24.6
Triglycerides, mg/dL	107.0 [83.0–143.0]	107.0 [79.0–153.0]	107.0 [74.0–148.0]	111.0 [78.0–149.0]
Lipoprotein(a), μIU/mL	8.9 [3.7–35.0]	9.0 [3.0–44.2]	11.5 [3.6–28.7]	11.3 [4.3–32.5]
Kidney function parameters				
Serum creatinine, mg/dL	0.93 ± 0.2	0.93 ± 0.2	0.94 ± 0.2	0.95 ± 0.2
Albuminuria, μg/min	8.0 [3.7–44.5]	8.5 [3.5–39.5]	9.0 [4.2–35.5]	11.4 [3.6–45.2]
GFR, mL/min/1.73 m^2[d]	97.2 ± 22.1	96.4 ± 22.4	101.3 ± 27.0	99.8 ± 26.1

Data are mean (SD) or median (IQR) for continuous variables and numbers (percentages) for dichotomous variables. For all parameters considered, we reported data from patients who completed 6 months of treatment.
[a] $P < 0.05$ *vs* baseline, *t* test or Wilcoxon rank-sum test.
[b] $P < 0.01$ *vs* baseline, *t* test or Wilcoxon rank-sum test.
[c] Data from a subgroup of trial participants: 25 allocated to ALC and 25 patients allocated to placebo.
[d] Data from a subgroup of trial participants: 36 allocated to ALC and 38 allocated to placebo.

ALC compared with those to placebo. However, the most frequent serious adverse events were cancer (n = 3) and cardiovascular disorders (n = 3) likely related to age, concomitant chronic conditions, and disease progression in this high-risk population (Table 4).

3. Discussion

Treatment with ALC for 6 months on top of simvastatin did not significantly affect SBP, insulin resistance, and lipid profile in patients with hypertension, dyslipidemia, and T2D on stable background antihypertensive and hypoglycemic therapy. No treatment effect was observed in the study group as a whole, as well as in the two strata on short- and long-term statin therapy considered separately.

The pathogenesis of arterial hypertension in T2D is multifactorial and involves the renin–angiotensin–aldosterone and endothelin-1 systems, increased oxidative stress, and inflammatory processes. Among these pathogenetic mechanisms, impaired insulin sensitivity appeared to play a pivotal role [30]. Owing to this complexity, reduction of SBP to the normal range is seldom achievable in diabetic patients despite multidrug therapy.

Previous studies in patients with diabetes demonstrated that intravenous L-carnitine administration could improve insulin sensitivity [12, 13]. Our pilot study also found that 2 g per day of oral ALC improved insulin sensitivity in patients with higher insulin resistance and effectively decreased SBP in all nondiabetic hypertensive participants with a high cardiovascular risk profile [14]. However, the results from the current trial challenge the findings of these studies.

It is intriguing to speculate why ALC failed to decrease BP and/or to influence GDR whereas significant improvements were demonstrated in patients both with and without diabetes [12–14]. The dose of ALC was identical to that of the pilot study and other trials reporting benefits of oral L-carnitine in different clinical settings [17–21, 23]. Moreover, patient compliance to the study drug was close to 90%.

Nevertheless, some differences among patients' baseline characteristics in the two studies may justify the inconsistency of the present results. First, our study population consisted of patients with T2D on hypoglycemic treatment compared with patients without diabetes in the pilot study. Despite that, GDR in the present trial was slightly higher than in the pilot study and was within the range (3.21 ± 0.99 and 6.93 ± 1.47 mg/kg) that was found to be associated with a significant BP-lowering effect of ALC. Thus, the severity of insulin resistance is an unlikely explanation for treatment failure in our present study. Second, patients with diabetes were older (mean age, 64.7 ± 7.6 *vs* 44.3 ± 9.3 years for the DIABASI and the pilot study, respectively), likely implying increased resistance to the antihypertensive drugs due to increased vascular stiffness. Third, the higher proportion of current/former smokers in this study compared with that of the pilot study (60.4% *vs* 40.6%, respectively) might have contributed to endothelial dysfunction and arterial stiffness [31], thus attenuating the effect of ALC on SBP. Finally, recruited patients had lower SBP values as compared with those initially assumed for sample size estimation, which might have reduced the statistical power of the analyses.

Another crucial difference concerned statin use: all patients in the current study received simvastatin whereas only one subject was on statin in the pilot study. Statin therapy is known to potentiate the effect of antihypertensive drugs [32, 33] through vasodilation, which is due to increased nitric oxide synthase activity [34], downregulation of angiotensin II-type 1 receptors [35], and endothelin-1 production [36]. Thus, pretreatment with simvastatin might have prevented any possible additional beneficial antihypertensive effect of ALC. Additionally, simvastatin has been shown to increase HbA_{1c} levels and to worsen insulin sensitivity [37, 38]. Actually, we observed a significant increase in HbA_{1c} after 6 months in both ALC and placebo groups, which was particularly evident in the short-term simvastatin stratum. This confirms that initial treatment with simvastatin may worsen HbA_{1c}, and that ALC cannot counteract this detrimental effect. We could not detect the same effect in patients on long-term statins, because HbA_{1c} values in both ALC and placebo groups were virtually identical throughout the study. However, even in these patients, ALC failed to improve the glycemic profile.

Despite finding no change in the GDR, we could observe some signs of improvement in insulin sensitivity with a significant decrease of HOMA-IR in both ALC and placebo groups at 6 months. In the short-term simvastatin stratum, ALC reduced HOMA-IR along with a significant decrease in insulin concentration, suggesting a possible initial metabolic effect of the study drug. However, the difference between groups was not significant, and this effect was not observed in the group of patients on ALC in the long-term statin stratum, implying that long-term statin therapy might have negated any beneficial effect of ALC on insulin sensitivity.

To avoid any potential confounding effect of the duration of statin therapy on study findings, we *a priori* stratified patients according to previous statin therapy YES or NO. Moreover, we found no relationship between duration of statin therapy and treatment effect (data not shown). Notably, the type and dose of statins used in the statin YES stratum before the enrolment were homogeneous between study groups, with the sole exception of rosuvastatin, which was more frequently used in the ALC group than in the placebo group [13 patients *vs* 4 patients ($P = 0.0001$), respectively]. Thus, whether previous treatment with rosuvastatin might have contributed to mask the metabolic effects of ALC cannot be definitely excluded.

The tentative lipid-lowering action of L-carnitine and ALC has been linked to increased fatty acid β-oxidation and reduced oxidative stress due to mitochondrial dysfunction improvement [7, 9]. Although the results from some small trials exploring the effect of the

Table 3. Effects of 6 Months of Treatment With ALC or Placebo According to Previous Treatment With Statins (YES or NO)

	Previous Statin Therapy YES (n = 112)			
	ALC (n = 57)		Placebo (n = 55)	
	Baseline	6 Months	Baseline	6 Months
Demographic characteristics				
Weight, kg	83.2 ± 14.0	83.1 ± 15.0	87.2 ± 17.0	86.8 ± 16.8
BMI, kg/m^2	30.1 ± 4.4	30.2 ± 4.4	31.0 ± 5.5	31.0 ± 5.6
Clinical characteristics				
SBP, mm Hg	136.2 ± 13.0	134. 0 ± 13.7	138.6 ± 19.3	135.8 ± 16.4
DBP, mm Hg	78.8 ± 6.6	77.4 ± 7.4	78.8 ± 10.3	78.7 ± 9.9
Mean BP, mm Hg	97.9 ± 7.0	96.3 ± 7.5	98.7 ± 11.4	97.7 ± 10.7
Laboratory parameters				
Serum glucose	149.1 ± 45.3	145.6 ± 43.9	154.2 ± 44.6	143.5 ± 52.9
HbA_{1c}, mmol/mol	53.2 ± 12.9	54.2 ± 13.0	52.8 ± 12.7	52.7 ± 15.9
HbA_{1c}, %	7.0 ± 1.18	7.1 ± 1.19	7.0 ± 1.16	7.0 ± 1.45
Insulin, μIU/mL	8.6 [6.8–14.0]	8.7 [6.1–17.8]	10.3 [6.4–16.6]	9.6 [5.7–16.7]
HOMA-IR	3.2 [2.0–5.7]	3.1 [1.9–5.8]	4.1 [2.0–5.7]	3.2 [1.4–6.6][a]
GDR, mg/kg/min[c]	5.6 [3.9–7.8]	4.9 [3.3–6.0]	5.6 [5.1–6.5]	6.1 [5.1–7.1]
Total cholesterol, mg/dL	167.9 ± 32.4	168.8 ± 30.2	170.7 ± 35.7	171.3 ± 32.4
HDL cholesterol, mg/dL	42.7 ± 10.8	41.4 ± 11.7	45.6 ± 12.2	43.7 ± 14.0
LDL cholesterol, mg/dL	101.5 ± 27.3	101.3 ± 22.8	103.0 ± 27.6	102.4 ± 22.8
Triglycerides, mg/dL	126.0 [90.0–173.0]	135.0 [93.0–165.0]	111.0 [83.0–184.0]	125.5 [85.0–175.0]
Lipoprotein(a), μIU/mL	11.3 [4.3–36.0]	13.8 [3.9–44.2]	14.7 [3.6–30.6]	12.6 [4.1–30.6]
Kidney function parameters				
Serum creatinine, mg/dL	0.92 ± 0.2	0.93 ± 0.2	0.95 ± 0.2	0.96 ± 0.2
Albuminuria, μg/min	7.8 [3.7–41.8]	8.1 [3.5–29.1]	17.1 [4.2–55.2]	12.2 [3.8–49.9]
GFR, mL/min/1.73 m^2[d]	99.3 ± 20.4	96.6 ± 20.4	99.3 ± 29.5	99.6 ± 27.8

combined therapy with L-carnitine and simvastatin on lipid profile in T2D were encouraging [21–23], our results suggest that the effects of ALC on lipid profile parameters are limited when the drug is used as an add-on statin therapy.

Consistent with earlier studies, our data confirm that treatment with ALC was remarkably well tolerated with no treatment-related serious adverse event requiring treatment interruption and/or patient withdrawal. Of note, no adverse event could be directly attributed to the study drug.

The prospective, randomized, placebo-controlled design of the trial together with the gold standard methods used for insulin sensitivity and GFR measurements in a subgroup of patients are the major strength of our study. We also formally tested the effect of ALC on top of standardized simvastatin therapy to prevent the confounding effect on metabolic profile of the eventual previous YES or NO statin therapy.

Finding that body weight and body mass index (BMI) were comparable at baseline and remained unchanged in different groups and strata during the study reasonably excludes the possibility that study results were confounded by systematic changes in diet and physical activity introduced during the trial.

We intentionally did not standardize BP-lowering therapy during the run-in, because we wanted to test the BP lowering effect of ALC in a context that reflects real life. Thus, the distribution of different BP-lowering medications (and of their different combinations) in our study population reflected the distribution in the average population of patients referred to a diabetology unit. This enhanced the generalizability of the study findings. Alternatively, finding that the proportion of patients using antihypertensive medications and the distribution of different antihypertensive agents (and of their different combinations) were

Table 3. Continued

	Previous Statin Therapy NO (n=107)			
	ALC (n = 52)		Placebo (n = 55)	
	Baseline	6 Months	Baseline	6 Months
Demographic characteristics				
Weight, kg	82.7 ± 15.0	83.1 ± 15.9	82.1 ± 13.3	82.0 ± 14.0
BMI, kg/m^2	29.9 ± 5.3	30.0 ± 5.6	29.1 ± 4.6	29.1 ± 4.7
Clinical characteristics				
SBP, mm Hg	134.6 ± 14.9	132.6 ± 13.9	137.6 ± 17.4	133.2 ± 13.6[a]
DBP, mm Hg	81.2 ± 6.6	79.5 ± 7.5	79.9 ± 9.7	77.8 ± 8.3
Mean BP, mm Hg	99.6 ± 7.3	97.2 ± 8.0	99.1 ± 10.9	96.3 ± 8.8[a]
Laboratory parameters				
Serum glucose	145.1 ± 37.7	142.3 ± 40.4	155.7 ± 39.8	149.5 ± 38.8
HbA_{1c}, mmol/mol	48.9 ± 14.9	54.7 ± 17.3[b]	48.2 ± 11.5	53.3 ± 12.0[b]
HbA_{1c}, %	6.6 ± 1.36	7.2 ± 1.58[b]	6.6 ± 1.05	7.0 ± 1.10[b]
Insulin, μIU/mL	9.1 [5.7–14.5]	6.8 [4.5–11.4][b]	7.9 [5.9–12.0]	8.0 [5.4–10.6]
HOMA-IR	3.0 [1.8–5.8]	2.2 [1.5–4.1][b]	2.9 [2.1– 4.7]	2.8 [1.8–4.7]
GDR, mg/kg/min[c]	6.3 [4.3–8.4]	5.7 [5.5–8.7]	5.9 [4.2–7.9]	6.0 [4.9–7.7]
Total cholesterol, mg/dL	148.4 ± 27.8	151.8 ± 30.4	141.0 ± 24.0	151.3 ± 27.6[b]
HDL cholesterol, mg/dL	47.5 ± 15.6	45.8 ± 15.8	44.2 ± 11.9	43.0 ± 10.8
LDL cholesterol, mg/dL	84.9 ± 25.4	86.5 ± 27.0	80.0 ± 19.3	87.3 ± 24.0[b]
Triglycerides, mg/dL	97.0 [63.0–119.0]	85.5 [65.0–116.5]	101.0 [64.0–126.0]	105.0 [69.0–141.0]
Lipoprotein(a), μIU/mL	6.1 [3.2–34.7]	7.0 [2.4–44.2]	9.4 [3.5–28.6]	10.8 [4.5–33.2]
Kidney function parameters				
Serum creatinine, mg/dL	0.94 ± 0.2	0.93 ± 0.2	0.93 ± 0.2	0.93 ± 0.2
Albuminuria, μg/min	10.4 [3.7–66.2]	8.8 [3.5–54.5]	5.7 [3.6–31.2]	8.4 [3.1–4.3]
GFR, mL/min/1.73 m^2[d]	94.4 ± 24.9	96.1 ± 25.7	102.8 ± 25.6	99.9 ± 25.4

Data are mean (SD) or median (IQR) for continuous variables and numbers (percentages) for dichotomous variables. For all parameters considered, we reported data from patients who completed 6 months of treatment.

[a] $P < 0.05$ *vs* baseline, *t* test or Wilcoxon rank-sum test.

[b] $P < 0.01$ *vs* baseline, *t* test or Wilcoxon rank-sum test.

[c] Data from a subgroup of trial participants: 11 and 13 patients allocated to ALC and 8 and 17 patients allocated to placebo when stratifications to statin YES and statin NO were considered, respectively.

[d] Data from a subgroup of trial participants: 23 and 15 patients allocated to ALC and 17 and 22 patients allocated to placebo when stratifications to statin YES and statin NO were considered, respectively.

comparable between groups can be taken to suggest that data were very unlikely confounded by concomitant BP-lowering therapy, even if it was not standardized.

Although concomitant medication changes were not recommended throughout the study period, adjustments in antihypertensive and antidiabetic treatments were conceded in selected cases to avoid acute clinical complications during the trial. However, the adherence pattern to chronic treatments was stable and the differences in antihypertensive and antidiabetic medication changes during the study period between treatment groups and strata were not statistically significant.

A potential limitation of this study was the unavailability of baseline and follow-up measurements of plasma carnitine levels. However, it is well known that plasma carnitine is low in patients with T2D, especially in the presence of dyslipidemia or microvascular complications [39]. This evidence strengthens the rationale of ALC use in this cohort. Alternatively, we wanted to test the possible BP-lowering effect of ALC above and beyond that of available medications in everyday clinical practice, a context in which serum carnitine level is a parameter that cannot be considered routinely for selection of potential candidates for treatment. In the case of encouraging findings, the role of serum carnitine as a tool to identify patients who may benefit the most from ALC therapy could have been evaluated in further studies.

Table 4. Adverse Events in the Study as Whole and in the Two Treatment Groups

	Overall	ALC	Placebo
Total	**193**	**95**	**98**
Serious adverse events			
Total	**12**	**7**	**5**
Any cancer	3	2	1
Metastatic squamous cell carcinoma of the hypopharynx	1	1	0
Monofocal hepatocellular carcinoma	1	1	0
Melanoma	1	0	1
Any cardiovascular event	3	2	1
Dilatative and hypocynetic cardiomyopathy	1	1	0
Ischemic cardiopathy	1	1	0
Angina pectoris	1	0	1
Other events	6	3	3
Respiratory distress	1	1	0
Posttraumatic subarachnoid hemorrhage	1	1	0
Adductor magnus muscle strain	1	1	0
Shoulder tendinous lesion	1	0	1
Nonserious adverse events (system organ classification)			
Total	**181**	**88**	**93**
Musculoskeletal disorders and trauma	36	21	15
Gastrointestinal and hepatobiliary disorders	32	14	18
Respiratory and thoracic disorders	19	11	8
Cardiac and vascular disorders	18	8	10
Metabolism, nutritional, and endocrinology disorders	18	6	12
Laboratory abnormalities	15	9	6
Ocular, ear, and labyrinth disorders	13	3	10
Dermatology and allergic disorders	11	5	6
Nervous system and psychiatric disorders	7	3	4
Urologic disorders	6	4	2
General disorders	6	4	2

4. Conclusion

Oral ALC does not improve either SBP control or the lipid and glycemic profile in diabetic hypertensive patients on stable statin therapy. We hypothesized that the possible hypotensive and hypolipidemic effect of ALC is blunted by statin use. It is worth exploring this objective in patients with and without diabetes and with hypertension who do not require treatment with statins.

Appendix 1

DIABASI Study Organization: Members of the DIABASI Study Organization were as follows: Principal Investigator—N. Perico [IRCCS - Mario Negri Institute for Pharmacological Research, Clinical Research Center for Rare Diseases “Aldo e Cele Daccò”, Ranica (Bergamo), Italy]; Scientific Study Coordinators—P. Ruggenenti, G. Remuzzi (IRCCS - Mario Negri Institute for Pharmacological Research, Clinical Research Center for Rare Diseases “Aldo e Cele Daccò”); Coordinating Center—IRCCS - Mario Negri Institute for Pharmacological Research, Clinical Research Center for Rare Diseases “Aldo e Cele Daccò”; Participating Centers—N. Perico, S. Rota, B. Ruggiero, A. Panozo, M. Abbate, B. Pahari, K. Courville, S. Prandini, V. Lecchi, G. Gherardi (IRCCS - Mario Negri Institute for Pharmacological Research, Clinical Research Center for Rare Diseases “Aldo e Cele Daccò”); R. Trevisan, A. Corsi, A.R. Dodesini, R. Rota, C. Aparicio (UO Malattie Endrocrine e Diabetologia–ASST Papa Giovanni XXIII, Bergamo, Italy); A. Bossi, A. Parvanova, I. Iliev, S. Yakymchuk [UOC Malattie Endocrine e Centro Regionale per il Diabete Mellito–ASST Bergamo Ovest–Ospedale Treviglio-Caravaggio, Treviglio (Bergamo), Italy];

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Appendix 2

Scientific Writing Academy 2015—Tutor: David G. Warnock, MD, Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama. Participants: Matias Trillini, MD, IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Bergamo, Italy; Aneliya Parvanova, MD, IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Bergamo, Italy; Sreejith Parameswaran, MD, Jawaharlal Institute of Postgraduate Medical Education and Research, India; Jonathan S. Chávez-Iñiguez, MD, Hospital Civil de Guadalajara "Fray Antonio Alcalde," Servicio de Nefrología, Guadalajara, Jalisco, Mexico; Fahrudin Masnic, MD, Clinic for Hemodialysis, University Clinical Center Sarajevo; Sidy Mohamed Seck, MD, Department of Nephrology, Faculty of Health Sciences, University Gaston Berger, Saint-Louis, Senegal; Teerayuth Jiamjariyaporn, MD, Bhumirajanagarindra Kidney Institute, Bangkok, Thailand; Monica Cortinovis, IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Bergamo, Italy; Luca Perico, PhD, IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Bergamo, Italy; Kanishka Sharma, IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Bergamo, Italy. Note: The Scientific Writing Academy is a project sponsored by IRCCS - Istituto di Ricerche Farmacologiche Mario Negri (Bergamo, Italy) and endorsed by the International Society of Nephrology that aims to teach the tools necessary to succeed in publishing scientific papers in international journals to researchers and physicians from around the world.

Acknowledgments

Stefano Rota and Barbara Ruggiero helped in patient screening, inclusion, and monitoring. We thank Olimpia Diadei and Wally Calini for valuable work in monitoring the study, and the staff of the Clinical Research Center and Diabetology Units for contribution to patient care and conducting the study. We are also indebted to Andrea Panozo, Bishnu Pahari, Karen Courville, Patricia Espindola, Silvia Prandini, Veruscka Lecchi, and Svitlana Yakymchuk for care of the study participants.

Financial Support: This work was supported by Sigma-Tau Industrie Farmaceutiche Riunite S.p.A (Pomezia, Rome, Italy), including the costs of the study and freely supplying the study medication (ALC or placebo capsules). The funding source had no role in study design, data collection, analysis and interpretation, writing of the report, and decision to submit the article for publication.

Clinical Trial Information: ClinicalTrial.gov NCT00984750 (registered 31 January 2008) and ClinicalTrialsRegister.eu EUDRACT 2007-005925-31 (registered 23 September 2009).

Author Contributions: P.R. and G.R. had the original idea, wrote the main protocol, coordinated the study centers, and critically revised the manuscript. A. Parvanova, M.T., C.A., I.I., R.T., and A.C.B. contributed to patient selection, monitoring, and care. A. Perna and F.P. conducted the statistical analysis. N.R. monitored the study. F.G., A.C., and S.F. were responsible for the execution and interpretation of centralized laboratory measurements. D.G.W. with the Scientific Writing Academy attendants interpreted the data and wrote the first draft of the manuscript (Appendix 2). A. Perna, A. Parvanova, M.T., M.A.P., and P.R. contributed to data analyses and interpretation. M.A.P. revised

the first draft of the manuscript, and P.R., M.A.P., A. Parvanova, and M.T. wrote the final version. All authors critically revised the manuscript and approved the final draft. No medical writer or editor was involved in the writing of the manuscript.

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Disclosure Summary: The authors have nothing to disclose.

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