

Effect of Combined Treatment With α -Lipoic Acid and Acetyl-L-Carnitine on Vascular Function and Blood Pressure in Patients With Coronary Artery Disease

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Mitochondria produce reactive oxygen species that may contribute to vascular dysfunction. α -Lipoic acid and acetyl-L-carnitine reduce oxidative stress and improve mitochondrial function. In a double-blind crossover study, the authors examined the effects of combined α -lipoic acid/acetyl-L-carnitine treatment and placebo (8 weeks per treatment) on vasodilator function and blood pressure in 36 subjects with coronary artery disease. Active treatment increased brachial artery diameter by 2.3% ($P=0.008$), consistent with reduced arterial tone. Active treatment tended to decrease systolic blood pressure for the whole group ($P=0.07$) and had a significant effect in the subgroup with blood pressure above the median (151 ± 20 to 142 ± 18 mm Hg; $P=0.03$) and in the subgroup with the metabolic syndrome (139 ± 21 to 130 ± 18 mm Hg; $P=0.03$). Thus, mitochondrial dysfunction may contribute to the regulation of

blood pressure and vascular tone. Further studies are needed to confirm these findings and determine the clinical utility of α -lipoic acid/acetyl-L-carnitine as antihypertensive therapy. (J Clin Hypertens. 2007;9:249–255) ©2007 Le Jacq

In addition to serving as the site of oxidative phosphorylation, it is now clear that mitochondria regulate many cellular functions, in part by producing reactive oxygen species that signal the adaptive response to environmental stress and injury.¹ Excess production of mitochondria-derived oxidants, however, may have maladaptive effects.^{2,3} There is growing evidence that disturbances of mitochondrial function, including increased oxidant production and altered mitochondria-dependent signaling, contribute to the pathogenesis of vascular disease in atherosclerosis and other cardiovascular risk factors.^{1,4,5}

On the basis of these observations, we hypothesized that an intervention designed to improve mitochondrial function would have beneficial vascular effects in patients with cardiovascular disease. Recent experimental studies have shown that administration of α -lipoic acid and/or acetyl-L-carnitine can reduce oxidant production and improve mitochondrial function in models of aging.^{6,7} Furthermore, these compounds reduce blood pressure (BP) and improve endothelial function in animal models of hypertension^{8–12} and diabetes.^{13–16} The present study was designed to examine the

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Table I. Baseline Clinical Characteristics by Treatment Order

CHARACTERISTIC	PLACEBO FIRST (N=21)	ACTIVE FIRST (N=15)	P
Age, y	64±7	62±5	.32
Male	16 (76)	11 (73)	.85
African American	1 (5)	5 (33)	.06
History of type 2 diabetes mellitus	3 (14)	2 (13)	.84
Metabolic syndrome	12 (57)	12 (80)	.15
History of hypertension	16 (76)	13 (87)	.67
History of hypercholesterolemia	17 (81)	15 (100)	.13
Family history of premature coronary disease	12 (57)	4 (27)	.08
History of cigarette smoking	15 (71)	10 (67)	.99
Aspirin treatment	21 (100)	15 (100)	.99
ACEI/ARB treatment	11 (52)	8 (53)	.96
Lipid-lowering therapy	19 (91)	15 (100)	.50
β-Blocker therapy	16 (76)	11 (73)	.85
Diuretic therapy	2 (10)	2 (13)	.72
Systolic blood pressure, mm Hg	137±15	131±22	.39
Diastolic blood pressure, mm Hg	76±7	71±9	.11
Total cholesterol, mg/dL	172±34	174±29	.89
HDL cholesterol, mg/dL	50±19	42±5	.07
Triglycerides, mg/dL	156±95	197±144	.31
LDL cholesterol, mg/dL	90±21	92±30	.87
Glucose, mg/dL	103±14	118±33	.11
Body mass index, kg/m ²	30.0±5.3	30.1±5.5	.93

Data are presented as mean ± SD or number (percentage). ACEI/ARB indicates angiotensin-converting enzyme inhibitor or angiotensin receptor blocker; HDL, high-density lipoprotein; and LDL, low-density lipoprotein.

effects of combined α -lipoic acid/acetyl-L-carnitine treatment on vascular function and BP in patients with coronary artery disease.

METHODS

Study Subjects

We enrolled consecutive patients, 55 years or older, receiving care at Boston Medical Center for stable coronary artery disease. The presence of coronary disease was confirmed by coronary angiography, history of myocardial infarction,¹⁷ or an imaging stress test demonstrating myocardial ischemia. Exclusion criteria included supplementation with α -lipoic acid, acetyl-L-carnitine, vitamin E, or vitamin C. Since α -lipoic acid has the potential to increase insulin sensitivity,¹⁸ we excluded patients with a history of symptomatic hypoglycemia. Finally, we excluded patients if their physicians made a change in their antihypertensive regimen during the month before the study or during the study. All subjects provided informed consent and the Boston Medical Center Institutional Review Board approved the protocol.

Study Protocol

Eligible patients were enrolled into a double-blind, placebo-controlled, crossover study that evaluated

vascular function and BP before and at the end of two 8-week treatment periods. There was a 4-week washout period between treatments, and the order of treatments was determined by a computer-generated randomization scheme. Active treatment consisted of tablets containing 200 mg of α -lipoic acid and 500 mg of acetyl-L-carnitine (Juvenon Cellular Health Supplement; Juvenon, Inc, Incline Village, NV) and the placebo consisted of a similar-appearing tablet containing vehicle alone. Subjects were instructed to take 1 tablet twice daily. Study medications were packaged and dispensed in a blinded manner by the Research Drug Service of Boston Medical Center. We contacted subjects by telephone 1 and 4 weeks after initiation of treatment to monitor any unanticipated effects and we confirmed compliance by pill count (no more than 5% of pills were returned).

Assessment of Vascular Function and BP

Prior to each of the 4 visits, subjects were asked to withhold vasoactive medications (nitrates, calcium channel blockers, angiotensin-converting enzyme inhibitors, other vasodilators, and β -adrenergic blockers) for 24 hours and to fast and refrain from smoking (if applicable) overnight. Blood and urine

samples were collected. Patients lay supine in bed in a darkened room for 10 minutes. We then used an automatic physiologic monitor (Dynamap; Tele Atlas, Boston, MA) to measure pulse and BP in the left arm and record the average of 3 measurements made 2 minutes apart.

We then used vascular ultrasound to assess endothelium-dependent flow-mediated dilation and reactive hyperemia of the brachial artery in the right arm using established methodology.^{20–22} Briefly, 2-dimensional images and Doppler flow velocity signals were recorded from the brachial artery at baseline using a Toshiba Powervision 6000 ultrasound system (Toshiba Medical, Inc, Tustin, CA). Reactive hyperemia was induced by 5-minute occlusion of arterial flow with a narrow-gauge BP cuff positioned on the upper arm. Doppler signals were recorded immediately after cuff release and 2-dimensional images were recorded from 55 to 65 seconds following cuff release. After a 10-minute rest period, we recorded 2-dimensional images of the brachial artery before and 3 minutes after administering sublingual nitroglycerin (0.4 mg). Five minutes after nitroglycerin administration, we repeated the BP measurement before allowing subjects to get out of bed. We did not administer nitroglycerin to subjects with a history of migraine headaches or with adverse effects to nitroglycerin in the past.

We measured brachial artery diameter using commercially available software (Brachial Analyzer; Medical Imaging Applications, Inc, Coralville, IA) and measured the flow velocity integral with public domain image analysis software (Image J). Flow-mediated dilation was expressed as percentage change from baseline and as actual change in millimeters.

Blood and Urine Markers

Serum lipids, insulin, and glucose levels were measured in the Boston Medical Center clinical laboratory and low-density lipoprotein cholesterol levels were calculated using the Friedewald formula.²¹ Serum total carnitine levels were measured in a blinded manner using an established fluoroscopic method that has a published coefficient of variation less than 4.3%.²² Serum C-reactive protein was measured using a high-sensitivity nephelometric method as previously described with a limit of detection of 0.17 mg/L.²³ Urinary F2-isoprostanes were measured using a commercially available ELISA kit (Cayman, Ann Arbor, MI).²⁴

Statistical Analyses

We compared the clinical characteristics of the subjects randomized to the placebo-first group

and the active treatment–first group using the unpaired *t* test and the chi-square test for continuous and categorical variables, respectively. We used repeated-measures analysis of variance (ANOVA) to examine the effect of treatment on brachial artery diameter, brachial artery flow-mediated dilation, nitroglycerin-mediated dilation, extent of reactive hyperemia, systolic and diastolic BP, lipoprotein levels, and blood and urine markers. This analysis considered the effects of visit (baseline vs after treatment) and treatment (placebo vs active) with treatment order (active first or placebo first) included as a between-subjects covariate. When the overall ANOVA was significant, we performed post hoc pairwise comparisons to compare mean values at baseline and during treatment. The study was powered to detect a difference in flow-mediated dilation of 1.8 percentage points (eg, 6.0% vs 7.8%) with 90% power ($\alpha=.05$) with a sample size of 36 subjects.

Since lipoic acid and acetyl-L-carnitine have been shown to have favorable effects on mitochondrial decay in the setting of aging,^{6,7,25} diabetes mellitus/metabolic syndrome,^{26,27} and hypertension,^{8,9,13,14} we completed secondary analyses in the subgroups of subjects above the median for age or systolic BP and in subjects with the metabolic syndrome as defined by the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (ATP III) or type 2 diabetes mellitus.²⁸ We examined the relation between baseline carnitine level and clinical characteristics, and the relation between change in carnitine level and BP response by calculating the Pearson correlation coefficients. All analyses were completed using SPSS 12.0 (SPSS Inc, Chicago, IL). Data are expressed as mean \pm SD, unless otherwise indicated. The criterion for statistical significance was $P<.05$.

RESULTS

Study Subjects

A total of 41 eligible subjects were entered into the study. Two subjects withdrew because of adverse reactions (one with a pruritic rash and one with nausea). Both were taking active α -lipoic acid/acetyl-L-carnitine at the time of these reactions, which resolved after discontinuation of study medication. Three subjects were withdrawn because they had significant changes in medical status while taking placebo (1 developed coronary restenosis, 1 developed unstable angina, and 1 suffered

Table II. Effect of Treatment on Physiologic Markers

MARKER, MEAN ± SD	NO.	PLACEBO		ACTIVE		<i>P</i> *
		BEFORE	AFTER	BEFORE	AFTER	
Total carnitine, μmol/L	32	101±23	99±20	104±24	112±23	.06
Total cholesterol, mg/dL	34	171±33	171±36	178±40	177±38	.94
LDL cholesterol, mg/dL	34	89±24	93±24	95±32	97±34	.60
HDL cholesterol, mg/dL	34	49±16	47±15	48±15	46±13	.78
Triglycerides, mg/dL	34	169±120	151±81	179±114	169±120	.50
Fasting glucose, mg/dL	34	104±14	105±24	106±25	104±20	.41
Insulin, IU/mL	32	9.6±7.6	9.1±6.2	12.0±7.9	13.4±11.4	.40
C-reactive protein, mg/L	27	3.2±3.4	3.3±4.8	2.1±2.6	2.8±3.3	.23
Urine F ₂ -isoprostanes, ng/mg creatinine	16	1.2±0.8	1.1±0.5	1.0±0.4	1.1±0.6	.96

*Overall *P* for repeated-measures analysis of variance. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein.

Table III. Effect of Treatment on Vascular Function

PARAMETER, MEAN ± SD	NO.	PLACEBO		ACTIVE		<i>P</i> *
		BEFORE	AFTER	BEFORE	AFTER	
Baseline diameter, mm	35	4.82±0.64	4.79±0.68	4.69±0.66	4.77±0.65†	.008
FMD, mm	35	0.29±0.16	0.34±0.17	0.31±0.14	0.33±0.16	.19
FMD, %	35	6.1±4.4	7.5±3.9	7.0±3.5	7.1±3.7	.11
NMD, %	20	10.0±4.1	9.6±3.6	11.0±4.2	10.0±4.1	.78
Baseline flow, mL/min	33	230±110	250±140	230±100	230±100	.20
Hyperemic flow, mL/min	31	1240±590	1330±700	1240±440	1240±470	.36

*Overall *P* for repeated-measures analysis of variance. †*P*=.01 compared with Active Before by post hoc analysis. There was no difference between Placebo Before and Active Before; *P*=.10 by post hoc analysis. FMD indicates flow-mediated dilation expressed as actual change (mm) or percentage increase over baseline (%); NMD, nitroglycerin-mediated dilation.

gastrointestinal bleeding). Thus, 36 subjects were included in the study; their clinical characteristics according to treatment order are presented in Table I. As shown, the majority of subjects were men with a high prevalence of risk factors for coronary artery disease. Subjects in the placebo-first and active treatment-first groups had similar clinical characteristics.

Effect of Treatment on Physiologic Markers

Table II displays the effects of placebo and α-lipoic acid/acetyl-L-carnitine treatment on total carnitine levels, lipid profiles, fasting glucose, serum insulin, C-reactive protein, and urinary F₂-isoprostanes. As shown, there was a strong trend for an effect of treatment on total carnitine levels, but no other significant effects on these blood markers.

Effect of Treatment on Vasodilator Function

For analysis of vascular function, we excluded 1 additional subject because of technically inadequate ultrasound images. As shown in Table III, α-lipoic acid/acetyl-L-carnitine treatment was associated with a significant 2% increase in baseline brachial artery diameter. There were no effects of

treatment on baseline flow or vasodilator function. There also was no effect of α-lipoic acid/acetyl-L-carnitine on flow-mediated dilation or hyperemic flow in the prespecified subgroups of older subjects (older than 62 years), subjects with higher BP (systolic BP ≥135 mm Hg), or among the 24 subjects with the metabolic syndrome (data not shown).

Effect of Treatment on BP

As shown in Table IV, there was no statistically significant effect of active treatment on systolic BP. Sublingual nitroglycerin produced a greater reduction in diastolic BP during treatment with active α-lipoic acid/acetyl-L-carnitine compared with placebo, with a trend for a similar effect on systolic BP after nitroglycerin.

As shown in Table IV, the subgroups of subjects with systolic BP above the median and subjects with the metabolic syndrome had significant decreases in systolic BP after active treatment. There was no effect in the subgroup of older subjects (data not shown).

Correlates of Carnitine Levels

Serum total carnitine levels correlated inversely with age ($r=-0.42$, $P=.006$), but did not correlate

Table IV. Effect of Treatment on Blood Pressure (BP)

BP, MEAN ± SD, MM HG	No.	PLACEBO		ACTIVE		P*
		BEFORE	AFTER	BEFORE	AFTER	
All subjects						
SBP	36	133±16	137±22	135±23	132±18	.07
DBP	36	72±8	73±8	73±10	72±8	.21
SBP after NTG	24	117±13	118±15	120±14	113±13	.09
DBP after NTG	24	65±7	67±8	68±7	64±8†	.04
SBP ≥135						
SBP	18	145±14	150±23	151±20	142±18‡	.03
DBP	18	76±7	75±9	78±8	74±9	.12
Metabolic syndrome						
SBP	24	132±15	137±21	139±21	132±15‡	.03
DBP	24	72±7	73±8	76±8	73±8	.06

*Overall P for repeated-measures analysis of variance. †P=.01 and ‡P=.04 compared with active before treatment by post hoc analysis. SBP indicates systolic BP; DBP, diastolic BP; and NTG, sublingual nitroglycerin.

with other risk factors, serum lipids, glucose, BP, or the metabolic syndrome (data not shown). There was no relation between change in carnitine level and change in BP in the group as a whole or in the subgroups with higher BP or the metabolic syndrome (data not shown).

DISCUSSION

This randomized, placebo-controlled, double-blind crossover study demonstrated that combined α -lipoic acid/acetyl-L-carnitine treatment was associated with an increase in baseline brachial artery diameter. Furthermore, we observed a nonsignificant trend for a BP-lowering effect of α -lipoic acid/acetyl-L-carnitine in all subjects and a significant reduction in systolic BP in subjects with systolic BPs above the median and in subjects with the metabolic syndrome. These findings suggest the possibility that these mitochondria-directed antioxidants reduce basal arterial tone, particularly in 2 clinically relevant subgroups. In contrast, we observed no effect of treatment on the dilator responses to increased flow, nitroglycerin, or ischemia (reactive hyperemia).

A prior study demonstrated a decrease in systolic BP and a direct vasodilator effect in nailfold capillaries after treatment with oral L-carnitine (3 g/d for 20 days) in patients with digital vasospastic disease.²⁹ An open-label study of patients with diabetic nephropathy reported that long-term α -lipoic acid treatment (600 mg/d for 18 months) prevented the increases in BP and urine albumin concentration observed in control patients.³⁰ Experimental studies have consistently demonstrated an antihypertensive effect of α -lipoic acid or L-carnitine in various rat models of hypertension, including spontaneously hypertensive rats,^{8,9}

uninephrectomized deoxycorticosterone acetate-salt (DOCA-salt) hypertensive rats,¹⁰ and salt-loaded Dahl and Wistar-Kyoto rats.^{11,12}

Our study differs from several previous human studies that examined the effects of lipoic acid or carnitine on endothelial function. For example, Heitzer and colleagues²⁶ observed an acute improvement in endothelium-dependent dilation of forearm microvessels following an intra-arterial infusion of lipoic acid (final concentration, 0.2 mmol/L) in patients with diabetes mellitus. Sola and colleagues²⁷ recently reported improved brachial artery flow-mediated dilation following treatment with lipoic acid 300 mg/d for 4 weeks in young patients with the metabolic syndrome (mean age, 46 years). Intravenous administration of L-carnitine (3-g bolus) enhanced reactive hyperemia in patients with peripheral arterial disease.³¹ The apparent discrepancies between the results of those prior studies and the present study likely relate to the marked differences in dose, route of administration, vascular bed studied, and/or patient population.

The mechanisms accounting for the increased brachial artery diameter and suggestive antihypertensive effects of α -lipoic acid and acetyl-L-carnitine in our study remain undefined. We observed no effect of treatment on serum lipids, glucose, and insulin, which might have influenced endothelial function or arterial diameter. Experimental studies indicate that α -lipoic acid and acetyl-L-carnitine play important and potentially synergistic roles in normal mitochondrial function, and that reduced levels of these compounds are associated with increased mitochondrial oxidant production.^{6,25,32} In addition, lipoic acid supplementation has favorable effects on cellular redox state and has been

shown to decrease lipid peroxidation and cellular production of reactive oxygen species.^{6,7,32,33} The effects of α -lipoic acid and acetyl-L-carnitine on oxidative stress that contributes to the pathogenesis and cardiovascular complications of hypertension suggest that these compounds may be useful adjuncts in treatment.³⁴ In the present study, we investigated the possibility that these compounds reduced oxidative stress and inflammation, but observed no effect of treatment on urinary F₂-isoprostanes or serum C-reactive protein. It is important to point out, however, that these systemic markers may not accurately reflect events in the vascular wall.

Despite the effects on BP and basal diameter, it is notable that we observed a trend only for increased total serum carnitine following treatment. It is known that acetyl-L-carnitine and α -lipoic acid are rapidly metabolized in human subjects, with plasma half-lives of 4.2 hours³⁵ and 30 minutes,³⁶ respectively. Thus, it is likely that acetyl-L-carnitine and lipoic acid metabolites and/or tissue levels may be more relevant for the observed effects. Similarly, the lack of effect of treatment on urinary F₂-isoprostanes does not rule out an effect of active treatment on oxidative stress at the tissue level.

We observed a particular benefit of α -lipoic acid/acetyl-L-carnitine in patients with the metabolic syndrome. This syndrome of insulin resistance is associated with hypertension; improvement of insulin sensitivity could have an antihypertensive effect. Consistent with our findings, several experimental studies suggest a BP-lowering effect in models of diabetes mellitus or insulin resistance.^{13–16} Those results and our findings are consistent with the growing evidence linking insulin resistance to mitochondrial dysfunction.^{37,38}

Our study has a number of limitations. First, we observed a significant effect on baseline arterial diameter in all subjects, but the observed effects on BP were significant only in subgroup analyses. These findings are difficult to reconcile, but could reflect a preferential effect on basal conduit artery tone. Further studies will be required to confirm these findings and elucidate the responsible mechanisms. Secondly, arterial tissue was not available for mechanistic analysis in this human study, so it remains speculative whether our findings actually reflect improved mitochondrial function and reduced oxidative stress. Third, we studied the combination of α -lipoic acid because these compounds may act synergistically,³² but it remains unknown whether either compound given alone would have had a similar effect. Finally, our observations

about BP were based on subgroup analyses, and as such could reflect chance findings. Balancing these limitations is the double-blind, prospective, crossover design of our study. Our results are consistent with prior experimental work, and the reduction in initial BP, BP after nitroglycerin, and the observed increase in brachial artery diameter all support an effect of active treatment on arterial tone and BP.

The findings of the present study could have clinical implications. Hypertension remains the most prevalent form of cardiovascular disease, and there is a growing need for new and well-tolerated therapeutic approaches.³⁹ The observed reduction in systolic BP during α -lipoic acid/acetyl-L-carnitine treatment (9 mm Hg in subjects with higher BP) could potentially have a major effect on cardiovascular risk.³⁹ Clearly, additional prospective studies are needed to confirm these findings and define the mechanism of benefit. Our results, however, appear to be consistent with the possibility that mitochondrial dysfunction contributes to the pathogenesis of hypertension, particularly in the setting of insulin resistance, and that therapy designed to restore mitochondrial function might prove useful for patient management.

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