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# Effect of L-carnitine on the size of low-density lipoprotein particles in type 2 diabetes mellitus patients treated with simvastatin

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**Abstract**

Therapeutic modulation of low-density lipoprotein (LDL) size could be of benefit in reducing the risk of cardiovascular events in diabetic patients. This study evaluated the efficacy of L-carnitine on the size of LDL particles in type 2 diabetes mellitus patients treated with simvastatin. Eighty diabetic patients were randomly assigned to 1 of 2 treatment groups for 3 months. The 2 groups received either simvastatin monotherapy 20 mg (n = 40) or L-carnitine 2 g/d and simvastatin 20 mg (n = 40). The following variables were assessed at baseline; after washout; and at 1, 2, and 3 months of treatment: body mass index, fasting plasma glucose, glycosylated hemoglobin, total cholesterol, LDL cholesterol, LDL subclasses, LDL size, high-density lipoprotein cholesterol, triglycerides, apolipoprotein A-1, and apolipoprotein B-100. After 12 weeks, comparing the 2 groups, we observed a decrease in fasting plasma glucose (1.45 vs 0.61 mmol/L, $P < .001$) and an increase in glycosylated hemoglobin (0.2% vs 0.4%, $P < .05$). Moreover, there was a decrease in total cholesterol (2.07 vs 1.45 mmol/L, $P < .001$), LDL (1.65 vs 1.29 mmol/L, $P < .001$), triglycerides (1.36 vs 0.41 mmol/L, $P < .001$), apo B-100 (49 vs 9 g/L, $P < .001$), and small-sized LDL proportion (10.8% vs 4.9%, $P < .001$), whereas LDL particle size increased (6 vs 3 Å, $P < .001$) and HDL increased (0.2 vs 0.11 mmol/L, $P < .001$). We observed that patients treated with carnitine and simvastatin showed a reduction in small-sized LDL proportion and an increase in LDL particle size.


## 1. Introduction

Type 2 diabetes mellitus represents a major public heath issue all over the world. Diabetes prevalence in adults 20 years and older was 4.0% in 1995, and it is expected to increase to 5.4% in 2030 [1].

The presence of an atherogenic lipid profile is common in diabetic patients and is characterized by elevated plasma triglyceride (TG) levels, low levels of high-density lipoprotein cholesterol (HDL-C), and a preponderance of low-density lipoprotein cholesterol (LDL-C).

Several studies have shown that small dense LDL particles are more atherogenic than large buoyant ones [2,3] and are associated with increased risk of coronary artery disease [4] or stroke [5].

It has been suggested that therapeutic modulation of LDL size could be of benefit in reducing the risk of cardiovascular events.

In a large prospective study (Quebec Cardiovascular Study), it was found that men with small dense LDL had an increased risk of ischemic heart disease compared with men with large buoyant LDL, independent of LDL-C and TG [6].

Statins are the cornerstone in the management of dyslipidemia. Statins competitively inhibit 3-hydroxy-3-methylglutaryl (HMG)–coenzyme A (CoA) reductase, which catalyzes the conversion of HMG-CoA to L-mevalonate, a key intermediate in cholesterol biosynthesis [7]. Because of their cholesterol-lowering effects, statins are used widely and clinically for the treatment of cardiovascular disease [8]. The major effect of statins is a reduction in LDL-

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C concentrations, primarily mediated by inhibition of the rate-limiting step in cholesterol biosynthesis, resulting in an increase in LDL receptors in the liver [9]. In addition, statins can reduce TG and increase HDL-C [10,11], all of which lead to a reduced risk for coronary heart disease in patients with type 2 diabetes mellitus [12-14].

Numerous studies suggested that statins lower all LDL subfractions, possibly as a result of the statin-induced stimulation of LDL-receptor–mediated catabolism; and thus, their net effect on LDL particle size is often none or only moderate [15-17].

L-carnitine is a natural constituent of higher organisms and in particular cells of animal origin [18]. Carnitine plays an important role in lipid metabolism, acting as an obligatory cofactor for $\beta$-oxidation of fatty acids by facilitating the transport of long-chain fatty acids across the mitochondrial membrane as acylcarnitine esters. Furthermore, because carnitine behaves as a shuttle for acetyl groups from inside to outside the mitochondrial membrane, it covers also a key role in glucose metabolism [19]. Carnitine acts as a carrier of activated acyl groups across the mitochondrial membrane of virtually all cells [20,21].

Specific enzymes (carnitine palmitoyltransferase I and II) catalyze the reversible formation of carnitine esters of long-chain fatty acids to which the mitochondrial membrane is impermeable. The role of carnitine in the regulation of fatty acids oxidation and ketogenesis is well characterized [22,23].

Carnitine deficiency, whether [24,25] or acquired [26], is associated with impaired fat oxidation and a number of functional abnormalities.

Carnitine can also bind acetyl residues in the mitochondrial matrix, where a specific carnitine acetyltransferase activity is present, and export them into the cytosolic compartment.

The aim of the present study was to compare the effects of simvastatin and L-carnitine coadministration vs simvastatin monotherapy on lipid profile and on LDL size in type 2 diabetes mellitus patients.

## 2. Study design

Eighty patients with type 2 diabetes mellitus (55 men and 25 women) were enrolled in the study. Baseline characteristics of these patients are shown in Table 1. Two groups were formed by randomly assigning the study patients. Group 1 received simvastatin monotherapy 20 mg tablet after dinner for 3 months; group 2 received L-carnitine 2 g/d and simvastatin 20 mg according to the same regimen and for the same duration. Patients were seen by a dietitian every month. At each visit, the dietitian provided instruction on dietary intake recording procedures as part of a behavior-modification program; and the patients' resulting food diaries were later used for counseling.

Patients' acceptance of the diet planned by the dietitian was good, and compliance with diet was judged to be excellent on the basis of subject feedback and daily records.

Table 1
Baseline characteristics at randomization

| | | |
|---|---|---|
| Sex | | |
| Men | 29 | 26 |
| Women | 11 | 14 |
| Age (y) | 45 ± 12 | 47 ± 13 |
| BMI (kg/m$^2$) | 26.5 ± 1.7 | 26.8 ± 1.3 |
| Heart rate (beat/min) | 80.2 ± 10.8 | 81.3 ± 10.7 |
| SBP (mm Hg) | 144.2 ± 15.2 | 145.1 ± 16.1 |
| DBP (mm Hg) | 81.8 ± 10.1 | 82.5 ± 10.3 |
| Nonsmokers | 12 | 11 |
| Smokers | 28 | 29 |

SBP indicates systolic blood pressure; DBP, diastolic blood pressure.

The following variables were assessed at baseline; after washout; and at 1, 2, and 3 months of treatment: body mass index (BMI), fasting plasma glucose (FPG), glycosylated hemoglobin ($HbA_{1c}$), total cholesterol (Tot-C), LDL-C, LDL subclasses, LDL size, HDL-C, TG, apolipoprotein (apo) A-1, and apo B.

### 2.1. Patients

During the run-in observation period, fasting serum lipid levels and LDL size were determined at least twice; and we enrolled patients with newly diagnosed (within 6 months) type 2 diabetes mellitus according to the American Diabetes Association criteria [27] who were managed through dietary restriction alone and those with hypercholesterolemia according to the National Cholesterol Education Program Expert Panel [28].

Standardized breakfast, lunch, and dinner were recommended based on a dietician-prescribed diet to each patient. Each patient received 1400 to 1600 kcal/d: 55% carbohydrates, 25% proteins, 20% lipids (7% saturated), 105 mg cholesterol, and 36 g fiber. This controlled-energy diet was continued, and the patients were instructed to maintain the same diet throughout the study. After enrollment, patients provided a medical history and underwent a physical examination with an electrocardiogram and a battery of biochemical/hematologic tests for the measurement of lipids and other components that were part of the safety assessment.

Patients were excluded from this study if they:

1. were younger than 40 years or older than 70 years;
2. had severe liver or kidney disease, including nephritic syndrome;
3. were or may have been pregnant or were lactating;
4. were hypersensitive to drugs;
5. had a history of myocardial infarction or recent or severe episodes of cerebrovascular disease;
6. had hypothyroidism, alcoholism, pancreatitis, multiple myeloma, malignant lymphoma, autoimmune diseases (eg, systemic lupus erythematosus), or Cushing syndrome;
7. had hyperlipidemia induced by drugs such as steroids;
8. were under therapy with statins.

### 2.2. Methods

Between January 2003 and 2006, outpatients with dyslipidemia and with type 2 diabetes mellitus that was managed through dietary restriction alone, attending the Department of Geriatrics, Catania University Hospital, Italy, were recruited for the study. After discontinuing all lipid-lowering drugs and supplements and/or treatment with other drugs known to affect mitochondrial metabolism, patients entered a 6-week dietary lead-in period. The study protocol was approved by the research ethics committee of Cannizzaro Hospital, Catania, Italy, and was performed in accordance with the Declaration of Helsinki principles and the Good Clinical Practice Guidelines [29]. After an initial 6 weeks of placebo washout, the patients were randomized into 2 groups using a drawing of envelopes containing randomization codes prepared by a statistician.

## 3. Clinical laboratory tests

Blood samples were obtained after the patients had fasted for 12 hours overnight. Venous blood samples were taken from all patients between 8:00 and 10:00 AM.

We used plasma obtained from the blood samples by the addition of EDTA and centrifugation at 3000*g* for 15 minutes at 4°C (Vacutainer SST II Advance; BD, Plymouth, United Kingdom). Immediately after centrifugation, the plasma samples were frozen and stored at −80°C.

The fasting plasma glucose (FPG) was assayed with the glucose-oxidase method with intra- and interassay coefficients of variation (CVs) of 0.8% to 2.1%, respectively. The $HbA_{1c}$ level was measured with the use of high-performance liquid chromatography with intra- and interassay CVs of 1.2% and 2.8%, respectively. The Tot-C and TG were determined using fully enzymatic techniques on a clinical chemistry analyzer; the intra- and interassay CVs were 1.1% and 2.2%, respectively, for the Tot-C measurement and 1.0% and 2.3%, respectively, for the TG measurement. The HDL-C level was measured after precipitation of plasma apo B–containing lipoproteins with phosphotungstic acid. The intra- and interassay CVs were 1.0% and 2.0%, respectively. Apolipoprotein A-1 and apo B were measured using immunoturbidimetric assays; the intra- and interassay CVs were 2.8% to 4.7%, respectively. All assays were carried out in our department laboratory.

### 3.1. Measurement of LDL particle size

The LDL particle size was determined by the LipoPrint LDL system (Quantimetrix, Redondo Beach, CA). The diameter of LDL particle was determined by gradient gel electrophoresis on 2% to 16% polyacrylamide gels.

In this study, we applied 2 kinds of polyacrylamide gel electrophoresis system. The former is simple; and it separates the lipoproteins into 3 major classes: very low-density lipoprotein, LDL, and HDL. The lipoprotein size can be assessed by the migration of LDL fraction: LDL-migration index. It was identified by the migration distance of the LDL relative to the HDL fraction.

#### 3.1.1. LDL subclass analysis

In brief, 25 $\mu$L of sample was mixed with 200 $\mu$L of LipoPrint loading gel and then placed upon the upper part of 3% polyacrylamide gel. After 30 minutes of that photopolymerization in room temperature, 60 minutes of electrophoresis with 3 mA for each gel tube followed. Scanning was done 30 minutes after completion of electrophoresis.

The LipoPhor system (Quantimetrix) was used as quality control. Each chamber had 2 quality controls. For quantification, scanning was done at 610 nm with an Artixscan 1100 scanner (Microtek, Cerritos, CA) and iMac (Apple, Cupertino, CA) personal computer. After scanning, electrophoretic mobility and area under the curve were analyzed with National Institutes of Health Image program version 1.62 (US National Institute of Health, Bethesda, MD).

*Low-density lipoprotein 1* and *LDL 2* are defined as large LDL, and *LDL 3 to LDL 7* are defined as small LDL. *Small-sized LDL proportion* was defined as the percentage of small-sized LDL (from band 3 to band 7) over the whole LDL.

The intra- and the interassay CVs of specimen with 51% of small-sized LDL proportion were 1.6% and 2.1%, respectively; and those of specimen with 8% of small-sized LDL proportion were 5.1% and 5.5%, respectively. In addition, the linearity of small-sized LDL proportion was validated with 5-level serum specimens according to the guidelines of NCCLS EP 06-A. The method passed the lack of fit test and proved to be linear.

### 3.2. Efficacy and tolerability assessment

During treatment, patients were advised to continue diet and physical activity if they had already been doing so (although they were not instructed to begin these lifestyle changes for the purpose of the study). Azotemia, creatininemia, hemochrome, liver enzymes, myoglobin, creatine kinase, and urine were measured before the start of therapy (week 0) and at weeks 4, 8, and 12 of treatment (study end).

Patients were advised to immediately report unexplained muscle pain, low urine output, irritable bowel, and any other adverse effects, whether thought to be caused by the study drug or by cardiovascular problems.

## 4. Statistical analysis

The data were analyzed by using the Statistical Analysis System software version 6.11 (SAS Institute, Cary, NC). Independent *t* tests were performed on all baseline data between groups. Differences in variables at baseline and after treatment were assessed with a repeated-measures analysis of variance that included a time × treatment interaction. Tukey post hoc tests were used to assess differences between the treatment groups. Differences

between means were evaluated by using analysis of variance or paired *t* tests where appropriate. Data were further analyzed with a Bonferroni-adjusted *t* test for multiple comparisons. The Mann-Whitney *U* test was used to compare nonparametric data. A 2-factor analysis of covariance was used to compare LDL size and other plasma variable between the 2 groups with change in BMI and in $HbA_{1c}$ as covariates. Simple Pearson correlations were computed in the total sample of diabetic patients between the initial carnitine intake and the initial values of all dependent variables. To adjust the analyses for confounding variables, simple correlations were repeated by using residual scores for all dependent variables derived from linear regression analysis after adjustment for BMI and $HbA_{1c}$ as the independent variables. $P < .05$ was considered significant. The primary population for statistical analysis was an intention-to-treat population of all randomly assigned subjects.

## 5. Results

### 5.1. Baseline values

The groups had similar general characteristics. Lipid profile was not significantly different before the treatment. No differences were observed between group 1 and group 2 in mild hypertension (12% compared with 10%), moderate hypertension (14% compared with 13%) smoking (72.5% compared with 70%), stage 1 retinopathy (11% compared with 12%), and stage 0 retinopathy (89% compared with 88%). All patients completed the treatment program and maintained the prescribed diet throughout the study.

### 5.2. Effects on fasting glucose and $HbA_{1c}$

After 12 weeks, compared with baseline, in group 1, we observed a significant decrease in $HbA_{1c}$ of 0.40% ($P < .001$) and in plasmatic glucose of 0.61 mmol/L ($P < .05$); in group 2, we found a significant decrease in $HbA_{1c}$ of 0.30% ($P < .05$) and in plasmatic glucose of 1.45 mmol/L ($P < .05$) (Table 2).

Comparing the groups at the end of treatment, we observed a decrease in FPG (1.45 vs 0.61 mmol/L, $P < .001$) and an increase in $HbA_{1c}$ (0.2% vs 0.4%, $P < .05$).

### 5.3. Effects on blood lipids

Compared with baseline, in group 1, there was a significant decrease in Tot-C of 1.45 mmol/L ($P < .001$), LDL of 1.29 mmol/L ($P < .001$), TG of 0.41 mmol/L ($P < .001$), and apo B-100 of 9.00 mg/dL ($P < .001$), whereas HDL increased by 0.11 mg/dL ($P < .001$); small-sized LDL proportion and LDL particle size were not significantly different.

In group 2, we observed a decrease in Tot-C of 2.07 mmol/L ($P < .001$), LDL of 1.65 mmol/L ($P < .001$), TG of 1.36 mmol/L ($P < .001$), apo B-100 of 49.00 g/L ($P < .001$), and small-sized LDL proportion of 10.80% ($P < .001$), whereas LDL particle size increased by 6 Å ($P < .001$) and HDL levels increased by 0.20 mmol/L ($P < .001$).

After 12 weeks, comparing the 2 groups, we observed a decrease in Tot-C (2.07 vs 1.45 mmol/L, $P < .001$), LDL (1.65 vs 1.29 mmol/L, $P < .001$), TG (1.36 vs 0.41 mmol/L, $P < .001$), apo B-100 (49 vs 9 g/L, $P < .001$), and small-sized LDL proportion (10.8% vs 4.9%, $P < .001$), whereas LDL particle size increased (6 vs 3 Å, $P < .001$) and HDL increased (0.2 vs 0.11 mmol/L, $P < .001$).

### 5.4. Tolerability and adverse effects

Both treatments were well tolerated in all patients. A total of 7 patients treated with simvastatin reported gastrointestinal tract complaints, with 2 leading to withdrawal, whereas during simvastatin plus carnitine treatment, 3 patients reported gastrointestinal tract complaints, with 1 leading to withdrawal.

Biochemical and hematologic safety was considered excellent in both groups. The only changes of importance were in 4 patients who demonstrated significant increases in creatine kinase level, 3 under treatment with simvastatin and

Table 2
Laboratory parameters in patients treated with L-carnitine plus simvastatin 20 mg and simvastatin 20 mg alone before and after 12 weeks of treatment

| | Simvastatin (n = 40) | | Carnitine + simvastatin (n = 40) | | *P* for time[a] | *P* for group × time[a] |
|---|---|---|---|---|---|---|
| | Before treatment | After 12 wk | Before treatment | After 12 wk | | |
| BMI (kg/m$^2$) | 27.7 ± 2.4 | 26.9 ± 2.8 | 28.0 ± 2.1 | 26.8 ± 2.6 | NS | NS |
| Glucose (mmol/L) | 7.82 ± 1.3 | 7.21 ± 1.0 | 7.99 ± 1.1 | 6.54 ± 1.4 | $P < .05$ | $P < .001$ |
| $HbA_{1c}$ (%) | 7.1 ± 0.5 | 6.9 ± 0 .4 | 7.1 ± 0.4 | 6.8 ± 0.8 | NS | $P < .05$ |
| Tot-C (mmol/L) | 6.72 ± 0.8 | 5.27 ± 0.7 | 6.72 ± 0.9 | 4.65 ± 0.8 | $P < .001$ | $P < .001$ |
| HDL-C (mmol/L) | 1.08 ± 0.09 | 1.19 ± 0.14 | 1.04 ± 0.08 | 1.24 ± 0.08 | NS | $P < .001$ |
| LDL-C (mmol/L) | 4.1 ± 0.4 | 2.81 ± 0.3 | 4.08 ± 0.4 | 2.43 ± 0.2 | $P < .001$ | $P < .001$ |
| TG (mmol/L) | 3.17 ± 0.3 | 2.76 ± 0.4 | 3.50 ± 0.4 | 2.14 ± 0.3 | $P < .001$ | $P < .001$ |
| Apo A-1 (g/L) | 154 ± 15 | 156 ± 24 | 156 ± 21 | 160 ± 19 | NS | NS |
| Apo B-100 (g/L) | 169 ± 23 | 160 ± 19 | 171 ± 19 | 122 ± 20 | $P < .001$ | $P < .001$ |
| Small-sized LDL proportion (%) | 23.8 ± 13.1 | 18.9 ± 12.1 | 23.6 ± 13.8 | 12.8 ± 10.2 | $P < .05$ | $P < .001$ |
| LDL particle size (Å) | 254 ± 6.8 | 257 ± 6.8 | 255 ± 4.1 | 261 ± 5.7 | $P < .05$ | $P < .001$ |

All values are x ± SD. NS indicates not significant.

[a] Determined with analysis of variance.

1 under treatment with simvastatin plus carnitine. No clinically important changes were observed in serial blood pressure, pulse rate, and body weight measurements.

## 6. Discussion

Small-dense LDL particles are a major risk factor for coronary artery disease in diabetes because they have increased sensitivity to oxidative modification, particularly when glycated. Simvastatin may reduce the plasma concentration of both small and large LDL particles, while having no effect on the mean distribution of LDL particle diameter. Treatment with simvastatin has also been shown to lower the concentration of large LDL particles in hypercholesterolemic patients with no effect on smaller LDL subfractions [30]. The results of the present study suggest that simvastatin, when added to L-carnitine supplementation, may reduce TG, Tot-C, and small-sized LDL-C and improve HDL-C. Combined treatment with simvastatin and L-carnitine may have not only accelerated the utilization of intracellular fatty acids by improving fatty acids oxidation capacity but may have also inhibited the influx of long-chain fatty acids by decreasing serum TG [31]. At the end of the treatment, we observed in the L-carnitine and simvastatin combined group an important additive effect on small-sized LDL-C compared with simvastatin. L-carnitine covers an important role in the mitochondrial uptake of long-chain fatty acids by facilitating their transportation across the inner mitochondrial membrane to undergo $\beta$-oxidation and may reasonably reduce level of fatty acid inflow for small LDL, thus distinctly lowering levels in the subjects presumably affected by excess production of this atherogenic lipoprotein. Fatty acids longer than 10 carbons in length need to traverse the mitochondrial membrane with carnitine. Mitochondrial fatty acid oxidation defects occur with carnitine, and carnitine cycles enzymes deficiencies. The consumption of free fatty acids by mitochondrial $\beta$-oxidation causes a decrease in TG synthesis in liver, which is increased in diabetes. Small dense LDL particles are cleared from plasma at a slower rate compared with larger LDL particles and appear to be more susceptible to oxidative damage and accumulation in the artery wall [32]. Howard and Howard [33] found a significant relation between insulin sensitivity and LDL size among nondiabetic men and women. Mykkanen et al [34] found an association between insulin resistance and the preponderance of small dense LDL particles in normoglycemic middle-aged men. Recent research suggests that carnitine is also crucial in the regulation of carbohydrate metabolism in addition to its role in the oxidation of fatty acids [35].

L-carnitine has a substantial effect on improving mean levels of LDL particle size and reducing small dense LDL particle mass.

As both small dense LDL mass and LDL peak particle diameter are important determinants of LDL content in endothelial plaque, it is important not only to increase the average size of LDL particles but also to reduce small dense LDL particle mass.

Another possible explanation for the TG-lowering effect of L-carnitine leads to increased urinary excretion of acetylcarnitine. Dellow et al [36] suggested that poor glycemic control is associated with an increase in urine acetylcarnitine excretion in diabetic patients. L-carnitine supplementation also has cholesterol-lowering effects. This may depend on its TG-lowering effect that consequently alters the lipoprotein composition that is adversely changed in diabetes. The decreased synthesis of TG lowers the TG content of LDL, which results in an enhanced uptake of LDL by its receptors.

Limitations of the current study include the open-label design, the relatively short duration, and the usefulness of the gradient electrophoresis for LDL size assay. Nonetheless, effective randomization of subjects according to clinical and laboratory characteristics and compliance with lifestyle modifications in both groups and medications in the L-carnitine– and simvastatin-treated group suggested a small likelihood of bias in the interpretation of the results. Furthermore, because statins are known to have a rapid onset of action, the time point chosen for efficacy and safety measurements (12 weeks) was sufficient to observe the maximal lipids effects for the studied drug. In our previous study, we observed that L-carnitine treatment reduced lipid peroxidation indices in diabetic hyperlipidemic patients [37]. The combination of HMG-CoA reductase inhibitors and carnitine is an appealing option for patients with diabetic dyslipidemia because such therapy would result not only in lower serum LDL-C levels and in reduction of small dense LDL proportion and TG but also in significant improvement in serum levels of HDL-C and in LDL particle size.

The investigation demonstrates a significant reduction in total and LDL-C but no change in LDL peak particle diameter in response to simvastatin treatment. The effect of simvastatin on LDL subclass distribution appears to have no consistent effect. In the patients treated with carnitine and simvastatin, we observed a reduction on small-sized LDL proportion and an increase in LDL particle size.

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