Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 60 (2011) 421 – 429
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Effects of combination of sibutramine and L-carnitine compared with sibutramine monotherapy on inflammatory parameters in diabetic patients

Giuseppe Derosa[a,*], Pamela Maffioli[a], Sibilla A.T. Salvadeo[a], Ilaria Ferrari[a], Alessia Gravina[a], Roberto Mereu[a], Angela D'Angelo[a], Ilaria Palumbo[a], Sabrina Randazzo[a], Arrigo F.G. Cicero[b]

[a]*Department of Internal Medicine and Therapeutics, University of Pavia, 27100 PAVIA, Italy*
[b]*"G Descovich" Atherosclerosis Study Center, Department of Internal Medicine, Aging and Kidney diseases, University of Bologna, 43100 Bologna, Italy*

Received 11 January 2010; accepted 17 March 2010

Abstract

The aim of the study was to evaluate the effects of 12-month treatment with sibutramine plus L-carnitine compared with sibutramine alone on body weight, glycemic control, insulin resistance, and inflammatory state in type 2 diabetes mellitus patients. Two hundred fifty-four patients with uncontrolled type 2 diabetes mellitus (glycated hemoglobin [HbA_{1c}] >8.0%) in therapy with different oral hypoglycemic agents or insulin were enrolled in this study and randomized to take sibutramine 10 mg plus L-carnitine 2 g or sibutramine 10 mg in monotherapy. We evaluated at baseline and after 3, 6, 9, and 12 months these parameters: body weight, body mass index, HbA_{1c}, fasting plasma glucose, postprandial plasma glucose, fasting plasma insulin, homeostasis model assessment of insulin resistance index, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, leptin, tumor necrosis factor–α, adiponectin, vaspin, and high-sensitivity C-reactive protein. Sibutramine plus L-carnitine gave a faster improvement of fasting plasma glucose, postprandial plasma glucose, lipid profile, leptin, tumor necrosis factor–α, and high-sensitivity C-reactive protein compared with sibutramine alone. Furthermore, there was a better improvement of body weight, HbA_{1c}, fasting plasma insulin, homeostasis model assessment of insulin resistance index, vaspin, and adiponectin with sibutramine plus L-carnitine compared with sibutramine alone. Sibutramine plus L-carnitine gave a better and faster improvement of all the analyzed parameters compared with sibutramine alone without giving any severe adverse effect.

1. Introduction

The prevention and treatment of overweight and obesity are critical because these factors are associated with an increased prevalence of cardiovascular risk factors, insulin resistance, impaired glucose tolerance, type 2 diabetes mellitus (T2DM), dyslipidemia, and hypertension [1-4]. The current recommendations for the treatment of overweight and obese people include increased physical activity and reduced calorie intake [5,6]. When the behavioral approach is not sufficient to get the optimal target of weight and metabolic control, a pharmacologic treatment is recommended [7].

Sibutramine hydrochloride monohydrate is 1 of the 2 molecules licensed for use as antiobesity drugs together with orlistat [8]; sibutramine is a norepinephrine and serotonin reuptake inhibitor approved for the long-term management of obesity, in conjunction with a reduced-calorie diet and behavior modification, in patients unable to lose weight with diet and lifestyle changes alone. In previous clinical trials, sibutramine has been reported to be related to headache, dry mouth, insomnia, constipation, hypertension, and tachycardia [9,10].

Carnitine, or L-β-hydroxy-γ-*N*-trimethylaminobutyric acid, instead, is synthesized primarily in the liver and

Declaration of interest: The study was not sponsored and was carried out with funding from the University of Pavia. All authors contributed to the draft manuscript and approved the final version of the manuscript. All the authors certify that they have no affiliation with, or financial involvement in, any organization or entity with a direct financial interest in the subject matter or materials discussed in the manuscript.

* Corresponding author. Tel.: +39 0382 526217; fax: +39 0382 526259.
E-mail address: giuseppe.derosa@unipv.it (G. Derosa).

0026-0495/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2010.03.010

kidneys. There is experimental evidence that L-carnitine stimulates the activity of the pyruvate dehydrogenase complex by decreasing the intramitochondrial acetyl–coenzyme A (CoA) to CoA ratio through the trapping of acetyl groups [11]. The simultaneous reduction of acetyl-CoA levels in the cytosol further contributes to activate the glycolytic pathway [12], which is why L-carnitine covers a role in the glucose metabolism and assists in fuel sensing.

Carnitine covers also an important role in lipid metabolism, acting as an obligatory cofactor for β-oxidation of fatty acids by facilitating the transport of long-chain fatty acids across the mitochondrial inner membrane as acylcarnitine esters. Its lack impairs the ability to use fat as fuel; this can result in an acute metabolic decompensation, most often early in life, with hepatic encephalopathy and hypoketotic hypoglycemia [13]. The positive effect of L-carnitine on lipid profile was confirmed by our group in a previous work where we demonstrated that L-carnitine lowered the plasma lipoprotein (a) level in hypercholesterolemic T2DM patients [14].

The aim of this study was to evaluate the effects of 12-month treatment with sibutramine plus L-carnitine compared with sibutramine monotherapy added to the usual antidiabetic therapy on body weight, glycemic control, lipid profile, and inflammatory parameters such as leptin, tumor necrosis factor–α (TNF-α), adiponectin (ADN), vaspin, and high-sensitivity C-reactive protein (Hs-CRP) in T2DM patients.

2. Material and methods

2.1. Study design

This multicenter, randomized, double-blind, controlled study was conducted at the Department of Internal Medicine and Therapeutics, University of Pavia (Pavia, Italy), and the "G Descovich" Atherosclerosis Study Center, Department of Internal Medicine, Aging and Kidney Diseases, University of Bologna (Bologna; Italy).

The study protocol was approved at each site by institutional review boards and was conducted in accordance with the Declaration of Helsinki and its amendments.

2.2. Patients

We enrolled 254 white patients aged at least 18 years of either sex (Table 1) with T2DM according to the European Society of Cardiology and European Association for the Study of Diabetes criteria [15] who were obese (body mass index [BMI] ≥30 kg/m^2) [16], with uncontrolled T2DM (glycated hemoglobin]HbA_{1c}] >8.0%], and in therapy with different oral hypoglycemic agents or insulin.

Suitable patients, identified from review of case notes and/or computerized clinic registers, were contacted by the investigators in person or by telephone.

Patients were excluded if they had a history of ketoacidosis or had unstable or rapidly progressive diabetic retinopathy, nephropathy, or neuropathy; *impaired hepatic function* (defined as plasma aminotransferase and/or γ-glutamyltransferase level higher than the upper limit of normal for age and sex); *impaired renal function* (defined as serum creatinine level higher than the upper limit of normal for age and sex); or severe anemia. Patients with serious cardiovascular disease (eg, New York Heart Association class I-IV congestive heart failure or a history of myocardial infarction or stroke) or cerebrovascular conditions within 6 months before study enrollment also were excluded. Women who were pregnant or breastfeeding or of child-bearing potential and not taking adequate contraceptive precautions were also excluded. All patients provided written informed consent to participate.

Table 1
General subject characteristics at baseline in the study

	Sibutramine group	Sibutramine + L-carnitine group
n	125	129
Sex (M/F)	63/62	65/64
Age (y)	51 ± 4	54 ± 5
Sm st (M/F)	24/18	22/19
Diab dur (y)	5 ± 2	6 ± 3
Height (m)	1.71 ± 0.06	1.69 ± 0.04
Concomitant disease, n (%)	112 (89.6)	119 (92.2)
Hypertension	96 (85.7)	98 (82.3)
Hypercholesterolemia	39 (34.8)	41 (34.4)
Hypertriglyceridemia	4 (3.6)	7 (5.9)
Combined dyslipidemia	25 (22.3)	32 (26.9)
Concurrent medications, n (%)	114 (91.2)	120 (93.0)
ACE-I	30 (26.3)	35 (29.2)
ARBs	31 (27.2)	39 (32.5)
Calcium-antagonists	24 (21.0)	20 (16.7)
β-Blockers	9 (7.9)	12 (10.0)
Diuretics	18 (15.8)	25 (20.8)
Statins	48 (42.1)	47 (39.2)
Fibrates	10 (8.8)	14 (11.7)
Omega-3	14 (12.3)	12 (10.0)
Acetylsalicylic acid	94 (82.5)	97 (80.8)
Ticlopidine	7 (6.1)	9 (7.5)

Data are expressed as means ± SD or number and percentage. Sm st indicates smoking status; Diab dur, diabetes duration; ACE-I, angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers.

At the beginning of the study and for all the observational periods, patients were taking different antidiabetic drugs; the complete list of the antidiabetic drugs taken is reported in Table 2, whereas the complete list of the other concurrent medications is reported in Table 1.

2.3. Treatments

Patients were assigned to receive, as addition to their current antidiabetic therapy, sibutramine 10 mg plus L-carnitine 2 g or sibutramine 10 mg in monotherapy for 12 months in a randomized, double-blind, controlled study. Both sibutramine and L-carnitine were supplied as identical, opaque, white capsules in coded bottles to ensure the blind status of the study. Randomization was done using a drawing

of envelopes containing randomization codes prepared by a statistician. A copy of the code was provided only to the responsible person performing the statistical analysis. The code was only broken after database lock, but could have been broken for individual subjects in cases of an emergency. Medication compliance was assessed by counting the number of pills returned at the time of specified clinic visits. At baseline, we weighed participants and gave them a bottle containing a supply of study medication for at least 100 days. Throughout the study, we instructed patients to take their first dose of new medication on the day after they were given the study medication. At the same time, all unused medication was retrieved for inventory. All medications were provided free of charge.

2.4. Diet and exercise

Subjects began a controlled-energy diet (nearly 600 kcal daily deficit) based on American Heart Association recommendations [17] that included 50% of calories from carbohydrates, 30% from fat (6% saturated), and 20% from proteins, with a maximum cholesterol content of 300 mg/d and 35 g/d of fiber. Patients were not treated with vitamins or mineral preparations during the study.

Table 2
Antidiabetic agents before and during the study

	Sibutramine group	Sibutramine + L-carnitine group
n	125	129
OHA, n (%)	116 (92.8)	120 (93.0)
Sulfonylureas, n (%)	25 (21.5)	26 (21.7)
Glyburide	7 (28.0)	9 (34.6)
Glimepiride	14 (56.0)	15 (57.7)
Gliclazide	4 (16.0)	2 (7.7)
Biguanides, n (%)	77 (66.4)	70 (58.3)
Metformin	77 (100.0)	70 (100.0)
Glinides, n (%)	20 (17.2)	14 (11.7)
Repaglinide	15 (75.0)	10 (71.4)
Nateglinide	5 (25.0)	4 (28.6)
α-Glucosidase inhibitors, n (%)	12 (10.3)	22 (18.3)
Acarbose	12 (100.0)	22 (100.0)
Thiazolidinediones, n (%)	64 (55.2)	67 (55.8)
Pioglitazone	34 (53.1)	41 (61.2)
Rosiglitazone	30 (46.9)	26 (38.8)
Incretin mimetics, n (%)	11 (9.5)	13 (10.8)
Exenatide	11 (100.0)	13 (100.0)
DPP-4 inhibitors, n (%)	17 (14.6)	15 (12.5)
Sitagliptin	11 (64.7)	10 (66.7)
Vildagliptin	6 (35.3)	5 (33.3)
Insulin, n (%)	13 (10.4)	16 (12.4)
Analog, n (%)	9 (69.2)	12 (75.0)
Lispro	7 (77.8)	8 (66.7)
Glulisine	2 (22.2)	4 (33.3)
Long acting, n (%)	7 (53.8)	9 (56.2)
Glargine	2 (28.6)	4 (44.4)
NPH	5 (71.4)	5 (55.6)

Data are expressed as number or percentage. OHA indicates oral hypoglycemic agents; DPP-4, dipeptidyl peptidase-4 inhibitors; NPH, neutral protamine Hagedorn.

Standard diet advice was given by a dietitian and/or specialist physician. The dietitian and/or specialist physician periodically provided instruction on dietary intake recording procedures as part of a behavior modification program and then later used the subject's food diaries for counseling. Individuals were also encouraged to increase their physical activity by walking briskly for 20 to 30 minutes, 3 to 5 times per week, or by cyclette. The recommended changes in physical activity throughout the study were assessed at each visit using the subject's activity diary.

2.5. Assessments

Before starting the study, all patients underwent an initial screening assessment that included a medical history, physical examination, vital signs, and a 12-lead electrocardiogram. We evaluated at baseline and after 3, 6, 9, and 12 months these parameters: body weight, BMI, HbA_{1c}, fasting plasma glucose (FPG), postprandial plasma glucose (PPG), fasting plasma insulin, homeostasis model assessment of insulin resistance index (HOMA-IR), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (Tg), leptin, TNF-α, ADN, vaspin, and Hs-CRP.

To evaluate the tolerability assessments, all adverse events were recorded. All plasmatic parameters were determined after a 12-hour overnight fast, with the exception of PPG, determined 2 hours after a standardized meal. Venous blood samples were taken for all patients between 8:00 and 9:00 AM. We used plasma obtained by addition of Na2-EDTA, 1 mg/mL, and centrifugation at 3000*g* for 15 minutes at 4°C. Immediately after centrifugation, the plasma samples were frozen and stored at −80°C for no more than 3 months. All measurements were performed in a central laboratory.

Body mass index was calculated as weight in kilograms divided by the square of height in meters. Glycated hemoglobin level was measured by a high-performance liquid chromatography method (DIAMAT; Bio-Rad, Richmond, CA; normal values, 4.2%-6.2%) with intra- and interassay coefficients of variation (CVs) of less than 2% [18]. Plasma glucose was assayed by glucose-oxidase method (GOD/PAP; Roche Diagnostics, Mannheim, Germany) with intra- and interassay CVs of less than 2% [19]. Plasma insulin was assayed with Phadiaseph Insulin RIA (Pharmacia, Uppsala, Sweden) by using a second antibody to separate the free and antibody-bound 125 I-insulin (intra- and interassay CVs: 4.6% and 7.3%, respectively) [20].

The HOMA-IR index was calculated as the product of basal glucose (in millimoles per liter) and insulin levels (in microunits per milliliter) divided by 22.5 [21,22].

Total cholesterol and Tg levels were determined using fully enzymatic techniques [23,24] on a clinical chemistry analyzer (HITACHI 737; Hitachi, Tokyo, Japan); intra- and interassay CVs were 1.0 and 2.1 for TC measurement and 0.9

Table 3
Body weight, glycemic profile, and insulin resistance data during the study

	Sibutramine group					Sibutramine + L-carnitine group				
	Baseline	3 mo	6 mo	9 mo	12 mo	Baseline	3 mo	6 mo	9 mo	12 mo
n	125	119	116	112	110	129	124	120	115	113
Sex (M/F)	63/62	61/58	59/57	58/54	56/54	65/64	63/61	61/59	59/56	57/56
Sm st (M/F)	24/18	22/18	21/18	21/17	21/17	22/19	21/19	21/19	21/18	20/17
Weight (kg)	97.7 ± 11.4	96.5 ± 10.7	94.2 ± 9.2	90.4 ± 7.1*	88.6 ± 6.0†	96.9 ± 10.8	93.1 ± 8.9	91.7 ± 8.6	88.0 ± 5.8*	86.0 ± 5.1$^{\dagger,\parallel}$
BMI (kg/m^2)	33.4 ± 3.2	33.0 ± 3.0	32.2 ± 2.7	30.9 ± 2.1*	30.3 ± 1.9†	33.9 ± 3.5	32.6 ± 2.9	32.1 ± 2.6	30.8 ± 2.0*	30.1 ± 1.8†
HbA_{1c} (%)	8.7 ± 1.5	8.4 ± 1.3	7.8 ± 1.0*	7.5 ± 0.8†	7.3 ± 0.6‡	8.8 ± 1.6	8.1 ± 1.2	7.6 ± 0.9*	6.8 ± 0.5$^{\ddagger,\parallel}$	6.4 ± 0.3$^{\S,\parallel}$
FPG (mg/dL)	144 ± 20	135 ± 15	128 ± 12	124 ± 10*	120 ± 9†	146 ± 21	136 ± 16	124 ± 10*	118 ± 8†	114 ± 6‡
PPG (mg/dL)	185 ± 29	174 ± 24	169 ± 22	165 ± 20*	161 ± 21†	187 ± 30	174 ± 24	166 ± 21*	159 ± 18†	155 ± 16‡
FPI (μU/mL)	24.9 ± 7.2	24.0 ± 6.8	23.3 ± 5.9	22.4 ± 5.4	21.2 ± 5.0*	24.1 ± 6.9	23.5 ± 6.0	22.6 ± 5.5	21.1 ± 4.9*	19.2 ± 4.1$^{\dagger,\parallel}$
HOMA-IR	8.9 ± 5.1	8.0 ± 4.5	7.4 ± 4.1	6.9 ± 3.6*	6.3 ± 3.5†	8.8 ± 5.0	8.0 ± 4.5	7.0 ± 3.7*	6.2 ± 3.4†	5.4 ± 2.8$^{\ddagger,\parallel}$

Data are means ± SD. FPI indicates fasting plasma insulin.
* $P < .05$ vs baseline.
† $P < .01$ vs baseline.
‡ $P < .001$ vs baseline.
§ $P < .0001$ vs baseline.
$^{\parallel}$ $P < .05$ vs sibutramine group.

and 2.4 for Tg measurement, respectively. High-density lipoprotein cholesterol level was measured after precipitation of plasma apolipoprotein B–containing lipoproteins with phosphotungstic acid [25]; intra- and interassay CVs were 1.0 and 1.9, respectively. Low-density lipoprotein cholesterol level was calculated by the Friedewald formula [26].

Leptin concentrations were assessed in duplicate by commercially available enzyme-linked immunosorbent assay (ELISA) kits (TiterZyme EIA kit; Assay Designs, Ann Arbor, MI) according to the commercial supplies. Intraassay CV was 4.5%, and the interassay CV was 6.5% [27].

The TNF-α level was assessed using commercially available ELISA kits according to manufacturer's instructions (TiterZyme EIA kit, Assay Designs). Intraassay CVs were 4.5% for low- and 3.6% for high-concentration samples, whereas the interassay CVs were 6.0% for low- and 11.8% for high-concentration samples, respectively, [28].

Adiponectin level was determined using ELISA kits (B-Bridge International, Sunnyvale, CA). Intraassay CVs were 3.6% for low- and 3.3% for high-control samples, whereas interassay CVs were 3.2% for low- and 7.3% for high-control samples, respectively [29].

Vaspin was measured by a 2-site ELISA method using commercially available ELISA kits (Adipogen, Seoul, Korea); the intra- and interassay CVs were 1.74% e 8.32%, respectively [30].

High-sensitivity C-reactive protein was measured with use of latex-enhanced immunonephelometric assays on a BN II analyzer (Dade Behring, Newark, DE). The intra- and interassay CVs were 5.7% and 1.3%, respectively [31].

2.6. Statistical analysis

An intention-to-treat analysis was conducted in patients who had received at least 1 dose of study medication and had a subsequent efficacy observation. Patients were included in the tolerability analysis if they had received at least 1 dose of trial medication and had undergone a subsequent tolerability observation. Considering as clinically significant a difference of at least the 10% compared with the baseline and an α error of 0.05, the actual sample size was adequate to obtain a power higher than 0.80 for all measured variables. Continuous variables were tested using repeated-measures analysis of variance. Intervention effects were adjusted for additional potential confounders using analysis of covariance. Analysis of variance was also used to assess the significance within and between groups. The statistical significance of the independent effects of treatments on the other variables was determined using analysis of covariance. Paired tests were also used: a 1-sample t test to compare values obtained before and after treatment administration, and 2-sample t tests for between-group comparisons [32]. Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 14.0 (SPSS, Chicago, IL). Data are presented as mean ± SD. For all statistical analyses, $P < .05$ was considered statistically significant.

3. Results

3.1. Study sample

A total of 254 T2DM patients were enrolled in the study. Of these, 223 completed the study; and 110 (49.3%) were allocated in sibutramine group and 113 (50.7%) in sibutramine plus L-carnitine group. There were 31 patients (15 men and 16 women) who did not complete the study; and the reasons for premature withdrawal included adverse effects as headache (1 man and 2 women after 3 months, and 1 man after 6 months in sibutramine group; and 1 man after 4 months and 2 women after 9 months in sibutramine plus L-carnitine group), constipation (1 man after 6 months

and 1 man after 9 months in sibutramine group, and 1 man after 3 months and 1 woman after 6 months in sibutramine plus L-carnitine group), insomnia (2 women after 9 months and 1 man after 12 months in sibutramine group, and 1 man after 6 months and 1 man after 12 months in sibutramine plus L-carnitine group), dry mouth (1 man after 3 months in sibutramine group; and 1 man after 3 months, 1 woman after 9 months, and 1 man after 12 months in sibutramine plus L-carnitine group), increased blood pressure (1 woman after 3 months and 1 woman after 6 months in sibutramine group, and 1 woman after 3 months in sibutramine plus L-carnitine group), increased heart rate (1 woman after 9 months and 1 man after 12 months in sibutramine group, and 1 woman after 3 months in sibutramine plus L-carnitine group), malaise (1 woman after 3 months in sibutramine group, and 1 woman after 3 months and 1 man after 9 months in sibutramine plus L-carnitine group), and palpitation (1 woman after 6 months and 1 man after 9 months in sibutramine plus L-carnitine group). In particular, no patients reported depression during the study. The characteristics of the patient population at study entry are shown in Table 1.

3.2. Body weight and BMI

We observed a significant decrease of body weight and BMI compared with baseline after 9 and 12 months ($P < .05$ and $P < .01$, respectively, for both) in both groups, but the body weight value reached with sibutramine plus L-carnitine was significantly lower than the value reached with sibutramine alone after 12 months ($P < .05$). No differences were registered between the 2 treatments regarding BMI (Table 3).

3.3. Glycemic parameters

Glycated hemoglobin was improved after 6, 9, and 12 months compared with baseline in both groups ($P < .05$, $P < .01$, and $P < .001$, respectively, for sibutramine alone and $P < .05$, $P < .001$, and $P < .0001$, respectively, for sibutramine plus L-carnitine) even if the value recorded with sibutramine plus L-carnitine was significantly better than the value recorded with sibutramine alone after 9 and 12 months ($P < .05$ for both) (Table 3).

A statistically significant decrease of FPG was obtained after 9 and 12 months ($P < .05$ and $P < .01$, respectively) compared with baseline with sibutramine, and after 6, 9, and 12 months with sibutramine plus L-carnitine ($P < .05$, $P < .01$, and $P < .001$, respectively), without differences between the 2 groups (Table 3).

We obtained a significant decrease of PPG after 9 and 12 months ($P < .05$ and $P < .01$, respectively) compared with baseline with sibutramine, and after 6, 9, and 12 months with sibutramine plus L-carnitine ($P < .05$, $P < .01$, and $P < .001$, respectively). No differences between the 2 groups were observed (Table 3).

Fasting plasma insulin improved after 12 months ($P < .05$) compared with baseline with sibutramine, and after 9 and 12 months with sibutramine plus L-carnitine ($P < .05$ and $P < .01$); the value reached with sibutramine plus L-carnitine was significantly better than the value obtained with sibutramine alone after 12 months ($P < .05$) (Table 3).

3.4. Lipid profile

We registered a significant decrease of TC and LDL-C after 12 months ($P < .05$ for both) with sibutramine, and after 9 and 12 months with sibutramine plus L-carnitine ($P < .05$ and $P < .01$, respectively, for both). Only sibutramine plus L-carnitine gave a decrease of Tg after 12 months ($P < .05$), even if there were no differences between the 2 groups. We did not observe any variations of HDL-C neither with sibutramine plus L-carnitine nor with sibutramine alone (Table 4).

3.4.1. Insulin resistance parameters

A statistically significant improvement of HOMA-IR was obtained after 9 and 12 months ($P < .05$ and $P < .01$, respectively) compared with baseline with sibutramine, and after 6, 9, and 12 months with sibutramine plus L-carnitine ($P < .05$, $P < .01$, and $P < .001$); and the value recorded with sibutramine plus L-carnitine was significantly better than the value recorded with sibutramine alone after 12 months (Table 3).

Table 4
Lipid profile data during the study

	Sibutramine group					Sibutramine + L-carnitine group				
	Baseline	3 mo	6 mo	9 mo	12 mo	Baseline	3 mo	6 mo	9 mo	12 mo
n	125	119	116	112	110	129	124	120	115	113
Sex (M/F)	63/62	61/58	59/57	58/54	56/54	65/64	63/61	61/59	59/56	57/56
Sm st (M/F)	24/18	22/18	21/18	21/17	21/17	22/19	21/19	21/19	21/18	20/17
TC (mg/dL)	224 ± 28	218 ± 23	211 ± 21	206 ± 17	197 ± 15*	222 ± 27	217 ± 23	209 ± 20	198 ± 16*	185 ± 11†
LDL-C (mg/dL)	160 ± 15	156 ± 13	147 ± 9	142 ± 7	138 ± 6*	157 ± 14	154 ± 12	144 ± 9	140 ± 8*	127 ± 6†
HDL-C (mg/dL)	43 ± 7	42 ± 6	43 ± 7	44 ± 7	41 ± 6	44 ± 8	43 ± 7	44 ± 8	42 ± 6	43 ± 7
Tg (mg/dL)	105 ± 42	99 ± 40	107 ± 44	101 ± 40	91 ± 36	107 ± 44	101 ± 40	93 ± 36	81 ± 29	75 ± 24*

Data are means ± SD.

* $P < .05$ vs baseline.

† $P < .01$ vs baseline.

Table 5
Inflammatory parameters during the study

	Sibutramine group					Sibutramine + l-carnitine group				
	Baseline	3 mo	6 mo	9 mo	12 mo	Baseline	3 mo	6 mo	9 mo	12 mo
n	125	119	116	112	110	129	124	120	115	113
Sex (M/F)	63/62	61/58	59/57	58/54	56/54	65/64	63/61	61/59	59/56	57/56
Sm st (M/F)	24/18	22/18	21/18	21/17	21/17	22/19	21/19	21/19	21/18	20/17
Leptin (ng/mL)	33.7 ± 17.1	30.1 ± 16.3	27.9 ± 14.5	25.2 ± 12.9*	23.7 ± 12.2†	32.6 ± 16.9	28.9 ± 14.8	26.0 ± 13.4*	23.9 ± 12.4†	22.2 ± 11.4‡
TNF-α (pg/mL)	4.9 ± 2.2	4.3 ± 1.7	4.2 ± 1.6	3.7 ± 1.3*	3.2 ± 1.1‡	4.8 ± 2.1	4.2 ± 1.6	3.5 ± 1.2*	3.0 ± 1.0‡	2.6 ± 0.9§
ADN (μg/mL)	4.8 ± 1.1	5.2 ± 1.4	5.6 ± 1.6	5.9 ± 1.9*	6.2 ± 2.0†	4.7 ± 1.0	5.3 ± 1.4	6.1 ± 2.1*	6.7 ± 2.3‡‖	7.5 ± 2.7§,¶
Vaspin (ng/mL)	1.1 ± 0.6	1.0 ± 0.5	1.0 ± 0.5	0.9 ± 0.4	1.1 ± 0.6	1.3 ± 0.8	1.0 ± 0.5	0.9 ± 0.4	0.9 ± 0.4	0.7 ± 0.3*
Hs-CRP (mg/L)	2.6 ± 1.8	2.2 ± 1.4	2.1 ± 1.3	1.9 ± 1.1	1.7 ± 1.1†	2.6 ± 1.8	2.3 ± 1.5	2.1 ± 1.3	1.7 ± 1.1†	1.5 ± 1.0‡

Data are means ± SD.
* $P < .05$ vs baseline.
† $P < .02$ vs baseline.
‡ $P < .01$ vs baseline.
§ $P < .001$ vs baseline.
‖ $P < .05$ vs sibutramine group.
¶ $P < .01$ vs sibutramine group.

3.4.2. Inflammatory state

A decrease of leptin compared with baseline was observed after 9 and 12 months ($P < .05$ and $P < .02$) with sibutramine, and after 6, 9, and 12 months ($P < .05$, $P < .02$, and $P < .01$, respectively) in the group treated with sibutramine plus L-carnitine. There were no differences between the 2 groups (Table 5 and Fig. 1).

Tumor necrosis factor–α significantly decreased after 9 and 12 months ($P < .05$ and $P < .01$, respectively) compared with baseline in the group treated with sibutramine, and after

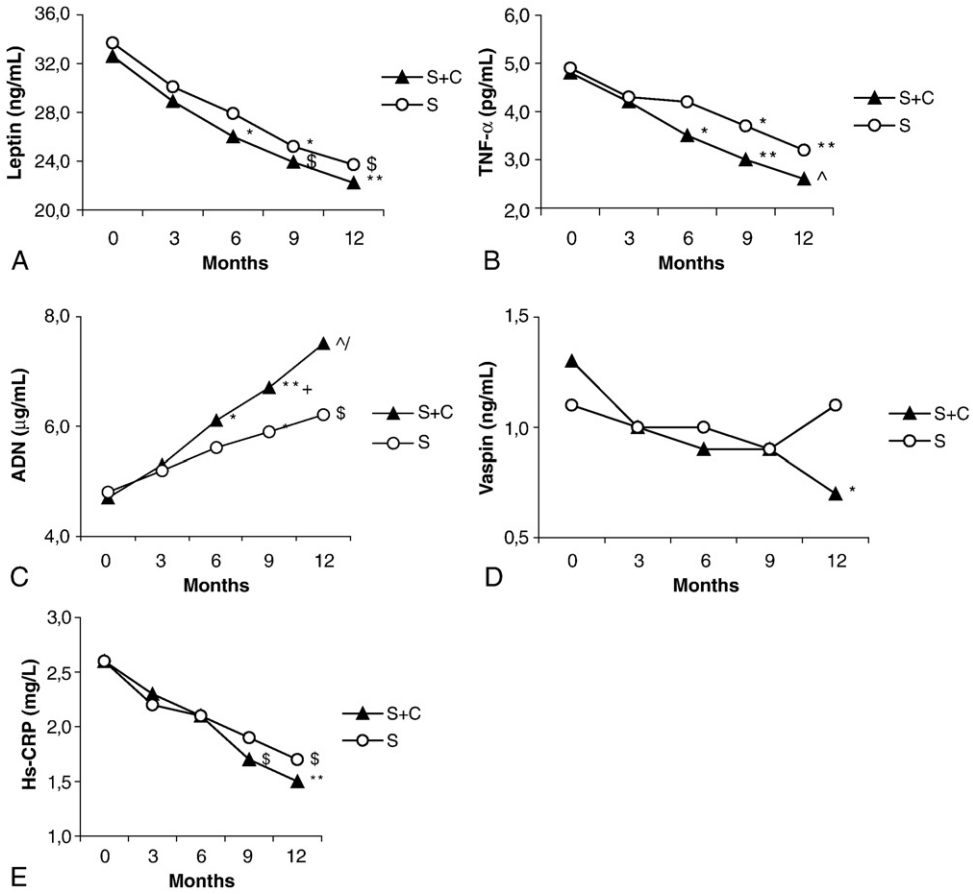

Fig. 1. Inflammatory parameters variations during the study. * $P < .05$ vs baseline; $ $P < .02$ vs baseline; ** $P < .01$ vs baseline; ^ $P < .001$ vs baseline; + $P < .05$ vs sibutramine group; / $P < .01$ vs sibutramine group. S+C = sibutramine plus L-carnitine; S = sibutramine.

6, 9, and 12 months in the group treated with sibutramine plus L-carnitine ($P < .05$, $P < .01$, and $P < .001$, respectively). We did not observe any significant differences between the 2 groups (Table 5 and Fig. 1).

An increase of ADN compared with baseline was observed after 9 and 12 months ($P < .05$ and $P < .02$, respectively) with sibutramine and after 6, 9, and 12 months ($P < .05$, $P < .01$, and $P < .001$, respectively) in the group treated with sibutramine plus L-carnitine. Sibutramine plus L-carnitine gave a better improvement of ADN compared with sibutramine alone after 9 and 12 months ($P < .05$ and $P < .01$, respectively) (Table 5 and Fig. 1).

A significant decrease of vaspin was recorded after 12 months ($P < .05$) with sibutramine plus L-carnitine, but not with sibutramine alone, even if no differences were registered between the 2 groups (Table 5 and Fig. 1).

High-sensitivity C-reactive protein was decreased after 9 and 12 months with sibutramine plus L-carnitine ($P < .02$ and $P < .01$, respectively), and after 12 months with sibutramine alone ($P < .02$) compared with baseline, without significant differences between the 2 groups (Table 5 and Fig. 1).

4. Correlations

Stepwise multilinear regression analysis was undertaken to establish which anthropometric and metabolic factors could best predict the insulin resistance (HOMA) improvement changes or which metabolic factors could best predict the anthropometric (BMI) improvement change. Significant predictors of change in insulin resistance (HOMA) were TNF-α and ADN concentration in sibutramine plus L-carnitine group ($r = 0.63$, $P < .001$ and $r = -0.58$, $P < .01$, respectively), and significant predictors of change in anthropometric value (BMI) were TNF-α and ADN concentration in sibutramine plus L-carnitine group ($r = 0.65$, $P < .001$ and $r = -0.60$, $P < .01$, respectively). We did not observe any correlation in sibutramine group.

5. Discussion

We have already conducted a study on L-carnitine where we showed that L-carnitine improved lipoprotein (a) levels in hypercholesterolemic T2DM patients [14], confirming what was already reported by Sirtori et al [33]. Our group has also conducted several studies on sibutramine where sibutramine appeared to be a tolerable and efficacious drug when added to pioglitazone for the global management of obese diabetic patients [34,35].

In the current study, we have recorded that sibutramine plus L-carnitine gave a better improvement of body weight and of glycemic and lipid profile compared with sibutramine alone, confirming the already known role of L-carnitine on glycemic and lipid metabolism [11-13].

Compared with our previous studies, we have also evaluated some inflammatory parameters, such as leptin, TNF-α, ADN, vaspin, and Hs-CRP.

Leptin acts as an afferent signal in the brain and peripheral organs to regulate feeding and energy expenditure [36]. Leptin also links energy stores to hormonal adaptations during fasting and feasting [36]; it also mediates structural and functional changes in neuronal circuits and immune and cardiovascular systems [36]. On the other side, TNF-α was the first adipose-secreted product proposed to represent a molecular link between obesity and insulin resistance [37,38]; TNF-α is also a macrophage-derived inflammatory factor. It alters insulin signaling in cultured cells and in vivo [39], and it has been reported that chronic exposure of cells or whole animals to TNF-α induces insulin resistance [37]. In our study, we observed that sibutramine plus L-carnitine, added to the previously taken antidiabetic therapy, gave a decrease of these parameters faster than sibutramine alone.

Regarding ADN, it is a protein exclusively synthesized by adipocytes. It is decreased in obesity and inversely related to glucose and insulin [40]. Ablation of the ADN gene in mice resulted in insulin resistance, glucose intolerance, dyslipidemia, and increased susceptibility to vascular injury and atherosclerosis [41-43]. Adiponectin reverses these abnormalities by stimulating oxidation of fatty acids; suppressing gluconeogenesis; and inhibiting monocyte adhesion, macrophage transformation, proliferation, and migration of smooth muscle cells in blood vessels [29,41,44]. In our study, sibutramine plus L-carnitine was better than sibutramine alone in increasing ADN.

On the other side, vaspin (visceral adipose tissue–derived serpin) is a member of the serine protease inhibitor family isolated by from the visceral white adipose tissue (WAT) of Otsuka Long-Evans Tokushima fatty rats, a model of abdominal obesity, insulin resistance, and diabetes [30]. Vaspin expression increases by 30 weeks, coinciding with obesity and high insulin levels in Otsuka Long-Evans Tokushima fatty rats, and declines as diabetes worsens and the rats lose weight by 50 weeks. Vaspin is restored by insulin or pioglitazone treatment [30]. Importantly, administration of vaspin to obese mice improves glucose tolerance and insulin sensitivity and reverses the expression of half of the number of genes induced in WAT by diet-induced obesity [30]. The reference range of normal vaspin values is 0.77 ± 0.42 ng/mL in normal glucose–tolerant, lean subjects [45]. Vaspin baseline levels were increased in our patients, probably because of the obesity and the insulin resistance; it has been previously postulated [46,47] that the induction of vaspin messenger RNA expression in human adipose tissue could represent a compensatory mechanism associated with obesity, severe insulin resistance, and T2DM. In our study, we observed that sibutramine plus L-carnitine, but not sibutramine alone, resulted in a decrease of vaspin to normal levels. This effect is probably due to the role of L-carnitine on lipid metabolism: facilitating the transport of long-chain fatty acids across the mitochondrial inner membrane, L-carnitine

improves the ability to use fat as fuel. In this way, fat WAT, which produces vaspin, is reduced; and so are vaspin levels.

Regarding Hs-CRP, it has been shown to independently predict myocardial infarction, stroke, and peripheral artery disease [48]. In our study, sibutramine plus L-carnitine improved Hs-CRP faster than sibutramine alone.

Regarding adverse reactions, we did not observe any significant differences between the group treated with sibutramine and the group treated with sibutramine plus L-carnitine. All the events were reported as mild or moderate. This was in line with what was already reported by our group in 2 previous studies [49,50]; sibutramine intake was not associated to any cardiovascular effects and was generally well tolerated.

Of course, our study has some limitations: for example, we did not evaluate if the beneficial effects on glycemic control, body weight, lipid profile, insulin resistance, and inflammatory parameters were sustained after the cessation of therapy. Another limitation is that we dosed a limited number of inflammation biomarkers, concentrating our attention on a few of these.

However, to the best of our knowledge, this is the first study investigating the effect of sibutramine plus L-carnitine on inflammatory and insulin resistance parameters.

6. Conclusions

Sibutramine plus L-carnitine gave a better and faster improvement of all the analyzed parameters compared with sibutramine alone without giving any severe adverse effect.

References

[1] Fried M, Hainer V, Basdevant A, et al. Interdisciplinary European guidelines on surgery for severe obesity. Rozhl Chir 2008;87:468-76.
[2] Pagotto U, Vanuzzo D, Vicennati V, Pasquali RG. Pharmacological therapy of obesity. G Ital Cardiol (Rome) 2008;9(4 Suppl 1):83S-93S.
[3] Marović D. Elevated body mass index fatty liver. Srp Arh Celok Lek 2008;136:122-5.
[4] Lavie CJ, Artham SM, Milani RV, Ventura HO. The obesity paradox: impact of obesity on the prevalence prognosis of cardiovascular diseases. Postgrad Med 2008;120:34-41.
[5] American Diabetes Association. Nutrition principles and recommendations in diabetes. Diabetes Care 2004;27(S1):36-46.
[6] American Diabetes Association. Physical activity/exercise and diabetes. Diabetes Care 2004;27(S1):58-62.
[7] Lee M, Aronne LJ. Weight management for type 2 diabetes mellitus: global cardiovascular risk reduction. Am J Cardiol 2007;99:68B-79B.
[8] Padwal RS, Majumdar SR. Drug treatments for obesity: orlistat, sibutramine, and rimonabant. Lancet 2007;369:71-7.
[9] McNeely W, Goa KL. Sibutramine: a review of its contribution to the management of obesity. Drugs 1998;56:1093-124.
[10] Abbott Laboratories limited. Sibutramine, summary of product characteristic. Abbot Park (Ill): Abbott laboratories; 2000.
[11] Uziel G, Garavaglia B, Di Donato S. Carnitine stimulation of pyruvate dehydrogenase complex (PDHC) in isolated human skeletal muscle mitochondria. Muscle Nerve 1988;11:720-4.
[12] Derosa G, Cicero AFG, Gaddi A, Mugellini A, Ciccarelli L, Fogari R. The effect of L-carnitine on plasma lipoprotein(a) levels in hypercholesterolemic patients with type 2 diabetes mellitus. Clin Ther 2003;25:1429-39.
[13] Amat di San Filippo C, Taylor MR, Mestroni L, Botto LD, Longo N. Cardiomyopathy carnitine deficiency. Mol Genet Metab 2008;94:162-6.
[14] Newsholme EA, Leech AR. Biochemistry for medical sciences. Chichester: John Wiley and Sons; 1983. p. 318-21.
[15] Rydén L, Standl E, Bartnik M, et al. Guidelines on diabetes, prediabetes, and cardiovascular diseases: executive summary. The Task Force on Diabetes and Cardiovascular Diseases of the European Society of Cardiology (ESC) and of the European Association for the Study of Diabetes (EASD). Eur Heart J 2007;28:88-136.
[16] World Health Organization. Obesity: preventing and managing the global epidemic. Report of WHO consultation on obesity. Geneva: WHO; 1997.
[17] Summary of American Heart Association diet and lifestyle recommendations revision 2006. Arterioscler Thromb Vasc Biol 2006;26:2186-91.
[18] Bunn HF, Gabbay KH, Gallop PM. The glycosylation of haemoglobin. Relevance to diabetes mellitus. Science 1978;200:21-7.
[19] European Diabetes Policy Group. A desktop guide to type 2 diabetes mellitus. Diabet Med 1999;16:716-30.
[20] Heding LG. Determination of total serum insulin (IRI) in insulin-treated diabetic patients. Diabetologia 1972;8:260-6.
[21] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
[22] Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care 2004;27:1487-95.
[23] Klose S, Borner K. Enzymatische Bestimmung des Gesamtcholesterins mit dem Greiner Selective Analyzer (GSA II). J Clin Chem Clin Biochem 1978;15:121-30.
[24] Wahlefeld AW. Methods of enzymatic analysis: triglycerides determination after enzymatic hydrolysis. Nergmer HV ed. New York: Academic Press, Inc.; 1974. p. 18-31.
[25] Havel RJ, Edr HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest 1955;34:1345-53.
[26] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
[27] Misra A, Garg A. Leptin: its receptor and obesity. J Invest Med 1996;44:540-8.
[28] Zhang M, Tracey K. The cytokine handbook. 3rd ed. San Diego: Academic Press; 1988.
[29] Yamauchi T, Kamon J, Waki H, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nature Med 2001;7:941-6.
[30] Hida K, Wada J, Eguchi J, et al. Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. Proc Natl Acad Sci 2005;102:10610-5.
[31] Rifai N, Tracy RP, Ridker PM. Clinical efficacy of an automated high-sensitivity C-reactive protein assay. Clin Chem 1999;45:2136-41.
[32] Winer BJ. Statistical principles in experimental design. 2nd ed. New York: McGraw-Hill; 1971.
[33] Sirtori CR, Calabresi L, Ferrara S, et al. L-Carnitine reduces plasma lipoprotein(a) levels in patients with hyper Lp(a). Nutr Metab Cardiovas Dis 2000;10:247-51.
[34] Derosa G, D'Angelo A, Salvadeo SAT, et al. Sibutramine effect on metabolic control of obese patients with type 2 diabetes mellitus treated with pioglitazone. Metabolism 2008;57:1552-7.
[35] Derosa G, Mereu R, Salvadeo SAT, et al. Pioglitazone metabolic effect in metformin-intolerant obese patients treated with sibutramine. Inter Med 2009;48:265-71.
[36] Flier JS. Obesity wars: molecular progress confronts an expanding epidemic. Cell 2004;116:337-50.

[37] Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature 1997;389:610-4.
[38] Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87-91.
[39] Hotamisligil GS. The role of TNF-alpha and TNF receptors in obesity and insulin resistance. J Intern Med 1999;245:621-5.
[40] Jackson MB, Ahima RS. Neuroendocrine and metabolic effects of adipocyte-derived hormones. Clinical Science 2006;110:143-52.
[41] Berg AH, Combs TP, Scherer PE. ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends Endocrinol Metab 2002;13:84-9.
[42] Bouskila M, Pajvani UB, Scherer PE. Adiponectin: a relevant player in PPARγ-agonist–mediated improvements in hepatic insulin sensitivity? Int J Obes Relat Metab Disord 2005;29(Suppl 1):S17-23.
[43] Maeda N, Shimomura I, Kishida K, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med 2002;8: 731-7.
[44] Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 2001;7:947-53.
[45] Youn BS, Klöting N, Kratzsch J, et al. Serum vaspin concentrations in human obesity and type 2 diabetes. Diabetes 2008;57:372-7.
[46] Klöting N, Berndt J, Kralisch S, et al. Vaspin gene expression in human adipose tissue: association with obesity and type 2 diabetes. Biochem Biophys Res Commun 2006;339:430-6.
[47] Zvonic S, Lefevre M, Kilroy G, et al. Secretome of primary cultures of human adipose-derived stem cells: modulation of serpins by adipogenesis. Mol Cell Proteomics 2007;6:18-28.
[48] Zwacka TP, Hornbach V, Torzewski J. C-reactive protein-mediated lipoprotein uptake by macrophages. Circulation 2001;103:1194-7.
[49] Derosa G, Cicero AFG, Murdolo G, et al. Efficacy and safety comparative evaluation of orlistat and sibutramine treatment in hypertensive obese patients. Diabetes Obes Metab 2005;7:47-55.
[50] Derosa G, Cicero AF, Murdolo G, Ciccarelli L, Fogari R. Comparison of metabolic effects of orlistat and sibutramine treatment in type 2 diabetic obese patients. Diabetes Nutr Metab 2004;17:222-9.