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□ ORIGINAL ARTICLE □

Sibutramine and L-Carnitine Compared to Sibutramine Alone on Insulin Resistance in Diabetic Patients

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Abstract

Objective To evaluate the effects of one year of treatment with sibutramine plus L-carnitine compared to sibutramine on body weight, glycemic control, and insulin resistance state in type 2 diabetic patients.

Methods Two hundred and fifty-four patients with uncontrolled type 2 diabetes mellitus (T2DM) [glycated hemoglobin (HbA_{1c}) >8.0%] in therapy with different oral hypoglycemic agents or insulin were enrolled in this study and randomised 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 (BMI), glycated hemoglobin (HbA_{1c}), fasting plasma glucose (FPG), post-prandial plasma glucose (PPG), fasting plasma insulin (FPI), homeostasis model assessment insulin resistance index (HOMA-IR), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), triglycerides (Tg), retinol binding protein-4 (RBP-4), resistin, visfatin, high sensitivity-C reactive protein (Hs-CRP).

Results There was a decrease in body weight, BMI, HbA_{1c}, FPI, HOMA-IR, and RBP-4 in both groups, even when the values obtained with sibutramine plus L-carnitine were lower than the values obtained in sibutramine group. There was a faster decrease of FPG, PPG, TC, LDL-C, resistin and Hs-CRP with sibutramine plus L-carnitine even when no differences between the two groups were obtained. Furthermore, only sibutramine plus L-carnitine improved Tg, and visfatin.

Conclusion Sibutramine plus L-carnitine gave a faster improvement of lipid profile, insulin resistance parameters, glycemic control, and body weight compared to sibutramine.

Key words: L-carnitine, sibutramine, insulin resistance, type 2 diabetes, resistin

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Introduction

Obesity is reaching epidemic proportions worldwide; it is correlated with various comorbidities, among which the most relevant are dyslipidemia (1), diabetes mellitus (T2 DM) (2), fatty liver (3), and cardiovascular diseases (4).

Sibutramine hydrochloride monohydrate is, together with orlistat, one of the two molecules licensed for use as anti-obesity drugs (5); sibutramine is a norepinephrine and serotonin reuptake inhibitor approved for the long-term management of obesity, in conjunction with a reduced calorie diet and behaviour modification, in patients unable to lose weight with diet and lifestyle changes alone. Sibutramine is rapidly metabolised by the hepatic cytochrome P450 system (CYP) generating two pharmacologic active metabolites which affect both food intake and energy expenditure (6).

Carnitine, or L-β-hydroxy-γ-N-trimethylaminobutyric acid, instead, is synthesized primarily in the liver and kidneys. The essential amino acids, lysine and methionine, are re-

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quired for its biosynthesis (7). Carnitine covers 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 acute metabolic decompensation, most often early in life, with hepatic encephalopathy and hypoketotic hypoglycemia (8).

There is also experimental evidence that L-carnitine stimulates the activity of the pyruvate dehydrogenase complex by decreasing the intramitochondrial acetyl-CoA/CoA ratio through the trapping of acetyl groups (9). The simultaneous reduction of acetyl-CoA levels in the cytosol further contributes to activate the glycolytic pathway (10), so L-carnitine also covers a key role in glucose metabolism and assists in fuel-sensing.

The aim of this study was to evaluate the effects of one year of treatment with sibutramine plus L-carnitine compared to sibutramine monotherapy added to the usual antidiabetic therapy on body weight, glycemic control, lipid profile, and insulin resistance parameters such as retinol binding protein-4 (RBP-4), resistin, visfatin, and high sensitivity-C reactive protein (Hs-CRP) in obese type 2 diabetic patients.

Material and Methods

Study design

This multicenter, randomised, 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.

Patients

We enrolled 254 Caucasian patients, aged ≥18, of either sex (128 males and 126 females) with a diagnosis of T2DM according to the ESC (European Society of Cardiology) and EASD (European Association for the Study of Diabetes) Guidelines criteria (11), obese (body mass index [BMI] ≥30 kg/m^2) (12), and with uncontrolled T2DM [glycated hemoglobin (HbA_{1c}) >8.0%] 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 gamma-glutamyltransferase level higher than the upper limit of normal [ULN] for age and sex), impaired renal function (defined as serum creatinine level higher than the ULN for age and sex), or severe anemia. Patients with serious cardiovascular disease (CVD) (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 enrolment also were excluded. Women who were pregnant or breastfeeding or of childbearing potential and not taking adequate contraceptive precautions were also excluded. All patients provided written informed consent to participate.

The sibutramine plus L-carnitine group was composed of 129 patients (65 males and 64 females) with a mean age of 54±5 years, with a diagnosis of T2DM for 6±3 years; 22 males and 19 females were smokers. One hundred and nineteen of these patients (92.2%) were affected by concomitant disease [98 (82.3%) hypertension; 41 (34.4%) hypercholesterolemia; 7 (5.9%) hypertriglyceridemia; 32 (26.9%) combined dyslipidemia]. One hundred and twenty (93.0%) of these patients were taking concurrent medications [35 (29.2%) angiotensin-converting enzyme-inhibitors (ACE-I); 39 (32.5%) angiotensin receptor blockers (ARBs); 20 (16.7%) calcium-antagonists; 12 (10.0%) β-blockers; 25 (20.8%) diuretics; 47 (39.2%) statins; 14 (11.7%) fibrates; 12 (10.0%) omega-3; 97 (80.8%) acetylsalicylic acid; 9 (7.5%) ticlopidine]. One hundred and twenty (93%) patients were also taking oral hypoglycemic agents {26 (21.7%) patients were taking sulphonylureas [9 (34.6%) glyburide, 15 (57.7%) glimepiride, and 2 (7.7%) gliclazide]; 70 (58.3%) patients, biguanide [70 (100%) metformin]; 14 (11.7%) patients glinide derivatives [10 (71.4%) repaglinide, 4 (28.6%) nateglinide]; 22 (18.3%) patients α-glucosidase inhibitor [22 (100%) acarbose]; 67 (55.8%) patients thiazolidinediones [41 (61.2%) pioglitazone, 26 (38.8%) rosiglitazone]; 13 (10.8%) incretin-mimetics [13 (100%) exenatide]; 15 (12.5%) dypeptidil peptidasi-4 (DPP-4) inhibitors [10 (66.7) sitagliptin, 5 (33.3% vildagliptin]} in monotherapy or in combination therapy. Sixteen (12.4%) patients were taking insulin {12 (75.0% analogue [8 (66.7% lispro, 4 (33.3%) glulisine], 9 (56.2%) long-acting [4 (44.4%) glargine, 5 (55.6%) NPH]}.

The sibutramine group was composed of 125 patients (63 males and 62 females) with a mean age of 51±4 years, with a diagnosis of T2DM for 5±2 years; 24 males and 18 females were smokers. One hundred and twelve of these patients (89.6%) were affected by concomitant disease [96 (85.7%) hypertension; 39 (34.8%) hypercholesterolemia; 4 (3.6%) hypertriglyceridemia; 25 (22.3%) combined dyslipidemia]. One hundred and fourteen (91.2%) of these patients were taking concurrent medications [30 (26.3%) ACE-I; 31 (27.2%) ARBs; 24 (21.0%) calcium-antagonists; 9 (7.9%) β-blockers; 18 (15.8%) diuretics; 48 (42.1%) statins; 10 (8.8%) fibrates; 14 (12.3%) omega-3; 94 (82.5%) acetylsalicylic acid; 7 (6.1%) ticlopidine]. One hundred and sixteen (92%) patients were also taking oral hypoglycemic agents {25 (21.0%) patients were taking sulphonylureas [7 (28.0%)

glyburide, 14 (56.0%) glimepiride, and 4 (16.0%) gliclazide]; 77 (66.4%) patients, biguanide [77 (100%) metformin]; 20 (17.2%) patients glinide derivatives [15 (75.0%) repaglinide, 5 (25.0%) nateglinide]; 12 (10.3%) patients α-glucosidase inhibitor [12 (100%) acarbose]; 64 (55.2%) patients thiazolidinediones [34 (53.1%) pioglitazone, 30 (46.9%) rosiglitazone]; 11 (9.5%) incretin-mimetics [11 (100%) exenatide]; 17 (14.6%) dypeptidil peptidasi-4 (DPP-4) inhibitors [11 (64.7) sitagliptin, 6 (35.3% vildagliptin]} in monotherapy or in combination therapy. Thirteen (10.4%) patients were taking insulin {9 (69.2% analogue [7 (77.8% lispro, 2 (22.2%) glulisine], 7 (53.8%) long-acting [2 (28.6%) glargine, 5 (71.4%) NPH]}.

Treatments

Patients were assigned to receive, as an addition to their current antidiabetic therapy, sibutramine 10 mg plus L-carnitine 2 g or sibutramine 10 mg for 12 months in a randomised, 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. Randomisation was done by drawing envelopes containing randomisation 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 the case 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 the 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.

Diet and exercise

Subjects began a controlled-energy diet (near 600 kcal daily deficit) based on American Heart Association (AHA) recommendations (13) that included 50% of calories from carbohydrates, 30% from fat (6% saturated), and 20% from proteins, with a maximum cholesterol content of 300 mg/day and 35 g/day of fiber. Patients were not treated with vitamins or mineral preparations during the study.

Standard diet advice was given by a dietitian and/or specialist doctor. Dietitian and/or specialist doctor periodically provided instruction on dietary intake recording procedures as part of a behaviour modification program and then later used the subject's food diaries for counselling. 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.

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 the following parameters at baseline, and after 3, 6, 9, and 12 months: body weight, body mass index (BMI), HbA_{1c}, fasting plasma glucose (FPG), postprandial plasma glucose (PPG), fasting plasma insulin (FPI), homeostasis model assessment insulin resistance index (HOMA-IR), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), triglycerides (Tg), RBP-4, resistin, visfatin, Hs-CRP.

In order to evaluate the tolerability assessments, all adverse events were recorded. All plasmatic parameters were determined after a 12-h overnight fast, with the exception of PPG, determined 2 hours after a standardized meal. Venous blood samples were taken for all patients between 08.00 and 09.00. We used plasma obtained by addition of Na2-EDTA, 1 mg/ml, and centrifuged at 3,000 g for 15 minutes at 4℃. Immediately after centrifugation, the plasma samples were frozen and stored at -80℃ 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 an HPLC method (DIAMAT, Bio-Rad, Richmond, CA, USA; normal values 4.2-6.2%), with intra- and interassay coefficients of variation (CsV) of <2% (14). Plasma glucose was assayed by glucose-oxidase method (GOD/PAP, Roche Diagnostics, Mannheim, Germany) with intra- and interassay CsV of <2% (15). 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 CsV: 4.6 and 7.3%, respectively) (16). The HOMA-IR index was calculated as the product of basal glucose (mmol/L) and insulin levels (μU/mL) divided by 22.5 (17, 18).

Total cholesterol and Tg levels were determined using fully enzymatic techniques (19, 20) on a clinical chemistry analyzer (HITACHI 737; Hitachi, Tokyo, Japan); intra- and interassay CsV were 1.0 and 2.1 for TC measurement, and 0.9 and 2.4 for Tg measurement, respectively. High density lipoprotein-cholesterol level was measured after precipitation of plasma apo B-containing lipoproteins with phosphotungstic acid (21) intra- and interassay CsV were 1.0 and 1.9, respectively; LDL-C level was calculated by the Friedewald formula (22).

Retinol binding protein-4 was measured using a RBP-4 (Human) EIA kit (Phoenix Pharmaceuticals, Inc., Burlingame, CA). The intra- and interassay CsV were less than 5.0% and less than 14.0%, respectively (23).

Resistin value was measured by a commercially available enzyme-linked immunoassay (ELISA) kit (BioVendor Laboratory Medicine, Brno, Czech Republic). Intraassay CsV

was 3.4% and interassay CsV was 6.9%, respectively (24).

Visfatin levels were measured by enzyme immunoassay (EIA) kit obtained from Phoenix Pharmaceuticals, Inc., (Burlingame, CA, USA). The intra- and interassay CsV were 10% and less than 14%, respectively (25). High sensitivity C-reactive protein was measured with use of latex-enhanced immunonephelometric assays on a BN II analyser (Dade Behring, Newark, DE, USA). The intra- and interassay CsV were 5.7% and 1.3%, respectively (26).

Statistical analysis

An intention-to-treat analysis was conducted in patients who had received $\geq$1 dose of study medication and had a subsequent efficacy observation. Patients were included in the tolerability analysis if they had received $\geq$1 dose of trial medication and had undergone a subsequent tolerability observation. Considering as clinically significant a difference of at least the 10% compared to the baseline and an alpha error of 0.05, the actual sample size was adequate to obtain a power higher than 0.80 for all measured variable. Continuous variables were compared by analysis of variance (ANOVA). Intervention effects were adjusted for additional potential confounders using analysis of covariance (ANCOVA). ANOVA 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 ANCOVA. A 1-sample t test was used to compare values obtained before and after treatment administration; 2-sample t tests were used for between-group comparisons. The Bonferroni correction for multiple comparison was also carried out (27). Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 11.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± standard deviation (SD). For all statistical analyses, $p<0.05$ was considered statistically significant.

Results

Study sample

A total of 254 type 2 diabetic 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 males and 16 females) who did not complete the study and the reasons for premature withdrawal included side effects as headache (1 male and 2 females after 3 months, and 1 male after 6 months in sibutramine group, and 1 male after 4 months and 2 females after 9 months in sibutramine plus L-carnitine group), constipation (1 male after 6 months and 1 male after 9 months in sibutramine group, and 1 male after 3 months and 1 female after 6 months in sibutramine plus L-carnitine group), insomnia (2 females after 9 months and 1 male after 12 months in sibutramine group, and 1 male after 6 months and 1 male after 12 months in sibutramine plus L-carnitine group), dry mouth (1 male after 3 months in sibutramine group, and 1 male after 3 months, 1 female after 9 months and 1 male after 12 months in sibutramine plus L-carnitine group), increased blood pressure (1 female after 3 months and 1 female after 6 months in sibutramine group, and 1 female after 3 months in sibutramine plus L-carnitine group), increased heart rate (1 female after 9 months and 1 male after 12 months in sibutramine group, and 1 female after 3 months in sibutramine plus L-carnitine group), malaise (1 female after 3 months in sibutramine group, and 1 female after 3 months and 1 male after 9 months in sibutramine plus L-carnitine group), and palpitation (1 female after 6 months and 1 male after 9 months in sibutramine plus L-carnitine group). It is noteworthy that no patients reported depression during the study.

Body weight and BMI

There was a decrease of body weight, and BMI compared to baseline after 9, and 12 months ($p<0.05$, and $p<0.01$, respectively for both) in both groups. The body weight value obtained with sibutramine plus L-carnitine was significantly lower than the value obtained in sibutramine group after 12 months ($p<0.05$), while no differences were recorded between the two groups regarding BMI (Table 1).

Glycemic parameters

We observed a statistically significant improvement of HbA_{1c} after 6, 9, and 12 months compared to baseline in both groups ($p<0.05$, $p<0.01$, and $p<0.001$, respectively for sibutramine group and $p<0.05$, $p<0.001$, and $p<0.0001$, respectively for sibutramine plus L-carnitine group) even when the value obtained in sibutramine plus L-carnitine group was significantly lower than the value obtained in sibutramine group after 9 and 12 months ($p<0.05$ for both) (Table 1).

There was a statistically significant decrease of FPG, and PPG after 9 and 12 months ($p<0.05$, and $p<0.01$, respectively) compared to baseline in sibutramine group, and after 6, 9, and 12 months in sibutramine plus L-carnitine group ($p<0.05$, $p<0.01$, and $p<0.001$, respectively). No differences between the two groups were recorded (Table 1).

Lipid profile

Total cholesterol, and LDL-C were significantly decreased after 12 months ($p<0.05$, for both) with sibutramine, and after 9, and 12 months with sibutramine plus L-carnitine ($p<0.05$, and $p<0.01$, respectively, for both). A decrease of Tg was recorded after 12 months ($p<0.05$) in sibutramine plus L-carnitine group but not in sibutramine group, even when there were no differences between the two groups. We did not observe any variations of HDL-C with sibutramine or sibutramine plus L-carnitine (Table 2).

Insulin resistance parameters

A statistically significant decrease of HOMA-IR was re-

Table 1. Body Weight, Glycemic Profile, and Insulin Resistance Data during the Study

	Sibutramine group					Sibutramine+L-carnitine group				
	Baseline	3 month	6 month	9 month	12 month	Baseline	3 month	6 month	9 month	12 month
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**$^{+}$
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^$^{+}$	6.4±0.3°$^{+}$
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^

Data are means ± SD

*p< 0.05 vs baseline; **p< 0.01 vs baseline; ^p< 0.001 vs baseline; °p< 0.0001 vs baseline

$^{+}$p< 0.05 vs Sibutramine group

Table 2. Lipid Profile Data during the Study

	Sibutramine group					Sibutramine+L-carnitine group				
	Baseline	3 month	6 month	9 month	12 month	Baseline	3 month	6 month	9 month	12 month
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< 0.05 vs baseline; **p< 0.01 vs baseline

corded after 9 and 12 months (p<0.05, and p<0.01, respectively) compared to baseline in the group treated with sibutramine, and after 6, 9, and 12 months with sibutramine plus L-carnitine (p<0.05, p<0.01, and p<0.001). The value obtained with sibutramine plus L-carnitine was significantly lower than the value obtained with sibutramine after 12 months (p<0.05) (Table 3 and Fig. 1A).

A significant decrease of FPI was present after 12 months (p<0.05) compared to baseline in the group treated with sibutramine, and after 9, and 12 months in the group treated with sibutramine plus L-carnitine (p<0.05, and p<0.01); the value recorded with sibutramine plus L-carnitine was significantly lower than the value obtained with sibutramine after 12 months (p<0.05) (Table 3 and Fig. 1B).

We observed a significant decrease of RBP-4 after 9 and 12 months (p<0.05, and p<0.02, respectively) with sibutramine, and after 6, 9, and 12 months (p<0.05, p<0.01, and p <0.001, respectively) with sibutramine plus L-carnitine. The improvement of RBP-4 obtained with sibutramine plus L-carnitine was significantly better than the improvement obtained with sibutramine after 9, and 12 months (p<0.05, and p<0.01) A significant decrease of FPI was present after 12 months (p<0.05) compared to baseline in the group treated with sibutramine, and after 9 and 12 months in the group treated with sibutramine plus L-carnitine (p<0.05, and p< 0.01); the value recorded with sibutramine plus L-carnitine was significantly lower than the value obtained with sibutramine after 12 months (p<0.05) (Table 3 and Fig. 1C).

Resistin value was significantly decreased after 9 and 12 months (p<0.05, and p<0.01, respectively) compared to baseline in the group treated with sibutramine, and after 6, 9, and 12 months in the group treated with sibutramine plus L-carnitine (p<0.05, p<0.01, and p<0.001, respectively). We did not observe any significant differences between the two groups. A significant decrease of FPI was present after 12 months (p<0.05) compared to baseline in the group treated with sibutramine, and after 9, and 12 months in the group treated with sibutramine plus L-carnitine (p<0.05, and p< 0.01); the value recorded with sibutramine plus L-carnitine was significantly lower than the value obtained with sibutramine after 12 months (p<0.05) (Table 3 and Fig. 1D).

We observed a significant decrease of visfatin after 12

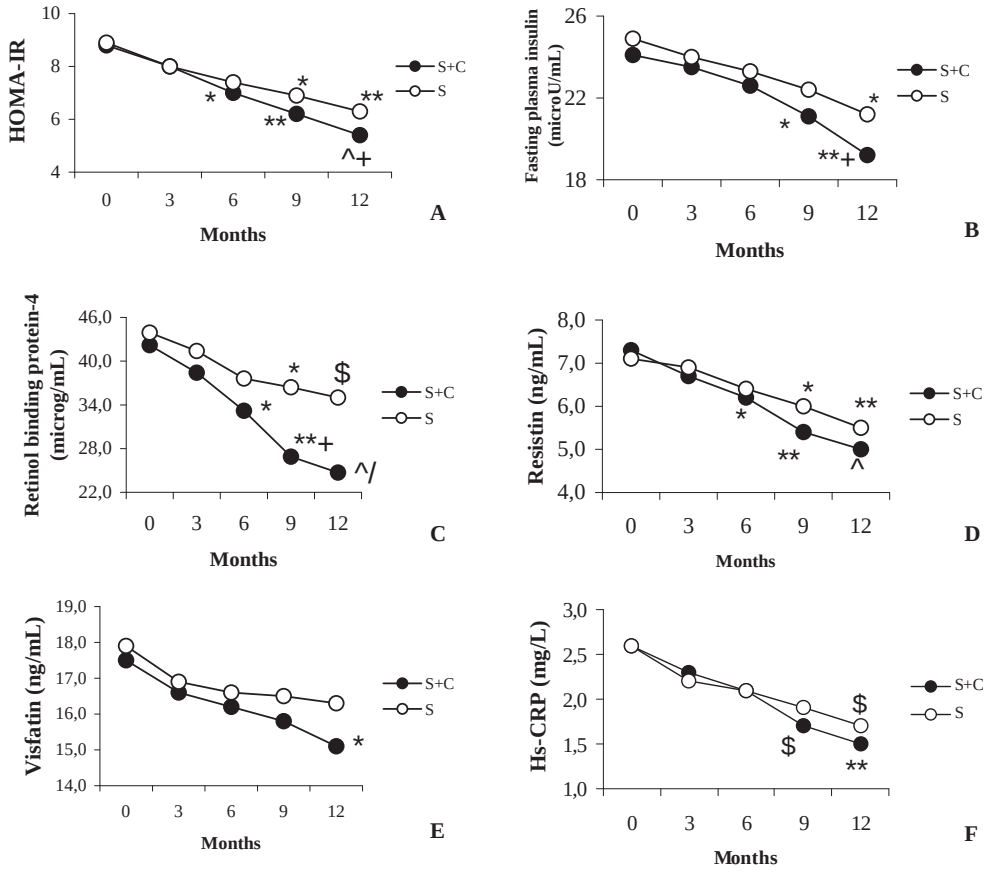
Table 3. Insulin Resistance and Inflammatory Parameters during the Study

	Sibutramine group					Sibutramine+L-carnitine group				
	Baseline	3 month	6 month	9 month	12 month	Baseline	3 month	6 month	9 month	12 month
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
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**$^{+}$
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^$^{+}$
RBP-4 (μg/mL)	43.9±11.8	41.4±10.2	37.6±9.4	36.4±9.0*	35.0±8.6$^{\$}$	42.2±10.8	38.4±9.5	33.2±8.1*	26.9±7.5**$^{+}$	24.7±6.8^$^{/}$
Resistin (ng/mL)	7.1±2.5	6.9±2.3	6.4±1.9	6.0±1.7*	5.5±1.5**	7.3±2.7	6.7±2.1	6.2±1.8*	5.4±1.4**	5.0±1.2^
Visfatin (ng/mL)	17.9±6.5	16.9±6.0	16.6±5.8	16.5±5.7	16.3±5.5	17.5±6.3	16.6±5.8	16.2±5.4	15.8±5.2	15.1±4.9*
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< 0.05 vs baseline; $^{\$}$p< 0.02 vs baseline; **p< 0.01 vs baseline; ^p< 0.001 vs baseline

$^{+}$p< 0.05 vs Sibutramine group; $^{/}$p< 0.01 vs Sibutramine group



*p< 0.05 vs baseline; $^{\$}$p< 0.02 vs baseline; **p< 0.01 vs baseline; ^p< 0.001 vs baseline

$^{+}$p< 0.05 vs Sibutramine group; $^{/}$p< 0.01 vs Sibutramine group

S+C: sibutramine plus L-carnitine; S: sibutramine

Figure 1. Inflammatory and insulin resistance parameters variations during the study.

months (p<0.05) with sibutramine plus L-carnitine, but not with sibutramine, even when no differences were recorded between the two groups. A significant decrease of FPI was present after 12 months (p<0.05) compared to baseline in the group treated with sibutramine, and after 9, and 12 months in the group treated with sibutramine plus L-carnitine (p<0.05, and p<0.01); the value recorded with sibutramine plus L-carnitine was significantly lower than the

value obtained with sibutramine after 12 months ($p<0.05$) (Table 3 and Fig. 1E).

Inflammatory state

A significant decrease of Hs-CRP value was obtained after 9 and 12 months in sibutramine plus L-carnitine group ($p<0.02$, and $p<0.01$, respectively), and after 12 months in sibutramine group ($p<0.02$) compared to baseline without significant differences between the two groups. A significant decrease of FPI was present after 12 months ($p<0.05$) compared to baseline in the group treated with sibutramine, and after 9 and 12 months in the group treated with sibutramine plus L-carnitine ($p<0.05$, and $p<0.01$); the value recorded with sibutramine plus L-carnitine was significantly lower than the value obtained with sibutramine after 12 months ($p <0.05$) (Table 3 and Fig. 1F).

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 RBP-4 and resistin concentration in sibutramine plus L-carnitine group ($r=0.61$, $p<0.01$, and $r=0.66$, $p<0.001$, respectively), and significant predictors of change in anthropometric value (BMI) were RBP-4 and resistin concentration in sibutramine plus L-carnitine group ($r=0.63$, $p<0.001$, and $r=0.67$, $p<0.001$, respectively).

Discussion

In one of our previous studies we already demonstrated that sibutramine is more effective than diet and physical activity alone in reducing body weight in obese patients with T2DM treated with pioglitazone (28), while we did not observe any differences between the two treatments regarding lipid profile, glycemic control and blood pressure (28). We also demonstrated that sibutramine appears to be a tolerable and efficacious drug when added to pioglitazone for the global management of obese diabetic patients (28, 29). Regarding the effects of L-carnitine alone in T2DM, Mingrone et al (30) already reported that L-carnitine constant infusion improves insulin sensitivity in insulin-resistant diabetic patients; a significant effect on whole body insulin-mediated glucose uptake is also observed in normal subjects. In diabetics, glucose taken up by the tissues, appears to be promptly utilized as fuel since glucose oxidation is increased during L-carnitine administration. The significantly reduced plasma levels of lactate suggest that this effect might be exerted through the activation of pyruvate dehydrogenase, whose activity is depressed in the insulin resistant status. Also in an earlier study on L-carnitine (31), we showed that L-carnitine improved Lp(a) levels in hypercholesterolemic type 2 diabetic patients, confirming what was already reported by Sirtori et al (32).

In the current study we recorded that sibutramine plus L-carnitine gave a better improvement of body weight and BMI compared to sibutramine alone. We also observed that both sibutramine and sibutramine plus L-carnitine added to the usual antidiabetic therapy taken before the beginning of the study, gave an improvement of glycemic control, even if L-carnitine addition gave a faster, and better improvement of HbA_{1c}. The better improvement of HbA_{1c} with L-carnitine is probably due to L-carnitine role in stimulating the activity of the pyruvate dehydrogenase complex by decreasing the intramitochondrial acetyl-CoA/CoA ratio through the trapping of acetyl groups (9), but it is [MBS1] a short-term effect. We have also observed that sibutramine plus L-carnitine gave a better improvement of lipid profile probably because of the L-carnitine role in lipid metabolism; L-carnitine acts 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 (8) and this contributes to the sibutramine effect in the long term.

Regarding insulin resistance, it has been reported that in T2DM patients the HOMA-IR resulted to be increased compared to the normal glucose tolerance (NGT) subjects (33). It has also been reported that a decrease in liver fat content might be expected to increase hepatic sensitivity (34, 35) and that a decrease in hepatic fat content might also improve insulin clearance (36, 37). Insulin resistance in fat cells reduces the effects of insulin and results in elevated hydrolysis of stored triglycerides in the absence of measures which either increase insulin sensitivity or which provide additional insulin. Increased mobilization of stored lipids in these cells elevates free fatty acids in the blood plasma. Insulin resistance in muscle cells reduces glucose uptake, whereas insulin resistance in liver cells results in impaired glycogen synthesis and a failure to suppress glucose production. Elevated blood fatty-acid concentrations (associated with insulin resistance and T2DM), reduces muscle glucose uptake, and increases liver glucose production, all of which contribute to an elevated blood glucose concentration (38).

Data from our study showed that sibutramine plus L-carnitine gave a better and faster decrease of HOMA-IR, and FPI compared to sibutramine alone. Compared to our previous studies, we have also evaluated some insulin resistance parameters, such as RBP-4, resistin, and visfatin. Regarding RBP-4, its concentration has been reported to be increased in subjects with obesity, insulin resistance or T2DM compared with lean subjects (39), even though the mechanisms by which RBP-4 induces insulin resistance are not well understood. On the other side, resistin is produced by mononuclear cells and activated macrophages: it has been demonstrated that overexpression of resistin decreases the ability of insulin to suppress hepatic glucose output or increase glucose uptake by muscle (40-42). Available data also support a role of resistin in determining an increase of inflammation and atherosclerosis (43). In the present study we

observed that sibutramine plus L-carnitine, added to the previously taken antidiabetic therapy, gave a better and faster improvement of RBP-4, and a faster decrease of resistin compared with sibutramine alone.

We have also analysed visfatin; visfatin was discovered to be a secretory protein highly enriched in human visceral adipocytes, yet this protein is also expressed by liver, muscle, bone marrow and lymphocytes, where it was first identified as PBEF (pre-B-cell colony stimulating factor) (44, 45). The expression and secretion of visfatin is increased during the development of obesity; however, in contrast with inflammatory cytokines, the rise in visfatin does not decrease insulin sensitivity. Instead, visfatin exerts insulin-mimetic effects in cultured adipocytes, hepatocytes and myotubes and lowers plasma glucose in mice (44). Visfatin binds to the insulin receptor with similar affinity but at a site distinct from insulin (44). In contrast with insulin, visfatin levels do not change with feeding and fasting (44). It remains to be determined if visfatin acts in concert with insulin to regulate metabolism and whether such interaction occurs via endocrine or paracrine mechanisms. In our study we recorded an improvement of visfatin with sibutramine plus L-carnitine but not with sibutramine alone.

Regarding inflammatory parameters, Hs-CRP has been shown to independently predict myocardial infarction, stroke and peripheral artery disease (46). In the present study both treatments improved this parameter. Regarding adverse reactions we did not observe any significant differences between sibutramine plus L-carnitine group, and sibutramine group. This was in line with what we already reported in two previous studies (47, 48); 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 whether the beneficial effects on glycemic control, body weight, lipid profile and insulin resistance parameters were sustained after the cessation of therapy. Another limitation is that we evaluated [MBS2] a limited number of insulin resistance biomarkers, concentrating our attention on a few of these. However, to the best of our knowledge, this is the first study to investigate the effect of sibutramine and L-carnitine on insulin resistance and inflammatory parameters.

Conclusions

Sibutramine plus L-carnitine gave a faster, and better improvement of lipid profile, insulin resistance parameters, glycemic control, and body weight compared to sibutramine monotherapy.

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