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ORIGINAL

Orlistat and L-carnitine compared to orlistat alone on insulin resistance in obese diabetic patients

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Abstract. Our study wants to evaluate the effects of one year treatment with orlistat plus L-carnitine compared to orlistat alone on body weight, glycemic and lipid control, and insulin resistance state in type 2 diabetic patients. Two hundred and fifty-eight 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 orlistat 120 mg three times a day plus L-carnitine 2 g one time a day or orlistat 120 mg three times a day. We evaluated at baseline, and after 3, 6, 9, and 12 months these parameters: body weight, body mass index (BMI), 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). We observed a faster, and better decrease of body weight, HbA_{1c}, FPG, PPG, LDL-C, HOMA-IR with orlistat plus L-carnitine compared to orlistat. A faster improvement of TC, Tg, FPI, resistin, RBP-4, visfatin, and Hs-CRP was reached with orlistat plus L-carnitine compared to orlistat. We can safely conclude that the association of orlistat plus L-carnitine was better than orlistat in improving body weight, glycemic and lipid profile, insulin resistance, and inflammatory parameters and no significant adverse events were recorded.

Key words: L-carnitine, Orlistat, Insulin resistance, HOMA-IR, Type 2 diabetes

OVERWEIGHT and obesity are increasing health problems associated with cardiovascular disorders and premature mortality [1]. Orlistat is the first prescription treatment for obesity that does not act as an appetite suppressant, but it works by interfering with the action of gastrointestinal (GI) lipase in the GI tract [2]. Orlistat has a unique molecular structure, which allows it to bind to the active site of GI lipase and block that enzyme activity. The enzyme is thus unable to break triglycerides (Tg) down into their component parts. As a result of this mechanism of action, 30% of ingested dietary fat remains undigested and unabsorbed, passing through the GI tract unchanged. Orlistat has mainly mild to moderate gastrointestinal side effects that usually attenuate with the prosecution of the treatment but often not acceptable from the patients [3] and some pharmacokinetic interactions that are rare but potentially relevant, with cyclosporin [4] and warfarin [5].

Carnitine, or the L-β-hydroxy-γ-N-trimethylaminobutyric acid, instead, is synthesized primarily in the liver and kidneys. The essential amino acids, lysine and methionine, are required for its biosynthesis [6]. 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 an acute metabolic decompensation, most often early in life, with hepatic encephalopathy and hypoketotic hypoglycemia [7].

There is also experimental evidence that L-carnitine stimulates the activity of the pyruvate dehydrogenase

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Table 1 General subjects characteristics at baseline in the study.

	Orlistat group	Orlistat+L-carnitine group
n	126	132
Sex (M/F)	62/64	65/67
Age (years)	53 ± 6	51 ± 4
Sm. st. (M/F)	21/25	20/23
Diab. Dur. (years)	6 ± 4	4 ± 2
Height (m)	1.69 ± 0.05	1.70 ± 0.06
Concomitant disease, n (%)	108 (85.7)	110 (83.3)
Hypertension	93 (86.1)	91 (82.7)
Hypercholesterolemia	43 (39.8)	48 (43.6)
Hypertriglyceridemia	5 (4.6)	6 (5.4)
Combined dyslipidemia	23 (21.3)	25 (22.7)
Concurrent medications, n (%)	110 (87.3)	109 (82.6)
ACE-I	29 (26.4)	34 (31.2)
ARBs	32 (29.1)	33 (30.3)
Calcium-antagonists	16 (14.5)	20 (18.3)
β-blockers	7 (6.4)	9 (8.2)
Diuretics	14 (12.7)	16 (14.7)
Statins	50 (45.4)	52 (47.7)
Fibrates	14 (12.7)	13 (11.9)
Omega-3	12 (10.9)	14 (12.8)
Acetylsalicylic acid	97 (88.2)	102 (93.6)
Ticlopidine	7 (6.4)	8 (7.3)

Data are expressed as means ± SD or n and %. Sm. st.: Smoking status; Diab. dur.: diabetes duration; ACE-I: angiotensin-converting enzyme-inhibitors; ARBs: angiotensin receptor blockers

complex by decreasing the intramitochondrial acetyl-CoA/CoA ratio through the trapping of acetyl groups [8]. The simultaneous reduction of acetyl-CoA levels in the cytosol further contributes to activate the glycolytic pathway [9], so L-carnitine covers also a key role in the glucose metabolism and assists in fuel-sensing.

The aim of this study was to evaluate the effects of one year treatment of orlistat plus L-carnitine compared to orlistat alone on body weight, glycemic and lipid control, and insulin resistance parameters such as retinol binding protein-4 (RBP-4), resistin, visfatin, 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 258 Caucasian type 2 diabetic patients aged ≥ 18 of either sex (Table 1) according to the ESC (European Society of Cardiology) and EASD (European Association for the Study of Diabetes) Guidelines criteria [10], obese (body mass index [BMI] ≥30 kg/m^2) [11], and with uncontrolled type 2 diabetes mellitus (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

Table 2 Antidiabetic agents before and during the study.

	Orlistat group n (%)	Orlistat+L-carnitine group n (%)
n	126	132
OHA	119 (94.4)	121 (91.7)
Sulphonylureas	22 (18.5)	24 (19.8)
Glyburide	8 (36.4)	7 (29.2)
Glimepiride	12 (54.5)	14 (58.3)
Gliclazide	2 (9.1)	3 (12.5)
Biguanides	75 (63.0)	81 (66.9)
Metformin	75 (100.0)	81 (100.0)
Glinides	19 (16.0)	22 (18.2)
Repaglinide	17 (89.5)	18 (81.8)
Nateglinide	2 (10.5)	4 (18.2)
α-glucosidase inhibitors	28 (23.5)	31 (25.6)
Acarbose	28 (100.0)	31 (100.0)
Thiazolidinediones	53 (44.5)	51 (42.8)
Pioglitazone	42 (79.2)	38 (74.5)
Rosiglitazone	11 (20.8)	13 (25.5)
Incretin-mimetics	9 (7.6)	12 (9.9)
Exenatide	9 (100.0)	12 (100.0)
DPP-4 inhibitors	13 (10.9)	17 (14.0)
Sitagliptin	9 (69.2)	11 (64.7)
Vildagliptin	4 (30.8)	6 (35.3)
INSULIN	14 (11.1)	17 (12.9)
Analogue	9 (64.3)	12 (70.6)
Lispro	5 (55.6)	8 (66.7)
Glulisine	4 (44.4)	4 (33.3)
Long-acting	5 (35.7)	6 (35.3)
Glargine	3 (60.0)	2 (33.3)
NPH	2 (40.0)	4 (66.7)

Data are expressed as n or %. OHA: oral hypoglycemic agents; DPP-4: dipeptidyl peptidase-4 inhibitors; NPH: neutral protamine Hagedorn

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.

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

Treatments

Patients were assigned to receive, as addition to their current antidiabetic therapy, orlistat 120 mg three times a day plus L-carnitine 2 g one time a day or orlistat 120 mg three times a day for 12 months in a randomised, double-blind, controlled study. Both orlistat and L-carnitine were supplied as identical, opaque, white capsules in coded bottles to ensure the blind status of the study. Randomisation was done using a drawing of 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 cases of an emergency. 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 [12] 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 cycle. 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 at baseline, and after 3, 6, 9, and 12 months these parameters: body weight, BMI, 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), 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 0800 h and 0900 h. We used plasma obtained by addition of Na_2-ethylenediaminetetraacetic acid (EDTA), 1 mg/ml, and centrifuged 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. HbA_{1c} level was measured by a high performance liquid chromatography (HPLC) method (DIAMAT, Bio-Rad, USA; normal values 4.2-6.2%), with intra- and interassay coefficients of variation (CsV) of < 2% [13]. FPG was assayed by glucose-oxidase method (GOD/PAP, Roche Diagnostics, Mannheim, Germany) with intra- and interassay CsV of < 2% [14]. FPI was assayed with Phadiaseph insulin radio immuno assay (RIA) (Pharmacia, Uppsala, Sweden) by using a second antibody to separate the free and antibody-bound 125 I-insulin (intra- and interassay CsV were 4.6 and 7.3%, respectively) [15].

The HOMA-IR index was calculated as the product of basal glucose (mmol/L) and insulin levels (μU/mL) divided by 22.5 [16-17].

TC and Tg levels were determined using fully enzymatic techniques [18-19] 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. HDL-C level was measured after precipitation of plasma apo B-containing lipoproteins with phosphotungstic acid [20] intra- and interassay CsV were 1.0 and 1.9, respectively; LDL-C level was calculated by the Friedewald formula [21].

RBP-4 was measured using a RBP-4 (Human) enzyme immunoassay (EIA) kit (Phoenix Pharmaceuticals, Inc., Burlingame, CA). The intra- and interassay CsV were less than 5.0% and less than 14.0%, respectively [22].

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 [23].

Visfatin levels were measured by EIA kit obtained from Phoenix Pharmaceuticals, Inc., (Burlingame, CA, USA). The intra- and interassay CsV were 10% and less than 14%, respectively [24].

Hs-CRP was measured with use of latex-enhanced immunonephelometric assays on a BN II analyser (Dade Behring, Newark, Delaware, USA). The intra- and interassay CsV were 5.7% and 1.3%, respectively [25].

Statistical Analysis

An intention-to-treat analysis was conducted in patients who had received ≥ 1 dose of study medication and had a subsequent efficacy observation. Patients were included in the tolerability analysis if

they had received ≥ 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 [26]. Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 11.0 (SPSS Inc., Chicago, Illinois, USA). Data are presented as means ± standard deviation (SD). For all statistical analyses, $p < 0.05$ was considered statistically significant.

Results

Study sample

A total of 258 patients were enrolled in the study. Of these, 227 completed the study and 113 (49.8%) were allocated in orlistat group and 114 (50.2%) in orlistat plus L-carnitine group. There were 31 patients (13 males and 18 females) who did not complete the study and the reasons for premature withdrawal included side effects as flatulence (2 males after 3 months, 1 male after 6 months in orlistat group, respectively, and 1 male and 1 female after 3 months, 1 male after 6 months, and 1 male after 12 months in orlistat plus L-carnitine group, respectively), constipation (1 female after 3 months, 1 female after 9 months in orlistat group, respectively, and 1 male in orlistat plus L-carnitine group after 3 months), abdominal pain (1 female after 3 months, 1 female after 9 months in orlistat group, respectively, and 1 male in orlistat plus L-carnitine group after 6 months), fatty/oily evacuation (1 female after 6 months, 2 males after 9 months in orlistat group, respectively, and 2 females after 6 months, 1 male and 1 female after 9 months, 1 female after 12 months in orlistat plus L-carnitine group, respectively), increased defecation (1 female in orlistat group after 12 months, and 2 females in orlistat plus L-carnitine group after 6 months), fecal urgency (1 female in orlistat group after 12 months, and 1 male in orlistat plus L-carnitine group after 12 months), malaise (1 female in orlistat group after 6 months, and 1 male after 9 months, 1 female after 12 months in orlistat plus L-carnitine group, respectively), lost to follow-up (1 female in orlistat plus L-carnitine group after 9 months), and withdrawn of informed consent (1 male in orlistat plus L-carnitine group after 6 months). The compliance to the therapy was very good, none of the patients was excluded for non compliance to therapy. The characteristics of the patient population at study entry are shown in Table 1; the patients ending the study and reporting adverse events are shown in Table 5.

Body weight and BMI

We observed a decrease of body weight after 9, and 12 months with both treatments ($p < 0.05$, and $p < 0.01$, respectively for both), even if at 12 months the value obtained with orlistat plus L-carnitine was significantly lower ($p < 0.05$) than the value obtained with orlistat alone (Tables 3 and 4).

Glycemic parameters

An improvement of HbA_{1c} was recorded after 6, 9, and 12 months in both groups ($p < 0.05$, $p < 0.001$, and $p < 0.0001$, respectively for orlistat plus L-carnitine group, and $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively in orlistat group) compared to baseline. The values obtained with orlistat plus L-carnitine were significantly lower than the values observed with orlistat at 9, and 12 months ($p < 0.05$ for both) (Tables 3 and 4).

FPG significantly decreased after 9, and 12 months with orlistat plus L-carnitine ($p < 0.05$, and $p < 0.01$, respectively), and after 12 months with orlistat ($p < 0.05$); furthermore the values recorded with orlistat plus L-carnitine were significantly better than those with orlistat after 9, and 12 months ($p < 0.05$ for both) (Tables 3 and 4).

There was an improvement of PPG after 9, and 12 months with both treatments ($p < 0.01$, and $p < 0.001$, respectively with orlistat plus L-carnitine, and $p < 0.05$, and $p < 0.01$, respectively with orlistat) even if the value reached with orlistat plus L-carnitine was significantly lower than the one obtained with orlistat at 9 months ($p < 0.05$) (Tables 3 and 4).

Lipid profile

Regarding lipid profile there was not any varia-

Table 3 Patients data during the study in orlistat group.

	Orlistat group				
	Baseline	3 month	6 month	9 month	12 month
n	126	122	119	115	113
Sex (M/F)	62/64	60/62	59/60	57/58	57/56
Sm. st. (M/F)	21/25	21/24	20/23	20/23	20/22
Weight (Kg)	94.5 ± 9.6	92.7 ± 9.4	90.3 ± 8.4	88.1 ± 7.2*	85.0 ± 5.9**
BMI (Kg/m^2)	33.1 ± 2.9	32.5 ± 2.3	31.6 ± 1.8	30.8 ± 1.5*	29.8 ± 1.2**
HbA_{1c} (%)	8.4 ± 1.4	8.1 ± 1.2	7.7 ± 0.9*	7.3 ± 0.6**	7.0 ± 0.5^
FPG (mg/dL)	136 ± 16	132 ± 14	129 ± 13	126 ± 12	121 ± 11*
PPG (mg/dL)	174 ± 24	170 ± 19	163 ± 17	156 ± 16*	149 ± 13**
FPI (μU/mL)	22.8 ± 5.7	22.1 ± 5.2	21.3 ± 4.7	20.2 ± 4.5*	19.3 ± 4.2$^{\$}$
HOMA- IR	7.7 ± 4.2	7.0 ± 3.6	6.6 ± 3.4	6.1 ± 3.2*	5.6 ± 2.8$^{\$}$
TC (mg/dL)	220 ± 24	212 ± 17	207 ± 15	194 ± 11*	186 ± 9$^{\$}$
LDL-C (mg/dL)	153 ± 15	149 ± 11	144 ± 8	134 ± 7*	126 ± 6$^{\$}$
HDL-C (mg/dL)	45 ± 7	44 ± 6	46 ± 8	45 ± 7	46 ± 8
Tg (mg/dL)	109 ± 48	95 ± 39	84 ± 30	76 ± 27	72 ± 25*
Resistin (ng/mL)	6.9 ± 2.5	6.1 ± 2.1	5.7 ± 1.7	5.3 ± 1.5*	5.0 ± 1.2$^{\$}$
RBP-4 (μg/mL)	42.1 ± 10.2	40.3 ± 9.9	37.1 ± 8.6	35.8 ± 8.3	33.6 ± 7.2*
Visfatin (ng/mL)	17.8 ± 6.4	16.9 ± 5.9	16.4 ± 5.4	16.1 ± 5.5	15.7 ± 5.1*
Hs-CRP (mg/L)	2.5 ± 1.6	2.3 ± 1.4	1.9 ± 1.1	1.7 ± 0.9*	1.5 ± 0.6$^{\$}$

Data are means ± *SD.* * $p < 0.05$ *vs.* baseline; $^{\$}p < 0.02$ *vs.* baseline; ** $p < 0.01$ *vs.* baseline; ^ $p < 0.001$ *vs.* baseline
Sm. st.: Smoking status; BMI: body mass index; HbA_{1c}: glycated hemoglobin; FPG: fasting plasma glucose; PPG: post-prandial plasma glucose; FPI: fasting plasma insulin; HOMA-IR: homeostasis model assessment insulin resistance index; TC: total cholesterol; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; Tg: triglycerides; RBP-4: retinol binding protein-4; Hs-CRP: high sensitivity-C reactive protein.

Table 4 Patients data during the study in orlistat+L-carnitine group.

	Orlistat+L-carnitine group				
	Baseline	3 month	6 month	9 month	12 month
n	132	129	122	118	114
Sex (M/F)	65/67	63/66	60/62	59/59	57/57
Sm. st. (M/F)	20/23	20/21	19/20	19/19	18/19
Weight (Kg)	95.1 ± 10.3	92.2 ± 9.2	88.7 ± 7.4	86.9 ± 7.0*	83.8 ± 4.2**$^{+}$
BMI (Kg/m^2)	32.9 ± 2.8	31.9 ± 2.0	30.7 ± 1.6	30.1 ± 1.4*	29.0 ± 1.3**
HbA_{1c} (%)	8.7 ± 1.6	8.5 ± 1.5	8.0 ± 1.2*	6.9 ± 0.4^$^{+}$	6.5 ± 0.3°$^{+}$
FPG (mg/dL)	140 ± 19	134 ± 15	126 ± 12	119 ± 9*$^{+}$	112 ± 7**$^{+}$
PPG (mg/dL)	178 ± 27	172 ± 23	163 ± 18	145 ± 12**$^{+}$	137 ± 10^
FPI (μU/mL)	23.1 ± 6.0	21.5 ± 4.8	20.1 ± 4.5*	19.4 ± 4.3$^{\$}$	18.6 ± 3.9**
HOMA- IR	8.0 ± 4.6	6.9 ± 3.6	6.1 ± 3.2*	5.5 ± 2.7$^{\$}$	5.0 ± 2.4**$^{+}$
TC (mg/dL)	223 ± 25	207 ± 15	193 ± 10*	185 ± 9$^{\$}$	179 ± 7**
LDL-C (mg/dL)	159 ± 17	142 ± 8	131 ± 7*	125 ± 6$^{\$}$	114 ± 5**$^{+}$
HDL-C (mg/dL)	44 ± 6	46 ± 8	45 ± 7	46 ± 8	44 ± 6
Tg (mg/dL)	102 ± 41	94 ± 38	83 ± 29	71 ± 24*	65 ± 23$^{\$}$
Resistin (ng/mL)	7.1 ± 2.7	6.9 ± 2.5	5.4 ± 1.6*	5.1 ± 1.1$^{\$}$	4.6 ± 0.8**
RBP-4 (μg/mL)	43.3 ± 10.7	38.6 ± 9.2	35.7 ± 8.2	32.4 ± 6.9*	30.1 ± 6.3**
Visfatin (ng/mL)	18.0 ± 6.7	17.4 ± 6.1	16.2 ± 5.6	15.5 ± 5.0*	15.3 ± 4.8$^{\$}$
Hs-CRP (mg/L)	2.7 ± 1.8	2.3 ± 1.4	1.7 ± 0.9*	1.5 ± 0.6$^{\$}$	1.2 ± 0.4**

Data are means ± *SD.* * $p < 0.05$ *vs.* baseline; $^{\$}p < 0.02$ *vs.* baseline; ** $p < 0.01$ *vs.* baseline; ^ $p < 0.001$ *vs.* baseline; ° $p < 0.0001$ *vs.* baseline; $^{+}p < 0.05$ *vs.* Orlistat group
Sm. st.: Smoking status; BMI: body mass index; HbA_{1c}: glycated hemoglobin; FPG: fasting plasma glucose; PPG: post-prandial plasma glucose; FPI: fasting plasma insulin; HOMA-IR: homeostasis model assessment insulin resistance index; TC: total cholesterol; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; Tg: triglycerides; RBP-4: retinol binding protein-4; Hs-CRP: high sensitivity-C reactive protein.

tion of HDL-C in neither group, while we observed an improvement of TC, and LDL-C after 6, 9, and 12 months ($p < 0.05$, $p < 0.02$, and $p < 0.01$ for both, respectively) with orlistat plus L-carnitine, and after 9, and 12 months with orlistat ($p < 0.05$, and $p < 0.02$ for both, respectively). Furthermore the LDL-C value obtained with orlistat plus L-carnitine was significantly lower than the value recorded with orlistat after 12 months ($p < 0.05$); no differences between the two treatments were obtained in group to group comparison regarding TC (Tables 3 and 4). A significant improvement of Tg was reached after 9, and 12 months ($p < 0.05$, and $p < 0.02$) with orlistat plus L-carnitine, and after 12 months with orlistat ($p < 0.05$) without any differences between the two groups.

Insulin resistance parameters

The values of FPI, and HOMA-IR significantly improved after 6, 9, and 12 months ($p < 0.05$, $p < 0.02$, and $p < 0.01$, respectively) with orlistat plus L-carnitine, and after 9, and 12 months with orlistat ($p < 0.05$, and $p < 0.02$, respectively). In group to group comparison there was not any difference regarding FPI, while HOMA-IR value recorded with orlistat plus L-carnitine after 12 months was significantly lower that with orlistat ($p < 0.05$) (Tables 3 and 4).

Regarding resistin there was a decrease after 6, 9, and 12 months ($p < 0.05$, $p < 0.02$, and $p < 0.01$, respectively) with orlistat plus L-carnitine and after 9, and 12 months ($p < 0.05$, and $p < 0.02$) with orlistat (Tables 3 and 4).

RBP-4 significantly improved after 9, and 12 months ($p < 0.05$, and $p < 0.01$, respectively) with orlistat plus L-carnitine, and after 12 months ($p < 0.05$) with orlistat (Tables 3 and 4).

We recorded a decrease of visfatin after 9, and 12 months ($p < 0.05$, and $p < 0.02$, respectively) with orlistat plus L-carnitine, and after 12 months with orlistat ($p < 0.05$) (Tables 3 and 4).

Comparing the two groups we did not recorded any differences regarding resistin, RBP-4, nor visfatin.

Inflammatory state

We observed a decrease of Hs-CRP after 6, 9, and 12 months with orlistat plus L-carnitine ($p < 0.05$, $p < 0.02$, and $p < 0.01$, respectively) and after 12 months ($p < 0.05$) with orlistat compared to baseline, without any differences between the two treatments (Tables 3 and 4).

Discussion

Our group has conducted several studies on orlistat, where orlistat appeared to be effective on anthropometric variables and on metabolic pattern during the treatment a tolerable and efficacious drug in hypercholesterolemic and obese patients [27-28], and in hypertensive obese patients [29]. We have also conducted a study on L-carnitine where we showed that L-carnitine improved Lp(a) levels in hypercholesterolemic type 2 diabetic patients [30] confirming what already reported in literature by Sirtori *et al*. [31]. On the other side L-carnitine did not give an improvement of body weight, glycemic and lipid profile compared to placebo [30].

In the current study we demonstrated that orlistat plus L-carnitine gave a better improvement of body weight, glycemic and lipid profile compared to orlistat. This can be due to the synergistic effects of orlistat and L-carnitine: the positive effect of L-carnitine on β-oxidation of fatty acids [7] and its activity of the pyruvate dehydrogenase complex [8], and the positive effects of orlistat on ingested dietary fat, gave a better improvement of these parameters compared to the drugs alone. Regarding insulin resistance, it has been reported in literature that in T2DM patients the HOMA-IR resulted to be increased compared to the normal glucose tolerance (NGT) subjects [32]. Data from our study showed that orlistat plus L-carnitine improved insulin resistance better and faster than orlistat 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 type 2 diabetes compared with lean subjects [33], even if 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 [34-36]. Available data support also a role of resistin in determining an increase of inflammation and atherosclerosis [37].

We have also analysed visfatin; visfatin was discovered as 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 pre-B-cell colony stim-

Table 5 Adverse events in both groups during the study.

	Orlistat group n(%)	Orlistat+L-carnitine group n(%)
Flatulence	3 (2.4)	4 (3.0)
Constipation	1 (0.8)	1 (0.7)
Abdominal pain	1 (0.8)	1 (0.7)
Fatty/oily evacuation	8 (6.3)	13 (9.8)
Increased defecation	6 (4.8)	10 (7.6)
Fecal urgency	4 (3.2)	6 (4.5)
Malaise	11 (8.7)	15 (11.4)

Data are expressed as n or %

ulating factor (PBEF) [38-39]. 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 [38]. Visfatin binds to the insulin receptor with similar affinity but at a site distinct from insulin [38]. In contrast with insulin, visfatin levels do not change with feeding and fasting [38]. 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 observed that orlistat plus L-carnitine, added to the previously taken antidiabetic therapy, gave a faster improvement of these parameters compared to orlistat.

Regarding inflammatory parameters, Hs-CRP has been shown to independently predict myocardial infarction, stroke and peripheral artery disease [40]. In our study orlistat plus L-carnitine decreased Hs-CRP faster than orlistat.

Regarding adverse reactions (Table 5) we did not observe any significant differences between the group treated with orlistat plus L-carnitine, and the group treated with orlistat; the reported adverse effects were flatulence, constipation, abdominal pain, fatty/oily evacuation, increased defecation, fecal urgency, malaise. This was in line with what already reported by our group in two previous studies about orlistat [29, 41], orlistat was related to a major incidence of gastrointestinal effects even if all the events were reported as mild or moderate. However orlistat plus L-carnitine 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 and insulin resistance parameters were sustained after the cessation of therapy. Another limitation is that we dosed a limited number of insulin resistance biomarkers, concentrating our attention on a few of these.

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

Conclusions

The association of orlistat plus L-carnitine was better than orlistat in improving body weight, glycemic and lipid profile, insulin resistance, and inflammatory parameters and no significant adverse events were recorded. We think that these positive effects can be due to the synergistic effects of these two drugs.

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