

Impact of Weight Loss on Adipocytokines, C-Reactive Protein and Insulin Sensitivity in Hypertensive Women with Central Obesity

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Summary

Objective: To assess the impact of weight reduction on serum adipocytokines, C-reactive protein (CRP), and insulin sensitivity in hypertensive female patients with central obesity.

Methods: This study was performed using the database and stored serum samples of female patients who had participated in an intervention study focused on weight loss. Thirty hypertensive women aged 18 to 65, body mass index (BMI) ≥ 27 kg/m², and central obesity were selected. They were randomly assigned to receive either a low-calorie diet plus orlistat 120 mg three times daily or a low-calorie diet alone for 16 weeks. Patients who experienced weight loss greater than 5% (n = 24) were assessed for blood pressure, anthropometric parameters, visceral fat, insulin resistance (HOMA-R - homeostasis model assessment of insulin resistance) and sensitivity (ISI - Insulin Sensitivity Index) indices, plus serum lipids, adipocytokines (adiponectin, leptin, IL-6, and TNF- α) and CRP levels.

Results: After BMI had been reduced by approximately 5% in both groups, visceral fat, fasting glucose, triglycerides, and TNF- α decreased. Only the orlistat group, which was more insulin resistant at baseline, showed a significant reduction in blood glucose after oral glucose load, in addition to increased insulin sensitivity.

Conclusion: This study's findings indicate that a weight loss greater than 5% is associated with improved inflammatory status and decreased insulin resistance, regardless of changes in adiponectin and TNF- α levels. The greatest improvements in insulin sensitivity experienced by the orlistat-treated patients could not be attributed to the use of this drug because of the higher number of insulin-resistant subjects in this group. (Arq Bras Cardiol 2007;89(6):371-375)

Key words: Weight loss; inflammation; C-reactive protein; hypertension; obesity; insulin resistance.

Introduction

Recently, a body of evidence has emerged indicating that obesity is associated with a subclinical inflammatory process. It is now recognized that adipose tissue expresses and secretes a variety of bioactive peptides, known as adipocytokines, which act both *in loco* (autocrine or paracrine activity) and systemically (endocrine activity), and that many of these substances are involved in inflammatory phenomena¹.

It is important that obesity be viewed as an inflammatory state, because inflammation may be one of the links among obesity and insulin resistance, hypertension, and cardiovascular disease. Tumor necrosis factor- α (TNF- α) and interleukin 6 (IL-6) are among the most studied proinflammatory adipocytokines, both of which are associated with insulin resistance and hypertension². Adiponectin is another important adipocytokine, associated with insulin sensitivity and anti-inflammatory action, with the distinguishing characteristic of being inversely correlated with obesity².

A number of studies have shown that weight loss is associated

with a decline in insulin resistance and inflammatory markers³. Orlistat is an anti-obesity agent that can reduce dietary fat absorption up to 30% by inhibiting gastrointestinal lipases^{4,5}. In patients with impaired glucose tolerance, orlistat was effective in preventing type-2 diabetes mellitus⁶, although it has not been demonstrated whether this benefit was associated with other advantages besides weight loss, such as better profile of adipocytokines or inflammatory markers.

This study sought to assess the impact of weight reduction on adipocytokine levels and inflammatory state in hypertensive female patients with central obesity.

Methods

This study was performed using the database and stored serum samples of female patients who had participated in an intervention study focused on weight loss. Patients were recruited from the obesity/diabetes and hypertension outpatient clinic of the Departments of Endocrinology and Nephrology of the Federal University of São Paulo (UNIFESP). The study protocol was approved by the Research Ethics Committee of UNIFESP, and all participants signed an informed consent form.

At first, thirty women were selected based on the

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Manuscript received November 4, 2006; revised manuscript received March 1, 2007; accepted April 23, 2007.

following inclusion criteria: age between 18 and 65; body mass index (BMI) ≥ 27 kg/m²; central adiposity (waist-to-hip ratio > 0.85 or waist circumference > 80 cm); and hypertension (blood pressure $\geq 140/90$ mm Hg or use of antihypertensive medication). Patients with known diabetes or those on treatment for diabetes mellitus, uncontrolled hypertension (diastolic blood pressure ≥ 110 mmHg), active gastrointestinal disease, and a history of myocardial infarction or gastrointestinal surgery (for weight loss) were excluded from the study, as were those who had participated in any previous clinical trial of orlistat.

The study sample was randomized into two groups: low-calorie diet + orlistat or low-calorie diet alone. Patients received an individualized diet plan and systematic follow-up. Of the initial 30 patients, three dropped out before completing the study and three were not included in the data analysis because their decrease in BMI was less than 5% (two patients in the orlistat group and one patient in the diet group). Twenty-four patients were evaluated, 14 in the orlistat group and ten in the diet group. The orlistat group received 120 mg p.o three times daily taken with each of the three main meals (breakfast, lunch, and dinner). The study consisted of a 16-week treatment period, preceded by two weeks during which all patients underwent the initial interview, history taking, physical examination, additional tests, and diet prescription, with follow-up visits every fifteen days. A low-energy diet was prescribed based on resting energy expenditure (REE), as measured by indirect calorimetry. The dietary intake of macronutrients was as follows: 25% lipids, 55% carbohydrates, and 20% proteins.

All patients underwent the following measurements at baseline and study conclusion:

- Anthropometry: body weight, height, BMI, plus waist and hip circumferences.

- Body composition, measured by bioelectrical impedance analysis using a Quantum-BIA 101Q apparatus (Akern RJL Systems, Clinton Township, MI, EUA).

- Abdominal visceral fat assessed by ultrasonography: defined as the distance between the internal surface of the rectus abdominis muscle and the anterior wall of the aorta, using an HTL/HDI 3000 device⁷.

- Blood pressure: both office BP and 24-hour ambulatory blood pressure monitoring (ABPM) were performed using an indirect, automated, noninvasive monitor, which transmits data to the SpaceLabs ABP Model 90207 analysis system (Data Interface Unit).

- Biochemical analyses: oral glucose tolerance test (OGTT) with insulin levels determined at 0 and 120 minutes and insulin resistance assessed by the resistance (HOMA-R – homeostasis model assessment of insulin resistance)⁸ and sensitivity (ISI – Insulin Sensitivity Index) indices. The ISI reflects both hepatic and peripheral sensitivity to insulin, since it incorporates glucose and insulin values, both fasting and at 120-min of OGTT⁹. This is a relative index, in which patients with normal insulin sensitivity have ISI close to 1. Serum insulin was measured by radioimmunoassay (LINCO Research, Inc.). Serum total cholesterol, cholesterol fractions, and triglycerides were measured by enzymatic colorimetric method using an automated spectrophotometer (Abbott VP).

- Adipocytokines and CRP: adiponectin was measured using the ELISA kit from B-Bridge International Inc. (Sunnyvale, CA, EUA). TNF- α was measured using the ELISA kit from BD PharMingen (San Diego, CA, EUA), with a lower detection limit of 7.7 pg/ml. Leptin was measured using the ELISA kit from LINCO Research (St. Charles, Missouri, EUA). IL-6 was measured by chemiluminescence using the IMMULITE-DPC kit (Los Angeles, USA), and values lower than 2.0 pg/mL were corrected according to the IMMULITE manual. CRP levels were measured by chemiluminescence using the IMMULITE-DPC kit (Los Angeles, EUA). The intra- and interassay coefficients of variation (CV) were, respectively, 5.7% and 7.3% for adiponectin, 6.2% and 7.5% for IL-6, 4.6% and 6.2% for leptin, 4.2% and 6.8% for TNF- α , and 6.4% and 10% for CRP.

Statistical analysis - Student's *t*-test for dependent means was used for intragroup comparison between baseline and final values of quantitative variables, and the chi-squared test, for qualitative variables. Student's *t*-test for independent means was used for intergroup comparison. Correlations were tested by Pearson's correlation coefficient. Data are presented as mean and standard deviation, and the significance level was set at $p < 0.05$. Statistical analyses were performed using SPSS for Windows, version 11.5.

Results

Patient data, pre- and post-weight loss, are shown in Tables 1 and 2. It can be noted that, at baseline, patients in the orlistat group had higher blood pressure, both systolic and diastolic. All patients were taking antihypertensive medication, with no difference in drug class between groups (data not shown). The orlistat group was more insulin-resistant, as evidenced by higher HOMA-R values and increased baseline glucose levels (fasting and 120 min post-glucose load). There were no significant differences in adipocytokines and CRP levels between both groups at study entry (Table 2).

Both treatment groups experienced similar weight reduction, with a decrease in waist circumference, visceral fat, fasting glucose, and triglycerides. Only in the orlistat group did blood glucose levels show a significant decrease at 120 minutes after the glucose load, in addition to an increase in the ISI. Total cholesterol and LDL-cholesterol also decreased in the orlistat-treated group, but not in the diet-treated group.

TNF- α levels decreased in both groups (Figure 1), but IL-6 levels remained unchanged. Although leptin levels declined in both groups, only in the orlistat group did this difference reach statistical significance. No significant changes were found in adiponectin concentrations in both groups. The orlistat group also showed a tendency toward decreased CRP levels, though marginally significant in a statistical sense ($p = 0.08$). Taking all patients into account, an inverse correlation was found between CRP and ISI ($r = -0.44$; $p = 0.04$) (Figure 2). Changes in CRP after weight loss were correlated with changes in waist circumference ($r = 0.41$; $p = 0.05$), but not with changes in BMI.

Discussion

Table 1 - Baseline and post-treatment characteristics of the patients

Parameter	Pre-treatment diet (n = 10)	Post-treatment diet (n = 10)	Pre-treatment orlistat (n = 14)	Post-treatment orlistat (n = 14)
Age (years)	46.8 ± 5.8		47.9 ± 9.3	
BMI (kg/m ²)	36.0 ± 7.6	33.0 ± 7.0*	35.4 ± 5.9	32.5 ± 5.9*
Waist circumference (cm)	106.1 ± 12.7	97.7 ± 10.6*	102.0 ± 9.0	95.1 ± 11.1*
24-hour systolic BP (mm Hg)	126.5 ± 12.6	121.1 ± 12.6	139.5 ± 12.7**	132.9 ± 13.0*
24-hour diastolic BP (mm Hg)	79.8 ± 8.3	78.6 ± 10.6	88.3 ± 8.6**	86.2 ± 10.4
Visceral fat (cm)	5.5 ± 1.5	4.0 ± 1.2*	4.6 ± 1.2	3.9 ± 1.3*
Total cholesterol (mg/dL)	210.5 ± 35.5	207.9 ± 39.2	200.9 ± 33.1	180.3 ± 24.9*
HDL-cholesterol (mg/dL)	44.3 ± 14.1	47.4 ± 16.9	41.1 ± 12.2	43.6 ± 9.8
Triglycerides (mg/dL)	142.2 ± 46.2	117.9 ± 52.9*	137.5 ± 56.0	111.3 ± 42.9
LDL-cholesterol (mg/dL)	137.8 ± 31.5	136.8 ± 32.3	132.3 ± 35.4	114.0 ± 24.1

* $p < 0.05$ for pre- vs. post-treatment within groups; ** $p < 0.05$ for pre-treatment differences between groups. n - number of patients; BMI - body mass index; BP - blood pressure; HDL-cholesterol - high-density lipoprotein cholesterol; LDL-cholesterol - low-density lipoprotein cholesterol.

Table 2 - Baseline and post-treatment characteristics of the patients

Parameter	Pre-treatment diet (n = 10)	Post-treatment diet (n = 10)	Pre-treatment orlistat (n = 14)	Post-treatment orlistat (n = 14)
Fasting glucose (mg/dL)	92.7 ± 7.6	87.7 ± 8.8*	113.5 ± 28.1**	97.9 ± 12.2*
120-min glucose (mg/dL)	127.5 ± 23.0	114.7 ± 28.3	173.4 ± 60.8**	142.4 ± 47.1*
ISI	1.23 ± 0.23	1.23 ± 0.25	1.10 ± 0.28	1.23 ± 0.19*
HOMA-R	4.0 ± 2.4	3.9 ± 2.0	7.2 ± 4.2**	5.3 ± 2.3
Adiponectin (ng/mL)	7.4 ± 3.4	7.1 ± 3.5	6.5 ± 1.8	5.9 ± 2.1
IL-6 (pg/mL)	1.1 ± 0.3	1.1 ± 0.4	1.2 ± 0.4	1.6 ± 0.9
Leptin (ng/mL)	62.4 ± 31.9	52.1 ± 37.5	39.4 ± 27.1	31.6 ± 8.6*
CRP (ng/mL)	0.48 ± 0.45	0.44 ± 0.31	0.95 ± 0.81	0.51 ± 0.41
TNF- α (pg/ml)	18.8 ± 7.1	8.2 ± 1.2*	24.5 ± 13.4	9.7 ± 7.0*

* $p < 0.05$ for pre vs. post-treatment within groups; ** $p < 0.05$ for pre-treatment differences between groups. n - number of patients; ISI - insulin sensitivity index; HOMA-R - insulin resistance index (homeostasis model assessment of insulin resistance); IL-6 - interleukin-6; CRP - C-reactive protein; TNF- α - tumor necrosis factor-alpha.

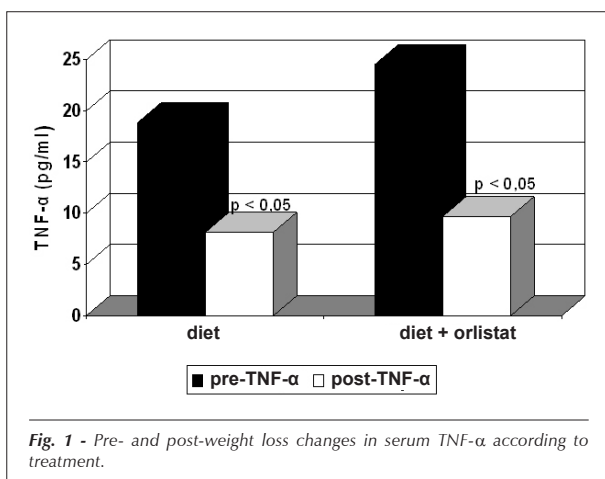
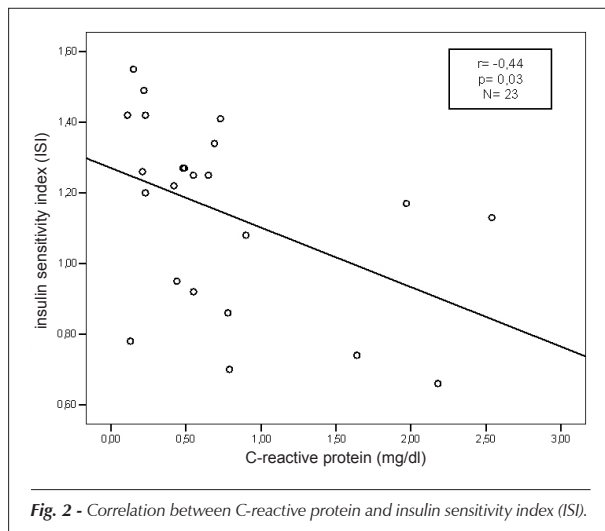


Fig. 1 - Pre- and post-weight loss changes in serum TNF- α according to treatment.

Our results show that an 8% decrease in BMI was associated with a decrease in parameters such as visceral fat, fasting glucose, triglycerides, and TNF- α . However, this amount of weight loss elicited different responses in the groups studied regarding glucose levels 120 minutes following oral glucose load and insulin sensitivity index. Although both groups were randomly selected, the orlistat-treated group was more insulin resistant and had higher fasting and post-load glucose levels. Therefore, the benefits found in this group may have resulted from the greater impact of weight loss in subjects with higher degree of insulin resistance, rather than being an effect of orlistat. The results of this study are in keeping with those of other studies comparing similar amounts of weight loss between groups with different degrees of insulin resistance at baseline, which have also shown significant improvement in insulin sensitivity only in patients who were more insulin



resistant before treatment^{10,11}. They also agree with the results from the XENDOS study (*XENical in the Prevention of Diabetes in Obese Subjects*)⁶, in which orlistat improved glucose parameters and even helped to prevent diabetes in patients with impaired glucose tolerance. Nevertheless, one cannot say that these effects were independent of the weight loss, since mean weight reduction in the orlistat group at the end of the XENDOS study was higher than in the placebo group.

Unlike other studies that reported increased levels of adiponectin with weight loss, in our study no significant changes were found in adiponectin concentrations in both treatment groups^{12,13}. On the other hand, some studies have shown no increase in adiponectin levels with weight loss either, yet insulin resistance improved, as was the case in our study¹⁴⁻¹⁶. Although at the beginning of treatment adiponectin was positively correlated with ISI and inversely correlated with body weight, the insulin sensitivity improvement found in the orlistat group was independent of adiponectin change. It has been recently found that serum adiponectin circulates in different molecular weight forms that may also have distinct biological functions¹⁷. Additionally, it has been demonstrated that weight reduction not only leads to changes in adiponectin levels, but also in the distribution of its circulating forms, with decreased levels of low molecular weight forms and increased levels of middle and high molecular weight forms, thereby improving insulin sensitivity¹⁷. Abbasi et al¹⁸, however, found no changes either in plasma adiponectin or in the distribution of adiponectin oligomers with weight loss, even though insulin sensitivity improved¹⁸. It is possible that weight loss may improve insulin sensitivity by mechanisms other than adiponectin action, such as mobilization of liver¹⁹ and muscle lipid content²⁰.

IL-6 remained unchanged in both groups after weight loss. Our findings differ from those reported by Bastard et al²¹. These authors demonstrated that, in obese, nondiabetic female patients, a modest weight loss (a decrease of about 5.0% in BMI) resulted in a significant reduction in serum IL-6 and the expression of IL-6 in subcutaneous adipose tissue²¹. In the present study, however, patients were more obese and

had significantly higher IL-6 levels, and these pretreatment differences might explain the differing results.

There was a significant decrease in TNF- α levels regardless of the treatment used, and changes in TNF- α were not correlated with changes in insulin resistance indices. Results of studies correlating weight loss with TNF- α are conflicting^{22,23}. Samuelsson et al²⁴ compared weight reduction with orlistat versus placebo and, taking into account patients from both groups who lost more than 10% of their baseline weight, concluded that TNF- α levels were lower in the orlistat than in the placebo group²⁴. In the present study, in which mean weight loss was 8%, there was no further reduction in TNF- α in the orlistat-treated patients and, therefore, the changes observed are not attributed to this drug. In the study by Monzillo et al²⁵, the decline in TNF- α levels with weight loss through caloric restriction and physical activity was found only in patients with impaired glucose tolerance, but not in diabetic subjects or those with normal glucose tolerance²⁵. Kopp et al²⁶ also assessed the impact of gastroplasty to treat obesity on adipocytokine levels according to the glucose tolerance status²⁶. In their study, mean body weight decreased by 32.3%, and no significant change was observed in TNF- α levels in either group. The reasons for these differences remain unclear. The baseline characteristics of the sample, such as body weight, degree of insulin resistance, and time of follow-up, may be important to determine adipocytokine response to weight loss.

Even though a tendency to decreased levels of CRP was seen in the orlistat-treated patients, these changes were not statistically significant in either group. The decrease in CRP levels showed no correlation with the decrease in body weight, but there was a positive correlation with the decrease in waist circumference. Moreover, CRP levels were negatively correlated with the insulin sensitivity index, suggesting that the subclinical inflammatory activity may play a role in the degree of insulin resistance and, thereby, in impaired glucose tolerance. The relevance of this association between inflammation and insulin resistance is corroborated by evidence indicating that increased levels of inflammatory markers may predict the development of type-2 diabetes mellitus^{27,28}. The elevated levels of CRP at baseline may contribute to establishing a clearer relationship between decreased CRP and weight reduction²⁶, and most studies have shown that the degree of CRP decrease was linearly related to the amount of weight lost^{24,29}. Our data suggest that decrease in CRP depends more on the decrease in abdominal fat than on weight loss itself, as corroborated by Marfella et al³⁰. In fact, patients with more centralized fat had also higher levels of inflammatory markers.

Conclusion

This study's findings suggest that a weight loss greater than 5% is associated with an improvement in inflammatory status and a decrease in insulin resistance, regardless of adiponectin increases and TNF- α reductions. Because of the differences in glucose tolerance between both groups studied, our data do not allow us to attribute the greater benefit found in the orlistat group to the use of this drug.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

This article is part of the thesis of master submitted by Rodolfo Leão Borges, from Universidade Federal de São Paulo.

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