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Behavior of Insulin Sensitivity and its Relation to Leptin and Tumor Necrosis Factor-Alpha in Obese Women Undergoing Liposuction: 6-Month Follow-up

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Background: Metabolic syndrome is a group of pathological processes which involve insulin resistance, a biochemical and molecular disorder. Obesity appears to be the most frequent clinical component in metabolic syndrome. Subcutaneous fat, independent from visceral fat, is still controversial as a marker of the pathophysiology of insulin resistance.

Methods: An open parallel-group clinical trial was performed of 12 women (age 30-40 years), with BMI from 30-33 kg/m^2 and fasting glucose ≤110 mg/dl. 6 women were included in the "liposuction plus diet" group, and 6 were included in the "diet-only" group. Metabolic profile, including insulin tolerance test (ITT), leptin and tumor necrosis factor alpha (TNFα), was performed at baseline, 1 and 6 months in both groups. Subcutaneous and visceral fat was quantified with spiral tomography at baseline and after 6 months. Friedman and Wilcoxon test were used for intra-group differences, Mann-Whitney U for differences between groups, and Spearman test for correlation, with significance set at $P<0.05$.

Results: No difference existed between groups regarding clinical characteristics and metabolic profile. In the liposuction group, the increase in insulin sensitivity was (3.8±0.86, 3.1±0.85, 4.5±1.02 %/min, $P=0.08$. Insulin sensitivity did not correlate with subcutaneous fat, leptin, or TNFα. Leptin diminished at 1 month (52.7±6.04 vs 31.6±11.9), $P=0.028$, and correlated with the subcutaneous fat (r=0.957). In the diet-only group, TNFα diminished at 6 months, $P=0.046$.

Conclusion: Subcutaneous abdominal fat correlates with leptin; nevertheless, it is a weak marker for TNFα and insulin sensitivity.

Key words: Obesity, liposuction, insulin resistance, subcutaneous fat, leptin, tumor necrosis factor alpha

Introduction

The metabolic syndrome consists of a group of pathological processes whose foundation involves a biochemical and molecular disorder called *insulin resistance*.[1] This syndrome is of particular interest because it is considered a predictor of cardiovascular disease, due to the fact that it is composed of pathologies such as diabetes mellitus type 2 (DM2), systemic arterial hypertension (SAHT), and obesity, plus the fact that it increases the risk of cardiovascular disease 5 times in subjects with DM2.[2]

Obesity seems to be the most frequent clinical component found in the metabolic syndrome, and although its importance in the physiopathology of insulin resistance is still controversial, it is accepted that adipocytokines are found to be related to this disorder.[3]

The distribution of body fat,[4] as well as the products of adipocytes such as leptin, TNFα, interleukin-6 (IL-6), resistin and adiponectin, among others, greatly influence the energetic balance, lipid metabolism and insulin sensitivity.[5] In this respect, abdominal fat and its two components – *visceral* and

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subcutaneous, has been considered an interesting marker directly related to insulin resistance. It is agreed that subcutaneous fat is the principal source of free fatty acids that are released into the systemic circulation and that subcutaneous fat is related to insulin resistance at the muscular level, and also regulates the action of insulin by means of a neuroendocrine network in which leptin participates.[6] On the other hand, visceral fat, even when it supplies small quantities of free fatty acids to the systemic circulation, is considered to play a fundamental role in insulin resistance, because visceral fat constitutes a metabolically more active fat than subcutaneous fat, and it directly influences hepatic insulin resistance.[7]

Based on the preceding data, there is no doubt that both fatty compartments have metabolic differences. However, the role that both types of fat play in insulin resistance is still controversial.[8]

Cross-sectional[9] and surgical studies with abdominoplasty[10] and liposuction [1] establish the importance of subcutaneous fat as a marker, independent of visceral fat, in the physiopathology of insulin resistance.

Therefore, the aim of this study was to determine, in a group of obese women, the effect of removal of abdominal subcutaneous fat by liposuction on the behavior of insulin sensitivity and the possible relationship of liposuction to adipocyte products like leptin and TNFα during a 6-month follow-up.

Materials and Methods

With approval of the Research and Ethics Committees of the participating Institutions, an open, randomized clinical trial was conducted with 12 women of ages 30-40 years and with a body mass index (BMI) between 30 and 33 kg/m^2. One group of 6 women was assigned liposuction and diet. The other 6 only received a diet and comprised the control group. All women had a fasting blood glucose of ≤110 mg/dl, and all signed their informed consents. Women with a systemic disease such as SAH, DM2 or polycystic ovarian syndrome, were on a medication which might affect insulin sensitivity, exercised >1 hour daily, or used tobacco or alcohol to any degree, were excluded from the study. Before the intervention and at 1 month and 6 months postoperatively, both groups underwent a metabolic profile, which included glucose, urea, creatinine, uric acid, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) cholesterol and triglycerides. Insulin sensitivity was evaluated in both groups at the above times, utilizing the insulin tolerance test (ITT). The abdominal subcutaneous and visceral adipose tissue was measured by spiral tomography at the beginning and at the end of the study.

The serum glucose concentration was determined using a glucose oxidase technique. The total cholesterol, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), high-density lipoprotein (HDL) triglycerides, and uric acid were measured using enzymatic methods. Creatinine levels were evaluated by colorimetry. The above parameters were completed with commercially available kits (Beckman Instruments Inc, Brea, CA, USA) with intra- and inter-assay coefficients of variation <3%. Enzyme-linked immunosorbent method (Diagnostic System Laboratories Inc, Webster, TX, USA) was used to assess serum leptin, with intra- and inter-assay coefficient of variation 4.4% and 4.9% respectively. The leptin and TNFα were determined with the ELISA technique, with an intra- and interassay error of <3%.

The ITT consisted of the following: 3 days prior to the test, an isocaloric diet of at least 250 g of carbohydrates per day was given. Following this, and after a 12-hour fast, an intravenous catheter was placed in a forearm vein and 250 ml of physiological saline at 0.9% was administered. A bolus IV injection of regular 0.1 U/kg of body weight. Blood samples were collected in tubes containing 1.5 ml of fluoride for the measurement of serum glucose at the following intervals: 5 minutes before and 3, 6, 9, 12 and 15 minutes after insulin injection. Dextrose (25 g) was injected after the last sample, to stop the decrease in serum glucose. This test determined the decrease of the glucose constant (K-ITT), and was reported in %/min. The K-ITT was calculated using Lundbaeck's formula (0.693/t?x100), wherein the greater the decrease in glucose, the greater the insulin sensitivity value.[12]

The subcutaneous and visceral fat was measured by third-generation spiral tomography (Somaton AR Star, Siemens®). Ten-millimeter cuts were obtained from the xiphisternum to the pubic symphysis with an intra-observer error <1%.

Both groups received the same reducing diet of 20 cal/kg, consisting of 60% complex carbohydrates, 25% fats, and 15% proteins, with a reduction of 100 calories every 4 weeks until reaching 1200 calories. The diet commenced immediately following the metabolic profile and baseline determinations. In order to determine adherence to the diet, a random telephone call asking for a 24-hour recall of food intake was made to the participants. In addition, the degree of appetite was determined using a visual analog scale of 1-10, where 0=no appetite, 5=normal appetite, and 10=considerable appetite. This scale was applied at the beginning of the study, at 1 month, and at 6 months.

Statistical Analysis

In order to determine the differences between the groups, the Mann-Whitney U test was utilized. In order to evaluate the intra-group differences, the Friedman test was employed, and when necessary, the Wilcoxon signed rank test. Spearman's test was used for correlation of the variables. A statistical significance of $P<0.05$ was established.

Results

Both groups had similar clinical characteristics prior to intervention (Table 1). After the intervention, the BMI significantly decreased in the liposuction plus diet group (31.9±1.0 vs 28.1±1.7; $P=0.028$), as well as in the diet-only group (31.9 ±1 vs 28.2 ±1.3; $P=0.028$). The metabolic profile was similar in both groups and was no different at the end of the study (Table 2).

During liposuction, 58% (4582±651 ml) of the subcutaneous abdominal fat was extracted.

In the liposuction plus diet group, insulin sensitivity increased with a statistical trend at the end of the study (3.8±.8; 3.1±.8; 4.5±1.0 %/min; respectively), $P=0.08$ (Figure 1). One month following liposuction, leptin significantly decreased (52.7±6.0 vs 31.6±11.9 ng/ml), $P=0.02$ (Figure 2). Appetite significantly diminished at 1 month following liposuction $P=0.02$, and this reduction was maintained during the 6-month period ($P=0.005$). There was no significant difference ($P=0.738$) in the median changes of the TNFα initially, at 1 month, and at 6 months following liposuction (0.019; 0.017; 0.015 pg/ml, respectively).

Table 1. Clinical characteristics between groups

	Liposuction plus diet (n=6)	Diet (n=6)	P*
Age (years)	34.0±3.7	34.6±3.6	0.69
BMI, kg/m^2	31.9±1.2	31.9±1.0	0.93
Subcutaneous fat, cm^3	7637.0±1342.0	6656.0±2108.0	0.58
Visceral fat, cm^3	1796.0±331.0	1311.0±421.0	0.09
Blood pressure (mmHg)	106/77	103/73	0.28

*U Mann-Whitney

Table 2. Metabolic profile in both groups

	Liposuction plus diet (n=6)		Diet only (n=6)	
Intervention	Before	After	Before	After
Glucose,mmol/L	5.0±.4	4.9±.3	5.1±.3	5.2±.5
Creatinine, µmol/L	63.3±11.7	63.3±8.6	58.9±9.1	61.8±7.9
Uric acid, µmol/L	317.1±54	309.2±55	267.6±38.2	277.5±55.1
Total cholesterol, mmol/L	4.4±.5	4.3±1.0	4.4±.4	4.0±.0
HDL cholesterol, mmol/L	1.2±.3	1.1±.5	1.0±.2	.9±.1
Triglycerides, mmol/L	1.3±.2	1.4±.2	1.3±.3	1.2±.3

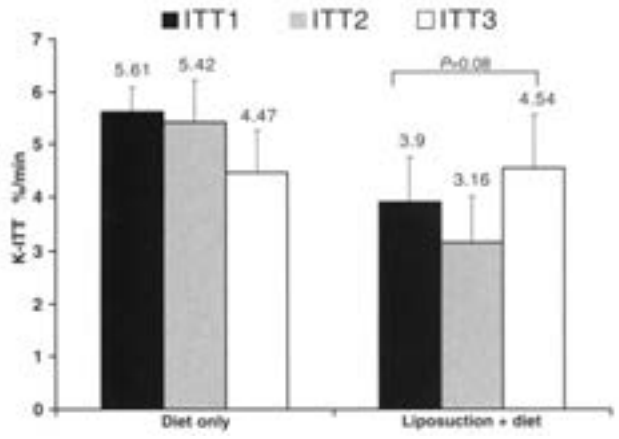

Figure 1. Behavior of insulin resistance in both groups. ITT (before), ITT2 (1 month), ITT3 (6 months).

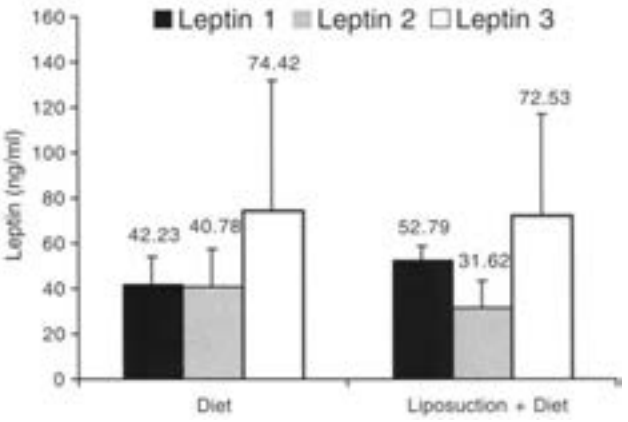

Figure 2. Behavior of leptin concentrations in both groups.

Insulin sensitivity was not significantly correlated with the leptin, TNFα, and BMI, nor with subcutaneous and visceral fat.

At the end of the study, leptin correlated directly with the change in subcutaneous fat (r=.943, P=0.005). In spite of this, leptin returned to its baseline values at the end of the study.

The TNFα did not correlate with the change of leptin (r=.029, P=0.957), nor with insulin sensitivity (r=-0.543, P=0.266) throughout the study. Likewise, this cytokine did not correlate with the decrease of BMI (r=-0.029, P=0.957), nor with the modification of the subcutaneous and visceral abdominal fat compartments, where the correlation was similar (r=.086, P=0.872).

At the end of the study, the subcutaneous fat was reduced by 36.7% and the visceral fat by 8.18%.

The correlation of the delta (change) in insulin sensitivity with the delta (change) in visceral fat was r=-.029.

In the diet-only group, the TNFα decreased significantly at 6 months (0.0454; 0.016; 0.0065 pg/ml respectively, P=0.046).

In this same group, there was no change in insulin sensitivity initially, at 1 month, and at 6 months: (5.6 ±.4; 5.4 ±.8; 4.4 ±.8 respectively P=0.513). Likewise, there was no change in the leptin concentrations during the 6 months (42.2 ±.11.8; 40.7± 16.8; 74.4 ±1.6; P=0.846).

The subcutaneous fat was reduced by 32.3% and the visceral fat by 14.9%.

Discussion

The objective of this study was to determine the importance of the abdominal subcutaneous fat as an independent marker of insulin sensitivity and to establish its possible relationship to leptin and TNFα. In the study, we controlled the amount of subcutaneous fat to be obtained by liposuction, because we established a range of from 55 to 65% of subcutaneous abdominal fat to be aspirated, based on previous quantification utilizing spiral tomography.

After removing 58% of the abdominal subcutaneous fat, we found a statistical tendency toward an increase in insulin sensitivity. This is explained by the considerable reduction of abdominal subcutaneous fat, which is the main source of free fatty acids for systemic circulation.[13]

One month following liposuction, we found a significant decrease in leptin, similar to that reported by Talisman.[15] This reduction correlated directly with the reduction in subcutaneous fat. Although leptin decreased significantly in the postoperative period, this protein appears to play a role in metabolism, independent of insulin sensitivity, because we did not find a correlation between these two markers, similar to the reports of Cnop et al[14] who concluded that leptin depends solely on subcutaneous fat, while insulin resistance depends solely on visceral fat.

In another study, where the possible relationship of leptin to insulin resistance was studied, it was found that the abdominal subcutaneous fat, the BMI, and muscle glucose utilization explain 72% of the variability in the serum concentrations of leptin;

nevertheless, the utilization of glucose had a negative correlation, independent of leptin.[15]

Despite the above, there are reports where leptin is indeed associated with insulin resistance. De Courten et al[16] studied 240 subjects in a model study of metabolic syndrome and hyperleptinemia wherein, following multiple regression analyses, they found a direct relationship between leptin and insulin resistance, independent of age, BMI, triglycerides, high-density lipoproteins, waist-hip ratio, and systemic arterial hypertension.[16] In another study of obese women in which HOMA was utilized for insulin sensitivity, resistin and leptin correlated in a positive fashion with insulin resistance.[17]

Obesity is considered to be a chronic inflammatory condition in which TNFα plays an important role. The importance of this cytokine in the development of insulin resistance is accepted.[18] In our study, the liposuction group did not show changes in the TNFα, and it did not correlate with either insulin sensitivity or leptin. It was not an expected result if we consider that the subcutaneous fat is an important source of this cytokine. The lack of change in TNFα probably is due to a metabolic response to surgical stress in the group that underwent liposuction, because in the diet-only group there was a decrease in TNFα, and it directly correlated with the decrease in BMI. Similar results have been obtained in women subjected to a diet for 1 year, where after decreasing the body weight by 10%, a significant decrease in this cytokine was seen.[19]

We found no correlation, either in the diet group or in the liposuction group, between the TNFα and leptin levels. In relation to this point, controversy still exists. In vitro studies of subcutaneous adipocytes obtained from obese women, have found a correlation in the expression of leptin and the cytokine.[20] In another study in which subcutaneous adipocytes with TNFα and other cytokynes were incubated, it was found that there was a decrease in the production of leptin by 30 to 50%.[21] On the other hand, in a study utilizing rats, leptin and TNFα did not correlate, and one concludes that both have independent routes for the regulation of insulin sensitivity.[22] The relationship of the behavior of this cytokyne in a group undergoing an acute reduction of the abdominal subcutaneous fat in an amount >50% has not been reported previously. In a bariatric surgery (gastric bypass) study of Molina et al[23] with a 6-month follow-up, an increase in TNFα was observed postoperatively with a subsequent decrease in TNFα baseline levels at 6 months. They found a correlation of TNFα with leptin and adiposity.[23]

Recent studies have found that subcutaneous, upper body fat has a greater role in systemic circulating free fatty acids than visceral fat and may contribute significantly to insulin sensitivity and the metabolic syndrome.[24,25] Our results contribute interesting data: 1) liposuction does not cause metabolic damage; 2) the significant acute reduction of subcutaneous fat allows for the study of the behavior of markers that are associated with insulin resistance, such as leptin and TNFα, thereby establishing the role of subcutaneous fat in the pathophysiology of the metabolic syndrome.

Conclusions

On the basis of our results, we conclude that subcutaneous fat is a weak marker for insulin resistance, it is associated in a significant way with leptin concentrations, and it does not correlate with TNFα concentrations.

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