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Effects of lifestyle modification on adipocytokine levels in obese patients

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Abstract. – *Objective:* **Adipose tissue is considered an active secretory organ of adipocytokines. The principal aim of our study was to examine the changes in adipocytokines levels after weigth reduction in obese patients.**

Matherials and Methods: **A population of 126 obese (body mass index > 30) non-diabetic outpatients was analyzed in a prospective way. Before and after three months of a lifestyle modification program, an indirect calorimetry, a tetrapolar electrical bioimpedance, blood pressure, a serial assessment of nutritional intake with 3 days written food records and a series of biochemical analyses were performed. The lifestyle modification program consisted of a daily hypocaloric diet (1520 kcal: 52% of carbohydrates, 25% of lipids and 23% of proteins). The exercise program consisted of an aerobic exercise for at least 3 times per week (60 minutes each).**

Results: **The mean age was 45.6 ± 16.9 years and the mean BMI 34.5 ± 5.2, with 33 males (26.2%) and 93 females (73.8%). A total of 88 patients completed the follow up during 3 months, with a percentage of weight loss of 3.1%. Seventy two patients lost weight after treatment (*responders*) with an average age of 45.4 ± 16.8 years and a percentage of weight loss of 4.2%. Sixteen patients did not respond (*no-responders*), with an increase in weight (88.9 ± 10.5 vs 88.7 ± 10.9 kg: $p < 0.05$) and BMI (34.7 ± 5.4 vs 35.5 ± 5.5 kg: $p < 0.05$). The average age (45.9 ± 15.9 years) of this group was similar than that of *responders*. In responders (weight loss), BMI, weight, fat mass, glucose, total cholesterol, LDL cholesterol and systolic blood pressure decreased and VO_2 increased. After treatment, no statistical differences were detected in energy intake: carbohydrate, fat, and protein. Exercise improved after treatment in responder group. Only serum leptin levels had a significant decrease in responder group (12%). After treatment, no responder group has similar values of all adipokines, including leptin (81.3 ± 70.6 vs 76.1 ± 43 ng/ml).**

Conclusion: **Three months of lifestyle modification significantly improved anthropometric and cardiovascular risk factors, regardless of their minimal decrease in energy intake and the weight loss. Additional studies will be need to clarify the contribution of lifestyle modification in changes of serum adipocytokine levels.**

Key Words:

Adipocytokines, Cardiovascular risk factors, Diet, Exercise, Obesity.

Introduction

According to the recent surveys, approximately two thirds of the Spain population is overweight[1]. In free-living individuals, long-term maintenance of body weight is determined by balance between energy intake and energy expenditure.

Several dietary approaches to this public health problem have been recommended. An accumulating body of evidence shows that modest weight loss (5%) through dietary changes and exercise is an effective treatment for managing obesity-associated disorders[2,3].

Adipose tissue is considered an active secretory organ, sending out and responding to signals that modulate appetite, insulin sensitivity, energy expenditure, inflammation and immunity. Adipocytokines are proteins produced mainly by adipocytes[4]. These molecules have been shown to be involved in the pathogenesis of the metabolic syndrome and of the cardiovascular disease. *Adiponectin* is an adipocyte-derived collagen like protein indentified through an extensive search of adipose tissue. Hypoadiponectinemia increases the risk of coronary artery disease togheter with the presence of multiple risk factors, indicating that adiponectin could be a key factor of the metabolic syndrome[5]. *Leptin* is a 16 KD protein secreted mainly from adipocytes.

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Leptin suppresses food intake and increases energy expenditure by enhancing thermogenesis and metabolic rate. Recent reports suggest that leptin contributes to the atherosclerosis and the cardiovascular disease in obese patients[6]. *Resistin* is a 12.5 KD, cysteine-rich protein indentified by screening for the genes that are induced during the differentiation of the adipocytes. However, the role of resistin in linking human obesity with type 2 diabetes mellitus is questionable[7]. *TNF-alpha* and *interleukin-6* are increased in obese individuals and in most experimental models with obesity and insulin resistance[8].

There are few studies addressing the effect of weight loss on circulating levels of the adipocytokines[9-12]. It has been shown consistently that leptin levels decrease with weight reduction[9,10], whereas data on the relationship among other adipocytokines and weight loss are controversial.

The major aim of this study was to examine the changes in serum adipocytokines levels after weigth reduction in obese patients.

Matherials and Methods

Subjects

One hundred and twenty-six volunteer obese non diabetic patients (body mass index > 30), 33 males (26.2%) and 93 females (73.8%) were enrolled in this prospective study. The mean age was 45.6 ± 16.9 years and the mean BMI 34.5 ± 5.2. Patients were divided in *responders* (with loss weight) and *no-responders* (without loss weight).

All patients enrolled in a Nutrition Clinic Unit and signed an informed consent. Exclusion criteria included history of cardiovascular disease or stroke during the previous 36 months, total cholesterol > 300 mg/dl, triglycerides > 400 mg/dl, blood pressure > 140/90 mmHg, fasting serum glucose > 110 mg/dl, as well as the use of sulphonylureas, thiazolidinediones, insulin, glucocorticoids, antitumor drugs, angiotensin receptor blockers, angiotensin converting enzyme inhibitors, psychoactive drugs, drinking and/or smoking habit.

Procedure

All patients with a 2 weeks weight-stabilization period before recruitment were enrolled. The lifestyle modification program consisted of a hypocaloric diet (1520 kcal: 52% of carbohydrates, 25% of lipids and 23% of proteins). The exercise program consisted of an aerobic exercise for at least 3 times per week (60 minutes each)[13].

Weight, blood pressure, basal serum glucose, lipoprotein (a), C-reactive protein (CRP), serum insulin, serum total cholesterol, LDL-cholesterol, HDL-cholesterol, serum triglycerides blood and adipocytokines (leptin, adiponectin, resistin, TNF-alpha, and interleukin-6) levels were measured at basal time and after three months.

Assays

Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA), while HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL cholesterol was calculated using Friedewald formula. Lipoprotein (a) was determined by immunonephelometry with the aid of a Beckman array analyzer (Beckman Instruments, CA).

Serum glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, CA). Insulin was measured by enzymatic colorimetry (Insulin, WAKO Pure-Chemical Industries, Osaka, Japan) and the homeostasis model assessment for insulin sensitivity (HOMA) was calculated by Matthews et al[14]. CRP has been measured by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a normal range of 0-7 mg/dl and analytical sensivity of 0.5 mg/dl.

Adipocytokines

Serum resistin was measured by ELISA (Biovendor Laboratory, Inc., Brno, Czech Republic) with a sensitivity of 0.2 ng/ml with a normal range of 4-12 ng/ml. Serum leptin was measured by ELISA (Diagnostic Systems Laboratories, Inc., TX) with a sensitivity of 0.05 ng/ml and a normal range of 10-100 ng/ml. Serum adiponectin was measured by ELISA (R&D systems, Inc., Minneapolis, USA) with a sensitivity of 0.246 ng/ml and a normal range of 8.65-21.43 ng/ml. Serum interleukin-6 and TNF-alpha were measured by ELISA (R&D systems, Inc., Minneapolis, USA) with a sensitivity of 0.7 pg/ml and 0.5 pg/ml, respectively. Normal values of IL-6 was 1.12-12.5 pg/ml and of TNF-alpha 0.5-15.6 pg/ml.

Indirect calorimetry

For the measurement of resting energy expenditure, the patients were admitted to a metabolic ward. After a 12 h overnight fast, resting metabolic rate was measured in the sitting awake subject in a temperature-controlled room over one 20 min period with an open-circuit indirect calorimetry system (standardized for temperature, pressure and moisture) fitted with a face mask (Med Gem; Health Tech, Golden, USA), coefficient of variation 5%. Resting metabolic rate (kcal/day) and oxygen consumption (ml/min) were calculated[15].

Anthropometric measurements

Body weight was measured to an accuracy of 0.5 kg and body mass index computed as body weight/(height^2). Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences to derive waist-to hip ratio (WHR) were measured, too. Bipolar body electrical bioimpedance was used to determine body composition[16]. An electric current of 0.8 mA and 50 kHz was produced by a calibrated signal generator (Biodynamics Model 310e, Seattle, WA) and applied to the skin using adhesive electrodes placed on right-side limbs. Resistance and reactance were used to calculate total body water, fat and fat-free mass.

Blood pressure was measured twice after a 10 minutes rest with a random zero mercury sphygmomanometer and averaged.

Dietary Intake and Habits

Patients received prospective serial assessment of nutritional intake with 3 days written food records. All enrolled patients received instruction to record their daily dietary intake for three days including a weekend day. Handling of the dietary data was by means of a personal computer equipped with personal software, incorporating use of food scales and models to enhance portion size accuracy. Records were reviewed by a registered dietitian and analyzed with a computer-based data evaluation system. National composition food tables were used as reference[17]. Regular aerobic physical activity (walking was allowed, no other exercises) was mantained during the period study (2-3 hours per week).

Statistical Analysis

The results were expressed as average ± standard deviation. The distribution of variables was analyzed with Kolmogorov-Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed, paired Student's-t test. Non-parametric variables were analyzed with the Wilcoxon test (Rank test). Qualitative variables were analyzed with the chi-square test and Fisher's test. Correlation analysis has been realized with Pearson's test and Spearman's test. A p-value under 0.05 was considered statistically significant.

Results

All subjects were weight stable during the 2 weeks period preceding the study (body weight change, 0.29 ± 0.1 kg). A total of 88 patients completed the follow up during 3 months, with a percentage of weight loss of 3.1%. Seventy-two patients lost weight after treatment (*responders*) (18 men/54 women) with an average age of 45.4 ± 16.8 years (Table I) and a percentage of weight loss of 4.2%. Sixteen patients (4 men/12 women) did not respond, with an increase in weight of 88.9 ± 10.5 vs 88.7 ± 10.9 kg: $p < 0.05$ and BMI of 34.7 ± 5.4 vs 35.5 ± 5.5 kg: $p < 0.05$. The average age (45.9 ± 15.9 years) of this group was similar than that of *responders*.

Table I shows the differences in anthropometric variables. In no responder group (n =16), a modest significant increase were detected in weight and BMI. In responder patients (n = 72, weight loss), BMI, weight, fat mass, and systolic pressure decreased and VO_2 increased. No differences were detected between the basal values in both groups.

Table II shows the differences in cardiovascular risk factors. In no responder group, no statistical differences were detected. In responder group, a decrease in serum glucose, serum total cholesterol and LDL cholesterol was detected. Differences were similar in responder group.

Table III shows the nutritional intake with 3 days written food records. After treatment, no statistical differences were detected in energy intake, carbohydrate, fat, and protein. Exercise improved after lifestyle modification in responder group.

Table IV shows differences between basal and after treatment levels of serum adipokines. Only leptin levels have a significant decrease in responder group (12%). After treatment, no-responder group has similar values of all

Table I. Changes in anthropometric variables.

Characteristics	No responders (n = 16)		Responders (n = 72)	
	Baseline	*3 months*	*Baseline*	*3 months*
BMI	34.7 ± 5.4	35.5 ± 5.5 *	34.6 ± 5.2	33.2 ± 5.2*
Weight (kg)	88.9 ± 10.5	88.7 ± 10.9 *	89.9 ± 18.1	86.2 ± 17.6*
Fat free mass (kg)	48.7 ± 14.9	47.9 ± 13.3	48.5 ± 14.1	47.9 ± 13.5
Fat mass (kg)	38.8 ± 11.9	41 ± 12.6	38.3 ± 11.5	36.2 ± 11.6*
Waist circunference	107.7 ± 15.1	109.7 ± 14.8	108.6 ± 15.1	104.3 ± 15.6*
Waist to hip ratio	0.92 ± 0.1	0.91 ± 0.09	0.93 ± 0.1	0.91 ± 0.1*
Systolic BP (mmHg)	133.8 ± 12.6	129 ± 11.5	131 ± 13.3	124.9 ± 13.3*
Diastolic BP (mmHg)	78.4 ± 12.8	80.6 ± 14.8	77.5 ± 14.1	80.4 ± 14.2
RMR (kcal/day)	1723 ± 417	1863.3 ± 4891	687.2 ± 401	1826.8 ± 489
VO_2 (ml/min)	245.6 ± 73.3	247 ± 72	239.3 ± 63.1	262 ± 77*

RMR: resting metabolic rate. O_2: Oxygen consumption. BP: Blood pressure. t Student test and Wilcoxon test were used as statistical methods. *$p < 0.05$, in each group with basal values.

adipokines. Sex differences were analyzed in adipocytokines response. Only basal levels of leptin was higher in women in both groups, (*no-responders*: 109 ± 36 vs 36.1 ± 16 ng/ml; $p < 0.05$) and (*responders*: 110.4 ± 30 vs 42 ± 20 ng/ml; $p < 0.05$). After treatment, leves of leptin remained higher in women than men (*non responders*: 92.9 ± 36 vs 36.1 ± 16 ng/ml; $p < 0.05$) and (*responders*: 90.5 ± 37 vs 47.4 ± 23 ng/ml; $p < 0.05$). IL-6, TNF-alpha, resistin and adiponectin did not have differences between the genders.

In the analysis of responder patients with BMI greater than 35, we detected an improvement in leptin levels (90.5 ± 73.8 vs 83.7 ± 87 ng/ml; $p < 0.05$) without differences in other adipokines. These results were similar in responder group with BMI greater than 40 (leptin: 131.7 ± 90.6 vs 107.6 ± 103 ng/ml; $p < 0.05$).

In correlation analysis, only leptin and IL-6 shows positive correlation with BMI, before and after treatment. Before treatment, statistical analysis shows: BMI and leptin: r = 0.5; $p < 0.05$ and BMI and IL-6: r = 0.3; $p < 0.05$. After

Table II. Classical cardiovascular risk factors.

Characteristics	No responders (n = 16)		Responders (n = 72)	
	Baseline	*3 months*	*Baseline*	*3 months*
Glucose (mg/dl)	100.1 ± 21	99.8 ± 16.8	100.5 ± 22.8	97.1 ± 20.4*
Total chol. (mg/dl)	210 ± 32	205.5 ± 36	211.5 ± 45.9	202.4 ± 38.8*
LDL-chol. (mg/dl)	128 ± 46	124.2 ± 44	130.7 ± 49	115.3 ± 49.4*
HDL-chol. (mg/dl)	53.3 ± 13.8	53.1 ± 13.1	55.1 ± 16.2	54.1 ± 14.8
TG (mg/dl)	134 ± 59.2	133.4 ± 64.3	126.7 ± 61.8	117.1 ± 59.7
Lp (a) (mg/dl)	32.9 ± 31.8	31.3 ± 31.4	34.2 ± 38.4	35.7 ± 37.4
Insulin (mUI/L)	12.8 ± 7.3	14.3 ± 8.8	14.9 ± 9.3	13.3 ± 8.8
HOMA	2.4 ± 1.1	2.3 ± 1.2	2.5 ± 1.8	2.2 ± 1.7
CRP (mg/dl)	6 ± 7.1	5.5 ± 6.9	6.7 ± 8.4	6.4 ± 7.4

LDL-chol: low density lipoprotein. HDL: high density lipoprotein. CRP C reactive protein: Chol: Cholesterol. Lp (a): lipoprotein a. TG: Triglycerides
t Student test and Wilcoxon test were used as statistical methods. *$p < 0.05$, in each group with basal values.

Table III. Dietary intake.

Characteristics	No responders (n = 16)		Responders (n = 72)	
	Baseline	*3 months*	*Baseline*	*3 months*
Energy intake (kcal/day)	1683.4 ± 452	1530 ± 478	1601 ± 361	1563 ± 388
CH (g/day)	169.7 ± 64	163 ± 59	162.4 ± 52	167.2 ± 57
Fat (g/day)	69.9 ± 25	65.1 ± 25.1	67.9 ± 20	63.9 ± 18.8
Protein (g/day)	79.8 ± 28	77.3 ± 22	81.5 ± 21	79.4 ± 19.7
Exercise (Hs./week)	0.69 ± 1.3	2.2 ± 2.2	0.72 ± 1.8	2.6 ± 2.8*

t Student test and Wilcoxon test were used as statistical methods. Hs: hours. * $p < 0.05$, in each group with basal values. CH: Carbohydrate.

treatment, results shows: BMI and leptin: r = 0.52; $p < 0.05$ and BMI and IL-6: r = 0.4; $p < 0.05$. In the correlation analysis with the variable changes, IL-6 and leptin remained with a positive correlation with BMI: r = 0.38; $p < 0.05$ and r = 0.5; $p < 0.05$, respectively.

Discussion

Three months of lifestyle modification through a caloric restriction and a moderate physical exercise significantly reduced BMI, weight, fat mass, waist circumference, waist to hip ratio, systolic pressure, serum glucose, serum total cholesterol, LDL-cholesterol and serum leptin, regardless of their minimal decrease in calory intake and the weight loss (3.8 kg in responder group). The *non responders* did not lose weight and did not improve cardiovascular risk factor due to a lack of increase in exercise as part of a lifestyle modification treatment.

The levels of serum leptin and serum IL-6 were positively correlated with BMI[18,19], before and after intervention. The most important variable that determines circulating leptin concentration is body fat mass[18]. It has been estimated that the adipose tissue contributes to 30% of circulating IL-6, with visceral adipose tissue producing higher levels of IL-6 compared with subcutaneous tissue, which is related with cardiovascular risk factors[19].

Our data are in agreement with other studies in which leptin has been consistenly shown to decrease in response to weight loss[9-12]. However the decrease in serum leptin in our patients was lower than referred previously. Some Authors have been reported a decrease of 22%[10] or 45%[11]. In these researches the percentage of weight loss was higher than ours, for example 7% in first study[10] and 10% in second one[11]. Our study shows a 4.2% of weight loss in responder group with a decrease of leptin levels of 12%. Monzillo et al.[20] detected a decrease of 14% in leptin levels with a weight loss of 7%, similarly to the present

Table IV. Circulating adypocitokines.

Characteristics	No responders (n = 16)		Responders (n = 72)	
	Baseline	*3 months*	*Baseline*	*3 months*
IL 6 (pg/ml)	2.04 ± 1.7	1.95 ± 2.8	2.01 ± 1.9	1.75 ± 2.14
TNF-alpha (pg/ml)	6.45 ± 2.9	6.5 ± 2.8	6.81 ± 2.7	6.6 ± 2.3
Adiponectin (ng/ml)	23.1 ± 17.1	22.1 ± 13.8	23.8 ± 17.3	21 ± 12.8
Resistin (ng/ml)	4.25 ± 1.8	4.23 ± 1.7	4.21 ± 1.6	3.9 ± 1.5
Leptin (ng/ml)	81.3 ± 70.6	76.1 ± 43	81.3 ± 70.6	71.6 ± 70*

IL-6: interleukin-6. t Student test and Wilcoxon test were used as statistical methods. * $p < 0.05$, in each group with basal values.

study. The variable differences present in the literature may partially explain by the differences in baseline BMI and leptin levels of the participant patients. A proportional greater decrease in leptin after weight loss is expected in individuals with greater BMI[21], as we have also detected in this study.

Our data showed no decrease in IL-6 and TNF-alpha concentrations after weight loss. Previously a decrease in interleukins levels with weight loss has been demonstrated[10,20], although other Authors have failed to report a change[11]. We observed that IL-6 and TNF-alpha tended to decrease even thought without statistical significance. Perhaps, our population had a low inflammatory background, being a group of obese patients without diabetes and cardiovascular disease in previous 36 months.

Several Authors[22], after 4-6 weeks of weight loss, detected marked improvement in glucose, insulin, leptin and triglycerides, whereas adiponectin and TNF-alpha concentrations did not change. Three studies[23-25] have demonstrated that a 10-20% weight reduction in obese individuals with very low calory diets (VLCD) or bariatric surgery was associated with a 36-51% increase in serum adiponectin levels. Conversely our data achieved a 6% reduction in BMI with diet and exercise, without no significant change in adiponectin levels, too[26]. These results were quite similar to that of Mousavinasab et al[27].

It is possible that a greater and sustained weight reduction is necessary to correct altered adipocyte function, represented by an increased adiponectin and reduced levels of other serum adipocytokines[28]. Perhaps, the dietary macronutrient composition, at least carbohydrate and fat contents, in part, could determine these differences. On the contrary, the results of Arvidsson et al[29] showed that the macronutrient composition of diets was not a major importance for the outcome of dietary treatment. In fact, in some trials, weight loss secondary to orlistat or sibutramine administration improved adipocytokines in a significant way[30]. However, new studies are needed to search the role of adipocytokine as a key factor of the metabolic syndrome[31].

In conclusion, additional studies will be needed to clarify the contribution of lifestyle modification in leptin levels and to clarify the mechanism of the changes of the other cytokines in obese patients. The presence of one control group will be necessary for a better understanding of other next works. In addition, three months of lifestyle modification significantly improved anthropometric and cardiovascular risk factors, regardless of their minimal decrease in calory intake and the weight loss.

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