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Effect of weight loss on some serum cytokines in human obesity: increase in IL-10 after weight loss

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Abstract

Obesity is a major risk factor for hypertension, coronary artery disease and type 2 diabetes. Weight loss is associated with significant metabolic benefits. Our objective was to examine changes in adipocytokines and interleukin (IL) 10 in obese subjects before and after weight loss. We measured anthropometric parameters, adipocytokine and IL-10 in 78 obese people who had visited obesity clinics at five university hospitals (Ajou, Ulsan, Catholic, Hanyang and Yonsei) in Korea. They restricted their caloric intake to less than their usual intake (by 500 kcal), were administered sibutramine and were given a program of exercise for 12 weeks. After 12 weeks, weight, body mass index, waist circumference, hip circumference, waist-to-hip ratio, total body fat, total cholesterol, triglyceride, tumor necrosis factor α (TNF-α), IL-6, resistin and leptin had significantly decreased, while adiponectin and IL-10 had significantly increased. A bivariate correlation analysis found that increment in IL-10 and baseline IL-10 levels significantly correlated with decrement in TNF-α ($P<.01$) and baseline adiponectin ($r=.52$, $P<.001$), respectively. These results were confirmed in a multiple regression analysis. The results suggest that weight loss after caloric restriction and medical treatment in obesity can improve metabolic risk factors through changes in some cytokines.

Keywords: Adipocytokines (adipokines); Interleukin (IL) 10; Weight loss

1. Introduction

Obesity, with its associated metabolic pathologies, is the most common and detrimental metabolic disease in adult humans. Moreover, obesity is a major risk factor for several disorders, including hypertension, coronary artery disease, dyslipidemias and type 2 diabetes. Adipocytes store excess energy in the form of lipids and are, thus, able to dramatically change in size in accordance with changing metabolic needs. Adipose tissue is also an active endocrine organ that secretes numerous proteins, including tumor necrosis factor α (TNF-α), interleukin (IL) 6, resistin, leptin and adiponectin. These adipocyte-specific or enriched proteins, termed adipocytokines, exert a variety of local, peripheral and central effects [1].

IL-10, which is secreted by activated monocytes/macrophages and lymphocytes, is known to possess multifaceted anti-inflammatory properties. It has been shown that IL-10 is expressed in advanced human atherosclerosis and is associated with low levels of apoptosis, further supporting a protective role for this anti-inflammatory cytokine [2]. In

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Table 1
Clinical characteristics of the study population and changes in anthropometric parameters and lipid profiles after 12 weeks

	Baseline	After 12 weeks	*P*
n	78	78	
Sex (male:female)	37:41		
Age (years)	38.5±11.8		
Weight (kg)	87.9±15.4	81.7±16.2	<.001
BMI (kg/m^2)	32.2±3.5	29.9±4.0	<.001
Waist circumference (cm)	100.6±9.8	95.2±13.9	<.001
Hip circumference (cm)	107.9±6.9	104.1±7.3	<.001
WHR	0.93±0.05	0.91±0.10	.038
Total body fat (%)	39.2±8.2	35.5±8.0	<.001
Total cholesterol (mmol/L)	5.09±0.9	4.80±0.93	<.001
Triglycerides (mmol/L)	1.88±1.24	1.54±1.06	.007
HDL-cholesterol (mmol/L)	1.17±0.26	1.18±0.27	.664
LDL-cholesterol (mmol/L)	3.12±0.82	3.01±1.28	.364

Data are presented as mean±S.D.

addition, endogenous IL-10 provides an important counterbalance to mechanisms that produce endothelial dysfunction during diabetes [3].

Since obesity is considered to be a pro-inflammatory state, serum levels of some cytokines may vary according to the degree of weight loss in obese individuals. In the present study, this hypothesis was tested by measuring several anthropometric and biologic parameters, together with serum concentrations of TNF-α, IL-6, resistin, leptin, adiponectin and IL-10, in obese subjects before and after weight loss.

2. Materials and methods

2.1. Subjects

Thirty-seven male and 41 female obese subjects [body mass index (BMI) ≥27 kg/m^2 with comorbid hypertension, diabetes or dyslipidemia; or BMI ≥30 kg/m^2], ranging in age from 18 to 65 years, who had visited obesity clinics at five university hospitals (Ajou, Ulsan, Catholic, Hanyang and Yonsei) in Korea were enrolled in the study. Exclusion criteria were as follows: a history of uncontrolled hypertension, uncontrolled diabetes, cardiovascular disease, malignancy, hepatic disease, renal disease and/or endocrine disease affecting body weight, and/or use of medication affecting body weight within the prior 3 months.

All participants gave written consent to participate in the study, which was approved by the local ethics committee.

2.2. Study design

The subjects visited an obesity clinic and restricted their caloric intake to less than their usual intake (by 500 kcal). They were also administered 10–15 mg of sibutramine (a potent reuptake inhibitor of noradrenaline and serotonin that inhibited food intake) and took part in an exercise program (physical activity of moderate intensity, such as brisk walking). Total daily calorie intake was determined using the 24-h recall method. Diet and physical activity were monitored by clinical dieticians.

Weight, BMI, waist circumference and total body fat (by dual X-ray absorptiometry) were measured on the first visit and after 12 weeks of follow-up. Serum levels of adipocytokines (TNF-α, IL-6, resistin, leptin and adiponectin) and IL-10 were measured at these times as well, as were total cholesterol, triglyceride and high-density lipoprotein (HDL) cholesterol levels. Low-density lipoprotein (LDL) cholesterol levels were calculated by applying the Friedewald formula: LDL-cholesterol=total cholesterol−(HDL-cholesterol+triglycerides/5).

TNF-α, IL-6 and IL-10 were measured using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA). Leptin and adiponectin were measured with a radioimmunoassay kit (Linco Research, St. Charles, MO, USA). Resistin was measured with ELISA (Linco Research).

2.3. Statistical analysis

Statistical analysis was performed using SPSS 12.0 (SPSS, Inc., Chicago, IL, USA). All data are presented as mean±standard deviation, and statistical significance was established as $P<.05$. A comparison between variables before and after weight loss was assessed by paired t test. Correlations between parameters were assessed using bivariate correlation analysis (Pearson correlation coefficient) and multiple regression analysis.

3. Results

The characteristics of study subjects and baseline anthropometric parameters (obese status, before weight loss) are shown in Table 1. The study population consisted of 78 individuals (37 men, 41 women) with an average age of 38.5±11.8 years (Table 1).

Weight, BMI, waist circumference, hip circumference, waist-to-hip ratio (WHR), total body fat, total cholesterol and triglyceride significantly decreased after 12 weeks of treatment with diet, medication and exercise (Table 1). LDL-cholesterol tended to decrease (P=.364), and HDL-cholesterol slightly but not significantly (P=.664) increased after 12 weeks (Table 1). The average weight reduction was 6.1 kg. Not only total body fat but also waist circumference

Table 2
Changes in biologic parameters after 12 weeks

	Baseline	After 12 weeks	*P*
TNF-α (pg/ml)	2.94±0.90	2.48±0.68	<.001
IL-6 (pg/ml)	2.50±0.81	2.20±0.86	.001
Resistin (ng/ml)	3.95±1.02	3.45±0.93	<.001
Leptin (ng/ml)	12.20±3.22	10.84±3.07	<.001
Adiponectin (μg/ml)	11.96±3.65	12.47±3.92	.001
IL-10 (pg/ml)	12.02±4.44	13.50±5.49	.041

Data are presented as mean±S.D.

Table 3
Correlation between changes in anthropometric and biologic parameters

	ΔTNF-α	ΔIL-6	ΔResistin	ΔLeptin	ΔAdiponectin	ΔIL-10
ΔWeight	.07	.24 **	.37 ***	.21	−.27 **	−.14
ΔBMI	.09	.14	.31 ***	.16	−.15	−.14
ΔWaist	.09	.07	.11	.12	−.05	−.03
ΔBody fat	.02	−.01	.01	−.18	−.02	−.13
ΔTNF-α	–	.33 ***	.20	.28 **	.15	−.32 ***
ΔIL-6	.33 ***	–	.12	−.04	.04	−.30 ***
ΔResistin	.20	.12	–	.13	.45 *	.03
ΔLeptin	.28 **	−.04	.13	–	−.04	−.05
ΔAdiponectin	.15	.04	.45 ***	−.04	–	.21 *
ΔIL-10	−.32 ***	−.30 ***	.03	−.05	.21 *	–

Data presented are Pearson correlation coefficients.
* P=.06.
** P<.05.
*** P<.01.

decreased significantly. Thus, the study subjects had both lower total body weight and improved central obesity.

At the end of 12 weeks, TNF-α, IL-6, resistin and leptin had significantly decreased in parallel with weight loss, while adiponectin and IL-10 had significantly increased (Table 2). These results led us to evaluate the correlation between anthropometric and biologic parameters.

Bivariate correlation analysis showed that decrement in weight (Δweight) significantly correlated with decrements in IL-6 (ΔIL-6) and resistin (Δresistin) and with increment in adiponectin (Δadiponectin) (Table 3). In addition, decrement in IL-6 significantly correlated with decrement in TNF-α (ΔTNF-α) (Table 3). The correlations between several other anthropometric and biologic parameters are shown in Table 3. Of particular interest is the correlation between increment in IL-10 (ΔIL-10) and changes in other adipocytokines. The IL-10 increment significantly correlated with the TNF-α decrement and showed a tendency to correlate with the adiponectin increment (P=.06; Table 3). Bivariate correlation was also used to analyze the relationship between baseline anthropometric parameters (obese status, before weight loss) and baseline biologic parameters. Baseline IL-6 significantly correlated with baseline TNF-α (Pearson correlation coefficient r=.29, P=.01), and baseline IL-10 very significantly correlated with baseline adiponectin (r=.52, P<.001). However, no correlation was detected between baseline levels of IL-10 and TNF-α.

Multiple regression analysis was then used to evaluate independent correlations and dependencies between parameters. The decrement in weight independently correlated with the decrement in resistin (P<.001) and with the increment in adiponectin (P<.001). In addition, the decrement in IL-6 independently correlated with the decrement in TNF-α (P=.02). The increment in IL-10 independently correlated with the decrement in TNF-α (P=.019) and showed a tendency to correlate with the increment in adiponectin (P=.054). Baseline IL-6 independently correlated with baseline TNF-α (P=.01), and baseline IL-10 independently correlated with baseline adiponectin levels (P<.001).

4. Discussion

IL-10 has numerous anti-inflammatory properties, including inhibition of the prototypic pro-inflammatory transcription factor nuclear factor κB, leading to suppressed cytokine production [4], inhibition of matrix-degrading metalloproteinase [5], reduction of tissue factor expression [6], inhibition of apoptosis of macrophages and monocytes after infection [7,8], and promotion of the phenotypic switching of lymphocytes to Th2 phenotype [9]. The secretion of IL-10 by activated monocytes/macrophages and lymphocytes and the involvement of these cells in human atherosclerosis have led to the hypothesis that IL-10 is produced locally in plaques and may offer protection from an excessive pro-inflammatory response that would result in further damage [2]. Heeschen et al. [10] reported that elevated serum IL-10 levels in patients with acute coronary syndromes are associated with a more favorable prognosis. Gunnet et al. [3] reported that IL-10 provided some protection against endothelial dysfunction during diabetes by inhibiting the increase in O_2^- in blood vessels. Therefore, IL-10 has been considered to be a "good" and protective cytokine in human metabolism [11].

Since obesity is classically viewed as a pro-inflammatory state, we hypothesized that serum IL-10 levels increase according to the degree of weight loss in obesity, with simultaneous improvement in accompanying metabolic derangements. In the present study of obese subjects, weight loss after caloric restriction and medical treatment has been shown to increase circulating levels of the anti-inflammatory cytokine IL-10. In 2003, Esposito et al. [12] showed that circulating levels of IL-10 were elevated in obese women and that low levels of the cytokine were associated with metabolic syndrome. These authors speculated that the higher IL-10 levels observed in obese women represented the body's attempt to inhibit continued pro-inflammatory cytokine production, which, however, failed in individuals with low innate IL-10 production, as occurs in metabolic syndrome [12]. Our study is not a case–control analysis, and

since our subjects were not specifically evaluated for metabolic syndrome, we could not determine whether baseline IL-10 level was elevated. Esposito et al. [12] also reported that changes in lifestyle aimed at reducing body weight and increasing physical activity over 1 year significantly reduced high circulating IL-10 levels in obese women without metabolic syndrome, while Manigrasso et al. [13] reported no significant change in IL-10 levels after an observed reduction in the body weight of android obese women. Recently, Ugochukwu and Figgers [14] reported that IL-10 levels significantly increased after caloric restriction in rats, and our results showed a significant increase in IL-10 levels after weight loss in humans. Several reasons may explain the differences between findings. The first is that, in the study of Esposito et al. [12], changes in IL-10 levels were not evaluated in 12 (46%) obese women who had metabolic syndrome at baseline but who were not considered to have metabolic syndrome after weight loss. However, it may well be that IL-10 levels in these 12 obese women were indeed elevated after weight loss. In our study population, we did not distinguish between metabolic syndrome and simple obesity. Nonetheless, our results suggest that IL-10 has "good" and protective effects, as evidenced by increases in this cytokine in accordance with the degree of weight loss and improvement in associated metabolic derangements. The second possible reason for the discrepancy is that, in the two abovementioned reports, the study populations were composed only of women, whereas nearly an equal number of men (47%) and women participated in our study. Since the degree of cardiovascular risk is known to be influenced by sex, differences in the gender composition of the study population may have led to differing conclusions. The third possible reason are the different study periods. The study of Esposito et al. [12] was carried out over 12 months, whereas our study period was only 12 weeks; thus, the length of the period of weight reduction may have influenced metabolic changes.

Wolf et al. [15] reported that the incubation of primary human monocytes and macrophages with adiponectin significantly induced IL-10 production. In our study, baseline IL-10 independently correlated with baseline adiponectin ($P<.001$), and the increment in IL-10 showed a tendency to correlate with that of adiponectin ($P=.054$). Our findings therefore provide additional evidence for the role of adiponectin in modulating human monocytes and macrophages toward an anti-inflammatory phenotype by inducing IL-10 in human obesity. It may also be the case that adiponectin modulates the immune response by modulating macrophage function.

Yokota et al. [16] studied adiponectin and TNF-α in human macrophages and reported that adiponectin attenuated TNF-α production in these cells. Although we did not find a correlation between adiponectin and TNF-α, it may well be that TNF-α is associated with IL-10 since both cytokines are secreted by macrophages and since adiponectin is known to be associated with IL-10 and TNF-α. Juge-Aubry et al. [17] demonstrated that IL-10 is secreted by explants of human white adipose tissues and is up-regulated by TNF-α in vitro, as well as in obese humans and rodents. They speculated that induction of IL-10 by TNF-α represents a counterregulatory effect, limiting the pro-inflammatory action of this cytokine. In our study, however, the increment in IL-10 independently correlated with the decrement in TNF-α ($P=.019$), suggesting that IL-10 closely correlated with TNF-α not only in terms of up-regulation but also as a counterregulatory effect. Accordingly, the improvement in metabolic derangements experienced by our obese study subjects following weight reduction may have been mediated by the increment in IL-10 and the decrement in TNF-α in macrophages, as was reported to be the case for adiponectin [16].

In another study, a decrease in IL-6 significantly correlated with a decrease in TNF-α after weight loss in morbidly obese patients [18]. Similarly, in our study, the decrement in IL-6 independently correlated with the decrement in TNF-α ($P=.02$). We therefore conclude that weight loss in obesity induces a significant decrease in the levels of the pro-inflammatory cytokines IL-6 and TNF-α through metabolic improvement.

In this study, the levels of total cholesterol and triglycerides decreased significantly following weight loss. In addition, LDL-cholesterol tended to decrease ($P=.364$) and HDL-cholesterol slightly but not significantly ($P=.664$) increased after 12 weeks (Table 1). Therefore, we think that weight loss after caloric restriction and medical treatment decreases obesity-related metabolic risk by improving metabolic parameters, such as lipid profile. These results concur with previous findings [19,20].

In summary, this study shows, for the first time, that weight loss after caloric restriction and medical treatment increases serum IL-10 levels in obese humans. Our results strongly suggest that weight reduction in obesity can improve metabolic risk factors through changes in some cytokines.

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References

[1] Rajala MW, Scherer PE. Minireview: the adipocyte — at the crossroads of energy homeostasis, inflammation, and atherosclerosis. Endocrinology 2003;144:3765–73.

[2] Mallat Z, Heymes C, Ohan J, Faggin E, Lesèche G, Tedgui A. Expression of interleukin-10 in advanced human atherosclerotic plaques: relation to inducible nitric oxide synthase expression and cell death. Arterioscler Thromb Vasc Biol 1999;19:611–6.

[3] Gunnet CA, Heistad DD, Faraci FM. Interleukin-10 protects nitric oxide-dependent relaxation during diabetes: role of superoxide. Diabetes 2002;51:1931–7.
[4] Wang P, Wu P, Siegel MI, Egan RW, Billah MM. Interleukin (IL)-10 inhibits nuclear factor kappa B (NF kappa B) activation in human monocytes. IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. J Biol Chem 1995;270:9558–63.
[5] Lacraz S, Nicod LP, Chicheportiche R, Welgus HG, Dayer JM. IL-10 inhibits metalloproteinase and stimulates TIMP-1 production in human mononuclear phagocytes. J Clin Invest 1995;96:2304–10.
[6] Lindmark E, Tenno T, Chen J, Siegbahn A. IL-10 inhibits LPS-induced human monocyte tissue factor expression in whole blood. Br J Haematol 1998;102:597–604.
[7] Arai T, Hiromatsu K, Nishimura H, et al. Endogenous interleukin 10 prevents apoptosis in macrophages during *Salmonella* infection. Biochem Biophys Res Commun 1995;213:600–7.
[8] Geng Y, Shane RB, Berencsi K, et al. *Chlamydia pneumoniae* inhibits apoptosis in human peripheral blood mononuclear cells through induction of IL-10. J Immunol 2000;164:5522–9.
[9] Pinderski LJ, Fischbein MP, Subbanagounder G, et al. Overexpression of interleukin-10 by activated T lymphocytes inhibits atherosclerosis in LDL receptor-deficient mice by altering lymphocyte and macrophage phenotypes. Circ Res 2002;90:1064–71.
[10] Heeschen C, Dimmeler S, Hamm CW, et al. CAPTURE Study Investigators. Serum level of the antiinflammatory cytokine interleukin-10 is an important prognostic determinant in patients with acute coronary syndromes. Circulation 2003;107:2109–14.
[11] Fisman EZ, Motro M, Tenenbaum A. Cardiovascular diabetology in the core of a novel interleukins classification: the bad, the good and the aloof. Cardiovasc Diabetol 2003;2:11.
[12] Esposito K, Pontillo A, Giugliano F, et al. Association of low interleukin-10 levels with the metabolic syndrome in obese women. J Clin Endocrinol Metab 2003;88:1055–8.
[13] Manigrasso MR, Ferroni P, Santilli F, et al. Association between circulating adiponectin and interleukin-10 levels in android obesity: effects of weight loss. J Clin Endocrinol Metab 2005; 90:5876–9.
[14] Ugochukwu NH, Figgers CL. Caloric restriction inhibits up-regulation of inflammatory cytokines and TNF-α, and activates IL-10 and haptoglobin in the plasma of streptozotocin-induced diabetic rats. J Nutr Biochem 2007;18:120–6.
[15] Wolf AM, Wolf D, Rumpold H, Enrich B, Tilg H. Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. Biochem Biophys Res Commun 2004;323:630–5.
[16] Yokota T, Oritani K, Takahashi I, et al. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. Blood 2000;96:1723–32.
[17] Juge-Aubry CE, Somm E, Pernin A, et al. Adipose tissue is a regulated source of interleukin-10. Cytokine 2005;29:270–4.
[18] Kopp HP, Kopp CW, Festa A, et al. Impact of weight loss on inflammatory proteins and their association with the insulin resistance syndrome in morbidly obese patients. Arterioscler Thromb Vasc Biol 2003;23:1042–7.
[19] Hung YJ, Chen YC, Pei D, et al. Sibutramine improves insulin sensitivity without alteration of serum adiponectin in obese subjects with type 2 diabetes. Diabet Med 2005;22:1024–30.
[20] Berkowitz RI, Fujioka K, Daniels SR, et al. Effects of sibutramine treatment in obese adolescents: a randomized trial. Ann Intern Med 2006;145:81–90.