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Research paper

Plasma Interleukin-6 levels, glutathione peroxidase and isoprostane in obese women before and after weight loss. Association with cardiovascular risk factors

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ABSTRACT

OBJECTIVE: To evaluate the levels of Interleukin-6 (IL-6), glutathione peroxidase and isoprostane in obese women and their association with markers of cardiovascular risk factors before and after weight loss. DESIGN: 36 healthy obese women of reproductive age (group A: age (mean ±SD) 35.4 ± 9.2 years, Body Mass Index (BMI) 38.5±7 kg/m^2) and 30 healthy, normal weight women (group B: age mean ±SD 34.9±7.4y., BMI 24±1.1 kg/m^2) were included in the study. Glucose tolerance was normal in all participating women. Il-6, glutathione peroxidase and isoprostane, C-Reactive Protein (CRP), insulin, fasting plasma glucose, HOMA-IR as well as the lipid profile were evaluated. Body weight, BMI, Waist to Hip ratio (W/H) ratio, Waist Circumference (WC), %free fat mass and the %fat mass were also measured. A hypocaloric diet was prescribed for the obese women and all participants were re-examined after six months. RESULTS: In obese women after weight loss, anthropometric obesity markers (BMI, W/H ratio), %fat, lipid profile, insulin levels and inflamation indices such as IL-6 and CRP, the oxidative stress index isoprostane, as well as glutathione peroxidase were significantly ameliorated. The levels of serum glutathione peroxidase activity were negatively correlated with IL-6 levels and were significantly increased after weight reduction. In obese women there was an association between IL-6 levels and the values of %fat, %free fat mass, insulin and HOMA-IR before and after weight loss. CONCLUSIONS: Weight loss is related to reduction of oxidative stress and inflammation; this beneficial effect could possibly be translated into reduction of cardiovascular risk in obese individuals.

Key words: Body mass index, C-reactive protein, Glutathione peroxidase, Interleukin-6, Isoprostane, Obesity

INTRODUCTION

Obesity is the most common metabolic disorder in developing countries and is characterized by a reduction in insulin sensitivity, both in animal models and in humans.[1] Obesity has been associated with increased cardiovascular morbidity and mortality[2-4] and is now considered a major independent risk factor for coronary heart disease.[5] Furthermore, the

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degree of abdominal adiposity conveys an independent prediction of risk beyond body mass index (BMI) for cardiovascular pathology.[4,6,7] Vascular endothelial dysfunction (VED) plays a pivotal role in the pathogenesis of atherosclerosis[8] and enhances the risk for future cardiovascular events. The presence of VED has been demonstrated in overweight patients with insulin resistance[9] and with visceral obesity.[9,10] Therefore, VED may be an important link between obesity per se and heightened cardiovascular risk. However, the molecular mechanisms involved in obesity-related insulin resistance are not yet well understood.[11] It has been clearly demonstrated that adipocytes are able to synthesize and secrete several cytokines, such as leptin,[12] tumor necrosis factor (TNFα)[13] and interleukin-6 (IL-6).[14] Recently an attractive hypothesis has emerged proposing that cytokines produced by adipose tissue may be responsible for insulin resistance in obesity.[2-5]

IL-6 is a cytokine produced by different cell types, including immune and adipose tissue cells, and mediates inflammatory responses. Unlike other cytokines, IL-6 is distinct in that its major effects take place at sites distant from its origin. For this reason IL-6 has come to be known as "the endocrine cytokine". Circulating IL-6 levels constitute a significant proatherogenic cytokine.

The role of chronic inflammation in atherosclerosis has become well established during the past decade. Numerous epidemiological studies have demonstrated an association of CRP levels with increased risk of myocardial infarction (MI), stroke, sudden cardiovascular death and peripheral vascular disease.[15] Furthermore, the synthesis of CRP by the liver has been shown to be largely regulated by IL-6.[15,16]

Recently reported population-based data from the Women's Health Study demonstrated that a single nonfasting measurement of CRP[1] is a stronger predictor of future cardiovascular events than LDL cholesterol,[2] identifies at-risk individuals with low LDL cholesterol levels and[3] adds prognostic information to the currently used Framingham risk algorithm.[17]

It has recently been reported that F2 isoprostanes are the most reliable indicators of oxidative stress, although their role in pathophysiologic processes is unknown.[18] The measurement of F2 isoprostanes may represent an important development in the assessment of free radical generation and oxidative stress *in vivo*. Glutathione peroxidase is an antioxidant enzyme that reduces hydrogen peroxide and lipid peroxides. In the reduced state, glutathione (GSH) is an indirect indicator of oxidative stress. GSH may also be an important antiatherogenic agent, is present in human plasma and intracellularly, has antioxidant properties by inhibiting free radical formation and generally functions as a redox buffer.[19]

The above data underline the need for further clarification of the metabolic and endocrine function of adipose tissue in obese subjects. We thus sought to investigate the relationships between cytokines, proinflammatory products and oxidative stress, as well as their relation to cardiovascular risk factor in obese women and their possible modification by weight reduction.

METHODS

Thirty-six obese women of reproductive age (group A: mean ±SD age 35.4±9.2yr, BMI 38.5±7 kg/m^2) and 30 normal weight women (group B: mean ±SD age 34.9±7.4yr, BMI 24±1.1 kg/m^2) were studied. All participants were informed about the objectives of the study and volunteered to participate. All individuals included in the study (groups A and B) had two menses in the 3 months before the testing and displayed a normal response to oral glucose tolerance test (OGTT).

Additional exclusion criteria included diabetes mellitus, hormone replacement therapy, pregnancy, lactation, psychiatric or neurological disorders, alcohol abuse, a history or the presence of malignancy, coronary heart disease and cerebrovascular disease. Continuing use of antihypertensive medication was permitted provided that the dose had been stable for at least 3 months before entry into the study.

None of the subjects was taking any medication known to influence lipid metabolism. Apart from obesity, all obese subjects were in good health and had maintained stable body weight for at least 12

months prior to entering the study. None was engaged in any type of exercise program or was excessively sedentary. All participants underwent physical examination, measurement of fat mass and complete biochemical investigations.

Anthropometric parameters such as Body Weight (BW), height, BMI, Waist Circumference (WC), Waist to Hip ratio (W/H) and %Body fat as well %Free fat mass (FFM) were also evaluated.

Weight in Kg was determined using a standard beam-balance scale and with the subjects barefoot and wearing light indoor clothing. Height in centimeters was measured using a meter rule built into the scale. BMI was calculated by dividing weight in Kg by height in meters squared. Obesity was defined as a BMI value greater than 30 kg/m^2. Waist circumference was measured with a flexible measuring tape, taking as a reference the midway line between the costal inferior border and the iliac crest. Body fat mass was evaluated by a bioelectric impedance analysis device (Bodystat Ltd, Isle of Man, Bodystat 1500).

Morning fasting blood samples were obtained both at entrance to the study and after the treatment period. All biochemical measurements were performed on frozen plasma samples obtained by centrifugation of the freshly drawn blood (3000 X *g* for 20 min at 4°C) and subsequent storage at -70°C.

The tests included measurement of insulin, IL-6, CRP, glutathione peroxidase activity and isoprostane.

Bio-electrical impedance analysis (BIA), a method that measures the impedance or opposition to the flow of an electric current through the body fluids, was evaluated as follows: a small constant current, typically 400 uA at a fixed frequency, usually 50 kHz, passed between electrodes spanning the body and the voltage drop between electrodes provided a measure of impedance.

Prediction equations, previously generated by correlating impedance measures against an independent estimate of TBW, were used subsequently to convert measured impedance to a corresponding estimate of TBW. Lean body mass was then calculated from this estimate using an assumed hydration fraction for lean tissue. Fat mass was calculated as the difference between body weight and lean body mass.[20,21]

OGTT was performed as follows: all subjects ingested a 75g glucose solution after an overnight fast. Serum samples were collected before and 30, 60, 90 and 120 min after the glucose load. The criteria developed by the World Health Organization Expert Committee on Diabetes Mellitus were used to determine whether a subject was not diabetic (2-h glucose concentration <140 mg/dl).[22] Insulin resistance was calculated based on homeostasis model assessment (HOMA-IR) index. The homeostasis model assessment (HOMA) has been suggested as a method to assess IR and is calculated using the formula HOMA-IR=fasting insulin (units/ml) x fasting glucose (mmol/l)/22.5).[23]

Obese women were subscribed a hypocaloric diet according to the following calculation: [18-40 y= 0.0621x weight in Kg+2.0357= —mJ/day x 240= — —Kcal/day, adjusting with daily activities: — —Kcal/day x —coefficient of daily activities= — —Kcal/day, in order to have hypocaloric diet — —Kcal/day-600Kcal/day].[24] The energy distribution of this diet was 50% carbohydrates, 30% fat and 20% protein and the duration of dietary intervention was 6 months.

Serum glucose levels were measured using the chromatographic method (bioanalyzer Wako Chemicals GmbH), cholesterol, triglyceride and HDL were measured by standardized laboratory methods and LDL-cholesterol level was calculated with the Friedewald formula. Insulin by immunoradiometric assay using a Sorin-Biomedica Kit (Saluggia, Italy). The lower limits of detection for insulin was 0.3 μIU/ml while inter-assay and intra-assay coefficients of variation (CV) were 6.9 and 6.4%, respectively. Normal range for our laboratory is 2-25 μIU/ml.

IL-6 levels were determined by enzyme-linked immunosorbent assay (Quantikine High Sensitivity IL-6; R&D Systems, Oxford, UK). The sensitivity of this assay was 0.70 pg/mL IL-6. Plasma levels of peroxidase of glutathione were determined by use of an enzymatic assay (BIOXYTECH of company *Oxis*Research, Inc Portland, USA) that allows a recovery of GSH >90% and has no appreciable inter-

ference with other thiols present in the plasma or in the reactive mixture; meanwhile, levels of isoprostane (8-iso-PGF2) were determined using the enzymatic method elisa by Assay Designs' Correlate-EIA™ Direct 8-iso-Prostaglandin F2kit.

Statistical methods

For statistical analysis the statistical package SPSS 11.5. (SPSS, Chicago, IL, USA) was used. Results are expressed as mean ± standard deviation (SD). Normality was assessed by the Kolmogorov-Smirnov test. The unpaired Student-t test was used for comparisons between normal and obese individuals. The paired Student-t test was used for comparisons between baseline and 6 months of treatment in obese women. For correlations the Pearson correlation coefficient was used. *P*-values <0.05 were considered statistically significant.

RESULTS

The results of this study showed significant differences between group A and group B with regard to the anthropometric parameters %body fat and %free fat mass (p <0.001) but not the age (Table 1).

The mean values of cholesterol, triglycerides and LDL cholesterol were not significantly different between the obese and the controls, whereas HDL levels were significantly lower in the obese group than in the normal weight group (Table 2).

The mean values of CRP, insulin, IL-6, HOMA-IR and isoprostane were significantly higher in the obese women as compared to the normal weight controls (p<0.001) (Table 2).

Table 1. Anthropometric indices of obese women at entrance to the study (group A) and of normal weight women (group B) (mean±SD)

	Group A (n=36)	Group B (n=30)	P value
Age (yr)	35.4±9.2	34.9±7.4	<0.456
Weight (kg)	102.1±18.7	60.2±5.3	<0.001
Body mass index (kg/m^2)	38.5±7	24±1.1	<0.001
Waist hip ratio	0.9±0.06	0.7±0.04	<0.01
Waist circumference (cm)	108.5±10.6	74.5±15	<0.001
%Fat mass	42.5±8.1	26.5±3.9	<0.001
%Free fat mass	57.9±8.2	73.4±3.8	<0.001

Table 2. Biochemical and hormonal values of obese women at entrance to the study (group A) and of normal women (group B) (mean±SD)

	Group A Obese Women (n=36)	Group B Controls (n=30)	P value
Insulin(IU/ml)	41±30	11,7±6,6	<0.001
Cholesterol (mg/dl)	209.5±43.7	185.7±38.5	<0.356
Triglycerides (mg/dl)	141.5±42.9	134.3±42	<0.667
HDL (mg/dl)	42.1±10.3	47.5±9.4	<0.05
LDL (mg/dl)	131.2±43.9	118.5±37.9	<0.335
IL-6(pg/ml)	83±17	2.34±1.7	<0.001
CRP(mg/l)	8.5±3.9	2.5±1.1	<0.001
HOMA-IR	10.1±8.2	2.5±1.6	<0.001
Isoprostane (pg/ml)	5166.4±1787.2	502±202.8	<0.001
Glutathione peroxidase (ng/ml)	22.3±9.5	67.5±21.3	<0.001

For SI units multiply for insulin by 7.175, for cholesterol, LDL and HDL by 0.02586, for triglycerides by 0.01536.

Glutathione peroxidase was significantly lower in the obese women than in the controls (p<0.001) (Table 2).

The biochemical and hormonal values as well as anthropometric indices in the obese women before and after weight reduction are presented in Table 3.

The mean weight, BMI, W/H ratio, waist circumference, %, body fat, cholesterol, triglycerides, CRP, insulin, HOMA-IR, interleukin-6 and isoprostane were significantly reduced after weight loss (p<0.001), whereas glutathione peroxidase was significantly increased after weight reduction (p <0.001).

The data presented in Table 4 indicate a positive correlation of BMI (group A) with W/H ratio (r=0.652, p<0.001), insulin (r=0.501, p<0.002), %fat (r=0.870, p<0.001), HOMA-IR (r=0.545, p<0.001), and glutathione (r=0.331, p<0.049), whereas it was negatively correlated with %free fat mass (r= -0.789, p<0.001) before weight reduction.

Table 5 shows a positive correlation of W/H ratio in group A with BMI (r=0.652, p=<0.001), %fat (r=0.627, p<0. 001) and a negative one of W/H ra-

Table 3. Anthropometric indices, biochemical and hormonal values in obese women before and after weight reduction (mean±SD)

	Before	After	P value
Weight (kg)	102.1±18.7	82.5±14.8	<0.001
Body mass index (kg/m^2)	38.5±7	30.9±5.7	<0.001
Waist hip ratio	0.9±0.06	0.8	<0.001
Waist circumference (cm)	108.5±10.6	94.1±9.4	<0.028
%Fat mass	42.5±8.1	34.7±7.3	<0.001
%Free fat mass	57.9±8.2	64.6±6.9	<0.001
Insulin (IU/ml)	41±30	26.9±18.3	<0.001
Cholesterol (mg/dl)	209.5±43.7	169.7±27.2	<0.001
Triglycerides (mg/dl)	141.5±42.9	125±40	<0.002
HDL (mg/dl)	42.1±10.3	33.9±8.2	<0.001
LDL (mg/dl)	131.2±43.9	116.9±28.5	<0.688
IL-6 (pg/ml)	83±17	11.7±3.1	<0.001
CRP (mg/l)	8.5±3.9	6.6±3.3	<0.007
HOMA-IR	10.1±8.2	6.5±4.6	<0.001
Isoprostane (pg/ml)	5166.4±1787.2	1472.2±506.4	<0.001
Glutathione peroxidase (ng/ml)	22.3±9.5	48.9±14.1	<0.001

For SI units multiply for insulin by 7.175, for cholesterol, LDL and HDL by 0.02586, for triglycerides by 0.01536.

Table 4. Correlations of BMI in obese women (group A) with W/H ratio, WC, % fat, % free fat mass, cholesterol, triglycerides, CRP, HOMA-IR, insulin and glutathione before weight reduction

	W/H	WC	%FAT	%FFM	CHOL	TRG	INS	HOMA	CRP	GLUT
r-pearson	0.652	0.533	0.870	-0.789	0.391	0.366	0.501	0.545	0.452	0.331
p	<0.001	<0.001	<0.001	<0.001	<0.020	<0.031	<0.002	<0.001	<0.001	<0.049

BMI: Body mass index; W/H: waist to hip ratio; WC: waist circumference; FFM: free fat mass; HOMA-IR: homeostasis model assessment index; CRP: C-reactive protein; ins: insulin; GLUT: glutathione peroxidase activity

tio with %FFM (r= -0.561, p<0.001) and LDL (r= -0.45, p<0.039) before weight loss.

Insulin levels of the obese women were positively correlated with BMI (r=0.381, p<0.022) and HOMA-IR (r=0.973, p<0.001) after weight loss as shown in Table 6.

IL-6 levels of the obese women (group A) correlated positively with %body fat (r=0.275, p<0.020), HOMA-IR (r=0.484, p<0.003) insulin (r=0.470, p<0.004) and negatively with %FFM (r=-0.279, p<0.019) and glutathione (r=-0.340, p<0.046) after weight loss (Table 7, Figure 1)

%Body fat correlated positively with BMI (r= 0.709, p<0.001), WC (r=0.400, p<0.001), insulin (r=0.388, p<0.001),CRP (r=0.382, p<0.001), IL-6 (r=0.275, p<0.020) and negatively with %FFM (r= -0.875, p<0.001) and HDL (r=-0.262, p<0.027) af-

Table 5. Correlations of W/H ratio in group A with BMI, %fat, %free fat mass, LDL before weight reduction

	BMI	%FAT	%FFM	LDL
r-pearson	0.652	0.627	-0.561	-0.45
p-value	<0.001	<0.001	<0.001	<0.039

Table 6. Correlations of insulin levels in group A with BMI, and HOMA-IR after weight reduction

	BMI	HOMA-IR
r-pearson	0.381	0.973
p-value	<0.022	<0.001

Table 7. Correlations of IL-6 levels in group A with HOMA-IR, %fat, %FFM, GLUT and insulin values after weight reduction

	HOMA – IR	INS	%FAT	%FFM	GLUT
r-pearson	0.484	0.470	0.275	-0,279	-0,340
p-value	<0.003	<0.004	<0.020	<0,019	<0,046

GLUT: glutathione peroxidase activity

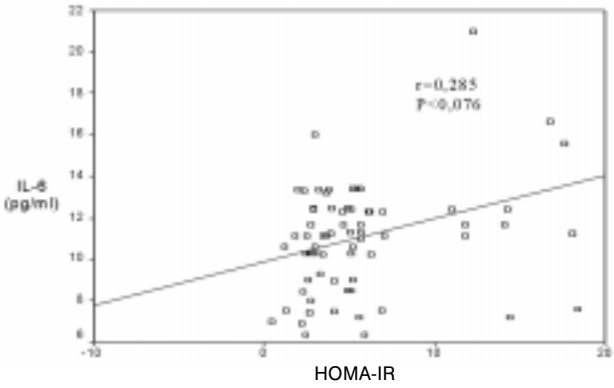

Figure 1. Correlation of IL-6 with HOMA-IR after weight reduction in group A.

ter weight loss (Table 8).

DISCUSSION

Obesity is associated with an increased risk of developing cardiovascular diseases.[2,4] The metabolic consequences of obesity can promote the process of atherosclerosis influencing endothelial function as well as the mechanisms of oxidative stress.[9,10] In our study the levels of adipose tissue related cytokines, oxidative and antioxidative substances were evaluated in obese women (group A) before and after weight reduction and in normal weight women (group B).

The obese women also presented visceral obesity as indicated by the values of WC and W/H ratio (108.9cm and 0.9cm, respectively) (Table 1). The women in group A had lower HDL values than those in group B with no difference in total cholesterol, LDL-cholesterol or triglycerides.

BMI values in group A correlated positively with W/H ratio, WC, %body fat, insulin, HOMA-IR, cholesterol, triglycerides, CRP and glutathione peroxidase activity and negatively with %free fat mass (Table 4). %Body fat was positively correlated with BMI, WC, plasma insulin concentration, CRP and IL-6. The latter correlation is a predictable relationship between the total body fat and risk factors, which are believed to be responsible for the chronic endothelial inflammation.[15-17] Fernandez-Real J.M. et al reported that the omental adipose tissue produces 3-fold higher levels of IL-6 than subcutaneous adipose tissue and this is the major mediator of inflammation, which induces the hepatic synthesis of CRP.[25] The findings of Lemieux et al[26] and Forouhi et al[27] l, suggested that fat distribution correlated with CPR levels as well as with other markers, such as BMI and WC. Although these studies show that there is a correlation between the low levels of inflammatory markers and the low body and splachnic adiposse tissue, the influence of weight reduction in the obese on the levels of these markers has not previously been studied. Previous studies have also suggested that CRP can predict future cardiovascular events independently of other more traditional cardiovascular risk factors, such as plasma lipid levels.[28-31] One possible link between subclinical inflammation and cardiovascular disease (CVD) may be insulin resistance. In the study of Festa et al, 1008 nondiabetic individuals were evaluated. In this study CRP levels were significantly correlated with cardiovascular risk factors.[33] Increased fasting levels of insulin and HOMA-IR seem to be mainly

Table 8. Correlations of %body fat in group A with BMI, WC, %FFM, insulin, CRP, HDL and IL-6 after weight reduction

	BMI	WC	%FFM	HDL	INS	CRP	IL-6
r-pearson	0.709	0.400	-0.875	-0.262	0.388	0.382	0.275
P-value	<0.001	<0.01	<0.001	<0.027	<0.001	<0.001	<0.020

related with the levels of the important proinflammatory cytokine IL-6.[34,36] This is in accordance with our results of increased levels of insulin, HOMA-IR and IL-6 in the obese group (A). Furthermore, fasting hyperinsulinemia is related with the production of free radicals.[37-39] The data of the present study indicate that serum CRP as well as IL-6 circulating levels decreased during weight loss, supporting the beneficial effect of weight reduction on cardiovascular risk factor. Our data are in accordance with other studies, indicating a possible link between adipose tissue and secretion of IL-6.[1] In our study IL-6 levels as well as isoprostane were significantly higher and glutathione peroxidase significantly lower in obese subjects compared to controls (group A) (Table 2). Furthermore, IL-6 levels positively correlated with isoprostane and negatively with glutathione peroxidase in the obese, a finding which has not been previously described (Table 7). After weight reduction the levels of insulin, CRP, IL-6 and isoprostane significantly decreased, whereas glutathione peroxidase significantly increased.

These results are in accordance with the beneficial effect of weight loss considering that increased glutathione peroxidase activity protects tissues from oxidative stress injury (Table 3). Accordingly, it is suggested that the improvement of inflammation factors results in a decrease of cellular destruction and protects from CVD. In other words, low values of glutathione peroxidase along with higher levels of isoprostane in obese women indicate defective protection mechanisms against atherosclerosis and oxidative stress. In obese women despite the improvement in BMI values (from 38.5 to 30.93 kg/m^2 $p < 0.001$), following therapeutic interventions, the levels of IL-6, insulin and antioxidative substances did not reach levels similar to the controls. It can be speculated that normalization of these parameters requires either greater BMI reduction and/or a longer period of dietary intervention. However, the approximately 20% decrease in body weight and the reduction in adiposity, as was reflected in the decreased ratio of fat to free-fat mass in group A, was associated with an improvement in all adverse parameters measured in this study.

It seems that adipose tissue produces peptides and cytokines which alter not only the hormonal milieu but also certain biochemical parameters and oxidative stress. The results of this study demonstrate that the elevated circulating IL-6, isoprostane and CRP levels and the decreased levels of glutathione peroxidase found in obese women correlated significantly with a number of cardiovascular risks factors and were ameliorated by weight reduction.

In conclusion, the data of the present study indicate that individuals with high BMI and central obesity have increased levels of markers associated with insulin resistance, vascular inflammation, oxidative stress and atherosclerosis. Weight reduction can improve all these parameters and, therefore, a possible reduction of the metabolic and cardiovascular morbidity of obesity is expected.

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