CLINICAL STUDY

Body weight and glucose metabolism have a different effect on circulating levels of ICAM-1, E-selectin, and endothelin-1 in humans

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Abstract

Background: Endothelial dysfunction and inflammation are present in both type 2 diabetes mellitus (T2DM) and obesity. In this paper we compared the role of weight loss and of glycaemic control in determining circulating levels of ICAM-1, endothelin-1 (ET-1), and E-selectin in patients with morbid (grade 3) obesity.

Methods and Results: ICAM-1, E-selectin, and ET-1 were higher in obese patients ($n = 96$) than in lean controls ($n = 30$); among obese patients, the three molecules were higher in T2DM patients ($n = 26$) than in patients with normal (NGT, $n = 43$) or impaired (IGT, $n = 27$) glucose tolerance. Sixty-eight obese patients had a significant weight loss induced by bariatric surgery, and showed a significant decrease in blood glucose, HbA1c and all molecules, so that ICAM-1, E-selectin, and ET-1 were not different in NGT, IGT and T2DM patients, and in lean controls; in 13 patients with a small weight loss induced by diet, changes were not significant, in spite of a significant reduction in blood glucose and HbA1c. At stepwise regression, changes in ICAM-1, ET-1, and E-selectin significantly correlated only with change in body mass index.

Conclusions: These data indicate that weight loss is more important than glycaemic control in regulating circulating levels of ICAM-1, ET-1, E-selectin in morbidly obese subjects.

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Introduction

Endothelial dysfunction and inflammation, as indicated by abnormal flow-dependent vasodilation and by increased circulating levels of adhesion molecules (ICAM-1 and E-selectin) (1, 2) are known to occur in type 2 diabetes mellitus (T2DM), and allegedly predict systemic atherogenesis (3). In addition, both hyperglycaemia and insulin administration increase circulating levels of endothelin-1 (ET-1), a potent vasoconstrictor peptide (4, 5). However, the majority of patients with T2DM are obese, and this raises the question of the importance of diabetes *per se* and of obesity *per se*. In fact, endothelium-dependent vasodilation is reduced in obesity (6, 7); in addition, ET-1 release can be elicited in lean subjects by co-administration of insulin and triglycerides, and is increased in non-diabetic obese subjects with 'metabolic syndrome' (8). Finally, neither short-term nor long-term improvements in glycaemic control lead to changes in adhesion molecules in

obese T2DM patients, suggesting that obesity is more important than glycaemic control in determining circulating levels of ICAM-1, ET-1 and E-selectin (9, 10). The aim of this study was to compare the effect of a sustained and persistent weight loss and of good metabolic control on circulating levels of ICAM-1, ET-1, and E-selectin in morbidly obese patients.

Materials and methods

Basal evaluations

Laparoscopic adjustable gastric banding (LAGB) is an on-going program approved by the local Ethics Committee at the IRCCS Ospedale San Raffaele and at Ospedale San Paolo since June 1996 (11), in patients with morbid (grade 3) obesity according to World Health Organisation (WHO) criteria (12). We considered 96 consecutive patients entering the program, recruited locally or referred from the Divisione di Malattie

Metaboliche, Università degli Studi di Napoli; inclusion criteria were age 18 to 65 years inclusive, body mass index (BMI, kg/m^2) >40.0 , or >35.0 in the presence of co-morbidities (T2DM or arterial hypertension) (13), history of at least two previous attempts to lose weight with dietary and medical measures followed by relapse of obesity; exclusion criteria were: obesity secondary to endocrinopathies (Cushing's disease or syndrome, hypothyroidism), gastrointestinal inflammatory diseases, risk of upper gastrointestinal bleeding, pregnancy, alcohol or drug addiction, previous or current malignancies, myocardial infarction or unstable angina during the preceding 6 months (13). Waist circumference was measured as an index of body fat distribution (14). Two oral glucose tolerance tests (OGTT, 75 g) were performed in all patients (15). A complete evaluation of micro- and macroangiopathic complications was performed in patients with T2DM (usually the diagnosis was the result of OGTT). Retinal photography revealed absence of retinopathy in all patients; microalbuminuria and proteinuria were absent in all patients, and ECG and medical history revealed no signs/symptoms of coronary heart disease. Blood pressure was measured by the same physician, using the same sphygmomanometer with an appropriate cuff. Patients were considered hypertensive when systolic/diastolic blood pressure was $>140/90$ mmHg or when patients were under stable anti-hypertensive medication. No T2DM patient was receiving insulin, and only 5/26 patients were receiving metformin, 850 mg b.i.d. As part of the program, obese patients underwent gastroscopy and a full blood examination, including markers of inflammation such as erythrocyte sedimentation rate (ESR) and white blood cells.

Control subjects

Thirty healthy lean volunteers with a normal glucose tolerance were recruited from the medical staff of the Divisione di Malattie Metaboliche, Università degli Studi di Napoli and underwent the same anthropometric and biochemical evaluations as the obese patients.

Follow-up evaluations

For the purpose of this study we considered 68 subjects (36 with normal glucose tolerance (NGT), 23 with impaired glucose tolerance (IGT), 9 T2DM) undergoing LAGB and 13 subjects (7 NGT, 4 IGT, 2 T2DM) refusing LAGB and followed-up with a traditional dietary treatment. After LAGB, the suggested diet was 1000/1100 kcal/day in women and men respectively, and included 48% carbohydrates (starch or bread), 33% proteins (fat free parts of different animals and fishes) and 19% lipids (olive oil). Diets were given by a dietitian and a physician with specific training in obesity treatment and nutrition; iron was supplemented on

the basis of blood examinations performed during the second month. Patients were also advised to take 30 min physical aerobic activity every day to avoid muscular loss, with a gradual increase over a one-month period. Physical activity could be gymnastic, swimming or dancing. Patients were scheduled for further assessment at three-month intervals for one year. Diet-treated patients were evaluated under basal conditions and at three-month intervals for one year, and their diet was 1000/1200 Kcal/day for women and men respectively (49% carbohydrates, 29% proteins, 22% lipids).

Laboratory methods

Patients were studied supine in the morning, during one of the two OGTTs, after an overnight fast and at least 1 h of bed rest. An i.v. 20-gauge cannula was inserted in the left antecubital vein, kept patent by a slow infusion of 0.9% NaCl solution. Specimens for ET-1 were collected in pre-chilled EDTA tubes with 200 μl aprotinin, and specimens for E-selectin and ICAM-1 were collected in pre-chilled tubes with no additives, centrifuged at 4 °C and stored at -70°C . Blood glucose levels were measured by a glucose-oxidase method (YSI, Yellow Springs, OH, USA). Insulin was assayed by a Mycoparticle Enzyme Immunoassay (MEIA, IMX, Abbott Laboratories, Abbott Park, IL, USA) with a monoclonal antibody without cross-reactivity with human pro-insulin; sensitivity was 6.0 pmol/l; intra-assay coefficient of variation (CV) was 3.0%, interassay CV was 5.0%. HbA1c was assayed by a routine HPLC method (16). ET-1 was assayed by a solid-phase monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) designed to measure ET-1 in extracted EDTA plasma (Human Endothelin-1 Immunoassay, R&D Systems, Minneapolis, MN, USA). Briefly, to 1 ml plasma, 1.5 ml of a mixture of acetone:1 M HCl:water (40:1:5, v:v:v) were added. After mixing by inversion and centrifugation (20 min at 2000 g at 4 °C), the supernatant was decanted and dried in a centrifugal evaporator at 37 °C. The pellet was reconstituted with 0.25 ml sample diluent and immediately assayed by ELISA technique following the manufacturer's instructions. ICAM-1 and E-selectin were assayed by the Human soluble ICAM-1 Immunoassay and by the Human soluble E-Selectin Immunoassay kits (R&D Systems), solid-phase ELISA kits designed to measure the above mentioned parameters directly on serum samples (17–19); inter- and intra-assay CV were between 4.3 and 7.6 for all molecules. The homostasis model assessment (HOMA) index was calculated as $\text{insulin } (\mu\text{U/ml}) \cdot \text{blood glucose } (\text{mmol/l}) \cdot 22.5^{-1}$ (20).

Sample size

The sample size allowed detection of 20% difference or more between obese and control subjects in the mean

level of ICAM-1, with a type 1 error of 0.05 and a power of 0.8. Assuming an ICAM-1 mean level of 250 ng/ml, a standard deviation of 44 ng/ml (10), and a rate of obese/control of 4, a number of at least 40 obese and 8 control subjects was required.

Calculations and statistical analysis

The difference among groups for all molecules under study and for clinical, endocrine and metabolic variables was assessed using the analysis of variance. Differences between groups were assessed using the Student's *t*-test for paired or unpaired samples. Significance of multiple comparisons was adjusted by the Bonferroni correction. Because normality had not been verified on all original variables, log-transformation of data was applied, when necessary. Fisher's exact test was used to compare absolute frequencies. Pairwise correlations between adhesion molecules and anthropometric and biochemical variables, and between their changes at one year, were also calculated. Stepwise regression analysis was further carried out to estimate the independent contribution of selected variables (variables significant at linear regression plus age, sex, type of intervention) on ICAM-1, E-selectin, and ET-1, and on their changes. *P* levels <0.05 were considered statistically significant.

Results

Table 1 shows clinical, endocrine, and metabolic variables (means±s.e.) in lean controls and in obese subjects; lean controls differed from obese subjects for all variables, except for age and sex; T2DM obese subjects differed from NGT and IGT obese subjects for HbA1c, ICAM-1, E-selectin, and ET-1; finally, obese NGT, IGT, and T2DM subjects showed progressively higher

glucose levels. No obese subject had abnormal gastroscopy or inflammation markers, and no differences were found between men and women in ICAM-1, E-selectin or ET-1. Of 96 obese patients, 15 T2DM patients did not undergo the follow-up study; these two groups of T2DM patients did not differ for any clinical or metabolic variable.

After 1 year the 68 patients undergoing LAGB had a greater weight loss (BMI = -8.2 ± 0.61 kg/m²) than diet-treated patients (BMI = -0.4 ± 1.04 kg/m²) (*P* < 0.01), while weight loss was not statistically significant at 3 and 6 months (not shown); Table 2 shows that in the LAGB group all variables significantly declined, including ICAM, E-selectin, and ET-1, with no differences among NGT, IGT, and T2DM subjects (not shown); in the second group, only glucose and HbA1c decreased, with no change in ICAM-1, E-selectin and ET-1; the behaviour of BMI, waist circumference, arterial blood pressure, insulin, HOMA, ICAM-1, E-selectin and ET-1 was significantly different in LAGB-treated as compared with diet-treated subjects (*P* < 0.05 to *P* < 0.001). After slimming, the 68 obese subjects still differed from lean controls for BMI, waist circumference and HbA1c, while ICAM-1, E-selectin and ET-1 were not different.

Table 3 shows that under basal conditions ICAM-1, ET-1 and E-selectin correlated with HbA1c, HOMA, insulin, blood glucose and BMI; at stepwise regression the three molecules correlated with HbA1c, and ICAM-1 and E-selectin also correlated with HOMA. After 1 year, ICAM-1 correlated with insulin and HOMA, and E-selectin correlated with HOMA, HbA1c, insulin and BMI; at stepwise regression, both ICAM-1 and E-selectin only correlated with HOMA. Change in ICAM-1 and E-selectin correlated with change in BMI, and change in ICAM-1 and E-selectin also correlated with change in HOMA and insulin; at stepwise regression, change in the three molecules only correlated with change in BMI.

Table 1 Clinical, endocrine and metabolic variables evaluated in control (lean) and obese subjects with normal (NGT) or abnormal (IGT) glucose tolerance, or T2DM under basal conditions. Results are means±s.e. or absolute frequencies; *n* = number of subjects.

	Lean	Obese		
	NGT (<i>n</i> = 30)	NGT (<i>n</i> = 43)	IGT (<i>n</i> = 27)	T2DM (<i>n</i> = 26)
Age (years)	38.0±1.22	39.2±1.37	45.7±1.74	43.6±1.46
Gender (male/female)	12/18	10/33	9/18	10/16
Arterial hypertension	0*	16	9	12
BMI (kg/m ²)	22.5±0.21*	44.5±1.09	44.9±1.32	42.9±1.21
Waist circumference (cm)	82.1±0.41*	124.7±2.49	125.9±2.41	122.0±2.29
HbA1c (%)	3.9±0.09*	6.0±0.11	6.6±0.22	8.0±0.32†
Blood glucose (mmol/l)	5.3±0.07*	5.5±0.11	6.3±0.20	8.2±0.31**
Insulin (pmol/l)	63.9±2.63*	127.4±15.75	107.1±10.99	109.1±8.13
HOMA§	2.1±0.11*	4.6±0.68	4.6±0.54	5.7±0.49
ICAM-1 (ng/ml)	255.0±7.98*	300.9±11.67	309.2±15.88	372.3±15.63†
E-selectin (ng/ml)	39.6±1.31*	58.0±4.99	62.0±5.42	91.1±8.06†
ET-1 (pg/ml)	0.94±0.05*	1.27±0.09	1.12±0.09	1.61±0.08†

§ Insulin (μU/ml).glucose (mmol/l).22.5⁻¹.

Lean vs obese subjects: **P* < 0.001. Obese subjects: NGT vs IGT vs T2DM: ***P* < 0.001; T2DM vs NGT and IGT: †*P* < 0.001.

Table 2 Clinical, endocrine and metabolic changes at one year in obese subjects undergoing laparoscopic gastric banding (LAGB) and in subjects treated with diet. Means $\pm$ S.E.

	Group 1 (LAGB) (n = 68)		Group 2 (no-LAGB) (n = 13)		P value group 1 vs group 2
	Before	After	Before	After	
BMI (kg/m ²)	45.5 $\pm$ 0.79	37.1 $\pm$ 0.68*	41.4 $\pm$ 2.54	41.0 $\pm$ 2.21	<0.001
Waist circumference (cm)	125.7 $\pm$ 1.69	109.3 $\pm$ 1.66*	121.3 $\pm$ 2.15	120.5 $\pm$ 2.49	<0.01
Systolic BP (mmHg)	132.7 $\pm$ 1.82	126.3 $\pm$ 1.56*	137.5 $\pm$ 3.77	142.1 $\pm$ 3.17	<0.05
Diastolic BP (mmHg)	84.1 $\pm$ 1.15	79.7 $\pm$ 1.24*	86.2 $\pm$ 1.52	91.7 $\pm$ 2.91	<0.05
HbA1c (%)	6.4 $\pm$ 0.15	5.6 $\pm$ 0.09*	6.6 $\pm$ 0.62	5.7 $\pm$ 0.65*	NS
Insulin (pmol/l)	128.1 $\pm$ 10.57	70.1 $\pm$ 4.76*	94.5 $\pm$ 9.52	97.5 $\pm$ 9.47	<0.01
Blood glucose (mmol/l)	6.3 $\pm$ 0.18	5.3 $\pm$ 0.11*	5.9 $\pm$ 0.42	4.9 $\pm$ 0.35*	NS
HOMA β	5.1 $\pm$ 0.28	2.4 $\pm$ 0.18*	3.5 $\pm$ 0.36	3.3 $\pm$ 0.52	<0.01
ICAM-1 (ng/ml)	322.6 $\pm$ 10.68	274.3 $\pm$ 6.79*	258.4 $\pm$ 15.32	280.1 $\pm$ 14.87	<0.01
E-selectin (ng/ml)	65.5 $\pm$ 4.50	42.4 $\pm$ 2.12*	56.6 $\pm$ 8.64	53.9 $\pm$ 4.32	<0.01
ET-1 (pg/ml)	1.31 $\pm$ 0.07	1.10 $\pm$ 0.05*	1.04 $\pm$ 0.09	1.21 $\pm$ 0.13	<0.01

§ Insulin (μ U/ml).glucose (mmol/l).22.5⁻¹.Before vs after: * $P < 0.01$. NS, not significant.

Discussion

In this paper we found that obese subjects with T2DM have higher ICAM-1, E-selectin and ET-1 levels than those seen in obese NGT or IGT subjects, in spite of similar BMI and waist circumference measurements; in addition, ICAM-1, E-selectin, and ET-1 levels correlated with blood glucose and HbA1c; all these data indicate the importance of the glycaemic milieu in determining circulating levels of ICAM-1, E-selectin and ET-1 (1, 2); however, the three molecules were significantly higher in obese subjects as a group than in lean controls, and also correlated with BMI, HOMA and insulin; at stepwise regression ET-1 correlated with HbA1c, and ICAM-1 and E-selectin also correlated with HOMA. Therefore, we should assume that obesity and the

glycaemic milieu are at least equally important in determining circulating levels of the three molecules (6–8).

In trying to dissect out the role of glycaemic milieu and of body weight, in the prospective study we analysed separately LAGB-treated and diet-treated patients and found that significant weight loss is accompanied by a significant decrease in HbA1c and in ICAM, E-selectin and ET-1; in diet-treated patients who only obtained improvements in blood glucose and HbA1c and had no decrease in body weight, no change in ICAM, E-selectin, and ET-1 was observed. In the 68 patients with a significant weight loss, ICAM-1, ET-1 and E-selectin were not different from lean controls in spite of differences in BMI, waist circumference and HbA1c. In contrast to what happened under basal conditions when the three molecules correlated with

Table 3 Correlations (linear regression and stepwise regression) between ICAM-1, ET-1 and E-selectin (dependent variables, log-transformed) and selected anthropometric and metabolic indexes. In A and B correlations are between absolute values; in C correlations are between changes. Only significant regressions are indicated.

Dependent variable	Independent variable	A: under basal conditions			B: follow-up			C: changes (Δ)		
		Linear regression		Stepwise regression P	Linear regression		Stepwise regression P	Linear regression		Stepwise regression P
		r	P		r	P		r	P	
ICAM-1	HbA1c	0.41	0.0001	0.001	0.29	0.01	0.01	0.24	0.0387	
	HOMA	0.29	0.0011							
	Insulin	0.21	0.0193							
	Glucose	0.39	0.0001							
	BMI	0.23	0.0111							
ET-1	HbA1c	0.47	0.0001	0.001				0.30	0.0073	0.01
	HOMA	0.38	0.0001							
	Insulin	0.31	0.0004							
	Glucose	0.41	0.0001							
	BMI	0.37	0.0001							
E-selectin	HbA1c	0.49	0.0001	0.001	0.25	0.0299	0.01	0.23	0.0444	0.01
	HOMA	0.36	0.0001							
	Insulin	0.31	0.0006							
	Glucose	0.40	0.0001							
	BMI	0.35	0.0001							

HbA1c and with HOMA, after one year, at stepwise regression, ICAM-1 and E-selectin correlated only with HOMA, indicating that after a significant weight loss, differences between NGT, IGT and T2DM disappear, and only insulin resistance still affects these molecules. Finally, at stepwise regression, changes in the three molecules only correlated with change in BMI. These findings point to an important role of obesity, and agree with the findings of Bagg *et al.* (10); in agreement with our data, they and others found that improvements in HbA1c are not associated with changes in ICAM, E-selectin and ET-1 (9, 10). In our study, body fat distribution changed in a manner similar to body weight, as assessed by waist circumference, and therefore we cannot rule out an additional role of fat distribution in the change in ICAM-1, E-selectin and ET-1, in view of the relationship between endothelial dysfunction and fat distribution (6).

With substantial slimming, triglycerides decrease significantly (11), and this might be one of the reasons why ET-1 levels decrease; tumour necrosis factor- α (TNF- α) is produced in the adipocyte (21), and is able to directly stimulate ICAM and E-selectin release from endothelial cells (22); since substantial slimming is accompanied by a significant decrease in circulating TNF- α (23), it is possible that this is responsible for the decrease in ICAM and E-selectin levels.

The limitation of our study is that we analysed ICAM-1, ET-1 and E-selectin only under basal conditions and after 1 year, and therefore we cannot answer the question of a possible time-relation between change in BMI, insulin, HbA1c and glucose, and change in the three molecules under study.

We conclude that these molecules are affected by body weight, insulin resistance and abnormal glucose metabolism; all these features are present in both obesity and T2DM, and decrease after weight loss (23–25); when weight loss is substantial, only insulin resistance still affects these molecules, while if only glucose metabolism is improved, these molecules do not decrease. The practical implication of this study is that significant and durable weight reduction in obese subjects, especially when complicated by diabetes, not only improves glucose metabolism, that can be obtained also through diet and no change in body weight, but also reduces the long-term cardiovascular risk factor represented by raised circulating levels of ICAM, E-selectin and ET-1.

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