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Involvement of leptin in the association between percentage of body fat and cardiovascular risk factors

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Abstract

Objectives: Recent epidemiologic studies have shown that obesity is associated with elevated blood concentrations of prothrombotic-proinflammatory factors and markers of endothelial dysfunction such as fibrinogen, C-reactive protein (CRP), von Willebrand factor (vWF), and homocysteine. We have assessed whether these markers are associated with percentage of body fat (BF), insulin sensitivity as well as with leptin concentrations.

Design and methods: Twenty-five men aged 49.6 ± 12.7 yr (mean ± SD) underwent whole-body air displacement plethysmography (Bod-Pod®) for estimating BF. Blood analyses for leptin and several other metabolic and cardiovascular markers were carried out.

Results: Obese subjects had higher levels as compared to controls of BF (37.5 ± 5.1 vs. 26.0 ± 6.6, $p < 0.01$), fibrinogen (3.30 ± 0.43 vs. 2.67 ± 0.11, $p < 0.01$), vWF (136.4 ± 50.4% vs. 81.6 ± 12.6%, $p < 0.05$), and leptin (17.6 ± 8.7 vs. 6.2 ± 3.3, $p < 0.01$), lower concentrations of HDL-cholesterol (1.09 ± 0.20 vs. 1.51 ± 0.10, $p < 0.001$) and lower QUICKI ($1/[\log(Ins_0) + \log(Glu_0)]$) (0.31 ± 0.03 vs. 0.34 ± 0.02, $p < 0.05$). No significant changes were observed in CRP (5.7 ± 3.4 vs. 3.8 ± 1.6, $p = 0.327$) and homocysteine (9.4 ± 4.2 vs. 8.3 ± 0.9, $p = 0.749$). A positive correlation was observed between BF and fibrinogen ($r = 0.67$, $p = 0.0003$). Plasma leptin concentrations were correlated with fibrinogen ($r = 0.71$, $p = 0.0001$) and CRP ($r = 0.43$, $p = 0.044$). After adjustment for BF leptin emerged as a significant predictor of fibrinogen ($\beta = 0.47$, $p = 0.023$; $R^2 = 0.59$, $p < 0.001$). QUICKI was positively correlated with HDL-cholesterol ($r = 0.59$, $p = 0.010$) and negatively with fibrinogen ($r = -0.53$, $p = 0.025$), CRP ($r = -0.52$, $p = 0.028$) and vWF ($r = -0.56$, $p = 0.013$).

Conclusions: Increased BF and impaired insulin sensitivity are associated with increased concentrations of cardiovascular risk factors. Leptin seems to be involved in this elevation and emerges as a predictor of circulating fibrinogen concentrations.

Keywords: Obesity; Cardiovascular risk; Leptin; Fibrinogen; Insulin sensitivity

1. Introduction

Obesity is the most prevalent nutritional disorder in industrialized countries and is a growing problem in developing countries [1,2]. It is defined by an excess of body fat (BF) and is generally accompanied by insulin resistance [3]. Both situations lead to an increase in circulating cardiovascular risk factors, which may be the origin of cardiovascular diseases (CVD).

The mechanisms by which increased adiposity leads to insulin resistance and CVD are poorly understood. Adipose tissue has been previously considered to be a passive storage depot of energy. However, current evidence shows that adipocytes play an active role in metabolism through the secretion of hormones and cytokines involved in whole-body energy homeostasis [4]. These include leptin, adipsin, adiponectin, angiotensinogen, resistin, plasminogen-activator inhibitor-1 (PAI-1), tumor

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necrosis factor-α (TNF-α), and interleukin-6 (IL-6), among others.

Leptin is a 16 kDa hormone secreted mainly by adipocytes, although expression in placenta, fetal tissues, stomach and other tissues has also been observed. Leptin informs the brain about the size of the fat stores and has a wide variety of central and peripheral actions, including effects on reproduction, immune system, blood pressure, and angiogenesis [5].

Recent evidence suggests that leptin may be involved in the development of CVD. Leptin has been associated with impaired fibrinolysis [6,7], hypertension [8] and calcification of vascular cells [9]. In addition, plasma leptin concentrations are raised in patients with congestive heart failure [10] and correlate with heart rate in heart transplant recipients [11]. Moreover, some authors have proposed hyperleptinemia as a component of a metabolic syndrome of cardiovascular risk [12] while others have shown that leptin is an independent risk factor for coronary events in hypercholesterolemic men [13].

The present study tests the hypothesis that the increase in blood concentrations of several cardiovascular risk factors in obese subjects could be related to high fat mass and high concentrations of leptin. Therefore, the aim of this work was to assess whether several cardiovascular risk factors are associated with percentage of BF, insulin sensitivity, as well as leptin concentrations.

2. Methods

2.1. Subjects

Patients were recruited from obese persons visiting the Endocrinology Department at the University Clinic of Navarra for weight loss treatment and were otherwise healthy. Sex- and age-matched healthy controls were recruited among hospital staff. The study included 25 men aged 49.6 $\pm$ 12.7 yr, represented by 20 obese patients and 5 nonobese controls. Nonobese subjects were defined as having a body mass index (BMI) < 30 kg/m^2, whereas subjects with a BMI of 30 or more were classified as obese. The examinations and blood collections were carried out in the morning after a 12-h fast. All participants gave written informed consent to participate in the present study, which was approved by the Hospital's Ethical Committee.

2.2. Anthropometric measurements

Body weight and height were measured using standardized procedures. BMI was calculated as weight in kg divided by the square of height in meters. The 1998 clinical guidelines were used to define obesity (BMI $\geq$ 30) [14]. Body fat was estimated by air-displacement-plethysmography (Bod-Pod®, Life Measurements, Concord, CA, USA) [15 to 17]. This method has been reported to agree closely with the traditional gold standard hydrodensitometry (underwater weighing). Furthermore, the Bod-Pod® has been shown to predict fat mass and fat-free mass more accurately than dual-energy X-ray absorptiometry and bioelectrical impedance [15–17].

2.3. Blood analyses

Serum glucose was analyzed by an automated analyzer (Hitachi 717), with quantification being based on the enzymatic colorimetric reactions described by Trinder [18]. Insulin was measured by means of an enzyme-amplified chemiluminescence assay (IMMULITE®, Diagnostic Products Corp., Los Angeles, CA, USA). Total cholesterol and triglyceride concentrations were determined by enzymatic spectrophotometric methods (Boehringer Mannheim, Mannheim, Germany). High-density lipoprotein (HDL-cholesterol) was quantified by a colorimetric method in a Beckman Synchron® CX analyzer (Beckman Instruments, Ltd., Bucks, UK). Low-density lipoprotein (LDL-cholesterol) was calculated by the Friedewald formula [19].

Fibrinogen concentrations were determined according to the method of Clauss [20] using a commercially available kit (Hemoliance®, Instrumentation Laboratory, Barcelona, Spain). Measurement of von Willebrand factor (vWF) antigen was performed by a micro-latex immunoassay (Diagnostica Stago, Inc., Parsippany, NJ, USA). A standard curve was prepared with a universal reference (NISBC 91/666) and the results were expressed as percentage of the standard. Intra-and inter-assay coefficients of variation were between 4.0% and 8.0%. The concentrations of C-reactive protein (CRP) were analyzed by means of an immunoturbidimetric assay (Boehringer Mannheim) after calibration with the standard CRM 470. Homocysteine was determined applying a fluorescence polarization immunoassay (Axis Biochemicals ASA, Oslo, Norway), using an IMx® analyzer (Abbott Laboratories, Abbott Par, IL, USA). Leptin was measured by a double-antibody RIA method (Linco Research, Inc., St. Charles, MO, USA). Intra-and inter-assay coefficients of variation were 5.0% and 4.5%, respectively.

2.4. Calculation of insulin sensitivity

Insulin sensitivity was calculated by using the quantitative insulin sensitivity check index (QUICKI) [21]. This index represents a simple accurate method for assessing insulin sensitivity in humans and is defined as $1/(\log[\text{insulin}_0] + \log[\text{glucose}_0])$. QUICKI has been shown to correlate better than the homeostasis model assessment (HOMA) with the minimal model index of insulin sensitivity (SI_{MM}) and the gold standard method, namely the hyperinsulinemic isoglycemic glucose clamp (SI_{clamp}).

2.5. Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD). Differences between groups were analyzed by Mann-Whit-

Table 1
Characteristics of the obese and non-obese subjects

	Obese ($n = 20$)	Non-obese ($n = 5$)	p[a]
Age (years)	47.8 ± 13.3	56.8 ± 7.6	0.1531
Body fat (%)	37.5 ± 5.2	26.0 ± 6.6	0.0022
BMI (kg/m^2)	35.1 ± 4.8	25.4 ± 3.4	0.0014
Glucose (mmol/L)	6.1 ± 2.5	5.3 ± 0.5	0.8364
Insulin (pmol/L)	130.6 ± 92.6	71.0 ± 43.8	0.0604
QUICKI	0.31 ± 0.03	0.34 ± 0.02	0.0465

Data are mean ± SD. BMI = body mass index; QUICKI = quantitative insulin sensitivity check index.
[a] Mann-Whitney U-test comparing values between obese and non-obese groups.

ney U tests. Pearson's correlation coefficients (r) were computed to explore the correlations between two variables. Interrelationships were assessed using a multivariate regression analysis. The calculations were performed using the SPSS/Windows version 9.0.1 statistical package (SPSS, Chicago, IL, USA). A p value lower than 0.05 was considered statistically significant.

3. Results

Clinical characteristics of the patients are summarized in Table 1. No statistically significant differences for age were found between the groups. As expected, BF as well as BMI were significantly higher ($p < 0.01$) in the obese subjects. No differences were found in circulating concentrations of glucose and insulin between the two groups. However, the QUICKI index, a method for assessing insulin sensitivity, was significantly reduced ($p < 0.05$) in the obese individuals. Although total as well as LDL-cholesterol concentrations were higher in the obese group, they did not reach statistical significance. Triglycerides were significantly increased ($p < 0.01$) and HDL-cholesterol was significantly reduced ($p < 0.001$) in the obese group (Table 2).

Prothrombotic factors such as fibrinogen and vWF were significantly increased ($p < 0.01$ and $p < 0.05$, respectively) in the obese subjects. CRP, a marker of systemic inflammation, although being 50% increased in the obese group did not show significant differences. Concentrations of homocysteine were similar in both groups. As expected, leptin concentrations were significantly higher ($p < 0.01$) in the obese patients (Table 3).

Table 3
Cardiovascular risk factors of the obese and non-obese subjects

	Obese ($n = 20$)	Non-obese ($n = 5$)	p[a]
Fibrinogen (g/L)	3.30 ± 0.43	2.67 ± 0.11	0.0067
von Willebrand factor (%)	136.4 ± 50.4	81.6 ± 12.6	0.0108
C-reactive protein (mg/L)	5.7 ± 3.4	3.8 ± 1.6	0.3269
Homocysteine (μmol/L)	9.4 ± 4.2	8.3 ± 0.9	0.7489
Leptin (ng/mL)	17.6 ± 8.7	6.2 ± 3.3	0.0028

Data are mean ± SD.
[a] Mann-Whitney U-test comparing values between obese and non-obese groups.

To analyze the influence of BF, leptin, and insulin resistance in the concentrations of the cardiovascular risk factors studied, correlations and multivariate regression analysis were performed. Circulating concentrations of fibrinogen were positively correlated with BF (Fig. 1A). Furthermore, a stronger correlation between fibrinogen and leptin concentrations was found (Fig. 1B). Although no statistical correlation between BF and CRP was found (Fig. 2A), the latter was positively correlated with leptin (Fig. 2B). The QUICKI index was negatively correlated with fibrinogen, CRP and vWF, and positively correlated with HDL-cholesterol (Figs. 3 and 4).

To assess the importance of the correlation of leptin with both fibrinogen and CRP and to discard the potential underlying effect of increased BF, a multiple linear regression analysis was carried out, with both fibrinogen and CRP as dependent variables and leptin and BF as independent variables. Leptin emerged as a significant predictor of fibrinogen concentrations after adjustment for BF ($\beta = 0.47$, $p = 0.023$; $R^2 = 0.59$, $p < 0.001$) contributing to 55% of its variability. However, this was not the case with CRP, whose correlation with leptin disappeared after correction for BF.

Table 2
Lipid profile of the obese and non-obese subjects

	Obese ($n = 20$)	Non-obese ($n = 5$)	p[a]
Triglycerides (mmol/L)	2.50 ± 2.36	0.93 ± 0.17	0.0016
Total-Cholesterol (mmol/L)	5.99 ± 1.41	5.05 ± 1.00	0.0596
LDL-Cholesterol (mmol/L)	3.75 ± 1.03	3.14 ± 0.97	0.1259
HDL-Cholesterol (mmol/L)	1.09 ± 0.20	1.51 ± 0.10	0.0007

Data are mean ± SD.
[a] Mann-Whitney U-test comparing values between obese and non-obese groups.

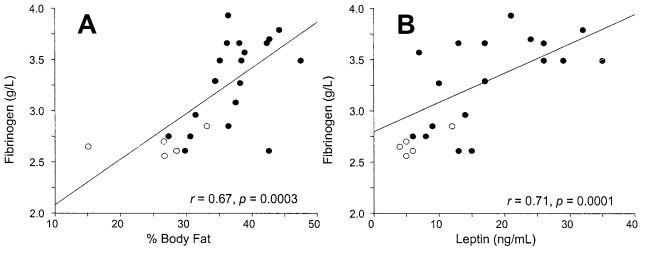

Fig. 1. Correlations of BF (A) and leptin (B) with circulating concentrations of fibrinogen of obese (●), or nonobese (○) patients. Pearson's correlation coefficient (r) and p values are indicated.

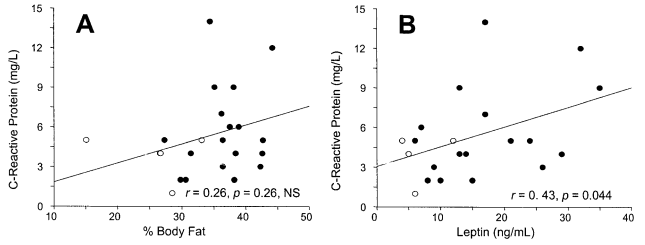

Fig. 2. Correlations of BF (A) and leptin (B) with circulating concentrations of C-reactive protein of obese (●), or nonobese (○) patients. Pearson's correlation coefficient (*r*) and *p* values are indicated.

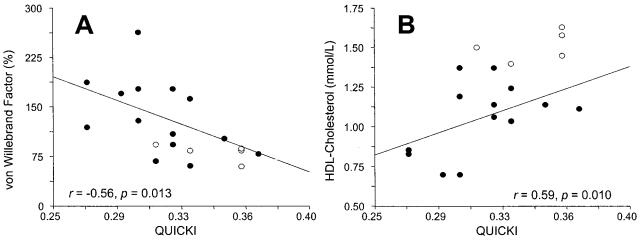

Fig. 4. Correlations of von Willebrand factor (A) and HDL-cholesterol (B) with the QUICKI of obese (●), or nonobese (○) patients. Pearson's correlation coefficient (*r*) and *p* values are indicated.

4. Discussion

Growing scientific and medical data supports the evidence that increased fat mass is associated with development of CVD [22–24]. In line with this fact, the American Heart Association included obesity as a major risk factor for coronary heart disease in 1998 [25]. The present study shows that increased BF and insulin resistance are related to several markers of inflammation and endothelial dysfunction, and that leptin is probably involved in this association.

Our results confirm earlier data reporting increased fibrinogen and vWF concentrations in obesity [26–28], thus reinforcing the observation that increased fat mass may contribute to the development of CVD. In addition, fibrinogen concentrations were strongly associated with BF. It is well know that fibrinogen concentrations correlate with BMI and BF [29,30], but the nature of this association is unknown. Although correlations do not imply causality, it is feasible that white adipose tissue plays a role in the regulation of fibrinogen concentrations. This might be explained by the increased production of IL-6 in adipose tissue of obese patients [31], given the fact that this cytokine has been shown to induce fibrinogen expression in the liver [32]. If this is the case, however, a correlation between BF and CRP, would have been expected since this marker has also been shown to be regulated by IL-6 [33]. Another possible explanation resides in the potential regulation of fibrinogen concentrations by leptin through the induction of hepatic expression of fibrinogen. In our study, this possibility is supported by the strong correlation observed between circulating leptin and fibrinogen concentrations. In addition, the main finding of this work is that blood leptin concentrations may be a predictor of blood fibrinogen concentrations, given the fact that the association between them is conserved after adjustment for BF. This possibility would be in accordance with the proinflammatory role attributed to leptin [34]. An alternative explanation is that leptin resistance and not leptin itself increases fibrinogen concentrations. This possibility is reinforced by the fact that obese Zucker rats, which lack functional leptin receptors, and hence are a model of leptin resistance, have higher concentrations of fibrinogen than normal rats [35]. Whether the association of fibrinogen and leptin concentrations is due to a direct effect of leptin or to leptin resistance requires further experiments in which leptin resistance in humans is assessed. The association between fibrinogen and leptin has been previously observed in two studies [7,29]. However, in both of them, the relation was not observed after adjustment for BF or BMI. The present study suggests that increased BF contributes to the development of a prothrombotic-proinflammatory state and that leptin specifically correlates with fibrinogen, suggesting that high leptin concentrations lead to the achievement of this condition, therefore leading to obesity-related disorders.

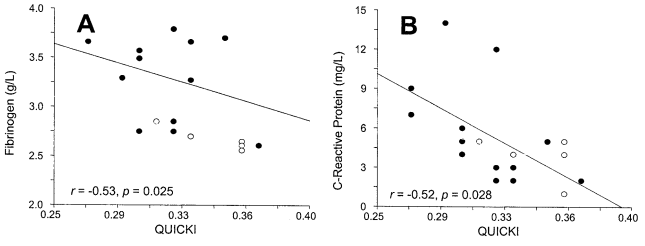

Fig. 3. Correlations of fibrinogen concentrations (A) and C-reactive protein (B) with the QUICKI of obese (●), or nonobese (○) patients. Pearson's correlation coefficient (*r*) and *p* values are indicated.

Despite a 50% increase in CRP concentrations in obese patients compared to controls, no statistical significance was reached. Several studies have shown that CRP is elevated in human obesity [36,37] and that it is correlated with indicators of adiposity [37–41]. These discrepancies may be explained taking into account several methodological aspects of the present study. First, the limited sample size raises the possibility of a lack of statistical strength. Second, several factors such as smoking, hypertension, and treatment with lipid-lowering or antihypertensive drugs may be confounding factors. In addition, our population sample of obese subjects may not be representative of central obesity, in which CRP concentrations are clearly correlated with BF [42]. However, in our study, CRP was significantly correlated with leptin concentrations. This association, which was not observed after adjustment for BF in multivariate regression analysis, is in agreement with previous studies [13,41,43] and suggests that, in addition to leptin, another factor altered in obesity may be involved in the obesity-induced increase in CRP.

The pronounced increase in vWF observed in obese

patients confirms that obesity has an adverse effect on the endothelium [27,28]. However, vWF did not show a significant correlation with either BF or leptin. Therefore, the increased vWF concentrations may be secondary to the dyslipidemia or insulin resistance observed [44]. Our results do not support an effect of increased adipose mass on plasma homocysteine concentrations. These results are in agreement with other published work [45,46], although some authors have found a weak correlation between plasma homocysteine and BMI [47].

Fibrinogen, CRP and vWF were negatively correlated with the QUICKI index. Interestingly, HDL-cholesterol was positively correlated with the QUICKI. Our data corroborate previous findings [48] and indicate that insulin resistance may, by itself or as a consequence of increased fat mass, induce endothelial dysfunction and inflammation. Taken together, these results confirm the marked involvement of insulin resistance associated to increased fat mass in the development of CVD. Nevertheless, prospective studies are needed to asses whether there is a simple association or a causal relationship between insulin resistance and high concentrations of fibrinogen, CRP and vWF and low concentrations of HDL-cholesterol.

In conclusion, these results indicate that men with high levels of BF have increased circulating prothrombotic-proinflammatory factors. Our work further strengthens the possibility that this relation is, at least in part, mediated by hyperleptinemia and insulin resistance. In addition, our results underline the role of leptin as an independent predictor of plasma fibrinogen concentrations.

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