Turkish Journal of Endocrinology and Metabolism, (2006) 2 : 39-44 **ORIGINAL ARTICLE**

Obese Subjects Have Low Global Fibrinolytic Capacity Associated with Insulin Resistance

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Obese subjects frequently have insulin resistance and they are at particularly risk of cardiovascular complications, possibly related to haemostatic and fibrinolytic system dysfunction. The aim of this study was to determine the effects of obesity on global fibrinolytic capacity (GFC) which is a new test used to assess fibrinolytic activity, and to evaluate the relationship of GFC with cardiovascular risk (CVR) factors.

Fifty obese subjects, with a body mass index (BMI)> 30 kg/m^2 (36 women, 14 men; mean age, 30±7 years; mean BMI, 34±3 kg/m^2); and 30 non-obese subjects, with a BMI<25 kg/m^2 (19 women, 11 men; mean age, 30±6 years; mean BMI, 22±2kg/m^2) were enrolled the study.

Anthropometric measurements (weight, height, hip and waist circumferences) were recorded down. Plasma fasting glucose, insulin, lipid profiles, fibrinogen levels, D-dimer and GFC were determined. Insulin resistance was calculated by homeostasis model assessment (HOMA-IR).

The gender and age were well matched in the two groups. Mean GFC was significantly lower in obese subjects than non-obese ones (7.6±7.5 µg/ml, 16.3±11.9 µg/ml, P< 0.001). However, there was no difference between mean D-dimer levels of the two groups. Mean plasma levels of fasting glucose (P< 0.05), insulin (P< 0.001), HOMA-IR (P< 0.01), fibrinogen (P< 0.001) in the obese group were higher than in the non-obese group. GFC showed inverse correlations with HOMA-IR (r=-0,41, P<0,001) and fasting insulin (r=-0,30, P<0,05).

These data showed that obese subjects have a net hypofibrinolytic state which is associated insulin resistance.

Keywords: Obesity, GFC, D-dimer, hypofibrinolysis, insulin resistance

Introduction

Obesity, and particularly abdominal obesity, is part of a metabolic syndrome (insulin resistance syndrome) including insulin resistance, impaired glucose tolerance or type-2-diabetes mellitus, hypertriglyceridemia and low HDL-cholesterol, hypertension (1, 2). Fibrinolytic disturbances have been reported in metabolic syndrome and obesity (3, 4). These disturbances might at least partly explain the increased cardiovascular risk (CVR) observed in these conditions, because hypo-fibrinolysis has been found to be a CVR factor in several epidemiological studies (5-10).

Fibrinolytic disturbances in insulin resistance syndrome and obesity usually have been demonstrated by determining changes in levels of molecules, mainly plasminogen activator inhibitor-1 (PAI-1), involved in fibrinolytic system (4, 7, 11, 12). Investigation of fibrinolytic status in these conditions with a test evaluating net fibrinolytic response can be aided to understanding of atherothrombosis pathogenesis in these subjects, more precisely. Global fibrinolytic capacity (GFC) is a recently developed method, which reflects the generation of D-dimer from plasma after the addition of a standardized amount of fibrin and a constant and a limited amount of exogeneous

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tissue-type plasminogen activator (t-PA). This test is sensitive to all the factors involved in the process of fibrinolysis (13-15). The pioneer studies by Amiral et al. (14) disclosed that GFC can help to identify individuals with a fibrinolytic dys-function. Recent studies also indicated that GFC is a sensitive method reflecting ongoing subclinical or overt prothrombotic / hypofibrinolytic states (15,16). As a result, this assay can help in the evaluation of obese subjects for net fibrinolytic response.

In this study, we compared obese subjects with non-obese ones for fibrinolytic system dysfunction with GFC assay and evaluated the relationships of GFC and CVR factors.

Materials and Methods

Fifty obese subjects, with body mass index (BMI) >30 kg/m^2 (36 women, 14 men; mean age, 30±7 years; mean BMI, 34±3 kg/m^2) and 30 non-obese subjects, with BMI<25kg/m^2 (19 women, 11 men; mean age, 30±6 years; mean BMI, 22±2 kg/m^2) were enrolled in the study. No subject in both groups had any known cardiovascular disease, diabetes, thrombotic-hemorrhagic disease and any devastating disease. No subject in the study has used any drug affecting coagulation or fibrinolysis within the 3 months period before the onset of the study. Informed consent was obtained from all subjects.

Weight and height were measured while the subjects were fasting overnight and wearing only underwear. Waist and hip circumferences were measured using a plastic tape meter at the level of the umbilicus and of the greater trochanters. In addition, waist-to-hip ratio (WHR) was also calculated. BMI was calculated as weight in kilograms divided by the square of height in meters. Blood pressure was measured with standard sphygmomanometer on the left arm after at least 10 min of rest. Mean values were determined by two independent measurements.

All sampling procedures were performed in the fasting state in the morning to avoid the effect of diurnal variation on the haemostatic system. Blood samples were drawn without using of a tourniquet, from large antecubital veins into vacuum tubes containing 3,8% trisodium citrate (1/9 dilution). Thereafter, the tubes were centrifuged at 4 °C at 3,000 rpm for 20 min and the supernatant plasma samples were stored in plastic tubes at -80 °C until analysis. Commercially available reagents, provided by the Diagnostica Stago were used to measure D-dimer (STA-Liatest D-Di kit) and fibrinogen (STA fibrinogen kit). Plasma GFC levels were measured by using a commercially available assay (Global Fibrinolytic Capacity STA Liatest D-Di; Diagnostica Stago, France). Briefly, after measurement of initial D-dimer plasma concentration with STA-Liatest D-Di, a standardized fibrin tablet was introduced into a 200 µl plasma sample supplemented with a constant and limited amount of t-PA. The mixture was incubated for 1 h at 37°C. Fibrinolysis is then stopped by introducing 50 µl of an excess of aprotinin and the generated D-dimer was measured by STA-Liatest D-Di again and generated D-dimer in the GFC test was calculated. Spectrophotometric method (Beckman Coulter LX20) was used to measure glucose, triglyceride, total cholesterol and HDL-cholesterol levels. LDL-cholesterol level was calculated according to Friedewald formula. Serum insulin levels were studies by chemiluminescent enzyme immunoassay (Immulite DPC 2000, Los Angeles, CA). Insulin resistance was assessed according to homeostasis model assessment (HOMA-IR = fasting serum glucose mmol/L x insulin µIU/mL/22.5) (17).

Statistical analysis was carried out using Statistical Package for Social Sciences software (SPSS for Windows, Version 10.0). Continuous variables were described as mean±SD. Statistical comparisons between groups were made using unpaired Student's t-test for normally distributed variables and Mann-Whitney U test for non-normally distributed variables. Chi-square test was used for comparison of gender between the groups. Relationships between continuous variables were examined using simple regressions and expressed as Pearson's correlation coefficients (r). Partial correlations were computed to take into account the effect of potential confounders. A P value of less than 0.05 was considered statistically significant.

Results

The demographic characteristics of both groups are shown in Table 1. The gender and age were well matched in the two groups. As expected, waist circumference and WHR were higher in obese group than non-obese group (P<0.001). Also,

systolic blood pressure, but not diastolic blood pressure was higher in obese group than non-obese group (P<0,01).

Table 1. The demographic characteristics of obese and non-obese subjects

	Obese (n: 50)	Non-obese (n: 30)	P
Age (years)	30±7	30±6	NS
Gender (% female)	72	63	NS
Waist circumference (cm)	104±11	75±9	<0,001
WHR	0,89	0,79	<0,001
BMI (kg//m^2)	34±3	22±2	<0,001
SBP (mmHg)	118±8	112±7	<0,01
DBP (mmHg)	76±5	72±7	NS

Numeric variables are expressed as means ± SD; BMI: Body Mass Index; WHR:waist-to-hip ratio; SBP:Systolic blood pressure; DBP: Diastolic blood pressure

Laboratory parameters of both groups are shown in Table 2. GFC was found to be significantly lower in the obese group compared with the non-obese group (P<0.001) (Fig.1). However, there was no difference in the mean D-dimer levels between the two groups. The mean plasma levels of fasting glucose (P<0.05), fasting insulin (P<0.001), HOMA-IR (P<0.01) and fibrinogen (P<0.001) in the obese group were higher than in the non-obese group. Although total cholesterol, LDL cholesterol and triglyceride levels were higher and HDL cholesterol was lower in the obese group than in the non-obese group, no statistically significant difference was noted. This finding demonstrated that obese subjects in the study have the some components of metabolic syndrome.

There were inverse correlations between GFC and athropometric parameters such as BMI, waist circumference and WHR. Furthermore GFC was correlated HOMA-IR (r=-0,41, P<0,001) (Fig. 2) and fasting insulin (r=-0,30, P<0,01), inversely. Moreover, after adjustment for BMI, the correlations between GFC and HOMA-IR remained significant (r=-0,33, P<0,01). There were no correlations between GFC and age, systolic and diastolic blood pressure, triglyceride, total, LDL and HDL cholesterol and fibrinogen.

We divided the subjects (all of obese and non-obese) according to HOMA-IR for direct evaluating of the effect of insulin resistance on GFC. The subjects which had higher HOMA-IR than the mean HOMA-IR (2,65) of the obese group were accepted to have insulin resistance. Thus, 13% and 44% of the none-obese and obese patients had insulin resistance, respectively. The subjects with insulin resistance had lower GFC than the subject without insulin resistance (mean, 5,47 µg/ml versus 13,5 µg/ml, P<0,001), in spite of not different plasma D-dimer levels between groups.

Table 2. The laboratory parameters of obese and non-obese subjects

	Obese (n: 50)	Non-obese (n: 30)	P
Fasting glucose (mg/dl)	91,6±8,5	86,4±9,9	<0,05
Fasting insulin (µIU/ml)	11,6±6	6,6±3	<0,001
HOMA-IR	2,65±1,4	1,82±1,01	<0,01
Total Cholesterol (mg/dl)	176±28	169±27	NS
Triglyceride (mg/dl)	88±49	72±53	NS
HDL-Cholesterol (mg/dl)	49±10	51±8	NS
LDL-Cholesterol (mg/dl)	109±24	103±21	NS
Fibrinogen (mg/dl)	294±56	251±52	<0,001
D-dimer (µl/ml)	0,35±0,5	0,42±1,1	NS
GFC (µg/ml)	7.6± 7.5	16.3±11.9	<0,001

Numeric variables are expressed as means ± SD; HOMA-R: Homeostasis model assessment; GFC: Global fibrinolytic capacity, NS: not significant

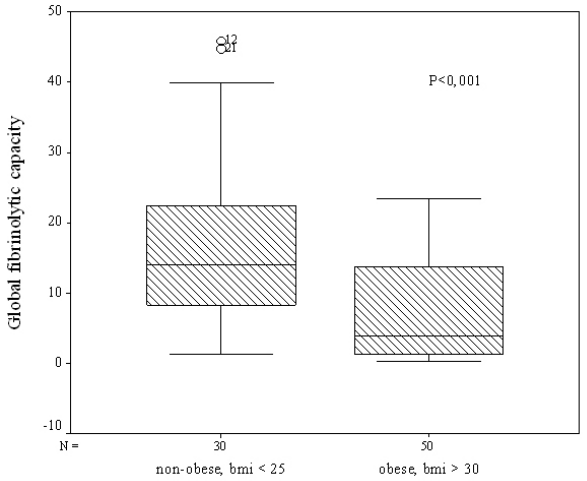

Fig. 1. The differences of the global fibrinolytic between obese and non-obese subjects

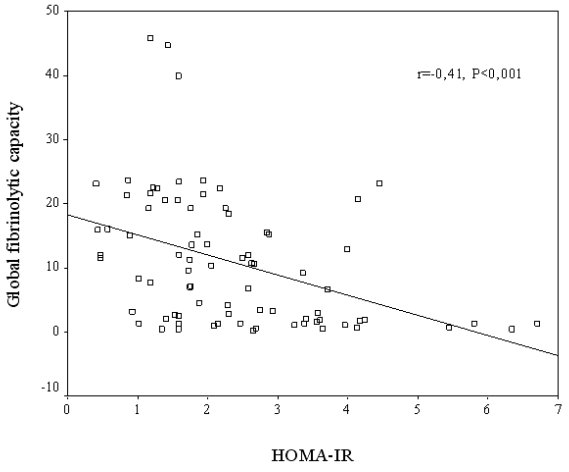

Fig. 2. Relationship between HOMA-IR and global fibrinolytic capacity in the study population

Discussion

In the present study, GFC - a new method for evaluating disturbances in plasma fibrinolytic activity- was lower in obese subjects than in non-obese subjects and there were inverse correlations between GFC and insulin resistance parameters in the study population.

The impairment of the fibrinolytic system, which leads fibrin deposition and is involved in proliferation and migration of vascular cells, could be a prime candidate for the development of atherothrombosis. Indeed, the results of several prospective studies are in favor of this hypothesis. It was reported that a decreased plasma fibrinolytic activity was predictive of coronary events in young men (5), an increased plasma concentration of PAI was predictive of myocardial infarction in patient with angina (6) and an increased t-PA antigen concentration (which reflects mainly t-PA/PAI 1 complexes) was predictive of myocardial infarction in healthy subjects and in angina patients (7-10). In line with these observations, showing hypofibrinolysis might be important for designating predisposition to atherothrombosis.

The biological process of fibrinolysis is a great dynamic puzzle. A wide variety of stimulatory and inhibitory molecules involved in haemostasis comprise the puzzle and affect some part of the overall fibrinolytic response, either in a negative or a positive way. Many previous studies evaluating fibrinolytic system, in any pathological event (including obesity) dissected the whole picture by focusing on a cluster of profibrinolytic and/or antifibrinolytic molecules such as PAI-1 and t-PA. Plasma levels of D-dimers are a direct measure of the extent of fibrinolysis, since they are a direct by product of cross-linked fibrin degradation. Significantly increased concentrations of D-dimers have been associated with enhanced intravascular fibrin degradation. This has been demonstrated in patients with acute venous thromboembolism, pulmonary embolism, disseminated intravascular coagulation, unstable angina pectoris and acute myocardial infarction (18-23). Furthermore, GFC is a new technique to evaluate the fibrinolytic system (14). The main principle of the GFC method is that, in the presence of a constant and limited amount of exogenous tissue-type plasminogen activator (tPA), D-dimer generated from a standardized fibrin quantity is measured. This test might be expected to be more sensitive than D-dimer for demonstrating fibrinolytic status without ongoing thrombosis. Indeed, it has been proposed that GFC reflects the net fibrinolytic response which is a result of the dynamic interactions of numerous stimulatory and inhibitory molecules (13, 14, 24). Thus, GFC can be important in indicating prothrombotic states like obesity.

In literature, although there are many studies showing a relation between obesity and fibrinolysis, especially an increase in PAI-1 level which is mainly secreted by adipose tissue (25-28), studies evaluating the net fibrinolytic status such as our study are only a few and relatively older methods were used in them (29). In with the present study, we showed obese subjects have lower GFC than in non-obese ones, in spite of the fact that the levels of D-dimer were comparable between the two groups.

Obesity, as observed partly our study, is strongly associated with CVR factors, including elevated level of serum total cholesterol, LDL-cholesterol, triglycerides, blood pressure, fibrinogen and insulin resistance (30). In our study, the differences of LDL-cholesterol, triglyceride, and HDL cholesterol between groups were not statistically significant, probably because of that the non-obese group was small and including four subjects with insulin resistance. Insulin resistance and subsequent hyperinsulinemia have been suggested as the mechanism linking the metabolic syndrome to cardiovascular disease (31). Hyperinsulinemia has predicted coronary heart disease in non-diabetic men in prospective studies (32). As expected, we found that the percent of subjects with insulin resistance in obese group was higher than the one of non-obese group. Moreover, the subjects with insulin resistance had lower GFC than the subject without insulin resistance. Furthermore, there was a inverse correlation between GFC and HOMA-IR even after adjusting for BMI in the study population. This finding suggests that hypofibrinolysis might be a reason of high CVR observed in insulin resistance states.

Consequently, our findings in this study showed that obese subjects have a net hypofibrinolysis state associated with insulin resistance.

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