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Negative correlation between plasma fibrinogen and insulin sensitivity measured with the minimal model technique

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Abstract. We aimed at investigating relationship between plasma fibrinogen and insulin sensitivity, which are two major determinants of metabolic Syndrome X (insulin resistance syndrome). We designed a prospective study of 27 non-diabetic, non-hypertensive subjects, presenting a wide range of body mass index BMI (10 men, 17 women; mean age$\pm$SEM: 35.9$\pm$2.2 years; BMI ranging from 21.1–45.2 kg/m^2). Insulin sensitivity was assessed with the minimal model procedure, over a 180 min intravenous glucose tolerance test with iterative sampling. Fibrinogen levels were determined by the method of Clauss. The insulin sensitivity index SI (i.e., the slope of the dose–response relationship between insulin increased above baseline and glucose disposal) ranged from 0.0009 to 16×10^{-4} min^{-1}/(μU/ml), with a mean value of $4.76 \pm 0.73 \times 10^{-4}$. Mean values of plasma fibrinogen were 3.33 ± 0.13 g/l, ranging from 2.21 to 5.07 g/l. There were highly significant negative correlations between SI and the level of plasma fibrinogen ($r = -0.61$, $p = 0.0007$) and between the basal effect of insulin BIE and plasma fibrinogen ($r = -0.521$, $p = 0.005$). Basal insulin was positively correlated to fibrinogen ($r = 0.386$, $p = 0.046$). When we analysed the data using partial correlation analysis, the negative relation between SI and fibrinogen was maintained independently from BMI ($r = -0.45$, $p < 0.05$). These data establish a strong negative association between insulin sensitivity and fibrinogen, involved in the increased cardiovascular risk of metabolic Syndrome X.

Keywords: Fibrinogen, insulin sensitivity, Syndrome X, rheology

1. Introduction

The syndrome of insulin resistance was described by Reaven as a cluster of metabolic abnormalities, including compensatory hyperinsulinemia, mildly impaired glucose tolerance, increased triglycerides and reduced high-density lipoprotein HDL-cholesterol [1]. This syndrome was termed Syndrome X and is different from the syndrome of the same name concerning microvascular angina. All of the above-mentioned factors associated with insulin resistance have been shown to be major risk factors for coronary heart disease (CHD) and mortality [2–4].

During the past decade, considerable evidence was obtained indicating that hemorheologic factors are important concomitants of Syndrome X [5]. Whole-blood viscosity is correlated to insulin resistance, both fibrinogen levels and plasma viscosity, which is strongly influenced by fibrinogen concentration, are positively associated with total cholesterol and triglycerides, and negatively associated with HDL

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levels [6]. The mechanism whereby hyperfibrinogenemia may accelerate CHD has not been totally clarified. Fibrinogen plays an important role in blood rheology as a prime determinant of plasma viscosity and inducer of a reversible red cell aggregation, but also in platelet aggregation and endothelial function. An interaction between metabolic risk factors for CHD and the fibrinolytic activity has been demonstrated, since levels of the plasminogen activator inhibitor-1 (PAI-1) are related to plasma triglycerides and insulin [7–9].

Two previous reports have suggested a relationship between fibrinogen and the glucose disposal rate during a glucose clamp [10,11]. However, there do not appear to be any studies in which a dose–response quantification of insulin sensitivity has been used to focus on relation with plasma fibrinogen. The present prospective study was conducted in order to evaluate the relationship between fibrinogen and insulin sensitivity, measured with the minimal model technique, which allows this approach.

2. Subjects and methods

27 non-diabetic, non-hypertensive subjects, presenting a wide range of body mass index (BMI), were explored in the study (10 men, 17 women; age 35.9 $\pm$ 2.2 years). Normotension and normal glucose tolerance were in all cases defined according to the WHO criteria. There was no family history of diabetes mellitus. Body weight and height were measured with the subjects in underwear and without shoes. BMI was calculated as body weight (kg) divided by height squared (m^2). Weight was 91.08 $\pm$ 4 kg, height 1.70 $\pm$ 0.02 m, BMI 31.3 $\pm$ 1.3 kg/m^2 ranging from 21.1 to 45.2 kg/m^2. No medication was taken on a regular basis. 23 subjects had never been tobacco users. 4 subjects were irregular and moderate smokers (less than 10 cigarettes per day).

Subjects underwent the intravenous glucose tolerance test (IVGTT) after a 12-hour fast. At 9:00 a.m., a cannula was set in the cephalic vein at the level of the cubital fossa for blood sampling at various times, while glucose was injected in the contralateral cephalic vein. Glucose (0.5 g/kg, solution at 30%) was slowly injected over 3 min. Insulin (0.02 units/kg body weight, i.e., 1 or 2 units) was injected intravenously immediately after time 19. Blood samples were drawn twice before the glucose bolus and at 1, 3, 4, 8, 10, 15, 19, 20, 22, 30, 41, 70, 90 and 180 min following the onset of the glucose injection. Times 1 and 3 min are used for the determination of insulin early secretory phase [12]. Times 4–19 are necessary for calculating an overall glucose tolerance index $K_{g_{4-19}}$ (i.e., the slope of the exponential decrease of blood glucose during this period), and times 8–180 are used for the minimal model calculations [13,14]. Giving insulin bolus at time 19 improves the reliability of the measurements, since a marked increase of plasma insulin above baseline (area under the curve higher than 1000 μU ml^{-1} min) is needed for a correct calculation of insulin sensitivity [15].

All samples were analysed for plasma insulin by radioimmunoassay (kit INSIK-5 from Sorin Biomedica France, 92160 Anthony) and plasma glucose with a Beckman glucose analyser. The within-assay coefficient of variation (CV) for insulin was determined by repetitive measurements of the same sample and ranged from 6.6% (low values) to 10.6% (high values). The between-assay CV for insulin ranged from 6.2% (low values) to 10.8% (high values). The sensitivity was 2 μU/ml.

Blood samples for fibrinogen were drawn at the beginning of the test, just after the arm was cannulated. Fibrinogen levels were determined by the method of Clauss [16].

Minimal model analysis of IVGTT was performed according to Bergman [15] with the software "TISPAG" from our department [17], which uses a non-linear least square estimation. This program

gave the values of insulin sensitivity as calculated from the following equations:

$$\mathrm{d}G(t)/\mathrm{d}t = -\big[p_1 + X(t)\big]G(t) + p_1 G_\mathrm{b}, \quad G(0) = G_0,$$

$$\mathrm{d}X(t)/\mathrm{d}t = -p_2 X(t) + p_3\big[I(t) - I_\mathrm{b}\big], \quad X(0) = 0,$$

where $G(t)$ and $I(t)$ are plasma glucose and insulin concentrations, $X(t)$ is the insulin in a compartment remote from plasma (insulin action), and p_1 to p_3 are model parameters. G_0 is the glucose concentration that would be obtained immediately after injection if there was instantaneous mixing in the extracellular fluid compartment. G_b and I_b are basal values of glucose and insulin. Parameter p_1 represents S_g, i.e., the fractional disappearance rate of glucose independent of any insulin response, p_2 and p_3 determine the kinetics of insulin transport, respectively, into and out of the remote insulin compartment where insulin action is expressed. SI is an index of the influence of plasma insulin to change the glucose effect *per se* on glucose concentration. Thus, SI is equal to $-p_3/p_2$.

S_g was divided into its two components: the contribution of hyperglycemia *per se* to tissue glucose utilisation and the effect of basal insulin on glucose uptake. The basal component of S_g is termed the basal insulin effect (BIE) and can be calculated as the product of basal insulin I_b and SI: BIE $= I_\mathrm{b} \times$ SI. Thus the contribution of non-insulin-dependent glucose uptake (glucose effectiveness at zero insulin, GEZI) to glucose uptake is the difference between total S_g and the BIE: GEZI $= S_\mathrm{g} - (I_\mathrm{b} \times$ SI).

The validity of our procedure using a reduced number of sampling times has been tested on 13 IVGTTs with values of SI ranging between 0.55 and 16.9 $\mathrm{min}^{-1}/(\mu\mathrm{U/ml}) \times 10^{-4}$. We compared the results given by the software with a classical protocol including 26 samples (1, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 24, 25, 27, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160, 180 min) [12] and with the reduced number of samples proposed by Steil and Bergman [13] and used here. Values of S_g ($r = 0.983$, slope $= 0.964$, intercept $=$ 0.13) and SI ($r = 0.974$, slope $= 0.863$, intercept $= 1.20$) were highly correlated. The mean relative deviation (defined as the percentage of difference between parameters calculated from the full sample protocol and parameters calculated from the reduced sample protocol) was -1.67 ± 2.46 for S_g and $-10 \pm 5\%$ for SI. This apparently high deviation for SI is explained by changes of less than 0.5 $\mathrm{min}^{-1}/(\mu\mathrm{U/ml}) \times 10^{-4}$ in non-insulin-dependent diabetes mellitus (NIDDM) patients with values of SI lower than 1 $\mathrm{min}^{-1}/(\mu\mathrm{U/ml}) \times 10^{-4}$. The mean absolute difference between SI calculated with the full protocol and SI calculated with the reduced sampling protocol was 0.91 ± 0.26 $\mathrm{min}^{-1}/(\mu\mathrm{U/ml}) \times 10^{-4}$. Intra-subject reproducibility of minimal model parameters and K_g has been investigated in 8 subjects (including 4 NIDDM patients) after they underwent two IVGTTs under the same conditions at an interval varying between 3 days and 1 year. Coefficients of variation (CV) for paired samples were calculated as mean SD divided by the mean of all the results. Mean SD is the square root of the sum of the SD for each pair divided by the number of paired observations minus one. The SD for each pair is the difference between tests divided by the square root of 2. The mean difference between the two tests is $+0.55 \pm 0.38$ $\mathrm{min}^{-1}/(\mu\mathrm{U/ml}) \times 10^{-4}$ for SI (i.e., a CV of 30.2%), $+0.36 \pm 0.08$ $\mathrm{min}^{-1} \times 10^{-2}$ for S_g (i.e., a CV of 18.3%), $+0.34 \pm 0.07$ $\mathrm{min}^{-1} \times 10^{-2}$ for GEZI (i.e., a CV of 19.4%) and $+0.29 \pm 0.08$ $\mathrm{min}^{-1} \times 10^{-2}$ for K_g (i.e., a CV of 21.4%). Concordance between the two sets of IVGTT parameters was also studied by their correlations: for SI ($r = 0.999$, slope $= 1.18$, intercept $= -0.08$), for S_g ($r = 0.879$, slope $= 0.824$, intercept $= 0.49$), for GEZI ($r = 0.912$, slope $= 0.704$, intercept $= 0.61$), for K_g ($r = 0.888$, slope $= 1$, intercept $= -0.22$).

3. Statistics

Data are presented as mean ± SEM. Correlations were tested by least square fitting for linear relationships. Partial correlation analysis was applied to compare variables independently related from age or BMI. For the accuracy of the SI index, the fractional standard deviation FSD was calculated [18]. The level of significance was set at $p < 0.05$.

4. Results

Mean (±SEM) data of basal and minimal model parameter values are given in Table 1. The insulin sensitivity index SI ranged from 0.0009 to 16 $\text{min}^{-1}/(\mu\text{U/ml}) \times 10^{-4}$, i.e., the whole range of insulin sensitivities [15]. One patient presented a near-zero SI value, two patients had SI values under 1 $\text{min}^{-1}/(\mu\text{U/ml}) \times 10^{-4}$. The mean fractional standard deviation FSD for SI, which represents the precision of minimal model fitting, was 9.05 ± 0.63%. Levels of plasma fibrinogen ranged from 2.21 to 5.07 g/l.

Table 1
Basal and IVGTT parameters

Parameters	Units	Mean values ± SEM
Fibrinogen	g/l	3.33 ± 0.13
G_b	mmol/l	4.3 ± 0.09
I_b	μU/ml	9.3 ± 0.7
$K_{\text{g}_{4-19}}$	$\text{min}^{-1} \times 10^{-2}$	2.2 ± 0.2
I_{1+3}	μU/ml	100.7 ± 13.7
SI	$\text{min}^{-1}/(\mu\text{U/ml}) \times 10^{-4}$	4.76 ± 0.73
S_g	$\text{min}^{-1} \times 10^{-2}$	2.9 ± 0.2
BIE	$\text{min}^{-1} \times 10^{-2}$	0.39 ± 0.05
GEZI	$\text{min}^{-1} \times 10^{-2}$	2.5 ± 0.24

Abbreviations: G_b = basal glucose, I_b = basal insulin, $K_{\text{g}_{4-19}}$ = slope of the exponential glucose decrease between 4 and 19 min after glucose infusion, I_{1+3} = sum of insulin values at 1 and 3 min after the end of glucose infusion, SI = insulin sensitivity, S_g = glucose effectiveness, BIE = basal insulin effect, GEZI = glucose effectiveness at zero insulin.

Table 2
Correlation analysis and partial correlation analysis

	I_b	$K_{\text{g}_{4-19}}$	I_{1+3}	SI	S_g	BIE	GEZI
FIB	$r = 0.386$	$r = 0.053$	$r = 0.095$	$r = -0.61$	$r = -0.03$	$r = -0.521$	$r = 0.1$
	$p = 0.046$	NS	NS	$p = 0.0007$	NS	$p = 0.005$	NS
BMI	$r = 0.484$	$r = 0.21$	$r = 0.387$	$r = -0.723$	$r = -0.18$	$r = -0.618$	$r = -0.05$
	$p = 0.01$	NS	$p = 0.045$	$p < 0.0001$	NS	$p = 0.0006$	NS
FIB/BMI	$r = 0.208$	–	–	$r = -0.45$	–	$r = -0.34$	–
	NS			$p < 0.05$		NS	

FIB = fibrinogen, BMI = body mass index, FIB/BMI = fibrinogen when the variable BMI is kept constant, NS = non-significant. Other abbreviations: see Table 1.

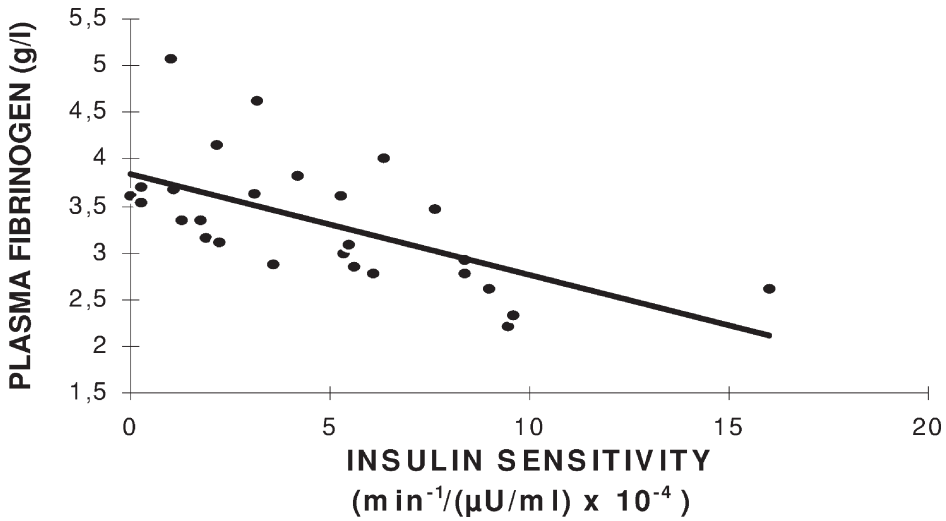

Fig. 1. Correlation between insulin sensitivity and plasma fibrinogen, $n = 27$, $r = -0.61$, $p = 0.0007$.

Linear regression analysis was carried out between fibrinogen, BMI and I_{b}, $K_{\mathrm{g}_{4-19}}$, I_{1+3}, SI, S_{g}, BIE, GEZI (Table 2). Fibrinogen was positively correlated to BMI ($r = 0.465$, $p = 0.014$) and I_{b}. There were highly significant negative relations between SI and fibrinogen (Fig. 1) and between BIE and fibrinogen. We used partial correlation analysis to assess the influence of BMI: the correlation remained significant only between SI and fibrinogen (Table 2). Fibrinogen was not correlated to age ($r = 0.075$, $p = 0.709$, NS).

5. Discussion

The aim of this study was to investigate the relationship between plasma fibrinogen and insulin sensitivity. The influence of cigarette smoking has to be discussed, since it may induce insulin resistance, although the mechanism remains unclear [19]. Furthermore, acute smoking is associated with increased fibrinogen levels [20]. The four smokers of our study were moderate and occasional tobacco users: they had normal levels of fibrinogen. Excluding these subjects left the strenght of the correlations unchanged.

Plasma fibrinogen concentrations increase gradually with age [20]. In the present study, this relation was minimized, because of homogeneity of our population according to age.

We found a strong negative correlation between plasma fibrinogen and the insulin sensitivity index SI, measured with the minimal model technique. Elevated levels of fibrinogen have been previously associated with decreased insulin-mediated glucose disposal, using the hyperinsulinemic euglycemic glucose clamp [10,11]. The insulin sensitivity index SI, determined from the IVGTT by resolving differential equations of the minimal model, is a useful measure of insulin sensitivity, which is capable of differentiating sensitivities among a normal population [21], and of detecting insulin resistance in obese or diabetic subjects [14,22]. Comparison with the glucose clamp, which is a reference method, has shown that the minimal model technique is equivalent [23].

Recently, a question concerning the validity of the minimal model analysis has emerged: it seems that the occurence of near-zero SI values is not negligible and its physiological significance remains unclear. We observed this phenomenon for one subject of this study, which leads to a moderate percentage of the

total population (3.7%). Near-zero SI values might be an artefact of the single-compartment assumption on the basis of this approach. However, there is a literature suggesting that near-zero SI corresponds to a real pathological state [24,25].

Landin's group studied different metabolic and hemostatic parameters, including glucose disposal rate and fibrinogen, in 22 non-obese middle-aged men, with normal blood pressure or untreated mild hypertension. Fibrinogen levels correlated negatively with the rate of glucose disposal [10]. However, the measurement of glucose disposal used in this study was only representative of the level of insulin concentration obtained during the glucose clamp and thus gave no information on either non-insulin-dependent glucose uptake or dose–response relationship between insulin and glucose uptake. For this reason, Moan's group, in a similar study, divided the glucose disposal rate by mean level of insulin during the last minutes of the clamp, to obtain the amount of glucose metabolized per unit of plasma insulin. There was also a significant negative correlation with the level of fibrinogen ($r = -0.66$, $p = 0.002$) [11]. Nevertheless, a frequent difficulty associated with the clamp procedure is the problem of obtaining a steady-state condition where the plasma insulin is at a predetermined value, and the glucose utilization rate is determinate. The minimal model approach has the advantage of accounting for insulin sensitivity, which is mostly a measurement of insulin-mediated glucose uptake, but also glucose effectiveness, that is, the ability of glucose itself to promote its own assimilation, independent of any change of insulinemia.

Our results establish this negative relationship between fibrinogen and insulin sensitivity, in a wide range of sensitivities, with a practicable and reproducible technique. The fractional standard deviation FSD for SI, which represents the precision of minimal model fitting, showed a guarantee of accuracy. It was not surprising to find a positive correlation between plasma fibrinogen and basal insulin. Hyperinsulinemia has been previously associated with elevated plasma levels of fibrinogen in young men with premature coronary artery disease [26]. Moreover, PAI-1 is positively correlated to basal insulin in normal and obese subjects [8]. Actually, baseline insulinemia is to some extent an index of insulin sensitivity, since there is an homeostatic feedback relationship between SI and insulin release. Insulin sensitivity and baseline insulin are non-linearly related to each other, according to a hyperbolic function $\mathrm{SI} = \alpha / I_{\mathrm{b}}$ with a being a constant [27]. Accordingly, it could be interesting to discuss whether fibrinogen levels are primarily dependent upon insulin levels or insulin sensitivity. To our knowledge, this distinction is not clearly elucidated in the current literature. We think that our results shed some light on this point. We observe that there is a closer correlation between fibrinogen and SI than between fibrinogen and insulinemia. The partial correlation analysis eliminates the variable "insulinemia" when BMI is kept constant. Thus, our findings are consistent with the hypothesis of insulin sensitivity being by itself a factor governing plasma fibrinogen levels.

However, there could be an alternative interpretation for this correlation, i.e., fibrinogen being a factor involved in the modulation of peripheral insulin action, perhaps via its effects on blood rheology and microcirculatory blood flow distribution. This explanation would be in agreement with a recent hypothesis that suggests to interprete insulin resistance as a consequence of endothelial dysfunction [28]. From our data, which are only correlative, it is not possible to discriminate between these two explanations.

In conclusion, this study provides further evidence for the association between insulin resistance and decreased fibrinolytic activity. It seems therefore likely that fibrinogen, either by its hemostatic or hemorheologic actions, is involved in the increased cardiovascular risk that accompanies insulin resistance in Syndrome X.

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