Relationships between fibrinogen and insulin resistance

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Abstract

A relationship between plasma fibrinogen levels and insulinemia, as well as the different parameters of the insulin resistance syndrome has been described. The aim of the present paper was to investigate whether plasma fibrinogen concentrations were linked to plasma insulin levels or to the degree of insulin resistance. For this purpose, 62 nondiabetic, nonhypertensive patients, 30 men and 32 women, with body mass indexes (BMIs) and ages ranging from 18.6 to 50.2 kg/m² and from 19 to 60 years, respectively, were studied. Insulin sensitivity was quantified by the minimal model procedure over a 180-min intravenous glucose tolerance test with iterative sampling. Plasma insulin was determined by radioimmunoassay without cross-reactivity to human proinsulin, and fibrinogen by the method of Clauss. Insulin sensitivity ranged from 0.009 to 23.2 min⁻¹ (mU·ml)⁻¹, covering the whole range of insulin sensitivities. Fibrinogen ranged from 1.70 to 5.07 g/l. There was a significant negative correlation between fibrinogen and insulin sensitivity (r = −0.76, P < 0.0001) and a positive correlation between fibrinogen and basal insulin (r = 0.56, P < 0.0001). After adjustment for BMI, body fat mass and waist-to-hip ratio, these two relationships remained significant. In addition, a multiple regression analysis was performed to assess the independent effect of the following related variables: fibrinogen, insulin sensitivity, insulinemia and BMI. Only insulin sensitivity appeared to account for the ability to predict fibrinogen values. Thus, we hypothesized it was likely that the state of insulin resistance rather than hyperinsulinemia per se was related to hyperfibrinogenemia. We proposed an interpretation of these data in connection with some factors like free fatty acids or tumor necrosis factor-α, which have been implicated in the pathogenesis of insulin resistance. Nevertheless, prospective and intervention studies are needed to assess whether there is a simple association or a causal relationship between insulin resistance and hyperfibrinogenemia. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

In recent years, plasma fibrinogen level has emerged as an important risk factor for coronary heart disease. Strong evidence for this comes from several extensive cross-sectional and prospective epidemiological studies, which have demonstrated that high plasma fibrinogen levels represent an independent marker for cardiovascular diseases in non-diabetic subjects [1]. Elevation of fibrinogen levels [2] and impaired fibrinolysis [3] are more common in diabetic patients than in non-diabetic subjects, although discordant results have been reported [4]. Moreover, a number of studies have linked plasma fibrinogen with different components of the metabolic syndrome or syndrome X, namely type II diabetes, hypertension, hypertriglyceridemia and hyperinsulinemia [5–7]. Nevertheless, there is no consensus about the impact of fibrinogen as a cardiovascular risk factor in the metabolic syndrome or in diabetes. Our knowledge about the relationship between hyperfibrinogenemia and hyperinsulinemia is still incomplete. Insulin resistance has been suggested as a potential pathogenetic link [5]. There are very few data on this topic. A study conducted on a relatively small sample of healthy young men showed a significant negative correlation between fibrinogen and the glucose disposal rate, which was used as an indicator of insulin sensitivity during an...
euglycemic hyperinsulinemic glucose clamp [8]. A similar association was reported on a pooled sample of 22 normotensive and untreated mild hypertensive patients [9]. Apart from these two previous studies, there are no consistent data on the relationship between plasma fibrinogen and insulin resistance. Recently, we gave preliminary evidence for this association [10,11]. The aim of the present paper was to elucidate whether plasma fibrinogen concentrations were linked in fact to plasma insulin levels or to the degree of insulin resistance.

2. Subjects

The study population was a sample of 62 normoglycemic patients (30 men and 32 women) who came to our Unit for a metabolic check-up. These subjects did not have diabetes according to the 1997 American Diabetes Association criteria [12]. No medication was taken on a regular basis. All subjects were non smokers. Anthropometric characteristics of the study sample are shown in Table 1. Blood pressure was measured on the right arm after 10 min in the supine position. Systolic blood pressure range was 100–140 mmHg (120.7 ± 2.5) and diastolic blood pressure 65–90 mmHg (71 ± 2) in the whole group: there was no significant difference between men and women. Informed consent was obtained from all subjects and the protocol was approved by the local Ethics Committee.

3. Anthropometric measurements

Weight and height measurements were performed in underwear and BMI was then calculated. Waist and hip circumference measurements were taken using a non-extensive flexible tape at the narrowest part of the torso and at the point of maximum extension of the buttocks, respectively. The WHR was then calculated. Body composition (body fat mass and percentage of body fat) was estimated by bioelectrical impedance analysis. All measurements were performed by a multi-frequency (1, 5, 10, 50, 100 kHz) device (Human IM-Scan from Dietosystem, Milano, Italy).

Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Whole group (n = 62) mean ± SD (range)</th>
<th>Men (n = 30) mean ± SD (range)</th>
<th>Women (n = 32) mean ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37 ± 11.6 (19–60)</td>
<td>38.9 ± 11 (19–56)</td>
<td>35.7 ± 13.1 (19–60)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.4 ± 7.4 (18.6–50.2)</td>
<td>30.3 ± 8.8 (18.6–50.2)</td>
<td>30.5 ± 6.4 (20.5–45.2)</td>
</tr>
<tr>
<td>WHR</td>
<td>0.87 ± 0.09 (0.7–1.1)</td>
<td>0.91 ± 0.11 (0.7–1.1)</td>
<td>0.83 ± 0.07 (0.7–1.05)*</td>
</tr>
<tr>
<td>Body fat mass (kg)</td>
<td>31.7 ± 14 (9–57.7)</td>
<td>30.5 ± 16 (9–57.7)</td>
<td>33.8 ± 14.4 (12.9–53)</td>
</tr>
<tr>
<td>Body fat mass (%)</td>
<td>35.8 ± 10.4 (12.1–58.4)</td>
<td>31.5 ± 11.4 (12.1–58.4)</td>
<td>40.1 ± 7.3 (22.7–48.5)*</td>
</tr>
</tbody>
</table>

* BMI, body mass index; WHR, waist-to-hip ratio; * women/men: * P < 0.05

4. Study protocol and analytical methods

The frequently sampled intravenous glucose tolerance test (FSIGTT) was performed as previously described [13]. After a 12-h overnight fast, an intravenous cannula was placed in the cephalic vein at the level of the cubital fossa for blood sampling, 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 19 min. 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. The 1 and 3 min samples were used for the determination of insulin early secretory phase [14]. The other samples were necessary for minimal model calculations [15,16]. Giving insulin bolus at time 19 min improved the reliability of the measurements, since a marked increase of plasma insulin above baseline is needed for a correct calculation of insulin sensitivity [17].

Plasma glucose was measured in duplicate by the glucose oxidase method with a Beckman Glucose Analyzer 2. Plasma insulin was determined by radioimmunoassay (kit Bi-Insulin IRMA from ERIA-Diagnostics Pasteur, France, without cross-reactivity to human proinsulin). All samples from a single patient were measured in the same assay. Blood samples for fibrinogen were drawn at the beginning of the test, after the arm was cannulated. Fibrinogen levels were determined by the method of Clauss.

Minimal model analysis of FSIGTT was performed according to Bergman [17] with the software ‘TISPAG’ from our unit [13], which uses a non-linear least square estimation. This program gave the values of insulin sensitivity (SI) and glucose effectiveness (SG) as calculated from the following equations:

\[
\frac{dG(t)}{dt} = - [p_1 + X(t)] G(t) + p_1 G_0
\]

\[G(0) = G_0\]

\[
\frac{dX(t)}{dt} = - p_2 X(t) + p_3 [I(t) - I_s]
\]

\[X(0) = 0\]
5. Statistical methods

Data are expressed as mean ± SD. Statistical significance was set at \( P < 0.05 \). The normal distribution of the variables was checked with the Kolmogorov–Smirnov test: if the variables were not normally distributed, they were ln-transformed. Data from men and women were compared by using a \( t \)-test. Relationships between fibrinogen and parameters of glucose assimilation were analyzed by using Pearson and partial correlation coefficients. Additionally, a multiple regression analysis was performed to assess the independent effect of related variables. All calculations were performed with the SigmaStat package for Windows (Jandel Scientific, Erkrath, Germany).

6. Results

Table 2 summarizes the mean data of basal and minimal model-derived parameter values. The insulin sensitivity index \( St \) ranging from 0.009 to 23.2 min/\((\mu U/ml) \times 10^{-4}\), covered the whole range of insulin sensitivities [17]. One patient presented a near-zero \( St \) value and three patients had \( St \) values under 1 min/\((\mu U/ml) \times 10^{-4}\). The mean fractional standard deviations FSD, which represent the precision of minimal model fitting, were 7.6 ± 1.2% for \( St \) and 13.9 ± 1.1% for \( Sg \). In this study, there was no significant difference between men and women.

Fig. 1 demonstrates the presence of a significant negative relationship between plasma fibrinogen and insulin sensitivity (\( r = -0.76, P < 0.0001 \)), and between plasma fibrinogen and basal insulin effect \( BIE (r = -0.62, P < 0.0001) \). Since the condition of insulin resistance leads to hyperinsulinemia, we examined the correlation between plasma fibrinogen and basal insulin values, which were not normally distributed and thus ln-transformed before analysis (\( r = 0.56, P < 0.0001 \)). We performed a partial correlation analysis to assess

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 \( p_1 \) and \( p_2 \) are model parameters. \( Go \) is the glucose concentration that would be obtained immediately after injection if there was instantaneous mixing in the extracellular fluid compartment. \( Go \) and \( I_0 \) are basal values of glucose and insulin. Parameter \( p_1 \) represents \( S_0 \), i.e. the fractional disappearance rate of glucose independent of any insulin response, \( p_3 \) and \( p_2 \) 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 \( -p_3/p_2 \).

\( Sg \) was divided into its two components [18]: the contribution of hyperglycemia per se to tissue glucose utilization and the effect of basal insulin on glucose uptake. The basal insulin component of \( Sg \) is termed the basal insulin effect (BIE) and can be calculated as the product of basal insulin \( I_0 \) and \( St \) (BIE = \( I_0 \times St \)). Thus the contribution of non-insulin-dependent glucose uptake (glucose effectiveness at zero insulin, \( GEZI \)) to glucose uptake is the difference between total \( Sg \) and the BIE (\( GEZI = Sg - [I_0 \times St] \)).

The validation of our procedure using a reduced number of sampling times has been published elsewhere [19]. For the accuracy of minimal model indices, the fractional standard deviations (FSD) were calculated in accordance with the criteria of Prigeon et al. [20].

In addition to the minimal model analysis, a separate approach using a classical monoeponential model of glucose disappearance was used. The least square slope of the log of absolute glucose concentration between 4 and 19 min after the glucose bolus, \( Kg_{4-19} \), was used as an index of glucose tolerance. The more classical \( Kg_{10-30} \), which measures the decrease in blood glucose between 10 and 30 min [21], could not be used in this study since its results may be influenced by the insulin injection at the 19th min.
the influence of body composition, i.e. BMI, body fat mass and WHR, on these data. The negative relationships between fibrinogen and SI, between fibrinogen and BIE, just as the positive correlation between fibrinogen and basal insulinenia, remained significant after adjustment for these three confounding factors. In this study, fibrinogen was not found to be correlated to age (Fig. 2).

By a multiple regression analysis with fibrinogen as the dependent variable and BMI, SI and basal insulinenia (ln-transformed) as independent variables, fibrinogen could be predicted from a linear combination of the variables BMI ($P = 0.0055$) and SI ($P < 0.0001$), but not basal insulinenia ($P = 0.1102$). The value of $r^2$ for the entire model was 0.66. In fact, only SI appeared to account for the ability to predict fibrinogen.

7. Discussion

The purpose of this study was to assess whether hyperfibrinogenemia was linked to the condition of insulin resistance or to hyperinsulinemia, in a sample of patients covering the whole range of insulin sensitivities. We used the minimal model analysis for determination of insulin sensitivity through computer modeling of glucose and insulin dynamics from the FSIGTT. This procedure gives measurements that correlate strongly with and are equivalent to those obtained with the glucose clamp [22]. Recently, the question of the occurrence of SI values indistinguishable from zero has arisen. We observed this phenomenon for only one subject of the study. Excluding this subject left the strength of the correlations unchanged. Although there is evidence for very low insulin sensitivity in type II diabetes, apparent near-zero values have emerged even in nondiabetic individuals [23]. Whether near-zero SI values represent a physiological state or a symptom of modeling deficiency, remains to be clarified.

We confirmed a highly significant negative correlation between fibrinogen and insulin sensitivity, and a positive correlation between fibrinogen and fasting insulin. These relationships remained significant after adjustment for BMI, body fat mass and WHR. Plasma fibrinogen levels are known to increase gradually with age [24]: in the present study, there was no influence from this factor, since we found no significant correlation between fibrinogen and age. Smoking is associated with higher fibrinogen levels and may induce insulin resistance [25]. All subjects were non smokers, so that this important confounding factor had not to be taken into account.

Elevated levels of fibrinogen have been previously associated with decreased insulin-mediated glucose disposal during the glucose clamp procedure, in two small samples of healthy young men ($n = 21$, $r = -0.66$, $P =$...
In the last-mentioned study, no correlation was found between fibrinogen and fasting insulin \( (r = -0.35, P < 0.05) \) [9]. Our data corroborate the conclusions of a large cross-sectional study by Imperatore et al. [7], where hyperfibrinogenemia is proposed as a new component of the metabolic syndrome. This assumption is based on the association between age-adjusted fibrinogen levels and the classical symptoms of the metabolic syndrome, i.e. high blood pressure, fasting plasma glucose and triglycerides, and low HDL cholesterol.

Our results should not be interpreted in terms of causality, since cross-sectional studies do not allow definition of causal relations. However, the conclusions of the multiple regression analysis lead us to argue that it is the state of insulin resistance rather than hyperinsulinemia per se that is related to hyperfibrinogenemia. This hypothesis is consistent with that of Imperatore et al. [7,26], who considers the correlation between hyperinsulinemia and hyperfibrinogenemia as an epiphenomenon of the state of insulin resistance underlying hyperinsulinemia. Moreover, it should be noticed that insulin does not increase fibrinogen synthesis in cell cultures [27] and does not seem to acutely regulate fibrinolysis [28]. It has been proposed that the increased free fatty acids release, observed in the pathogenesis of insulin resistance and type II diabetes, could stimulate hepatic fibrinogen synthesis [29]. A defective fibrinolysis, with high plasminogen activator inhibitor-1 (PAI-1) levels, has been found in type II diabetic patients: PAI-1 levels are closely related to the amount of visceral fat and thus seem to depend more on the insulin resistance syndrome that precedes type II diabetes than on diabetes itself [30]. Furthermore, plasma fibrinogen levels rise acutely in response to various stimuli, including release of cytokines such as tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)) during the inflammation process. Recent studies have shown that TNF-\( \alpha \) is implicated in the insulin resistance of human obesity [31] and it is well known that TNF-\( \alpha \) stimulates hepatic fibrinogen synthesis. Therefore, it is clear that some pathogenic conditions of the insulin resistance syndrome may lead to hyperfibrinogenemia. Additionally, the use of troglitazone, a new thiazolidinedione derivative that improves insulin resistance, produced a better glycemic control and a significant decrease of the levels of PAI-1 and fibrinogen in type II diabetic patients [32]. A similar result was obtained by a treatment with the angiotensin-converting enzyme inhibitor perindopril in overweight hypertensive subjects [33]. Nevertheless, it is difficult to firmly establish whether the decrease of plasma fibrinogen levels is related to the improvement of insulin sensitivity or due to direct effects of the drugs on the regulatory mechanisms that control hepatic synthesis of fibrinogen.

In summary, there is a clear association between hyperfibrinogenemia and the metabolic syndrome, and this association is probably mediated by insulin resistance rather than hyperinsulinemia per se. Large prospective studies, including quantification of insulin sensitivity and measurements of various metabolic and hemostatic parameters, as well as intervention trials, are needed to clarify the link between hyperfibrinogenemia and insulin resistance. Fibrinogen lowering can be achieved by drugs, exercise or improved metabolic control in diabetic patients. All of these drugs or lifestyle modifications influence other cardiovascular risk factors and it may be difficult to define precisely whether there is a simple association between fibrinogen and insulin resistance or a causal relationship.

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