Proinsulin, Intact Insulin, and Fibrinolytic Variables and Fibrinogen in Healthy Subjects

A population study

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OBJECTIVE — As high serum insulin predicts impaired fibrinolysis and proinsulin reacts in most conventional insulin assays, we hypothesized that proinsulin could link low fibrinolytic activity and hyperinsulinemic conditions.

RESEARCH DESIGN AND METHODS — We explored the relationship between fibrinolysis and plasma fibrinogen on the one hand and specific insulin and proinsulin on the other, in a healthy population sample of 165 men and women, 25–74 years of age, from the Northern Sweden MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) Study. Specific insulin and proinsulin were measured by enzyme-linked immunosorbent assay. Partial correlation coefficients, adjusted for age and sex, were calculated.

RESULTS — Plasma fibrinogen levels were related to insulin ($r = 0.25$, $P < 0.01$) and proinsulin ($r = 0.29$, $P < 0.001$), as was plasminogen activator inhibitor (PAI)-1 activity ($r = 0.36$ and $r = 0.29$, respectively; $P < 0.001$). Tissue plasminogen activator (tPA) activity correlated inversely to insulin ($r = -0.35$, $P < 0.001$) and proinsulin ($r = -0.36$, $P < 0.001$). In a multivariate analysis taking also smoking and anthropometric and metabolic measurements into account, fasting proinsulin was a significant predictor of high plasma fibrinogen level. Insulin and proinsulin levels were not related to tPA activity. High levels of postload insulin, triglycerides, and diastolic blood pressure, but not proinsulin, predicted high PAI-1 activity.

CONCLUSIONS — In a healthy population, the relationship previously described between high insulin levels and impaired fibrinolysis is not attributable to confounding from proinsulin. Elevated proinsulin levels are associated with high fibrinogen levels.

In prospective population studies, hyperinsulinemia has been implicated as a cause of cardiovascular disease in men (1,2), although strongly opposed by others (3,4).This has prompted a search for mediators of the possible adverse effects of endogenous insulin on atherothrombotic disease.

Impaired fibrinolysis increases the risk of myocardial infarction (5,6). The main regulator of fibrinolytic activity is plasminogen activator inhibitor (PAI)-1. High plasma PAI-1 activity predicts reinfarction and death in patients with infarction at early age (7). Elevated plasma fibrinogen levels are also strong predictors of myocardial infarction and stroke (8).

In subjects with hyperinsulinemia, PAI-1 activity is high and the activity of tissue plasminogen activator (tPA) is low (9). But acute exogenous hyperinsulinemia leads to unchanged (10) or decreased PAI-1 activity (11), although the circadian rhythm of the fibrinolytic system may obscure a true effect (12). Also, insulin treatment in patients with NIDDM leading to chronic peripheral hyperinsulinemia is associated with lower PAI-1 activity (13).

These inconsistencies may be partly explained by assay methodology. Hitherto, most conventional insulin assays measure total immunoreactive insulin (IRI), which to a great extent includes proinsulin and its conversion intermediates (14). As evidence of β-cell dysfunction, the fasting ratio of proinsulin to IRI increases with increasing hyperglycemia (15). Thus, it is possible that the epidemiological evidence for both the atherothrombotic effects of hyperinsulinemia and the apparent relationship between IRI and reduced fibrinolysis is due to the same confounder, a relationship with proinsulin and its conversion intermediates. This may draw some support from the results of clinical trials with human proinsulin in NIDDM patients who were prematurely terminated from the study because of a cluster of myocardial infarctions in the treated group (16). Recently, Båvenholm et al. showed high proinsulin levels to determine both the severity of coronary artery atherosclerosis in young men (17) and PAI-1 activity in healthy control subjects (18).

The aim of this study was to define, in a healthy population sample, the association between the plasma levels of fibrinogen, tPA, and PAI-1 activity on the one hand and insulin and proinsulin, measured by specific methods, on the other.

RESEARCH DESIGN AND METHODS

Subjects

The study was performed within the framework of the Northern Sweden MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) Project. In 1994, the population in the two northernmost provinces of Sweden was screened for cardiovascular risk factors. A total of 2,500 indi-

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CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; IRI, immunoreactive insulin; PAI, plasminogen activator inhibitor; tPA, tissue plasminogen activator; WHR, waist-to-hip ratio.

Table 1—*Partial correlations of concentrations of insulin and proinsulin in 165 healthy subjects, 25–74 years of age, to plasma fibrinogen and fibrinolytic variables, adjusted for age and sex*

	Fasting insulin	2-h insulin	Fasting proinsulin	2-h proinsulin
Fibrinogen	0.25 (0.10–0.39)†	0.20 (0.04–0.34)*	0.29 (0.14–0.42)‡	0.23 (0.08–0.38)†
tPA activity	−0.35 (0.21–0.48)‡	−0.30 (0.16–0.44)‡	−0.36 (0.21–0.48)‡	−0.33 (0.18–0.46)‡
PAI-1 activity	0.36 (0.22–0.49)‡	0.37 (0.23–0.50)‡	0.29 (0.14–0.42)‡	0.35 (0.21–0.48)‡

Data are partial correlation coefficients (95% CI). *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

viduals in the 25–74 year age range were randomly selected and invited by mail (from a total population of 367,000 in this age range). In total, 1,921 subjects participated in the study (76.8%). Initially, in this study, the 226 subjects who were sampled between 7:00 and 9:00 A.M. were included. After an overnight fast, a 75-g oral glucose tolerance test was performed. Five subjects (2.2%) were found to have previously unknown diabetes, and 14 (6.2%) subjects had impaired glucose tolerance. These 19 subjects were studied separately. Thereafter, subjects who were pregnant, had diabetes or a history of myocardial infarction or stroke, or were using estrogen replacement therapy, oral contraceptives, or antihypertensive agents were excluded. Complete data sets were available in 85 men and 80 women. Sampling procedures have been described previously (12).

Methods

Insulin was measured by enzyme-linked immunosorbent assay (ELISA) without cross-reactivity to human proinsulin (19). Split(32-33)- and des(31,32)-proinsulin do not react, whereas split(65-66)- and des(64,65)-proinsulin cross-react with an efficiency of 30 and 63%, respectively, on a molar basis. The detection limit was 5 pmol/l, and the working range was 5–600 pmol/l. Interassay coefficients of variation (CV) were 5–6% at 80–350 pmol/l and 11% at 30 pmol/l. Proinsulin immunoreactivity was measured by ELISA with a detection limit of 0.25 pmol/l and a working range of 0.25–100 pmol/l (20). The four major proinsulin conversion intermediates reacted 65–99% on a molar basis. C-peptide and insulin did not react. Interassay CVs were 6–7% at 5–30 pmol/l.

Plasma fibrinogen determinations were performed by a functional kinetic method (Fibrinogen Kinetic, Boehringer Mannheim, Indianapolis, IN) using a Hitachi 717 analyzer. The between-assay CV was 3.7%. A freeze-dried fibrinogen standard (21) with an assigned value of 2.4 g/l was analyzed eight times with a mean value of 2.36 g/l.

tPA and PAI-1 activity were determined by chromogenic assays: Spectrolyse/fibrin and Spectrolyse/pl kit, respectively (Biopool, Umeå, Sweden) (12). The interassay CV for tPA activity was 16% and for PAI-1 activity 11%.

Statistics

Means (geometric for transformed values) and standard deviations or 95% CI are presented. All the main study variables were positively skewed and, therefore, (ln) transformed values were used. Partial (Pearson's) correlations were calculated, adjusted for age and sex. Multiple linear regression analysis was performed by a stepwise method. Two-tailed tests were used and, because of the multiple comparisons in partial correlation analysis, significance levels >0.01 were considered insignificant. The program SPSS for Windows 6.1 was used (Chicago, IL).

RESULTS — The mean age of the participants was 48.0 ± 14.0 years and their mean BMI was 25.4 ± 3.9 kg/m². Mean fasting insulin was 35.8 pmol/l (95% CI 32.7–39.1) and proinsulin 6.9 pmol/l (95% CI 6.3–7.5). The average plasma fibrinogen level was 3.47 g/l (95% CI 3.35–3.59). Both fasting and postload levels of insulin and proinsulin correlated with fibrinogen levels (Table 1; Fig. 1). In a linear regression analysis, age, BMI, cigarette smoking, female sex, and fasting proinsulin were the significant predictors of high plasma fibrinogen levels and explained 32% of the variance in fibrinogen levels. Neither waist-to-hip ratio (WHR), blood pressure, cholesterol, HDL cholesterol, nor insulin contributed to this model. The partial regression coefficient for (ln)proinsulin on (ln)fibrinogen was 0.065 (95% CI 0.006–0.123, $P = 0.031$).

Mean tPA activity was 0.58 IU/ml (95% CI 0.52–0.65). All (pro)insulin measurements correlated inversely with tPA activity (Table 1). In a linear regression analysis, low tPA activity was independently predicted by high BMI and triglyceride levels, while increasing age predicted higher tPA activity, which could explain 31% of the variability of tPA activity. Neither insulin and proinsulin, sex, WHR, blood pressure, nor HDL cholesterol contributed significantly to the model.

Mean PAI-1 activity was 7.6 U/ml (95% CI 6.3–9.0). Both fasting and post-

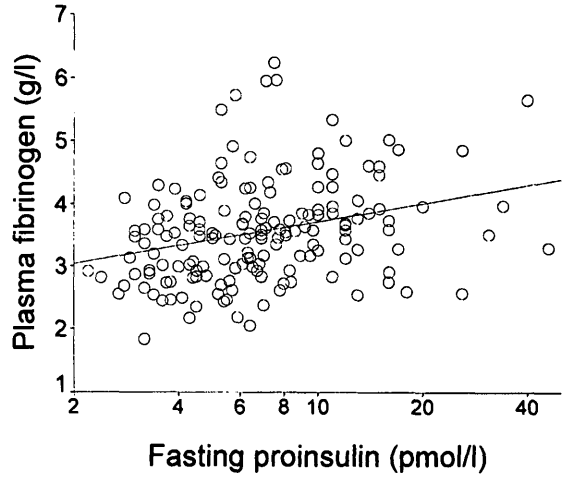

Figure 1—*Relationship between plasma fibrinogen levels and fasting proinsulin levels in 165 randomly selected healthy men and women, 25–74 years of age, in the Northern Sweden MONICA Study.*

load insulin and proinsulin correlated significantly with PAI-1 activity (Table 1). In a multiple regression analysis, high PAI-1 activity was predicted by high levels of triglycerides, postload insulin, diastolic blood pressure, and age (negatively). This model explained 29% of the variability in PAI-1 activity. Neither fasting insulin nor proinsulin levels were significant predictors of PAI-1 activity in this model.

The 19 subjects with impaired glucose tolerance or previously unknown diabetes had high insulin and proinsulin levels, 66 pmol/l (95% CI 51–85) and 14.4 pmol/l (95% CI 10.4–20.0), respectively. Fasting insulin correlated with fibrinogen ($r = 0.61$, 95% CI 0.22–0.83, $P = 0.009$), but not significantly with PAI-1 ($r = 0.39$, 95% CI −0.09 to 0.7, $P = 0.1$). High proinsulin levels were insignificantly associated with higher plasma fibrinogen levels ($r = 0.58$, 95% CI 0.17–0.82, $P = 0.014$), but stronger to low tPA activity ($r = -0.64$; 95% CI −0.26 to −0.84, $P = 0.006$) and high PAI-1 activity ($r = 0.68$, 95% CI 0.32–0.87, $P = 0.003$).

CONCLUSIONS — This is the first report regarding intact insulin and proinsulin and their relationship with fibrinolytic activity in a randomly selected healthy population sample, including women and a wide age range. Elevated levels of insulin and proinsulin were associated with higher levels of fibrinogen and PAI-1 activity and lower tPA activity, implicating a prothrombotic and hypofibrinolytic condition. Our results indicate that in a healthy population, the link between elevated insulin levels and high PAI-1 activity is attributable to that part of insulin-like immunoreactive substance that is true insulin and is not explainable by the cross-reaction by the immunoreactivity of proinsulin or its conversion intermediates in conventional IRI assay.

Only recently have more specific methods been able to show high proinsulin levels and proinsulin-to-IRI ratios in NIDDM subjects (15,22), and we found the proinsulin-to-IRI ratio to correlate strongly with the degree of β-cell dysfunction in NIDDM (15). Proinsulin and its conversion intermediates show stronger correlations than insulin itself with cardiovascular risk factors in subjects with normal glucose tolerance (23,24) and in NIDDM (25). We found proinsulin levels to be an independent predictor of increased plasma fibrinogen concentrations. These results corroborate a recent population study from the Yudkin group (23). In young men with myocardial infarction, des(31,32)-proinsulin concentrations accounted for clinically important variations in plasma fibrinogen level (18). Insulin does not increase fibrinogen synthesis in cell cultures (26), but it has been proposed that the increased nonesterified fatty acids release seen in subjects with insulin resistance would stimulate hepatic fibrinogen synthesis (27).

The present study shows an association between high intact insulin and proinsulin concentrations and fibrinolytic variables, which has not previously been shown in the general population. Although strong relationships were found between PAI-1 activity and insulin and its propeptides in young nondiabetic men with and without coronary artery disease, these were no longer significant after taking age, BMI, and lipid levels into account (18). In contrast, in patients with myocardial infarction, Gray et al. (28) found considerably stronger relations between PAI-1 and insulin propeptides than with insulin. Our hyperinsulinemic subset of subjects with impaired glucose tolerance or unknown diabetes showed much higher correlations between proinsulin and PAI-1, than between intact insulin and PAI-1, although this should be judged with caution due to the small numbers. In NIDDM patients, only split (32-33)-proinsulin was correlated with high PAI-1 activity (25).

Both insulin and proinsulin stimulates PAI-1 synthesis and release from human hepatocytes (26,29) and porcine endothelial cells (30). In rabbits, PAI-1 activity increased in response to insulin and proinsulin infusions (31). In a recent study, no effects of insulin or proinsulin were seen on PAI-1 synthesis by endothelial cells, and the proinsulin-mediated PAI release from hepatic cells was only 2–4% of the insulins' effect (32). Thus, the role of insulin-like molecules in the regulation of fibrinolysis is far from settled.

In conclusion, the relation between high PAI-1 activity and hyperinsulinemia in a healthy population is not attributable to confounding from cross-reactivity with proinsulin. Proinsulin may be important for the development of increased fibrinogen levels with subsequent higher cardiovascular risk.

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