Increased Molar Proinsulin-to-Insulin Ratio in Women With Previous Gestational Diabetes Does Not Predict Later Impairment of Glucose Tolerance

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OBJECTIVE — To evaluate if an increased proinsulin-to-insulin ratio (PI/I) in former gestational diabetes mellitus (GDM) subjects could be a marker for later impairment of glucose tolerance.

RESEARCH DESIGN AND METHODS — This study is a prospective follow-up. In a previous follow-up study of former GDM subjects 3–4 years after an index pregnancy, an increased PI/I was found also in normoglycemic nonobese former GDM subjects compared with control subjects. A 75-g oral glucose tolerance test (OGTT) was performed 3 years after the first follow-up, i.e., 6–7 years after the index pregnancy in 97 of the former GDM subjects and in 23 control subjects. A 75-g OGTT according to the World Health Organization was performed. Glucose, insulin, proinsulin, and C-peptide were determined at 0, 30, 60, 90, 120, 150, and 180 min after the glucose intake.

RESULTS — Since the first follow-up, an additional 3 in 97 (3.1%) and 15 in 97 (15.5%) of the former GDM subjects had NIDDM or impaired glucose tolerance (IGT), respectively. All control subjects still had a normal OGTT. The fasting PI/I at follow-ups 1 and 2 was significantly correlated in the former GDM subjects ($r = 0.41$, $P < 0.001$) and in the control group ($r = 0.46$, $P < 0.05$). There was no significant correlation between the PI/I in follow-up 1 and the fasting or 2-h glucose values at follow-up 2. If GDM subjects with a PI/I in the upper quartile in the first follow-up were compared with those with a lower PI/I, there were no significant differences in outcome of OGTT in the second follow-up.

CONCLUSIONS — The hypothesis that an increased fasting PI/I is a marker for later development of NIDDM or IGT in former GDM subjects could not be supported.

Women with previous gestational diabetes mellitus (GDM) run an increased risk of developing impaired glucose tolerance (IGT) or NIDDM later in life (1). Several studies have demonstrated disproportionally elevated levels of proinsulin in relation to insulin in NIDDM (2–4). In a previous study of women who formerly had GDM and control women who were examined 3–4 years after the index pregnancy, 3.4% had NIDDM and 22% had IGT (5). We also found that the fasting molar proinsulin-to-insulin ratio (PI/I) was significantly higher in normoglycemic nonobese former GDM patients compared with control subjects, while there was no difference in the level of insulin, glucose, and C-peptide or response to an oral glucose load. One could then speculate that an increased PI/I in a normoglycemic subject could be an early marker for later development of IGT and NIDDM.

To evaluate if an increased molar PI/I in former GDM could predict later development of NIDDM or IGT, a second follow-up study of the former GDM patients was performed.

RESEARCH DESIGN AND METHODS

— A previous study included a consecutive series of 239 pregnant women with GDM (6). All 239 with previous GDM were invited to participate in the first follow-up study (5). Altogether, 145 were willing to participate in the study. A group of 41 women volunteers with normal body weight and without any known risk factors for diabetes served as a control group in the first follow-up (5). All control participants had undergone normal pregnancies. During the index pregnancy, they had also been subject to a screening program for GDM including random capillary blood glucose measurements (6).

The 145 former GDM women and 41 control women participating in the first follow-up study 3–4 years after the index pregnancy were invited to participate in the present follow-up study, which was conducted 6–7 years after the index pregnancy. The five women with IDDM were not asked to participate in the second follow-up. Ninety-seven of the former GDM subjects and 23 of the control women were willing to participate in this second follow-up study. The women from the first follow-up study who were unwilling to participate in this second follow-up study were not different with regard to glucose tolerance during pregnancy, gestational age at the time of diagnosis of GDM, oral glucose tolerance test (OGTT) area under the curve, PI/I, prepregnancy weight, or infant birth weight, but had a significantly lower maternal age of 1 year younger (Table 1).

After an overnight fast, a 75-g OGTT was performed in the morning. Blood was withdrawn from an antecubital vein before and 30, 60, 90, 120, 150, and 180 min after glucose administration for determination of glucose, insulin, and

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GDM, gestational diabetes mellitus; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; PI/I, proinsulin-to-insulin ratio; WHO, World Health Organization.

Table 1—*Clinical characteristics from the index pregnancy of GDM subjects who did or did not participate in the second follow-up study*

Clinical characteristics	Participation in the second follow-up study	
	Yes	No
n	97	48
Age at OGTT (years)	31 (20–46)	30 (16–43)*
Prepregnancy weight (kg)	61 (40–120)	60 (39–130)
Gestational age at OGTT (days)	224 (54–278)	221 (70–294)
OGTT area under the curve (mmol/l)	44.3 (42.0–64.8)	44.9 (42.0–76.0)
Infant birth weight (g)	3,610 (2,120–4,830)	3,460 (1,655–4,820)
PI/I (%)	4.3 (1.5–60.3)	6.8 (1.0–146)

Data are medians (ranges) calculated by the Mann-Whitney *U* test. *$P < 0.01$.

proinsulin. The World Health Organization (WHO) criteria were used for the interpretation of the OGTT (6). Plasma glucose was measured enzymatically (glucose dehydrogenase, Merck, Darmstadt, Germany), and insulin and C-peptide were determined by radioimmunoassay (7). Proinsulin was determined by enzyme-linked immunosorbent assay (8). In this assay, C-peptide does not cross-react up to 10.0 $nmol^{-1}$. However, there was an 85–100% cross-reactivity between intact proinsulin, des 31,32, des 64,65, and split 65,66 proinsulin. Split 32,33 proinsulin cross-reacted 70%. The measured proinsulin immunoreactive material will therefore consist of conversion products as well as intact proinsulin. As the cross-reactivity of insulin with proinsulin was 90–100% in the insulin radioimmunoassay, the insulin concentrations were corrected for proinsulin immunoreactive material by subtracting the measured proinsulin concentration.

This study was approved by the regional Ethics Committee. All patients gave informed consent to participate in the study.

Statistical analysis

To compare samples not normally distributed from two independent groups, the Mann-Whitney *U* test was used. To measure associations between different variables, the Spearman rank correlation test was used. To compare group distributions, the χ^2 test was used. A *P* value <0.05 was considered to indicate a statistically significant difference.

RESULTS— Of the 97 former GDM patients who participated in this second follow-up study, 18 had IGT and 79 had normal glucose tolerance in the first follow-up study. The result of the OGTT according to WHO criteria is given in Fig. 1. Thus, 3 in 97 (3.1%) and an additional 15 in 97 (15.5%) subjects had developed NIDDM or IGT, respectively, since the first follow-up study. In total, 3 in 97 (3.1%) and 28 in 97 (28.9%) subjects had NIDDM or IGT, and 4 in 18 (22.2%) with IGT initially in the first follow-up were now classified as having a normal glucose tolerance. All the control subjects (n = 23) still had a normal OGTT.

In the former GDM subjects, there was a significant increment of blood glucose in the fasting state (0.7 mmol/l, $P < 0.001$) and at 2 h (0.7 mmol/l, $P < 0.001$) after the glucose load between the first and second follow-ups. There was no significant correlation between the PI/I at the first follow-up and the change in fasting (r = 0.02, NS) and 2-h (r = 0.06, NS) glycemia. There was a significant correlation between the fasting PI/I in the first and second follow-ups, both in the GDM group (r = 0.41, $P < 0.001$) and in the control group (r = 0.46, $P < 0.05$; Figs. 2 and 3). PI/I did not change significantly in the former GDM subjects, while there was a significant increase in the control subjects between follow-ups 1 and 2 (Table 3).

There was no correlation in the GDM group between the PI/I in the first follow-up and the fasting blood glucose value (r = 0.04, NS) or the 2-h blood glucose value in the OGTT at the second follow-up. If the GDM patients with a PI/I value in the first follow-up in the upper quartile (≥8.54%, n = 24) were compared with those with a PI/I value below the upper quartile (<8.54%, n = 73) with regard to outcome of OGTT in the second follow-up, there were no significant differences regarding fasting or 2-h blood glucose value or rate of IGT or NIDDM (Table 2).

CONCLUSIONS— This study confirms that women with previous GDM run an increased risk of developing impaired glucose tolerance or NIDDM later in life (1,5). Since the first follow-up study 3–4 years after the index pregnancy, a further 17.5% of the women in the follow-up had developed NIDDM or IGT at the second follow-up 6–7 years after the index pregnancy. This observation is in contrast to that in the control women, who all still had a normal OGTT. The diagnosis of IGT according to the WHO criteria should be interpreted with some caution because 4 in 18 patients who initially were classified as having IGT had a normal glucose tolerance at the second follow-up.

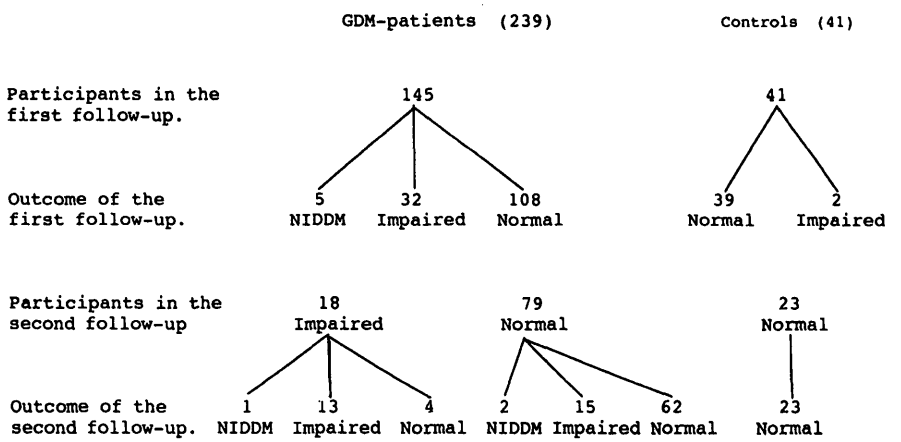

Figure 1—*Outcome of OGTT in the first and second follow-up studies of former GDM patients.*

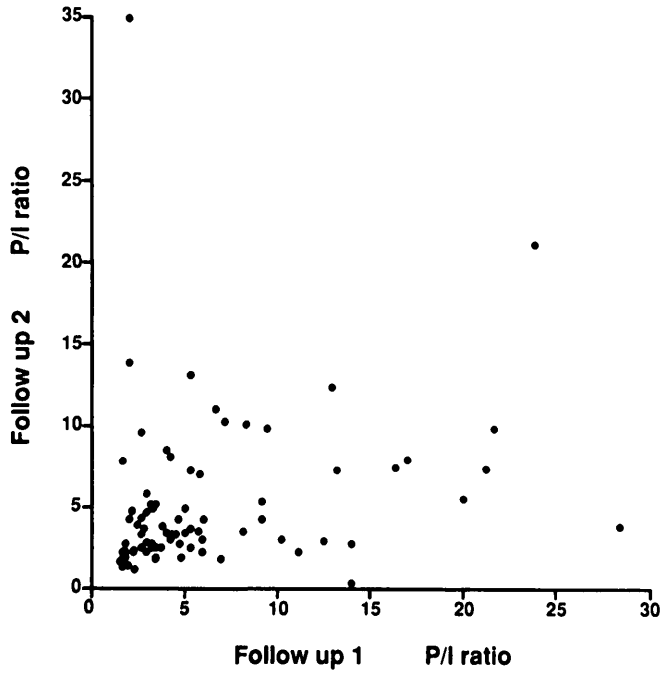

Figure 2—*The correlation between the PI/I in the first and second follow-ups among GDM patients (n = 97, r = 0.41, P < 0.001).*

Disproportionally elevated proinsulin in relation to insulin in patients with NIDDM has been reported (2–4). Increased demand for insulin has been proposed as one explanation (4). However, other conditions such as obesity associated with increased insulin demand have not been shown to be associated with an increased PI/I (10). It has also been proposed that an elevated PI/I is associated with increased deposition of amyloid in the β-cell and reflects a defect in β-cell function (11). Kahn et al. (10) have recently reported that in a primate model of diabetes, disproportional proinsulinemia was a manifestation of β-cell damage from streptozocin that was not exacerbated by insulin resistance or hyperglycemia (12).

In a previous study (5), we found an increased PI/I in nonobese normoglycemic former GDM patients compared with control subjects. When including all former GDM patients in multiple linear regression, we were unable to find any significant correlation between the PI/I and BMI, waist and hip circumference, or glucose area under the curve. Levy et al. (13) studied mild NIDDM and found subnormal insulin response but normal proinsulin response compared with a nondiabetic control group. They concluded that the relative increase in proinsulin in mild NIDDM was a consequence of impaired insulin release. Their findings could not explain our previous study (5) where nonobese former GDM subjects with impaired glucose tolerance had both elevated insulin and proinsulin levels compared with the nondiabetic control group. When nonobese, normoglycemic former GDM subjects were selected to match a control group regarding age, BMI, and area under the glucose curve, the PI/I was significantly increased in the former GDM subjects (5). The increased PI/I was due to increased proinsulin levels and normal insulin levels. Thus, the increased proinsulin secretion in former GDM subjects with impaired or normal glucose tolerance could not be explained by impaired insulin secretion. This observation suggested that the increased PI/I in former GDM patients only was associated with the variable former GDM per se and was of no predictive value for future glucose tolerance. The increased PI/I (or just increased PI) could be a marker of β-cell dysfunction that is stable in daily life but that during stress (e.g., pregnancy) results in impaired glucose tolerance or represents a family trait that is unrelated to disturbances in glucose metabolism. This last hypothesis is supported by the finding that healthy siblings of patients with IDDM who have been discordant for >6 years have elevated proinsulin levels independently of HLA identity (14). Similar findings have also been reported in nondiabetic monozygotic twins of IDDM patients (15).

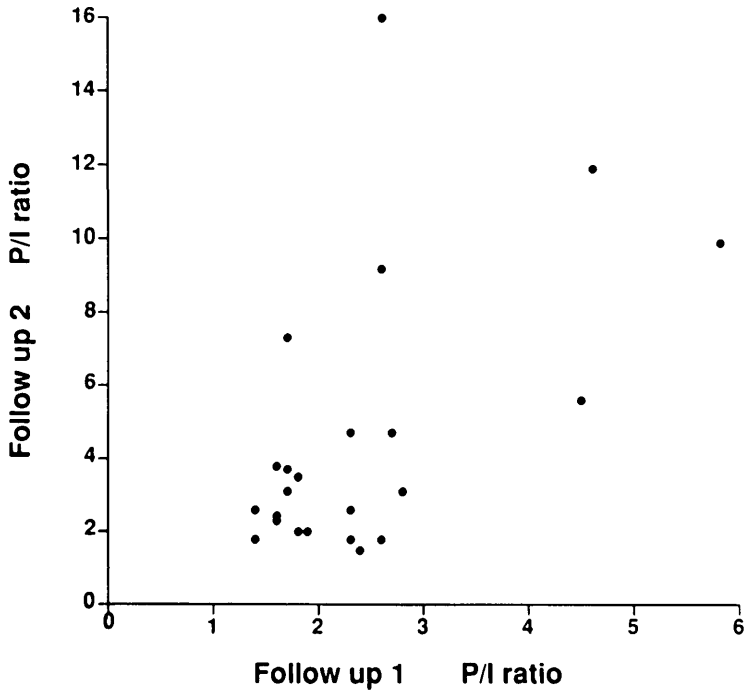

Figure 3—*The correlation between the PI/I in the first and second follow-up studies among control subjects (r = 0.46, P < 0.05).*

Table 2—*Outcome of the OGTT in the second follow-up in relation to the PI/I in the first follow-up: women with a PI/I value in the upper quartile compared with those with a PI/I value below the upper quartile*

OGTT in second follow-up	PI/I in first follow-up		
	≥8.54%	<8.54%	*P* value
n	24	73	—
Fasting β-glucose	4.8 (4.0–7.2)	5.0 (2.8–18.5)	NS*
2-h β-glucose	5.9 (4.0–8.3)	5.7 (3.4–30.5)	NS*
Normal	18 (75)	48 (66)	NS†
IGT	6 (25)	22 (30)	NS†
NIDDM	0 (0%)	3 (4%)	NS†

Data are medians (ranges) or *n* (%) calculated by *Mann-Whitney *U* test or †χ^2 test.

The results of the present study demonstrate that an increased PI/I in former GDM patients to some extent seems to be a consistent finding because there was a significant correlation between the PI/I in the first and second follow-up studies. Because there was no association between the PI/I in the first follow-up and the outcome of the OGTT in the second follow-up, we could not support the hypothesis that an elevated PI/I is a marker for future development of glucose intolerance in former GDM patients.

Table 3—*PI/I in the first and second follow-up in former GDM and control subjects*

	PI/I		
	First follow-up	Second follow-up	*P*
GDM subjects (%)	4.3 (1.5–60)	3.6 (0.4–35)	NS
Control subjects (%)	2.3 (1.4–5.8)	3.1 (1.5–16)	<0.01*
P	<0.001†	NS†	—

Data are medians (ranges). *Wilcoxon's. †Mann-Whitney *U* test.

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