Elevated Proinsulin Levels Related to Islet Cell Antibodies in First-Degree Relatives of IDDM Patients

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OBJECTIVE — To assess whether proinsulin levels are elevated in first-degree relatives of insulin-dependent diabetes mellitus (IDDM) patients and whether there is a relationship between proinsulin levels and the occurrence of immunological markers.

RESEARCH DESIGN AND METHODS — Fasting proinsulin concentrations were measured in 85 first-degree relatives (54 siblings, 20 parents, 11 children) of IDDM patients and in 90 age- and weight-matched control subjects with no family history of diabetes mellitus.

RESULTS — Fasting proinsulin levels (median, 25th, and 75th percentiles) were 8 pM (range 3.2–14 pM) in first-degree relatives and 1.7 pM (range 1.7–4 pM) in control subjects ($P < 0.0001$). Proinsulin was significantly elevated in siblings (7.2 pM, range 3.8–15 pM; $P < 0.0001$), parents (9.8 pM, range 6.4–13 pM; $P < 0.0001$), and children (6.6 pM, range 1.8–12 pM, $P = 0.04$) compared with control subjects but without differences between these groups. Islet cell antibody positive (ICA^+) IDDM relatives had significantly higher proinsulin levels than ICA^- (16 pM; range 7.2–25 vs. 6.9 pM, range 3.1–12 pM; $P = 0.02$). There was no difference between individuals with and without insulin autoantibodies. No difference in proinsulin levels was observed if the relatives were subdivided according to HLA-DR sharing with the diabetic proband.

CONCLUSIONS — Fasting proinsulin concentrations were raised not only in siblings but also in parents and children of IDDM patients. Because proinsulin is more elevated in ICA^+ than in ICA^- subjects, increased proinsulin levels could reflect minor β-cell damage due to previous immunological attack.

It is widely accepted that autoimmune mechanisms are involved in the pathogenesis of insulin-dependent diabetes mellitus (IDDM) (1). Islet cell antibodies (ICAs) reacting with antigens in the cytoplasm of islet cells are present years before the onset of clinical diabetes and serve as serological markers of ongoing β-cell destruction in predisposed individuals (2). Several studies in discordant monozygotic twins or first-degree relatives of IDDM patients provide evidence that ICA^+ relatives are more likely to develop overt diabetes than are ICA^- (3–6).

Recently, elevated fasting plasma proinsulin levels have been demonstrated in discordant monozygotic twins (7) and siblings (8) of IDDM patients. There are several possible explanations for this altered β-cell function. It could mean that these subjects are in a "prediabetic" state, supported by the finding of increased proinsulin levels in recent-onset IDDM patients (9,10). However, the fact that one-third of unaffected siblings of IDDM patients displayed elevated proinsulin levels suggests other explanations. Increased proinsulin secretion could, e.g., reflect sequelae after a previous β-cell attack not necessarily leading to diabetes, or alternatively, represent a family trait related to the development of diabetes (8).

Increased proinsulin secretion has also been described in poorly controlled non–insulin-dependent diabetes mellitus (11–13) and in some conditions with impaired glucose tolerance (14,15). In these circumstances, the hypersecretion of proinsulin relative to insulin is probably due to an increased drive on the β-cell.

The aim of this study was to evaluate whether fasting proinsulin levels are also elevated in children and parents of IDDM patients compared with the already demonstrated elevation in siblings and to investigate the relationship between proinsulin levels and the occurrence of ICA in these individuals.

RESEARCH DESIGN AND METHODS

— Eighty-five (46 male, 39 female) healthy first-degree relatives of IDDM patients from 45 families participating in the prospective Basel family study were investigated (16,17). Their

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RECEIVED FOR PUBLICATION 16 APRIL 1991 AND ACCEPTED IN REVISED FORM 25 SEPTEMBER 1991.

mean ± SD body mass index (BMI) was 22.5 ± 2.4 kg/m^2 and age was 38 ± 16 yr. The subjects were recruited over a period of 5 yr (April 1981 to April 1986), and follow-up was for an average of 58.6 mo (415 proband yr). The study population consisted of 54 siblings (age 33 ± 14 yr, BMI 22.4 ± 2.7 kg/m^2), 20 parents (age 57 ± 9 yr, BMI 23.7 ± 1.9 kg/m^2), and 11 children (age 27 ± 9 yr, BMI 20.8 ± 2.1 kg/m^2). Twelve (8 siblings, 3 parents, 1 child) were ICA$^+$ (mean titer 58 Juvenile Diabetes Foundation [JDF] U), and 73 were ICA$^-$. Probands were considered ICA$^+$ if ICAs were detected on three or more occasions during the observation period. Of the 12 ICA$^+$ subjects, 4 have been persistently positive (2–4 yr, mean titer 38 JDF U), 2 have acquired ICA during the study, and 5 who were initially ICA$^+$ (2–4 yr) lost them on follow-up (mean titer 72 JDF U). In one proband, ICAs have been found intermittently (80 JDF U). At the time of proinsulin determination, 7 were ICA$^+$ (mean titer 49 JDF U). Insulin autoantibodies were detected in 11 subjects, of which 1 was ICA$^+$ and 10 were ICA$^-$. Seventy-six relatives had been typed for HLA-DR specificities (17). Eighteen (4 ICA$^+$) were HLA-DR identical (12 siblings, 4 parents, 2 children), 54 (8 ICA$^+$) were haploidentical (30 siblings, 16 parents, 8 children), and 4 were HLA-DR nonidentical siblings with regard to the diabetic proband. The relatives were compared with 90 control subjects (39 male) matched for age (39 ± 16 yr) and BMI (22.1 ± 2.5 kg/m^2) and without family history of diabetes mellitus. All subjects were studied after an 8-h overnight fast. Blood was drawn into heparin tubes, and plasma was stored at −20°C until assayed.

Assays

Whole-blood glucose was analyzed by a glucose oxidase method (YSI, Yellow Springs, OH), HbA_{1c} was determined chromatographically. Plasma insulin was measured by an enzyme-linked immunosorbent assay (ELISA) with guinea pig anti-insulin polyclonal antibodies with a detection limit of 20 pM. Values below the detection limit were defined as 19 pM. Interassay coefficients of variation (C.V.) were 4.3 and 7.2% at 181 and 360 pM, respectively. Intra-assay C.V. was <5%. Because the cross-reactivity with intact proinsulin was 100%, insulin values were corrected for measured proinsulin values.

Proinsulin was measured by an ELISA technique in which anti-insulin antibodies were used as the coating antibody, and C-peptide F(ab)'$_2$ fragment peroxidase-coupled antibodies were used as detector antibodies (18). The detection limit of the assay was 1.8 pM when diluted 33%. Values below the election limit were defined as 1.7 pM. Interassay C.V. was 9.8% at 18 pM. Insulin >650 pM interfered in the assay. In this case, further dilution was performed. C-peptide did not cross-react <10,000 pM. The main intermediates of proinsulin conversion, 65–66 split, 32–33 split, des-64/65- and des-31/32-proinsulin all cross-react 80–100% in the assay.

Cytoplasmic ICAs were determined by indirect immunofluorescence on unfixed cryostat sections of blood group O human pancreas specimens (19). ICA titers were assessed by serial dilution to end point with a dilution of 1:2. Positive samples were further tested for complement-fixing ability with an anti-human C3 conjugate (20). Our ICA assay was compared in the ICA Proficiency Testing Program sponsored by the Juvenile Diabetes Foundation. By analysis of 40 coded serum samples, sensitivity, specificity, and laboratory validity were 100%. In our laboratory, 10 JDF U correspond to an ICA titer of 1:2–1:4.

Autoantibodies to human insulin were measured by means of a direct, immunospecific ELISA including displacement studies with an excess of human insulin as described previously (21).

Statistical analysis

Values are given as median, 25th, and 75th percentiles unless otherwise stated. Mann-Whitney rank-sum tests, Kruskal-Wallis tests, χ^2 tests, and Spearman's rank correlation coefficient tests were used where appropriate. $P \leq 0.05$ was considered significant.

RESULTS — Fasting proinsulin concentrations were significantly higher in first-degree relatives (8 pM, range 3.2–14 pM) than in control subjects (1.7 pM, range 1.7–4 pM; $P < 0.0001$). Proinsulin levels were equally elevated in siblings (7.2 pM, range 3.8–15 pM; $P < 0.0001$); parents (9.8 pM, range 6.4–13 pM; $P < 0.0001$); and children (6.6 pM, range 1.8–12 pM; $P = 0.04$) compared with control subjects but without differences between these groups (Fig. 1). Because many (48 of 90) control subjects had proinsulin values below the detection limit of the assay, IDDM relatives and control subjects were also compared by χ^2 analysis with cutoff values at the detection limit of the assay. Also, with this statistical approach, a significant difference ($P < 0.0001$) between these groups was found.

ICA$^+$ subjects had significantly higher proinsulin concentrations (16 pM, range 7.2–25 pM) than ICA$^-$ (6.9 pM, range 3.1–12 pM; $P = 0.02$; Fig. 2). If only the subjects who were ICA$^+$ at the time of proinsulin determination ($n = 7$) were considered for calculation, there was still a significant difference between the ICA$^+$ and ICA$^-$ groups (18 pM, range 5.2–28.2 vs. 6.9 pM, range 3.1–12 pM; $P = 0.02$). The difference in proinsulin levels between ICA$^+$ and ICA$^-$ individuals was also observed within the siblings subgroup, median proinsulin levels being 16 pM (range 7.2–25 pM) for ICA$^+$ siblings ($n = 8$) and 6.8 pM (range 3.2–12 pM) for ICA$^-$ siblings ($n = 46$, $P = 0.04$). In addition, despite the small number of ICA$^+$ parents, there was a tendency towards higher proinsulin values in the three ICA$^+$ (20 pM, range 12.2–36.5 pM) compared with the ICA$^-$ parents (8.1 pM, range 5.3–12 pM; $P = 0.10$). No difference was found between IAA$^+$ and

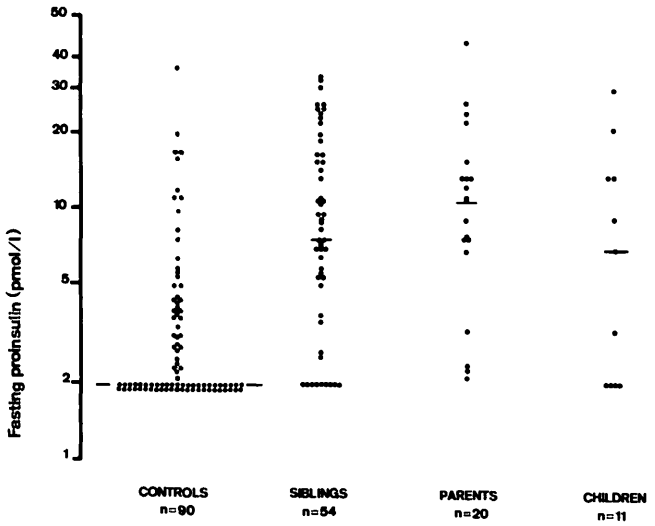

Figure 1—*Fasting proinsulin concentrations in healthy control subjects without family history of diabetes and first-degree relatives of insulin-dependent diabetic patients subdivided into parents, siblings, or children of the diabetic proband. All 3 groups had significantly higher proinsulin values than control subjects. Bars, medians.*

IAA$^-$ relatives (8.1 vs. 7.6 pM; Fig. 2). During the observation period, one ICA$^+$ monozygotic twin and one ICA$^-$ sibling progressed to overt diabetes. Their proinsulin levels were 14 and 6.6 pM, respectively.

To exclude a clustering effect due to inclusion of several members of the same family; data were also subjected to analysis selecting one member per family at random (n = 45). Analyzing the randomly selected data also gave proinsulin levels that were significantly higher in the relatives than in control subjects ($P < 0.0001$). The values of the ICA$^+$ (n = 8) and ICA$^-$ (n = 37) individuals were 17 pM (range 7.2–25.5 pM) and 6.7 pM (range 2.2–12 pM, $P = 0.03$), respectively.

If the relatives were subdivided according to HLA-DR sharing with the diabetic proband, each group had significantly elevated proinsulin levels compared with the control group. Median proinsulin values were 6.8 pM (range 3.2–14 pM, $P < 0.0001$) for HLA-DR identical, 7.2 pM (range 3.1–12 pM, $P < 0.0001$) for HLA-DR haploidentical, and 15.5 pM (range 9.5–20.5 pM, $P = 0.0003$) for nonidentical subjects with no significant differences between the groups (Kruskal-Wallis test; $P = 0.4$; Fig. 3). This was also the case in the siblings subgroup (6.5, 6.9; and 15.5 pM for HLA-DR identical, haploidentical and nonshared, respectively, $P = 0.39$).

Plasma insulin and blood glucose values did not differ between control subjects and IDDM relatives (Table 1). We found significant correlation between fasting proinsulin and glucose concentrations in neither the control subjects ($\rho = 0.20$, $n = 81$, $P = 0.07$), in the relatives as a whole group ($\rho = 0.07$, $n = 85$, $P > 0.1$), nor if subdivided according to ICA status (ρ = 0.11 and 0.02 for ICA$^+$ and ICA$^-$ individuals, respectively). There was no correlation between BMI and proinsulin levels in both the relatives and control subjects.

CONCLUSIONS— This study demonstrates that elevated fasting proinsulin levels are found not only in siblings of patients with IDDM but also in their parents and children, suggesting that this may be a feature of any first-degree relative of IDDM patients. Fasting proinsulin is increased irrespective of HLA-DR sharing with the diabetic family member. The most important finding of our study is that individuals who are or had been ICA$^+$ exhibited significantly higher proinsulin levels than ICA$^-$ individuals, despite similar blood glucose and insulin concentrations. Thus, our results indicate that, in this population, namely

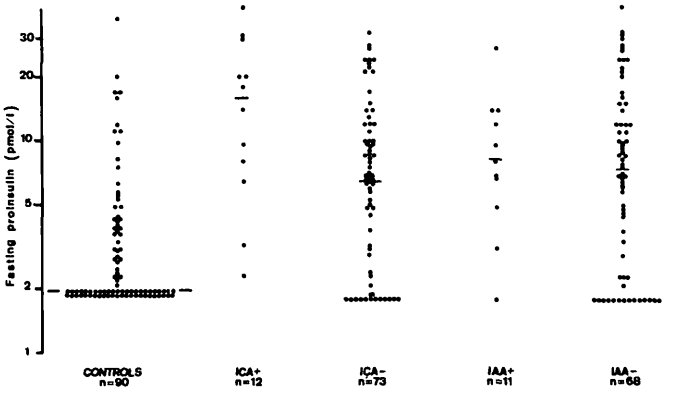

Figure 2—*Fasting proinsulin concentrations in healthy control subjects without family history of diabetes and first-degree relatives of insulin-dependent diabetic patients subdivided according to islet cell antibody (ICA) and insulin autoantibody status. All groups of relatives had significantly higher proinsulin concentrations than control subjects, with ICA$^+$ individuals having significantly higher levels than ICA$^-$ individuals. Bars, medians.*

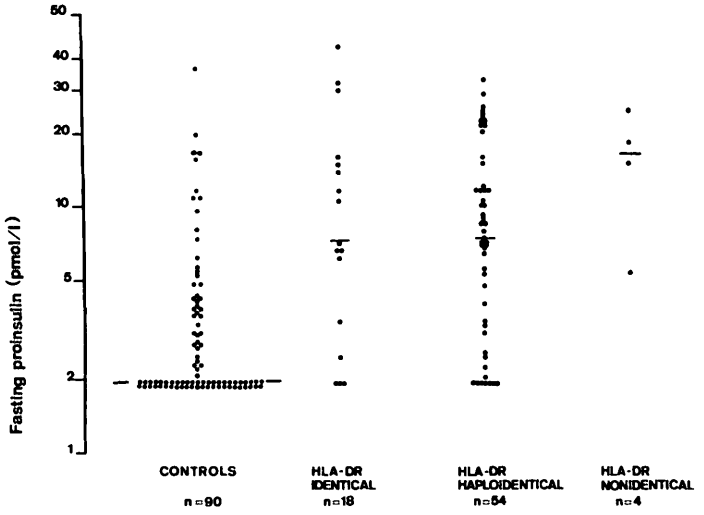

Figure 3—*Fasting proinsulin concentrations in healthy control subjects without family history of diabetes and first-degree relatives of insulin-dependent diabetic patients subdivided according to HLA-DR sharing with diabetic proband. All 3 groups had significantly higher proinsulin concentrations than control subjects with no difference between the 3 groups. Bars, medians.*

first-degree relatives of IDDM patients, increased proinsulin secretion could indeed reflect some degree of β-cell dysfunction due to previous immunological attack.

The fact that minor changes in β-cell function were—though to a lesser degree—also observed in "immunologically negative" IDDM relatives indicates an increased vulnerability of their β-cells (7,8). In these susceptible individuals, any exogenous or endogenous factor could easily affect β-cell function not necessarily resulting in the presence of cytoplasmic ICA (22). Furthermore, at this early stage of the disease process, with available techniques, immune markers may be absent or only transiently positive (23).

The first-degree relatives studied here have a low risk of progressing to overt diabetes because their follow-up time is long past the high-risk period. Furthermore, their average ICA titers were <40 JDF U, and, in 50% of the cases, ICA were only intermittently present. Thus, although raised proinsulin levels may be a marker of β-cell dysfunction, its predictive value for diabetes development is presumably low (24).

Our results are somewhat at variance with a study by Heaton et al. (7), who found proinsulin levels to be equally elevated in both ICA^+ (complement-fixing) and ICA^- identical twins of IDDM patients. However, the two studies are not fully comparable because the populations studied differ in both terms of age and immunological status. Heaton et al. investigated a small and homogenous group of children with or without complement-fixing ICA, whereas our study population comprised mainly adult first-degree relatives who were positive for cytoplasmic ICA in a long-term follow-up study. Furthermore, different proinsulin assays with different immunoreactive properties were used (7,18,25).

Because there is no evidence of a changed metabolic clearance rate of proinsulin in IDDM patients compared with nondiabetic individuals (26,27), it seems unlikely that elevated proinsulin levels in IDDM relatives are due to alterations in the metabolic clearance of proinsulin. The raised proinsulin concentrations in these individuals most probably result from an increased secretion of immature insulin granules or perturbed proinsulin conversion or both. We have recently demonstrated that interleukin-1, which may be involved in the pathogenesis of β-cell destruction in IDDM, adversely affects proinsulin conversion in isolated

Table 1—*Metabolic characteristics of first-degree relatives of insulin-dependent diabetic patients and control subjects*

	Glucose (mM)	HbA_{1c} (%)	Insulin (pM)	Proinsulin (pM)		
Relatives						
All	4.5 (4–4.9)	4.3 (3.9–4.6)	42 (21–70)	8 (3.2–14)		
ICA^+	4.6 (4.5–5.2)	4.2 (3.9–4.5)	44 (19–128)	16 (7.2–25)	$P = 0.02$	$P < 0.0001$
ICA^-	4.5 (4–4.9)	4.3 (3.9–4.6)	41 (25–69)	6.9 (3.1–12)		
Control subjects	4.6 (4–5.3)*	ND	40 (19–80)	1.7 (1.7–4)		

Data are median, 25th, and 75th percentiles.
ICA, islet cell antibody; ND, not determined.
*n = 81.

rat pancreatic islets (28). Thus, increased proinsulin levels in the circulation could reflect similar changes in humans and offers a possible explanation for the demonstrated relation to ICA positivity.

In summary, our data confirm and extend the observation that minor changes in β-cell function, reflected by increased fasting proinsulin levels, are a frequent finding in relatives of IDDM patients. These changes are more pronounced in ICA^+ individuals, suggesting that they could indeed be a consequence of previous β-cell attack.

Acknowledgments— We thank M. Spörri and M. Marti for secretarial help.

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