

## Proinsulin levels in newborn siblings of Type 1 (insulin-dependent) diabetic children and their mothers

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**Summary.** Elevated proinsulin levels have been observed in healthy first degree relatives of Type 1 (insulin-dependent) diabetic patients. This elevation could reflect a sequelae after a previous attack on the beta-cells not necessarily leading to diabetes, or represent a family trait related to the development of diabetes. When cord plasma levels of proinsulin, insulin and C-peptide from 14 newborn siblings of Type 1 diabetic patients were compared with 21 newborn control siblings unrelated to diabetic subjects, no differences were observed. Neither were any differences observed between their mothers at delivery when comparing the same parameters. In cord plasma the proinsulin levels (median and range) were higher than those in plasma from 35 adult fasting

women unrelated to diabetic subjects (10, 5–83 pmol/l vs 4, 2–33 pmol/l;  $p < 0.001$ ) whereas the C-peptide levels (median and range) were lower (0.20, 0.11–0.56 nmol/l vs 0.37, 0.21–0.69 nmol/l;  $p < 0.001$ ). No differences in insulin levels using a highly specific insulin assay were observed. The results suggest that newborn children have high proinsulin and low C-peptide levels unrelated to heredity of diabetes and that the previously described elevated proinsulin level observed in older first degree relatives of diabetic subjects occurs later in life.

**Key words:** Type 1 (insulin-dependent) diabetes mellitus, proinsulin, C-peptide, insulin, infant (newborn), siblings.

First degree relatives of patients with Type 1 (insulin-dependent) diabetes mellitus, in particular relatives who are HLA-identical with the diabetic proband or monozygotic twins, have an increased risk of developing Type 1 diabetes [1, 2].

Elevated proinsulin levels have been observed in healthy monozygotic twins [3], siblings [4–6], as well as parents and children [6] of Type 1 diabetic patients. The elevated proinsulin level may indicate that the subjects are in a late “prediabetic” phase, a concept supported by the findings of increased proinsulin levels in newly-diagnosed Type 1 diabetic patients [7, 8]. Another possibility is that the increased proinsulin levels may reflect the sequelae of a previous attack on the beta-cells not necessarily leading to diabetes. Supporting this view is our observation that 89 out of 93 healthy, 10–28-year-old, islet cell antibody (ICA)-negative siblings of Type 1 diabetic patients had fasting proinsulin levels that were above the median value for control subjects who had no diabetes among their first degree relatives [5]. Furthermore, the proinsulin levels were not affected by the HLA-status which the sibling shared with the diabetic proband. Only two of the ICA-negative siblings developed diabetes or impaired glucose tolerance during at least 2.5 years of fol-

low-up. A third possibility is that elevated levels of proinsulin represent a family trait related to development of diabetes but not to HLA-status. The latter question could be explored by studies of newborn siblings of Type 1 diabetic patients.

The aim of this study was to compare beta-cell activity assessed by determining proinsulin, insulin and C-peptide in newborn siblings of diabetic patients and their mothers at delivery with control subjects who, among their first degree relatives, had no diabetes. We also compared beta-cell function in a group of non-pregnant mothers of newly-diagnosed Type 1 diabetic patients with age-matched non-pregnant women without diabetes among their first degree relatives.

### Subjects and methods

#### Subjects

This study was approved by the ethics committee of the Karolinska Institute in Stockholm. Informed consent to participate in the study was given.

The study comprised 14 newborn siblings of Type 1 diabetic children and 21 newborn siblings with no diabetes among their first

**Table 1.** Characteristics of subjects – mothers of Type 1 diabetic patients and control subjects at delivery and their newborn children, fasting non-pregnant mothers of Type 1 diabetic patients and control subjects

	Subjects at delivery		Non-pregnant subjects	
	Relatives of diabetic patients	Control subjects	Mothers of diabetic patients	Control subjects
Number	14	21	30	35
<i>Adult women</i>				
Age (years)				
Mean	33.9	32.2	39.1	38.3
SD	4.3	3.4	5.6	7.5
Range	28.2–40.8	25.9–37.8	26.6–49.4	24.3–51.2
BMI (kg/m <sup>2</sup> )				
Mean	23.7 <sup>a</sup>	21.4	21.5	22.0
SD	3.2	2.6	1.4	1.7
Range	19.4–29.1	17.7–27.1	18.4–24.0	18.3–24.7
Gestational age (weeks)				
Mean		40.3		
SD	1.1	1.0		
Range	37.9–41.0	39.0–43.1		
<i>Children</i>				
Male	5	12		
Female	9	9		
Birthweight (kg)				
Mean	3.6	3.7		
SD	0.5	0.4		
Range	2.5–4.3	3.0–4.4		

<sup>a</sup>  $p = 0.02$  vs control subjects at delivery. The body mass index (BMI) values assessed before pregnancy

degree relatives. All their mothers had had normal and uneventful pregnancies, labours and deliveries. None, except one, had had glucosuria during the pregnancy. The mother with glucosuria had a normal glucose tolerance test in gestation week 34 (75-g oral glucose load, fasting blood glucose 4.5 mmol/l and after 120 min 7.2 mmol/l). All mothers were delivered vaginally. One child in the control group was delivered in breech presentation. For all the newborn children, the Apgar score was more than 8 at 1 min and 10 at 5 min after delivery. The characteristics of the mothers and their newborn children are summarized in Table 1.

We also examined 30 healthy, non-obese (body mass index < 25 kg/m<sup>2</sup>) mothers of children with Type 1 diabetes. Their children had become diabetic at an average age of 9.7 years (range 0.9–16.0 years) and at an average of 2.1 years (range 0.2–4.7 years) before the study. The control group comprised 35 healthy age-matched women without diabetes among their first degree relatives. None of the subjects studied was taking any medication. The time of blood-sampling in relation to their last menstrual period was not significantly different between the mothers of diabetic children and control women (16 ± 9 vs 12 ± 9 days, mean ± SD). Their age and body mass indices are given in Table 1.

## Methods

Mixed arterio-venous cord blood, and venous blood from the mother, were taken as close to the delivery as possible and always within 15 min. The venous blood samples from the other groups i.e. mothers of diabetic children and the control subjects were taken the morning after an overnight fast.

The samples were kept on ice until centrifugation. Plasma was frozen at –20°C until analysed. Plasma glucose was measured by a

glucose oxidase method with a Beckman Glucose Analyzer 2 (Beckman Instruments Inc, Fullerton, Calif., USA). C-peptide was determined by an RIA method using antibody M1230, and second antibody separation [9, 10]. Insulin was measured by an ELISA method with a detection limit of 10 pmol/l [11]. Interassay coefficient of variation was 8.6% at 38 pmol/l. Human C-peptide, proinsulin, split(32-33)- and des(31,32)-proinsulin did not crossreact in the assay whereas split(65-66)- and des(64,65)-proinsulin crossreacted to 30% and to 63% respectively. Proinsulin was measured by an ELISA technique [12]. The detection limit was 1.8 pmol/l when diluting serum 1:3. Interassay coefficient of variation was 9.8% at 18 pmol/l. Human C-peptide (10 nmol/l) did not crossreact in the assay. The cross-reactivities of split (32-33)-, des(31,32)-, split(65-66)-, and des(64,65)-proinsulin were 60–70%, 70–90%, 90–100% and 90–100%, respectively, from 5 to 80 pmol/l.

## Statistical analysis

Variables that were not normally distributed, C-peptide, insulin, proinsulin and the proinsulin/insulin and proinsulin/C-peptide ratios, were compared using non-parametric statistical methods, i.e. the Mann-Whitney U-test and Spearman's correlation coefficient test. Normally distributed variables were analysed using the Student's *t*-test. A *p*-value less than 0.05 was considered statistically significant. The number of newborn children studied was calculated to be sufficient to detect a difference equal to the variance or more with a power of 0.8 and a *p*-value of 0.05. As the hypothesis was that proinsulin values might be higher but not lower in relatives of Type 1 diabetic patients compared to control subjects a one-sided test was used, giving a necessary sample size of 13 in each group [13].

## Results

### *Beta-cell activity in newborn siblings of Type 1 diabetic patients and their mothers at delivery*

Individual values for insulin and proinsulin varied markedly. Glucose, C-peptide, insulin, proinsulin concentrations and the ratio proinsulin/insulin and proinsulin/C-peptide in cord plasma did not differ between newborn siblings of diabetic patients and control subjects (Table 2). At delivery, the values for glucose, C-peptide, insulin, proinsulin and the ratio of proinsulin/insulin and proinsulin/C-peptide did not differ between mothers of diabetic patients and control subjects (Table 2).

The maternal plasma glucose value correlated with the glucose value in cord plasma ( $r_s = 0.39$ ,  $p = 0.02$ ). Proinsulin values in maternal plasma and cord plasma correlated ( $r_s = 0.40$ ,  $p = 0.02$ ), but no significant correlations were observed for C-peptide or insulin.

### *Beta-cell activity in fasting non-pregnant mothers of Type 1 diabetic patients*

Glucose, C-peptide, insulin, proinsulin concentration and the proinsulin/insulin and proinsulin/C-peptide ratios did not differ between fasting non-pregnant mothers of diabetic patients and control subjects (Table 2). There was no correlation between proinsulin levels and the time that had elapsed since the diabetic proband developed diabetes.

**Table 2.** Glucose, C-peptide, insulin and proinsulin values and the proinsulin/insulin and proinsulin/C-peptide ratios of study subjects – mothers of Type 1 diabetic patients at delivery, their newborn children, healthy fasting non-pregnant mothers of Type 1 diabetic patients and control subjects

	Subjects at delivery		Newborn children		Non-pregnant subjects	
	Mothers of diabetic patients	Control subjects	Siblings of diabetic patients	Control subjects	Mothers of diabetic patients	Control subjects
Number	14	21	14	21 <sup>b</sup>	30	35
<i>Glucose (mmol/l)</i>						
Mean	5.7	6.3	4.6	5.0	5.0	5.0
SD	1.3	1.1	0.9	0.8	0.4	0.6
Absolute range	3.2–8.1	4.6–8.6	2.7–6.0	3.6–6.9	4.3–6.0	4.0–6.4
<i>C-peptide (nmol/l)</i>						
Median	0.79	0.72	0.25	0.20 <sup>a</sup>	0.36	0.37
Quartile range	0.58–0.80	0.62–0.88	0.21–0.32	0.16–0.29	0.32–0.44	0.28–0.42
Absolute range	0.46–2.84	0.43–1.44	0.14–0.40	0.11–0.56	0.24–0.49	0.21–0.69
<i>Insulin (pmol/l)</i>						
Median	123	92	25	16	23	21
Quartile range	67–192	75–155	10–34	10–25	13–30	10–26
Absolute range	51–771	21–475	10–82	10–58	10–44	10–56
<i>Proinsulin (pmol/l)</i>						
Median	12	14	13	10 <sup>a</sup>	4	4
Quartile range	5–20	7–22	11–16	9–15	3–7	2–7
Absolute range	3–38	4–75	4–28	5–83	2–68	2–33
<i>Ratio (%)</i>						
<i>proinsulin/insulin</i>						
Median	7	13	46	79 <sup>a</sup>	21	18
Quartile range	4–13	7–22	38–80	53–100	14–36	11–39
Absolute range	3–54	3–43	13–160	29–280	6–567	6–200
<i>Ratio (%)</i>						
<i>proinsulin/C-peptide</i>						
Median	1.2	1.8	5.5	4.9 <sup>a</sup>	1.2	1.3
Quartile range	0.6–2.8	1.0–2.7	3.8–6.9	4.5–8.6	0.8–2.0	0.6–1.8
Absolute range	0.5–6.6	0.7–10.4	1.5–10.7	2.7–25.5	0.4–15.8	0.26–10.5

<sup>a</sup>  $p < 0.001$  vs adult non-pregnant control subjects. <sup>b</sup>  $n = 20$  for C-peptide and ratio C-peptide/proinsulin

### Beta-cell activity in newborn children compared to adult subjects

Though the glucose and insulin levels did not differ the C-peptide values were lower and the proinsulin values higher in newborn children compared to adults (Table 2). The proinsulin/insulin ratio as well as the proinsulin/C-peptide ratio were higher in cord blood (Table 2). The median molar ratio of C-peptide to insulin was lower in cord plasma (11.8, range 5.8–21.3) compared to that of fasting adults (19.1, range 6.7–43.0)  $p = 0.003$ .

### Discussion

In the present study of newborn siblings of diabetic patients, no differences were found in indicators of beta-cell activity between these siblings and newborn control subjects who had no diabetes among their first degree relatives. Both C-peptide, insulin and proinsulin levels were similar in the groups compared. Thus, the increase in proinsulin concentration previously demonstrated in first degree relatives of diabetic patients [3–6] seems to occur later in life. This change in beta-cell function could reflect beta-cell damage as a result of environmental factors. It

could also be a defective maturing/aging process of the beta-cells in the siblings resulting in persistently higher levels of proinsulin later in life. In that case, a different composition of proinsulin immunoreactivity in newborn siblings and control newborns cannot be excluded.

The hypothesis that increased levels of proinsulin in first degree relatives could be a result of beta-cell damage are in accordance with the recent findings of Kahn et al. [14]. They found in a primate model with streptozotocin-induced diabetes elevated basal and arginine-induced proinsulin/insulin ratios.

Criticism could be raised that the groups in the present study are small. In our study [5] comparing 93 ICA-negative siblings of diabetic patients and 41 control subjects without diabetes among their first degree relatives the logarithmic values (mean  $\pm$  SD) for fasting proinsulin were  $0.97 \pm 0.28$  vs  $0.62 \pm 0.31$  ( $p < 0.01$ ) i.e. a 50% difference of the mean value, the difference being similar in magnitude to the standard deviation. In the present study the sample size was sufficient to detect a difference equal to the variance in a one-sided test with a power of 0.8 and a  $p$ -value of 0.05. Of course differences of smaller magnitude might not have been disclosed.

Mothers of newly-diagnosed Type 1 diabetic children did not differ in proinsulin values when compared to

women without diabetic relatives. Not even at delivery was a difference in proinsulin levels between the two groups of mothers observed. The glucose, C-peptide, insulin and proinsulin concentrations were, as expected, higher at delivery than those seen in fasting adults, a result of labour and increased insulin resistance during pregnancy [15]. In contrast to the present observation, in another study, elevated fasting proinsulin levels were reported in parents to Type 1 diabetic patients [6]. The reason for the discrepancy is unclear but may be attributed to differences in the study populations. Our patients were younger, had lower body mass indexes and included only females.

In cord plasma, the proinsulin levels were higher and the C-peptide levels lower compared to the levels in fasting adult subjects. Consequently the ratios of proinsulin/insulin and proinsulin/C-peptide were higher in newborn infants compared to adults. This observation was consistent with previous reports of increased proinsulin and decreased C-peptide levels in healthy infants [16, 17].

The molar ratio between C-peptide and insulin was lower in newborn infants than in adults. This low ratio has been described earlier but in these reports proinsulin and conversion intermediates have crossreacted in the insulin assay giving biased ratios [17, 18]. In the present insulin assay where proinsulin and most conversion intermediates did not crossreact, the lower C-peptide/insulin ratio might indicate a different metabolic clearance rate for insulin and/or C-peptide in newborns compared with adults. A diminished hepatic extraction of insulin in this age group has been suggested to explain this observation [17]. It may be noted, however, that the comparison is made between a non-steady-state (cord plasma) and a steady-state condition (fasting adult plasma).

In conclusion, beta-cell activity in newborn siblings of Type 1 diabetic patients does not differ from that of healthy newborn infants without diabetes among their first degree relatives. Thus, the raised proinsulin level earlier described in healthy siblings of Type 1 diabetic patients seems to occur later in life and may be caused by environmental factors.

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