Increased Plasma Pro-B-Type Natriuretic Peptide 
in Infants of Women with Type 1 Diabetes

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Background: Up to 40% of newborn infants of women with type 1 diabetes have echocardiographic signs of cardiomyopathy. Increased plasma concentrations of B-type natriuretic peptide (BNP) and its precursor (proBNP) are markers of cardiac failure and hypoxia in adults. In this study, we investigated whether plasma concentrations of proBNP and/or BNP are increased in infants of women with type 1 diabetes.

Methods: Plasma BNP and proBNP were measured with RIAs. The proBNP assay measures both intact proBNP and NH2-terminal fragments derived from this precursor, whereas the BNP assay measures only BNP-32 and not proBNP.

Results: Infants of women with diabetes and hemoglobin A1c (Hb A1c) >6.2% before delivery had a higher median plasma proBNP concentration (31 pmol/L; interquartile range, 21–47 pmol/L; n = 11549) than infants of healthy women (16 (9–32) pmol/L; n = 21; P = 0.01). Infants of women with diabetes and Hb A1c <6.2% (n = 15) had intermediate values. The plasma BNP and proBNP concentrations were closely associated (r² = 0.80; P <0.0001); within the group of infants of women with diabetes and Hb A1c <6.2%, both correlated with the degree of fetal stress during labor.

Conclusions: Maternal diabetes and suboptimal metabolic control may affect the fetal heart and predominantly stimulate proBNP secretion in conjunction with perinatal stress.

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Maternal diabetes is associated with increased risk of stillbirth (1), but the causes are not fully clarified. Up to 40% of newborn infants of women with type 1 diabetes have echocardiographic signs of cardiomyopathy with cardiac enlargement and asymmetric septal hypertrophy. Most often, these changes are asymptomatic and disappear within the first 6 months of life, but they can also lead to severe morbidity and mortality (2–4). It is thus conceivable that cardiac dysfunction could be a cause of stillbirths in pregnancies complicated by diabetes. During fetal life, infants of women with diabetes often have hyperglycemia, hyperinsulinemia (5), and increased concentrations of markers of hypoxic stress, e.g., plasma erythropoietin (6, 7). These factors could all have negative effects on the fetal heart in utero (8). It is well established that optimal metabolic control at conception and during the first trimester is essential to minimize the risk of congenital malformations in the offspring (9). In addition, suboptimal metabolic control in women with type 1 diabetes is associated with the risk of stillbirth (1). Nevertheless, the strength of a putative association between metabolic control in the mother and the extent of neonatal hypertrophic cardiomyopathy is not known (8, 10). At this stage, the diagnosis of neonatal diabetic cardiomyopathy is based on clinical observations and echocardiography.

B-Type natriuretic peptide (BNP) is a 32-amino acid peptide produced in excess by cardiac myocytes during cardiac stress (11). BNP is released as a 108-amino acid propeptide (proBNP), which is then cleaved into the active BNP (32 amino acids) and an N-terminal fragment (11). The biological roles of BNP include regulation of the extracellular fluid volume and blood pressure by increasing natriuresis and inhibiting the renin-angiotensin-aldosterone axis. In the fetus, BNP functions as a vasodilator in the placental circulation (12). BNP probably also has important protective autocrine effects in the heart by inhibiting fibrosis and hypertrophy (13).

Plasma measurements of proBNP and BNP are now used to diagnose cardiac dysfunction in adults. The
plasma concentrations of BNP and proBNP are increased in patients with heart failure, including those with hypertrophic cardiomyopathy (14, 15). Children with congenital heart disease and newborns with severe fetal distress also have increased plasma BNP concentrations (16–19). However, it is unknown whether the plasma BNP or proBNP concentrations are affected in newborn infants of women with diabetes and thus may be markers of neonatal cardiomyopathy.

In this study, we measured umbilical cord blood BNP and proBNP concentrations in newborn infants of women with type 1 diabetes and controls. The results suggest that maternal diabetes is associated with increased secretion of proBNP from the fetal heart and that this effect is augmented by fetal stress during labor.

Patients and Methods

PATIENTS

Thirty-seven pregnant women with type 1 diabetes and 23 healthy pregnant women were included in a prospective manner. Exclusion criteria were delivery before 34 weeks of gestation, preeclampsia, hypertension, and medically treated diseases other than type I diabetes. All women gave informed written consent, and the study protocol was approved by the local ethics committee (Ref. KF 01-048/-01). Basic data on the women, the course of labor, and the newborns were retrieved from medical charts and databases within the Department of Obstetrics.

Women with type 1 diabetes were divided into 2 groups according to their hemoglobin A1c (Hb A1c) fraction before delivery. Arbitrarily, women with an Hb A1c fraction below the median (6.2%) were considered to have good metabolic control (5.2%–6.1%), whereas women with a fraction at or above the median were considered to have suboptimal metabolic control (6.2%–7.2%). In the control group, the Hb A1c fraction ranged from 5.0% to 5.9%. All pregnant women in our department are screened for gestational diabetes by a risk factor–based procedure (20). Surprisingly, 1 woman in the control group had an Hb A1c of 6.8%. The result was first recognized postpartum, and her data were excluded.

PLASMA ANALYSIS

A venous blood sample for analysis of Hb A1c by HPLC (21) was obtained from each mother before elective delivery (i.e., induction of labor or elective cesarean section) or when the women entered the labor ward in spontaneous labor. The umbilical cord was doubly clamped immediately after delivery, and arterial blood was drawn for measurement of pH. Umbilical venous blood for proBNP and BNP measurements was drawn into EDTA-containing tubes (1.5 g/L). The tubes were immediately placed on ice, centrifuged, and stored at –20 °C for 1–3 days and then at –80 °C until analysis. ProBNP was measured with a processing-independent assay in which trypsin digestion of the plasma proteins is used to release the NH2-terminal fragment of proBNP and its derived peptides before measurement by an RIA with antibodies directed against the NH2 terminus of proBNP (22). The intraassay CVs of the proBNP assay are 12% at 13 pmol/L, 7% at 75 pmol/L, and 5% at 130 pmol/L (n = 10) (22). BNP was measured with a commercially available immunnoassay (Shionogi) that quantifies the bioactive BNP-32 peptide and has no cross-reactivity with atrial natriuretic peptide. According to the manufacturer, the intraassay CVs are 9.4% at 8.3 pmol/L and 12% at 168.9 pmol/L (23).

STATISTICAL ANALYSIS

Data are presented as the mean (SD) for gaussian-distributed variables and the median (interquartile range) for variables with a skewed distribution. Comparisons between groups were performed with ANOVA, followed by the Bonferroni multiple comparison tests where appropriate. Non-gaussian-distributed data were log-transformed before analysis. Associations between variables were examined with linear regression analysis. A 2-sided P <0.05 was considered statistically significant.

RESULTS

To examine the impact of maternal diabetes on plasma proBNP and BNP concentrations in the infant, we collected venous umbilical cord blood from infants of women with type 1 diabetes and healthy controls. A

<table>
<thead>
<tr>
<th>Table 1. Basic characteristics of women with type 1 diabetes and controls.</th>
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</thead>
<tbody>
<tr>
<td>Diabetes</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>Mean (SD) maternal age, years</td>
</tr>
<tr>
<td>Para &gt;1, n (%)</td>
</tr>
<tr>
<td>Mean (SD) prepregnancy BMI, kg/m²</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
</tr>
<tr>
<td>Mean (SD) Hb A1c, %</td>
</tr>
<tr>
<td>At weeks 6–10</td>
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<tr>
<td>At delivery*</td>
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</tbody>
</table>

* Women with type I diabetes were divided at the median Hb A1c (6.2%) into those with good and those with suboptimal metabolic control.
* BMI, body mass index (weight/height²); ND, not determined (Hb A1c was not measured in controls at weeks 6–10).
* P <0.0001, ANOVA.

* Women with type I diabetes were divided at the median Hb A1c (6.2%) into those with good and those with suboptimal metabolic control.

* BMI, body mass index (weight/height²); ND, not determined (Hb A1c was not measured in controls at weeks 6–10).

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* BMI, body mass index (weight/height²); ND, not determined (Hb A1c was not measured in controls at weeks 6–10).

* P <0.0001, ANOVA.
priori, we decided to subdivide the women with diabetes into 2 subgroups based on the Hb A1c fraction at term: Hb A1c at or above the median value (6.2%) was considered as suboptimal metabolic control and Hb A1c below the median as good metabolic control. Baseline variables were comparable in the 3 groups except for Hb A1c, for which diabetic women in suboptimal metabolic control had the highest values, controls the lowest values, and diabetic women in good metabolic control were intermediate (Table 1).

INCREASED UMBILICAL CORD PLASMA proBNP IN INFANTS OF WOMEN WITH SUBOPTIMALLY REGULATED TYPE 1 DIABETES

The proBNP plasma concentrations were significantly higher in cord blood from infants of women with type 1 diabetes and suboptimal metabolic control than in cord blood from infants born to control women (Fig. 1A and Table 2). The median plasma proBNP concentration in infants of women with diabetes and good metabolic control was intermediate and did not differ significantly from the other groups (Fig. 1A and Table 2). The plasma BNP concentration did not differ significantly among the 3 groups, although there was a trend toward a higher median concentration ($P = 0.08$) in the infants of women with an Hb A1c fraction above the median (Fig. 1B and Table 2). There was a strong association between plasma proBNP and BNP concentrations (Fig. 1C; $r^2 = 0.80$ for log-transformed data; $P < 0.0001$). The mean (SD) proBNP/BNP molar ratios were 8 (12), 6 (3), and 6 (5) in infants of control women and women with diabetes and good or suboptimal metabolic control, respectively.

Fifteen infants of healthy women (68%) vs 6 infants of women with diabetes (18%) were delivered by elective cesarean section (Table 2). To examine whether the method of delivery confounded the observed proBNP concentration difference between the diabetes and control groups, we stratified the infants according to method of delivery. The plasma proBNP concentration was increased to the same extent in infants of diabetic mothers whether they were delivered vaginally or by cesarean section (Fig. 2). In a 2-way ANOVA with plasma proBNP as the dependent variable and diabetes and method of delivery as the independent variables, the effect of maternal diabetes was statistically significant ($P = 0.047$), whereas the effect of the method of delivery was not ($P = 0.09$). Of note, this analysis did not differentiate between women with suboptimal or good metabolic control because of the small number of patients in the study.

The gestational age was slightly lower and, as expected, the birth weight was higher in the diabetes group than in the control group (Table 2). However, neither proBNP nor BNP concentrations were associated with gestational age or birth weight.

![Fig. 1. ProBNP and BNP concentrations in umbilical cord plasma from infants of nondiabetic women and infants of women with type I diabetes.](Image)
Table 2. Characteristics of deliveries and plasma BNP and proBNP concentrations in infants of women with type I diabetes and controls.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Good metabolic control</th>
<th>Suboptimal metabolic control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (SD) gestational age, a days</td>
<td>227 (7)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Mean (SD) birth weight, a g</td>
<td>3522 (380)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Mean (SD) umbilical cord blood pH</td>
<td>7.2 (0.1)c</td>
</tr>
<tr>
<td>Abnormal CTG, d n (%)</td>
<td>0 (0)d</td>
<td>4/25f</td>
<td>3/21f</td>
</tr>
<tr>
<td>Apgar score &lt;7 at 1 min, n</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Apgar score &lt;7 at 5 min, n</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Method of delivery (V/CSP/CSE), n</td>
<td>7/15/0</td>
<td>7/3/6</td>
<td>9/3/6</td>
</tr>
<tr>
<td>Mean (SD) duration of pushing, min</td>
<td>34 (30)</td>
<td>29 (27)</td>
<td>44 (31)</td>
</tr>
<tr>
<td>Mean (SD) total time of labor, min</td>
<td>477 (260)</td>
<td>385 (188)</td>
<td>385 (196)</td>
</tr>
<tr>
<td>Median (IQR) BNP, pmol/L</td>
<td>2.9 (2.1–5.0)</td>
<td>3.4 (2.4–6.0)</td>
<td>5.8 (3.4–9.5)</td>
</tr>
<tr>
<td>Median (IQR) proBNP, b pmol/L</td>
<td>16 (9–32)</td>
<td>23 (17–37)</td>
<td>31 (21–47)</td>
</tr>
</tbody>
</table>

a Women with type I diabetes were divided at the median Hb A1c (6.2%) into those with good and those with suboptimal metabolic control.

b P ≤ 0.02, ANOVA.
c n = 7.
d CTG, cardiotocography; V, vaginal delivery; CSP, elective cesarean section; CSE, emergency cesarean section; IQR, interquartile range.
e n = 10.
f n = 12.
g n = 14.

ASSOCIATION OF UMBILICAL CORD PLASMA proBNP AND BNP CONCENTRATIONS WITH PERINATAL STRESS

On regression analyses, cord plasma concentrations of proBNP and BNP were positively associated with the duration of pushing in the second stage of labor and inversely associated with cord blood pH and Apgar score after 1 min in the infants of women with diabetes and suboptimal metabolic control (Table 3). Within this group of infants, the cord plasma proBNP concentration was associated with maternal Hb A1c at weeks 6–10 of pregnancy but not with Hb A1c at time of delivery (Table 3). In contrast, we did not see any associations between proBNP or BNP and the indexes of perinatal stress or Hb A1c in infants of healthy women or women with diabetes and good metabolic control.

Discussion

The aim of this study was to assess the impact of maternal type I diabetes on expression of proBNP and BNP in the fetus. When interpreting the results, it should be noted that we selected women with a relatively uncomplicated pregnancy by excluding pregnancies with preeclampsia or early preterm delivery. The results suggest that uncomplicated diabetic pregnancy is associated with increased plasma proBNP concentrations in the fetus and that proBNP and BNP concentrations tend to be higher when the mother is in suboptimal as opposed to good metabolic control.

As in plasma from adults with cardiac dysfunction (23), the umbilical cord plasma proBNP and BNP concentrations were closely associated. Nevertheless, the effect of maternal diabetes was more pronounced for proBNP than for BNP. The proBNP assay used in this study measures both intact proBNP and NH2-terminal fragments derived from this precursor, whereas the BNP assay measures only BNP-32 and not proBNP. We recently suggested that plasma proBNP is a more sensitive marker for acute cardiac stress than BNP, whereas proBNP and BNP probably have similar diagnostic sensitivities in chronic cardiac dysfunction (24). Interestingly, the plasma proBNP/BNP molar ratio in the newborns (mean 6) was markedly higher than what is seen in adults [mean 2.5 in patients with cardiac disease and 1.1 in healthy individuals (23, 25)]. This may suggest that the conversion of proBNP to BNP is less efficient in fetal life than in adulthood, although the proposed proBNP-processing protease, corin, is expressed in the fetal heart, at least in mice (26).

The murine placenta contains BNP mRNA in the peripheral margin of the decidual layer (27). In this study, we examined BNP secretion in 3 term placentas from nondiabetic women with normal pregnancies (data not shown). We were unable to amplify the BNP mRNA transcript in any of 9 placental RNA preparations from the placentas with a real-time reverse transcription-PCR assay that detects BNP mRNA concentrations less than 1/1000th of those in adult human hearts (28). Moreover, proBNP immunoreactivity was not detectable (<0.2 pmol/g of tissue) when protein extracts from the 3 placentas were examined with our proBNP immunoas-
say. These findings are compatible with the theory that increased proBNP concentrations in umbilical cord blood of infants of women with diabetes reflect increased secretion from the fetal heart rather than from the placenta.

Previous studies have shown that severe perinatal stress causes an increase in umbilical cord plasma BNP and proBNP concentrations (17). We therefore investigated whether the plasma proBNP and BNP concentrations were associated with measures of perinatal stress. Importantly, duration of pushing, umbilical cord blood pH, and Apgar scores were similar in the control and diabetes groups, suggesting that differences in fetal stress could not account for the present results. Nevertheless, 3 indicators of perinatal stress (i.e., duration of pushing, cord blood pH, and Apgar score after 1 min) all displayed significant correlations with plasma proBNP and BNP within the group of infants of women with diabetes and suboptimal metabolic control. In contrast, none of the indexes of perinatal stress were associated with proBNP or BNP in the control group or the group of infants of women with diabetes and good metabolic control. These findings could reflect that maternal diabetes causes dysfunction of the fetal heart that precipitates primarily when the fetal circulation is under stress. This “2-hit” hypothesis, that tissue dysfunction in metabolic diseases needs a second insult before it becomes manifest, has parallels in adult diabetic cardiomyopathy and liver dysfunction (29).

Of note, poor metabolic control per se increases cardiac output by ~10% in adults with type 1 diabetes (30), and it is conceivable that the increase in fetal BNP secretion in infants of mothers with diabetes reflects increased pre- or afterload of the fetal heart, perhaps attributable to fetal hypervolemia.

In the infants of women with diabetes, the plasma

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**Table 3. Pearson correlation coefficients (r) for associations between plasma proBNP and BNP concentrations vs indexes of perinatal stress and Hb A1c.**

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>Controls</th>
<th>Good metabolic control</th>
<th>Suboptimal metabolic control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProBNP</td>
<td>Duration of pushing</td>
<td>0.21 (n = 7)</td>
<td>0.47 (n = 7)</td>
<td>0.76 (n = 7)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cord blood pH</td>
<td>−0.44 (n = 7)</td>
<td>−0.09 (n = 15)</td>
<td>−0.59 (n = 16)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Apgar score at 1 min</td>
<td>−0.21 (n = 20)</td>
<td>−0.02 (n = 15)</td>
<td>−0.55 (n = 16)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Hb A1c (weeks 6–10)</td>
<td>ND</td>
<td>0.46 (n = 12)</td>
<td>0.60 (n = 15)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Hb A1c (delivery)</td>
<td>0.08 (n = 19)</td>
<td>0.15 (n = 15)</td>
<td>0.24 (n = 16)</td>
</tr>
<tr>
<td>BNP</td>
<td>Duration of pushing</td>
<td>0.16 (n = 7)</td>
<td>0.17 (n = 7)</td>
<td>0.84 (n = 7)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cord blood pH</td>
<td>−0.07 (n = 7)</td>
<td>−0.09 (n = 15)</td>
<td>−0.65 (n = 16)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Apgar score at 1 min</td>
<td>−0.30 (n = 20)</td>
<td>−0.14 (n = 15)</td>
<td>−0.50 (n = 16)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Hb A1c (week 6–10)</td>
<td>ND&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.30 (n = 12)</td>
<td>0.42 (n = 15)</td>
</tr>
<tr>
<td></td>
<td>Hb A1c (delivery)</td>
<td>0.24 (n = 19)</td>
<td>0.11 (n = 15)</td>
<td>0.16 (n = 16)</td>
</tr>
</tbody>
</table>

<sup>a</sup> The correlation coefficients (r) are Pearson correlation coefficients from linear regression analysis of logarithmically transformed BNP or proBNP as the dependent variable and the indicated indexes of perinatal stress as the independent variable; values in parentheses are the number of samples.

<sup>b</sup> Women with type 1 diabetes were divided at the median Hb A1c (6.2%) into those with good and those with suboptimal metabolic control.

<sup>c</sup> P <0.05.

<sup>d</sup> P <0.01.

<sup>e</sup> ND, not determined (Hb A1c was not measured in controls at weeks 6–10).
proBNP concentration was more closely associated with Hb A1c concentrations early in pregnancy than with Hb A1c concentration at term. This finding could reflect that diabetes already imposes adverse effects on the fetal heart during its formation; however, it may also result from the fact that the larger variation in Hb A1c at weeks 6–10 yields more statistical power in the linear regression analysis.

Increased proBNP secretion in infants of women with diabetes may affect both the fetal circulation and have autocrine effects within the heart. The resistance in the placental vasculature is increased in maternal diabetes (31). Infusion of BNP into the fetal-placental circulation of sheep leads to dilation of the placental vasculature (12, 32). Thus, it is possible that increased proBNP secretion serves as a protective response and decreases the afterload on the fetal heart. It has recently become clear from studies of genetically modified mice that BNP (and atrial natriuretic peptide) in addition to its peripheral effects also has autocrine effects in the heart. Mice lacking the BNP or the BNP receptor genes display cardiac fibrosis and hypertrophy (33, 34). It is therefore tempting to speculate that increased proBNP secretion also constitutes a local defense mechanism that counteracts the signals causing fetal cardiac hypertrophy when the mother has diabetes and is in suboptimal metabolic control.

In conclusion, our results indicate that the cord plasma proBNP concentration is increased in infants of women with type I diabetes and suboptimal metabolic control and that the increase in proBNP is accentuated during perinatal stress.

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