The Influence of Maternal BMI and Age in Twin Pregnancies on Insulin Resistance in the Offspring

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OBJECTIVE — There is strong evidence that low birth weight is associated with glucose intolerance and diabetes in adults. We have carried out a twin study to distinguish among maternal influences, which affect both twins; fetoplacental influences, which are unique to each twin; and the genetic factors that may underlie this association.

RESEARCH DESIGN AND METHODS — We identified a sample of 423 twin pairs (250 monozygotic and 173 dizygotic) from the East Flanders Prospective Twin Survey who were born between 1964 and 1982. Data collected in this study included the mother’s body composition and weight gain during pregnancy, the twins’ birth weights, and gestational age. The twins (aged 18–34 years) attended a research center for measurement of height, weight, and waist-to-hip ratio as well as fasting glucose, proinsulin, and insulin concentrations.

RESULTS — Among twin pairs discordant for birth weight, we found little evidence that the lighter twin had abnormal glucose-insulin metabolism in adult life. However, both a low prepregnancy maternal BMI and older maternal age at delivery were associated with hyperinsulinemia and evidence of insulin resistance in the offspring. Fasting insulin increased by 1.3% (95% CI 0.1–2.6%) per unit fall in maternal BMI and by 1.1% (0.02–2.0%) per year increase in maternal age. These associations were independent of the twins’ BMI and waist-to-hip ratio and their zygosity.

CONCLUSIONS — These novel findings suggest that in twin pregnancies, maternal factors are more important than fetoplacental factors in determining glucose-insulin metabolism in the offspring.

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suggests that the inverse association between birth weight and diabetes cannot be entirely accounted for by genetic factors. Moreover, this together with two other studies using the same approach applied to blood pressure suggests that causes of variation in fetal growth between twin pairs are sufficient to exert programming effects and draws attention to the importance of the fetal supply line (9, 10). However, more recent studies have not confirmed these initial findings (11–13).

We have studied a sample of twins from East Flanders Prospective Twin Survey, a population-based twin register in which obstetric and perinatal data on all twins was collected. Because of the unique data available, we have been able to examine the relative contribution of maternal factors and the influence of the fetoplacental unit, as indicated by twin differences in size at birth to plasma glucose and insulin concentrations.

RESEARCH DESIGN AND METHODS

Subjects
The East Flanders Prospective Twin Survey includes details of all twins born in the Belgian Province of East Flanders since 1964. Birth weight and duration of gestation (calculated from the date of the last menstrual period) were abstracted from the obstetrical records, and placental examination was carried out within 24 h after delivery. Zygosity was determined through sequential analysis based on sex, fetal membranes, umbilical cord blood groups, placental alkaline phosphatase, and DNA fingerprints (14). Between July 1964 and May 1982, the East Flanders Prospective Twin Survey had registered 1,685 twin pairs with birth weight ≥500 g or gestational age ≥22 weeks, where both twins were alive and residents of the East Flanders area. We randomly contacted 803 pairs. Of these, 423 pairs (52.7%) agreed to participate, of which 179 pairs were both men (119 MZ and 60 dizygotic [DZ]), 187 were both women (131 MZ and 56 DZ), and 57 were of opposite sex. Of 46 pairs (11 MZ and 32 DZ), only one member participated. Perinatal characteristics of the participants are representative for the East Flanders twin population. The twins gave informed consent, and the project was approved by the Committee of Medical Ethics.

Methods
Between February 1997 and April 2000, the twins attended a 2-h examination, which took place in the morning after an overnight fast. Standing height (cm) was measured to the nearest 0.1 cm with a Harpenden fixed stadiometer and body weight (kg) on a SECA balance scale to the nearest 0.1 kg. BMI, as a measure of overall body composition, was calculated as body weight divided by the square of height (kg/m²). Blood samples were taken to measure glucose, insulin, and proinsulin concentrations. Plasma glucose was analyzed by using the hexokinase method (Olympus AU600), plasma insulin by using microparticle enzyme immunoassay (Abbott Axsym), and intact proinsulin by an ELISA (Merckodia, Uppsala, Sweden). Homeostasis model assessment was used to assess insulin resistance and β-cell function, based on fasting insulin and glucose levels as described by Matthews et al. (15). None of the subjects reported abnormal kidney or liver function. The mothers of the twins were contacted and given a questionnaire at the time the twins were invited to our research center, inquiring about weight before pregnancy, weight gain during pregnancy, and current height. Maternal BMI (kg/m²) was calculated as weight (before and at the end of pregnancy) divided by the square of current height. Of the 423 mothers, 13 were deceased and 78 did not recall their weights.

Statistical analyses
Linear regression was used to estimate the association between within-pair differences in adult glucose or insulin concentrations. These analyses were performed using SAS version 6.12. In the analysis of maternal effects, twins were analyzed as individuals using multilevel regression analysis to model the paired structure of the data (MLwiN 1.10.0006). Because the fasting concentrations of glucose, proinsulin, and insulin and the indexes of insulin resistance and β-cell function had skewed distributions, they were log transformed to normality before analysis. We adjusted for the twins’ sex, adult BMI, and age. All tests were two sided, and P values <0.05 were considered significant.

RESULTS — The characteristics of the twins according to sex and zygosity are shown in Table 1. Although there were no differences in the mothers’ age, height, or weight between MZ and DZ twins, MZ twins weighed significantly less at birth than DZ twins among both men (P = 0.003) and women (P = 0.025) and had a shorter gestation (P = 0.02). The twins’ adult age, height, weight, BMI, fasting concentrations of insulin and proinsulin, and insulin resistance were similar. Fasting glucose concentration was higher and β-cell function lower in MZ men. Four twins (two MZ and two DZ) had impaired fasting glyceremia (fasting glucose concentration ≥6.1 and <7.0 mmol/l).

Within-pair analysis: influence of the fetoplacental unit
The mean within-pair birth weight difference was 259 g (SD 214) in MZ twins and 314 g (SD 260) in DZ twins. Means as well as variances were significantly different between MZ and DZ twins (P = 0.02 and P = 0.005, respectively).

Table 2 shows the results of an analysis relating within-pair differences in adult glucose and insulin levels for every 100-g difference in birth weight. Among MZ twins, the within-pair birth weight difference was unrelated to within-pair differences in fasting glucose, proinsulin, and insulin concentrations as well as insulin resistance and β-cell function. Similar results were obtained in DZ twins, although there was evidence that fasting insulin concentrations (P = 0.04) were somewhat higher in the smaller twin at birth and β-cell function was increased in the smaller twin at birth when all pairs were combined. These results were similar for men and women and were not affected by adjustment for any maternal characteristic (weight or BMI before pregnancy, weight gain during pregnancy, and maternal age).

Between-individual analysis: influences of maternal environment
In a combined analysis of all 800 individual subjects, there were no statistically significant associations between birth weight and fasting glucose, proinsulin, insulin, concentrations, and insulin resistance, even after adjustment for gestational age, sex, adult BMI, and age (Table 3), with the exception of β-cell function, which fell with increasing birth weight.
Table 1—Maternal characteristics, birth size, and adult characteristics of the twins according to sex and zygosity

<table>
<thead>
<tr>
<th></th>
<th>MZ (n = 250)</th>
<th>DZ (n = 173)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother’s age at delivery (years)</td>
<td>27.2 ± 5.1</td>
<td>28.1 ± 4.6</td>
</tr>
<tr>
<td>Mother’s height (cm)</td>
<td>162.7 ± 6.2</td>
<td>163.2 ± 5.1</td>
</tr>
<tr>
<td>Mother’s prepregnancy weight (kg)</td>
<td>58.8 ± 9.7</td>
<td>59.0 ± 9.6</td>
</tr>
<tr>
<td>Mother’s prepregnancy BMI (kg/m²)</td>
<td>22.2 ± 3.4</td>
<td>22.1 ± 3.3</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>36.9 ± 2.5</td>
<td>37.5 ± 2.4</td>
</tr>
</tbody>
</table>

Table 2—Difference in fasting glucose, proinsulin, and insulin concentrations and insulin resistance and β-cell function for every 100-g within-pair difference in birth weight in MZ and DZ twins, and in all twins combined

<table>
<thead>
<tr>
<th></th>
<th>MZ (n = 239)</th>
<th>DZ (n = 138)</th>
<th>All (n = 377)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>0.01 (-0.004 to 0.02)</td>
<td>0.17</td>
<td>0.00 (-0.01 to 0.01)</td>
</tr>
<tr>
<td>Fasting proinsulin (pmol/l)</td>
<td>-0.02 (-0.14 to 0.10)</td>
<td>0.77</td>
<td>-0.04 (-0.23 to 0.22)</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>-0.12 (-0.70 to 0.47)</td>
<td>0.70</td>
<td>-1.03 (-2.01 to -0.05)</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>-0.004 (-0.03 to 0.02)</td>
<td>0.79</td>
<td>-0.02 (-0.08 to 0.01)</td>
</tr>
<tr>
<td>β-Cell function (%)</td>
<td>-2.55 (-5.31 to 0.21)</td>
<td>0.07</td>
<td>-2.75 (-6.39 to 0.90)</td>
</tr>
</tbody>
</table>

Data are means ± SD. HOMA, homeostasis model assessment. *Geometric means ± SD.

(P = 0.04). Similar results were obtained in separate analyses of MZ and DZ twins.

However, low maternal BMI before pregnancy was significantly associated with raised fasting insulin and proinsulin concentrations and increased insulin resistance and β-cell function. In analyses stratified by sex, the associations between maternal BMI and the offspring’s fasting insulin and insulin resistance were significant in women (P = 0.003 and P = 0.005, respectively) but not in men (P = 0.8 and P = 1.0, respectively). The interaction between sex and maternal BMI was tested in a regression model with insulin as the dependent variable and maternal BMI, sex, adult BMI, and the interaction term (sex × maternal BMI). The interaction term was significant at P = 0.02. Similar results were obtained with insulin resistance (P = 0.03).

Older maternal age was also related to higher fasting insulin concentrations, insulin resistance, and increased β-cell function in the offspring. Fasting insulin increased by 1.3% (95% CI 0.1–2.6%) per unit (kg/m²) fall in maternal BMI and by 1.1% (0.02–2.0%) per year increase in maternal age. The effect of maternal age was similar in both sexes.

The effects of maternal BMI and age were independent from each other. Table 4 shows that at any level of maternal BMI, older maternal age was associated with increased fasting insulin concentrations, while among both younger and older mothers, the twins’ insulin concentrations fell as their mothers’ BMI increased.

Mothers who were older and thinner had twin offspring with the highest insulin levels. Similar effects were observed with the indexes of insulin resistance and hyperinsulinemia (β-cell function). These effects were independent of other maternal characteristics (weight gain during pregnancy, gestational age, and birth order) (results not shown).

A multiple regression analysis was carried out with fasting insulin as the dependent variable and maternal BMI and age, current offspring’s BMI and age, birth weight, and zygosity as independent variables. This showed that in addition to maternal BMI before pregnancy (P = 0.04) and maternal age at delivery (P = 0.01), current BMI (β = 0.6% per kg/m², P < 0.001) and age (0.2% per year, P < 0.001), but not birth weight (−0.08% per 100 g, P = 0.09), gestational age (0.1% per week, P = 0.31), or zygosity (P = 0.63), were associated with fasting insulin concentrations in the twin offspring. Similar results were obtained for insulin resistance.

**CONCLUSIONS**—In this study, we found little evidence that low birth weight in twin pairs is associated with abnormal glucose-insulin metabolism in adult life. However, we report for the first time that maternal effects, specifically low maternal BMI and older age at delivery, are dominant in predicting insulin resistance in the twin offspring. These maternal effects were independent of the birth weights of the twins and were additive: the oldest
Programming of insulin resistance in twins

Table 3—Relationship between fasting glucose, proinsulin, and insulin concentrations and insulin resistance and β-cell function and birth weight and maternal characteristics in all 800 twins

<table>
<thead>
<tr>
<th></th>
<th>Birthweight</th>
<th>Maternal BMI before pregnancy</th>
<th>Maternal age at delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Change per 100 g</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>0.0% (−0.1 to 0.2%)</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>Fasting pro-insulin</td>
<td>−0.3% (−1.5 to 1.2%)</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>−0.8% (−1.6 to 0.1%)</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>−0.6% (−1.5 to 0.3%)</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>β-Cell function</td>
<td>−1.0% (−1.9 to −0.1%)</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

Data were adjusted for sex, gestational age, adult BMI, and age. *Interaction with sex: P = 0.02; men: 0.1% change per 1 kg/m², P = 0.82; women: −2.7% change per 1 kg/m², P = 0.003. †Interaction with sex: P = 0.03; men: 0.0% change per 1 kg/m², P = 0.99; women: −2.5% change per 1 kg/m², P = 0.005.

mothers with the lowest BMI had the most insulin-resistant offspring.

Our study is based on a substantial number of twin pairs who were identified through the East Flanders Prospective Twin Survey, which included accurate obstetric and perinatal data as well as zygosity determination. Although the mothers’ weight and height were self-reported, other studies have shown that these recalled anthropometric measurements correlate closely with actual measurements (16,17). In this study, insulin resistance was based on a single fasting measurement of insulin and glucose. These correlate well with measurements of insulin resistance by the euglycemic clamp, but they are less precise. As a result, the data may underestimate the strength of relationships between maternal age or BMI and the offspring’s insulin resistance (18). Our twins were young, with a narrow range of fasting insulin and glucose concentrations, but as there is good evidence that hyperinsulinemia in young adults is predictive of glucose intolerance and diabetes in later life, it is likely that these maternal influences will be associated with these adverse health outcomes in later life (19).

The adverse effect of low maternal BMI on insulin resistance in the offspring has never been described in twins, but it is consistent with findings in studies of singletons. Among middle-aged men and women in Scotland (3) and China (5), low maternal BMI in early pregnancy has been associated with raised fasting insulin concentrations in the offspring. In addition, the Dutch famine study showed that exposure to the famine in utero results in insulin resistance in adulthood, especially if the mothers had a low BMI in pregnancy (4). We found that the mother’s pregreg-
would provide a test of the importance of environmental versus genetic factors in explaining the link between birth size and glucose tolerance. Although there were substantial within-pair differences in birth weight in MZ twins, these differences did not correlate with within-pair differences in adult glucose-insulin metabolism. Similar findings have been reported in a study based in Birmingham (U.K.) (12).

Our data contrast with the substantial evidence from singleton studies that low birth weight is associated with insulin resistance and glucose intolerance later in life (26–28). There are a number of possible explanations. First, it is unlikely that the factors that limit fetal growth in singletons are the same as those operating in twins; for example, there is evidence that maternal factors constraining the size of the fetus act disproportionately in twins compared with singletons (29). Second, our assessment of insulin resistance was based on fasting insulin and glucose measurements rather than on the response to a glucose tolerance test. Although these measurements are interrelated and have a glucose tolerance test. Although these measurements did not correlate with within-pair birth weight in MZ twins, these differences did provide a test of the importance of fetoplacental factors in determining fetal size. Our findings suggest that within the limitations of the available data on fetal growth in this study and its interpretation, fetoplacental factors appear to be more important than maternal factors in predicting glucose-insulin metabolism in the offspring.

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