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Birth weight is nongenetically associated with glucose intolerance in elderly twins, independent of adult obesity

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Abstract. Grunnet L, Vielwerth S, Vaag A, Poulsen P (Steno Diabetes Center, Gentofte; and Rigshospitalet, Copenhagen, Denmark). Birth weight is nongenetically associated with glucose intolerance in elderly twins, independent of adult obesity. *J Intern Med* 2007; **262**: 96–103.

Objectives. Using a unique twin approach, we examined the extent to which birth weight is determined by genetic and nongenetic factors and whether associations between birth weight and measures of glucose metabolism are of genetic or nongenetic origin.

Setting/subjects. An oral glucose tolerance test (OGTT) was performed in a population-based cohort of twins including 138 same-sex monozygotic (MZ) and 214 dizygotic (DZ) twin pairs aged 55–73 years whose birth weight was known. Heritability of birth weight was determined and regression analyses with intra-twin pair differences of birth weight and measures of glucose metabolism, with and without adjustment for adult obesity, were performed.

Results. The heritability of birth weight was estimated to be 38%. We demonstrated significant nongenetic associations between birth weight and measures of glucose homeostasis in MZ twins, with a reduction in fasting plasma glucose, 120 min post-OGTT plasma glucose, fasting plasma insulin and HOMA-IR index of 15.7%, 25.5%, 26.4% and 37.2%, respectively, for every 1 kg increase in birth weight. The nongenetic negative associations between birth weight and measures of glucose intolerance were independent of adult obesity, whereas the nongenetic association between birth weight and insulin resistance persisted, although not as strongly, after adjusting for current body size.

Conclusions. We demonstrated a genetic component to birth weight in elderly twins. When adjusting for this influence, we found a nongenetic negative association between birth weight and glucose tolerance as well as insulin resistance that was partially independent of adult obesity. This implies that the foetal environment influences glucose homeostasis in elderly twins.

Keywords: birth weight, insulin resistance, twins, type 2 diabetes.

Introduction

The association between low birth weight and the development of type 2 diabetes (T2D) and associated defects of glucose metabolism is well established [1]. However, it is currently unknown whether this association is of genetic or nongenetic origin. Studies have indicated that a common genotype may result in both low birth weight and T2D, suggesting that a genetically determined inappropriate insulin secretion and action result in both impaired foetal growth and susceptibility to T2D later in life [2, 3]. In contrast, the thrifty phenotype hypothesis proposes that adverse intrauterine conditions due to an environmental insult during foetal life lead to impaired growth and development, and to permanent programming of several organs, including muscle, pancreas, liver and adipose tissue, which predispose to the subsequent development of T2D [4]. We and other researchers have previously demonstrated lower birth weights in twins with

T2D than in their genetically identical nondiabetic co-twins, suggesting that the association between T2D and insulin resistance, on the one hand, and on the other, low birth weight, is not merely a coincidence involving the putative T2D susceptibility genotype and a genetically determined low birth weight [5–7].

We have previously demonstrated an association between birth weight and 2-h plasma glucose level after an oral glucose load [7] and, more recently, nongenetic associations between birth weight and gold standard measures of the pathophysiological mechanisms underlying the development of T2D (i.e. insulin secretion and action) in elderly twins [8, 9]. However, the former study included mainly glucose-intolerant twins and the genetic influence was not adjusted for. In addition, the results in the latter studies were based upon a smaller sub-population of elderly glucose-tolerant twins.

Classical twin studies provide a unique method for distinguishing genetic and nongenetic aetiological factors by means of intraclass correlations. Furthermore, monozygotic (MZ) twins are genetically identical and regression analysis of within-twin pair differences in birth weight and other phenotypic variables allows us to differentiate associations of genetic and nongenetic origin. In addition, this type of analysis can control for common environmental and maternal factors, such as parental height, weight, social class and smoking behaviour that putatively influence the adult phenotype in both MZ and dizygotic (DZ) twins.

Adult obesity is closely involved in the development of T2D and insulin resistance [10–12]. In addition, low birth weight subjects show a higher degree of abdominal [10, 13] and truncal obesity [14] than that seen in normal birth weight controls. At least in theory, adult obesity may therefore mediate the relationship between birth weight and disorders of glucose metabolism [13, 15, 16].

In the present population-based study of elderly twins we aimed at extending our previous studies [7–9] on the association between birth weight and glucose metabolism to a larger elderly twin population including twins with both normal and impaired glucose tolerance (IGT) as well as T2D with available birth weight records. We determined the relative genetic and environmental influences on birth weight and, in particular, investigated the nongenetic effects of birth weight on measures of glucose metabolism (i.e. glucose tolerance, insulin secretion and sensitivity) using intra-twin pair regression analyses with adjustment for measures of overall and abdominal obesity.

Methods

Subjects

A population-based cohort of 606 elderly twins (born between 1921 and 1940) ascertained independently of disease status were identified through the Danish Twin Register [17]. The cohort includes MZ and same-sex DZ twin pairs and was examined in 1994–1995, primarily to determine the heritability of T2D and IGT as previously reported [18]. At that time, birth weight information was available mainly for 218 glucose-intolerant twins. In the present study, we obtained birth weight information of an additional 134 twins irrespective of their glucose tolerance status. Registration of birth weights was not obligatory before 1927, for which reason birth weight information was only traced for 352 (60%) of the twins. Pairs of twins with birth weight registration (64.8 ± 4.2 years) were therefore significantly younger than those without birth weight information (69.0 ± 4.8 years; $P < 0.0001$). Importantly, the prevalence of T2D (13.6%) and IGT (20.2%) amongst the investigated twins with available birth data was similar to that in twins without birth weight registration (12.2% and 23.6% respectively). Twins were asked about physical similarity and mistaken identity in order to establish zygosity (the similarity method) [19, 20]. This method has been evaluated through comparison with serological zygosity testing, and has a misclassification rate of less than 5% [19].

Clinical examination

Weight and height were measured with the subjects dressed in lightweight clothes with their shoes removed, and the results used to calculate body mass

index [weight (kg)/height (m^2)]. Waist circumference of standing subjects was measured using a tape measure placed midway between the lowest rib and the iliac crest. Hip circumference was measured over the widest part of the gluteus. The waist–hip ratio (WHP) was calculated from these values. A standardized 75 g oral glucose tolerance test (OGTT) was performed after an overnight fast. Peripheral venous blood was taken before oral glucose ingestion and 30 and 120 min after. Fasting plasma glucose, 120 min post-OGTT plasma glucose and $AUC_{glucose(0\text{-}120)}$ were used as measures of glucose tolerance. Three different indirect measures for insulin resistance were used. The homeostasis model assessment (HOMA) index of insulin resistance was calculated using the equation [21]:

$$\text{Fasting plasma insulin } (\text{pmol L}^{-1}) \times [\text{fasting plasma glucose } (\text{mmol L}^{-1})/22.5] \times 0.144.$$

An insulin sensitivity index proposed by Matsuda and DeFronzo [22] was calculated as:

$$\frac{10\ 000}{\sqrt{([\text{Fasting glucose} \times \text{fasting insulin}] \times [\text{mean glucose} \times \text{mean insulin during OGTT}])}}.$$

This index represents both hepatic and peripheral insulin sensitivity and is highly correlated with whole body glucose disposal during the euglycaemic hyperinsulinaemic clamp ($r = 0.73$, $P < 0.0001$) [22]. Finally, fasting plasma insulin was used as a measure for insulin resistance. To measure insulin secretion we calculated the HOMA β-cell function (%) index [21],

$$\frac{\text{Fasting plasma insulin}}{\text{Fasting plasma glucose} - 3.5} \times 20$$

and the ratio between the 30 min increment in plasma insulin and glucose concentration after oral glucose loading [23]:

$$\frac{\text{Insulin}_{30} - \text{insulin}_0}{\text{Glucose}_{30} - \text{glucose}_0}.$$

The study was approved by the regional ethics committees and the study was conducted according to the principles of the Helsinki Declaration.

Statistical analysis

All statistical analyses were carried out using SAS (Version 8.2; SAS Institute). Contingency tests were used to investigate possible significant associations between the prevalence of T2D and IGT amongst twins with and without available birth data. Phenotypic associations, in which the twins are included as individuals, were investigated using Spearman correlation.

Comparing phenotypes in MZ and DZ twins Monozygotic twins share approximately 100% of their entire genome and DZ twins on average share half of their segregating genes, for which reason twins cannot be considered as independent observations. The phenotypic parameters of MZ and DZ twins were compared by ANOVA, adjusting for the intra-twin pair relationship. The full ANOVA model includes a random-effects term for twin-pair membership and a fixed-effects term for zygosity. Data are summarized as mean (and SD). $P \leq 0.05$ was considered to be significant.

Regression analyses with intra-twin pair differences In order to investigate whether the associations between birth weight and measures of glucose metabolism were of genetic or nongenetic origin we performed linear regression analyses with intra-twin pair differences, allowing elimination of common environmental effects (maternal, placental and common postnatal environmental effects) in MZ and DZ twins. Importantly, any effects caused by genotype are absent in MZ twins, so that a significant regression with intra-twin pair differences between two phenotypes in MZ twin pairs indicates differences of a nongenetic origin. The designation of a member of a twin pair is arbitrary, i.e. there is no systematic method of assigning a particular twin to a particular group and its sibling to the other group. To avoid this problem, the intra-twin pair regression analyses were performed using each twin twice ($2n$), as previously recommended [24].

In the multiple regression of intra-twin pair differences, in which we controlled for body mass index (BMI) and WHR, the fitted regression line was constrained to pass through the origin as recommended [25]. An examination of the residuals indicated deviation from normality, and so all outcome variables were transformed by the natural logarithm (ln) to reduce this.

Intraclass correlations and heritability The relative contribution of genes and environment to phenotypic variance can be estimated by means of intraclass correlations that express the resemblance between MZ and DZ twin pairs respectively. Intraclass correlations and confidence intervals were calculated using the Mx program [26]. Statistical comparisons of intraclass correlations were made after applying Fisher's z-transformation. Heritability (h^2) was expressed as twice the difference in intraclass correlation between MZ and DZ twins [$2(r_{MZ}-r_{DZ})$] [26].

Results

Clinical characteristics

Monozygotic and DZ twins had similar clinical and biochemical characteristics except for age, for which MZ twins were slightly but significantly older than DZ twins, and insulin sensitivity index, which was significantly lower (indicating a higher degree of insulin resistance) in MZ than in DZ twins (Table 1).

Table 1 Clinical characteristics of the twin cohort

	MZ	DZ
n (m/f)	138 (70/68)	214 (106/108)
Prevalence of T2D/IGT	16.7/16.7	12.6/20.6
Age (years)	65.8 (3.5)*	64.1 (4.5)
Birth weight (kg)	2.682 (0.762)	2.662 (0.608)
BMI (m^2 kg^{-1})	26.0 (3.9)	25.8 (4.3)
Waist–hip ratio	0.88 (0.08)	0.86 (0.10)
Fasting glucose (mmol L^{-1})	6.22 (1.56)	6.06 (1.57)
Glucose 120 min (mmol L^{-1})	8.22 (4.39)	7.69 (4.16)
$AUC_{glucose(0\text{-}120)}$	318 (175)	283 (200)
Fasting insulin (pmol L^{-1})	48.02 (24.66)	45.4 (30.47)
HOMA-IR	2.00 (1.30)	1.84 (1.65)
Insulin sensitivity index	18.98 (11.63)*	22.43 (12.59)
HOMA β-cell (%)	55.31 (25.91)	55.25 (32.49)
Ratio $insulin_{0-30}/glucose_{0-30}$	77.6 (55.5)	79.7 (81.7)

Data are presented as mean (SD). T2D, type 2 diabetes; IGT, impaired glucose tolerance; BMI, body mass index; HOMA, homeostasis model assessment. *$P < 0.05$ (MZ twins versus DZ twins).

Relative impact of genetic and environmental factors on birth weight

The intraclass correlation for birth weight was significantly higher amongst MZ than DZ twins (MZ: 0.75 [0.64; 0.82]; DZ: 0.56 [0.42; 0.67], $P = 0.002$), yielding an estimate for the heritability of birth weight of 38% [27.7; 48.3].

Genetic versus nongenetic association between birth weight and glucose metabolism

Examination of the phenotypic correlations revealed that, except for a negative correlation between birth weight and 120 min plasma insulin ($r = -0.16$, $P = 0.049$) in MZ twins, none of the remaining parameters (fasting glucose, glucose 120 min, fasting insulin, HOMA-IR, insulin sensitivity index, HOMA β-cell function and insulin/glucose ratio) were significantly associated with birth weight (data not shown). However, regression of birth weight on measures of glucose metabolism, eliminating the effect of genotype in MZ twins, revealed significant negative associations between birth weight, and fasting blood glucose, 120 min post-OGTT glucose, fasting insulin and HOMA-IR in MZ twins. For every 1 kg difference in birth weight within a twin pair the heavier twin had 15.7% lower fasting plasma glucose, 25.5% lower 120 min post-OGTT plasma glucose, 26.5% lower fasting plasma insulin and a 37.2% lower HOMA-IR index than the lighter twin (Table 2). The equivalent regressions for DZ twins were also significant, although the differences in the outcome variables for every extra 1 kg of birth weight were of smaller magnitude (Table 2).

Genetic versus nongenetic association between birth weight, obesity and glucose metabolism

To investigate whether the nongenetic associations between birth weight, on the one hand, and insulin

Table 2 Difference in outcome variables for every 1-kg difference in birth weight within monozygotic (MZ) and dizygotic (DZ) twin pairs

Outcome variable	MZ (95% CI)	DZ (95% CI)
Fasting glucose (mmol L^{-1})	−15.7 (−23.0; −5.3)**	−6.8 (−12.8; −0.4)*
120 min post-OGTT glucose (mmol L^{-1})	−25.5 (−38.0; −10.5)**	−19 (−28.5; −8.3)**
$AUC_{glucose(0\text{-}120)}$	−22.6 (−42.3; 3.9)	−13.7 (−30.8; 7.7)
Fasting insulin (pmol L^{-1})	−26.5 (−43.3; −4.8)*	−16.9 (−28.8; −3.0)*
HOMA-IR	−37.2 (−54.4; −13.4)**	−22.5 (−64.2; −6.6)**
Insulin sensitivity index	30.6 (−7.0; 83.5)	23.4 (4.7; 45.5)*
HOMA β-cell (%)	−0.1 (−20.0; 24.5)	−5.1 (−19.4; 11.8)
Ratio insulin/glucose	1.9 (−27.3; 42.9)	18.7 (−14.0; 64.0)

Values are expressed in percentage. OGTT, oral glucose tolerance test; HOMA, homeostasis model assessment. Model: ln **Δ** fasting glucose/ln **Δ** glucose 120 min/ln **Δ** $AUC_{glucose(0\text{-}120)}$, ln **Δ** fasting insulin/ln **Δ** HOMA IR/ln **Δ** insulin sensitivity index/ln **Δ** HOMA β-cell and ln **Δ** ratio insulin/glucose = **Δ** birth weight. *$P \leq 0.05$; **$P \leq 0.01$.

Table 3 Adjusted difference in outcome variables within monozygotic twin pairs

Outcome variable	Birth weight (1 kg)	BMI (1 U)	WHR (0.1 U)
Fasting glucose (mmol L^{-1})	−12.7 (−20.5; −4.2)*	3.3 (1.5; 4.9)**	−2.3 (−12.5; 9.1)
120 min post-OGTT glucose (mmol L^{-1})	−22.8 (−34.4; −9.4)*	4.6 (1.8; 7.4)**	14.9 (−5.2; 38.9)
Fasting insulin (pmol L^{-1})	−17.8 (−37.0; 7.2)	10.2 (7.3; 13.5)**	13.7 (−8.8; 41.8)
HOMA-IR	−24.1 (−48.3; −0.9)*	13.4 (9.5; 17.4)**	12.9 (−14.7; 46.6)
Insulin sensitivity index	12.5 (−26.7; 72.7)	−11.8 (−17.1; −6.6)**	7.8 (−24.1; 53.1)

Adjustment for birth weight, body mass index (BMI) and waist–hip ratio (WHR) respectively. OGTT, oral glucose tolerance test; HOMA, homeostasis model assessment. Model: ln **Δ** fasting glucose/ln **Δ** glucose 120 min/ln **Δ** fasting insulin/ln **Δ** HOMA IR/ln **Δ** insulin sensitivity index/ = **Δ** birth weight **Δ** BMI **Δ** WHR. *$P \leq 0.05$ **$P \leq 0.01$.

resistance and glucose tolerance, on the other, were independent of adult obesity, we included within-pair differences of BMI and WHR in the multiple regression analyses. After adjustment for BMI and WHR, the associations between birth weight and HOMA-IR, fasting plasma glucose and 120 min post-OGTT plasma glucose, respectively, remained significant in MZ twins (Table 3). For a 1-kg difference in birth weight within a twin pair, the heavier MZ twin had a 24.1% lower HOMA-IR ($P = 0.04$), 12.7% lower fasting plasma glucose ($P = 0.049$), and a 22.8% lower 120 min post-OGTT plasma glucose level ($P = 0.03$) compared with the lighter twin, independent of BMI and WHR (Table 3). However, the associations of birth weight with fasting insulin and with the insulin sensitivity index were no longer significant after adjusting for BMI and WHR.

Body mass index *per se* had a significant nongenetic impact on all the outcome variables in MZ twins, including fasting plasma glucose and 120 min post-OGTT plasma glucose, fasting insulin, HOMA-IR and insulin sensitivity index. WHR was not significantly associated with any of the outcome variables when BMI was included as a covariate (Table 3). However, WHR had a significant negative effect on glucose 120 min post-OGTT, fasting insulin, HOMA-IR and insulin sensitivity index in MZ twins when BMI was not included in the analyses (data not shown).

In order to rule out the possibility that the nongenetic associations between birth weight and measures of glucose metabolism were determined solely by the inclusion of twins with T2D, the analyses were repeated on the data set from which twins with T2D and their co-twins had been excluded. The nongenetic

associations between birth weight and glucose tolerance persisted, whereas the associations between birth weight and measures of insulin resistance were no longer significant (data not shown).

Discussion

In the present study we investigated whether the associations between birth weight and measures of glucose metabolism are of genetic or nongenetic origin and whether they are mediated by adult obesity. We demonstrated nongenetic negative associations between birth weight and insulin resistance and glucose intolerance. This finding implies that the foetal environment influences insulin resistance and glucose intolerance in elderly twins. The association between birth weight and glucose intolerance was independent of current body size, whereas that between birth weight and insulin resistance was apparent, though weaker upon adjustment for adult obesity.

The heritability of 38% implies that there is a genetic component to birth weight. Although we cannot rule out the possibility that the greater similarity in birth weight amongst MZ than DZ twin pairs may be due not only to genes, but also to a similar prenatal environment, the present result is consistent with previous twin studies that have reported heritability estimates in the range of 20–50% [27–30]. Taken together with the previously reported genetic influence on glucose intolerance [18], our finding underlines the importance of adjusting for genetic factors when exploring putative associations between birth weight and measures of glucose metabolism in order to ensure that any demonstrated association is not merely a product of a common genotype, as previously proposed [3]. This may also explain why not all studies report an effect of birth weight on glucose intolerance and insulin resistance [reviewed in Ref. 1]. Accordingly, in the present study we did not find any significant correlations between absolute values of birth weight and measures of glucose metabolism, except between 120 min post-OGTT plasma insulin level and birth weight in MZ twins.

After adjusting for common environmental components, in particular for genotype amongst MZ twins, we found significant nongenetic associations between birth weight and measures of glucose tolerance and insulin resistance. The magnitude of the associations between birth weight and metabolic variables was smaller in the DZ than in the MZ twins. The genetic influence cannot be completely eliminated in the DZ twins but only reduced by an average of 50%, emphasizing the nongenetic origin of the associations between birth weight on the one hand and glucose tolerance and insulin resistance on the other. The nongenetic impact of birth weight on glucose metabolism was indeed of clinical importance, as for every 1-kg difference in birth weight within a twin pair the heavier twin had 15.7% lower fasting plasma glucose, 25.5% lower 120 min post-OGTT plasma glucose, 26.5% lower fasting plasma insulin and a 37.2% lower HOMA-IR index in comparison with the lighter twin. Interestingly, the nongenetic associations between birth weight and glucose tolerance remained significant after adjustment for BMI and WHR, indicating that the effect of birth weight is independent of, and not mediated by, an effect of overall and/or abdominal obesity in MZ twins. However, the nongenetic relationship between birth weight and insulin resistance was weakened when adjusting for current body size, proposing that this association may be influenced to some extent by adult obesity.

Body mass index *per se* had a significant nongenetic impact on both glucose tolerance and insulin resistance whereas WHR *per se* was not associated in a nongenetic manner with any of the metabolic variables when adjusting for BMI and birth weight. Several studies have concluded that abdominal obesity is associated with an increased risk of developing insulin resistance and glucose intolerance [11, 12], which makes the results presented here somewhat surprising. Nevertheless, we have previously demonstrated that WHR is not directly associated with measures for glucose tolerance and/or insulin resistance (fasting insulin), but rather through an association with BMI [31]. Consequently, when including and adjusting for BMI in the present analyses, we could not demonstrate an independent, nongenetic effect of WHR on measures of glucose metabolism.

In the present study we were unable to find any significant correlations between birth weight and the measures of insulin secretion (HOMA β-cell function or insulin/glucose ratio) in either MZ or DZ twins. This is in contrast to a previously demonstrated nongenetic association between birth weight and insulin secretion expressed as disposition index (insulin secretion capacity in relation to insulin sensitivity determined by gold standard methods) in elderly twins [8]. One obvious explanation for this discrepancy is that different methods, involving indirect measures of insulin secretion, were employed in the current study.

The extent to which we can generalize the conclusions of twin studies of the foetal origins of adult disease, including those of glucose metabolism, has been a matter of debate due to the special circumstances prevailing in twin pregnancies. Importantly, twin studies provide a unique means of assessing the origin of an association between birth weight and adult phenotype. Nevertheless, twins may differ from singletons with respect to foetal growth and growth restriction in particular, and, on average, they weigh less at birth. There is inconsistent evidence concerning whether and when twin growth is compromised during gestation compared with singleton pregnancies. Studies have demonstrated a degree of physiological down-regulation in twin foetal growth, and it has been hypothesized that early embryonic development in twins could be slightly differently timed or delayed by unidentified signals (e.g. nutritional requirements for growth and gene regulation) in order to avoid long-term negative effects of intrauterine growth retardation [32]. Consequently the fact that twins are smaller than singletons may not necessarily signify pathology [33].

In conclusion, we demonstrated a genetic component to birth weight in elderly twins. After adjusting for this genetic influence, we identified nongenetic negative associations between birth weight and measures of glucose intolerance that were independent of adult obesity. These findings imply that the foetal environment influences glucose homeostasis in elderly twins. In addition, we showed a nongenetic association between birth weight and insulin resistance, which was less pronounced after adjusting for current body size. We suggest that this association is partly mediated by adult obesity.

Conflict of interest statement

No conflict of interest was declared.

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