Birth Weight and Creatinine Clearance in Young Adult Twins: Influence of Genetic, Prenatal, and Maternal Factors

Marij Gielen,* Sara-Joan Pinto-Sietsma,† Maurice P. Zeegers,§ Ruth J. Loos,‖** Robert Fagard,¶ Peter W. de Leeuw,‡ Gaston Beunen,‖ Catherine Derom,§ and Robert Vlietinck**

Departments of *Population Genetics, Genomics and Bioinformatics, and 1Epidemiology, Maastricht University, and 1Department of Internal Medicine, Division of Vascular Medicine, University Hospital Maastricht, Maastricht, the Netherlands; ‡Comprehensive Cancer Institute Limburg and Department of General Practice, †Department of Sport and Movement Science, Faculty of Kinesiology and Rehabilitation Sciences, ¶Hypertension and Cardiovascular Rehabilitation Unit, and ‖Center for Human Genetics, Faculty of Medicine, Catholic University of Leuven, Leuven, Belgium; and **Human Genome Lab, Pennington Biomedical Research Center, Baton Rouge, Louisiana

Previous studies have shown that low birth weight (LBW) is a risk factor for renal impairment in adult life. The effects of LBW and renal function were studied by using twins, which allows distinguishing among fetoplacental, maternal, and genetic influences. Perinatal data were obtained at birth, and absolute creatinine clearance (not corrected for body surface area) was measured at a mean age of 25.6 yr in 653 individuals. Twins were considered both as individuals and as members of twin pairs. Statistical analyses were performed with and without adjusting for gestational age, zygosity, gender, age, body mass index, glucose level, BP, and smoking status. Creatinine clearance was 4 ml/min lower in twins with LBW (<2500 g) than in twins with a high birth weight (P < 0.04, adjusted). Intrapair birth weight difference correlated positively with the intrapair difference in creatinine clearance equally in monozygotic and dizygotic twins (r = 0.35, P < 0.0001; r = 0.43, P < 0.0001, respectively). This suggests that fetoplacental factors are related to renal function and that genetic factors are less important.

In the 1980s, Barker introduced his “fetal origins hypothesis.” This hypothesis states that undernutrition in utero permanently changes the body’s structure, physiology, and metabolism. These metabolic and endocrine adjustments are beneficial for short-term survival but may lead to cardiovascular and metabolic disease in adult life (1). To strengthen this hypothesis, experimental animal data suggest that low birth weight (LBW) contributes to the development of renal disease and hypertension (2–7).

In addition to these findings, epidemiologic data suggest that LBW is an independent risk factor for renal impairment in adult life (8). Lackland and co-workers (9,10) described that young adults with lower birth weights were more prone to develop ESRD. Furthermore Australian Aborigines and Pima Indians with LBW had higher rates of albuminuria, as a marker of renal impairment, compared with individuals with normal birth weight (11–13).

Twins allow distinguishing among fetoplacental, maternal, and genetic influences. Twins share the same maternal environment, and in the case of monozygotic twins, they are genetically identical. By comparing the heavier and the lighter twin member within a pair, the influence of fetoplacental environment can be estimated while potential confounding maternal and genetic characteristics are controlled for. By comparing twins concordant for LBW with twins discordant for high birth weight (HBW), the influence of maternal environment can be estimated. Furthermore, twins commonly display growth retardation and therefore may have a higher risk for developing an impaired renal function in adult life.

Until now, no large epidemiologic twin study has been performed to investigate the relationship between LBW and impaired renal function in young, healthy adults. In the present study, we examined the association between birth weight and renal function in young adult twins. We hypothesized that genetic, individual fetoplacental, and maternal factors influence renal function. We therefore studied the association of these factors with renal function in a large Prenatal Programming Twin Study recruited from the East Flanders Prospective Twin Survey (EFPTS; Belgium) (14).
Materials and Methods

Participants

The study sample consisted of 804 individuals (380 complete pairs and 44 pairs of whom only one sibling agreed to participate, aged 18 to 34 yr) who were randomly selected from the EFPTS. The EFPTS is a population-based survey that has prospectively registered all twins who were born in the Belgian Province of East Flanders since 1964. Perinatal data were collected at birth, and placental examination was performed within 24 h after delivery. Zygosity was determined through sequential analysis on the basis of gender, fetal membranes, umbilical cord blood groups, placental alkaline phosphatase, and DNA fingerprints (15,16). Between July 1964 and May 1982, the EFPTS had registered 2141 twin pairs who met the World Health Organization criteria for liveborn infants (birth weight of at least 500 g or gestational age of at least 22 wk, if birth weight unknown). Pairs of whom one or both members were stillborn, died in neonatal or later life, or had major congenital malformation were excluded. We randomly contacted 803 twin pairs using an envelope system. To ensure equally distributed groups, we stratified for birth year, gender, and zygosity/chiorionicity. Eventually, 424 twin pairs (804 individuals; overall response 52.8%) participated in the Prenatal Programming Twin Study. We were able to measure the creatinine clearance of 764 twins. None of the twins had ESRD or were on hemodialysis. After validation (see below), 111 individuals were excluded and 653 twins remained. These individuals were members of 265 complete twin pairs (530 individuals) and of 123 incomplete pairs (123 individuals). The twins gave informed consent, and the project was approved by the Local Committee of Medical Ethics.

Measurements

Blood samples were taken, and 24-h urine was collected to measure the volume and the creatinine levels. Before urine collection, participants were asked to select a day of normal routine, to drink normally and refrain from caffeine use, and to refrain from heavy exercise.

Creatinine clearance was measured with the formula \( (U \times V)/P \), where \( U \) is urine creatinine concentration, \( V \) is urine volume, and \( P \) is plasma creatinine concentration. Because 24-h urine collection might be inaccurate as a result of collection errors, we first performed a validation step in which we compared the measured creatinine clearance with the estimated clearance as derived from the modified Cockcroft-Gault formula (17). For this purpose, we used the Bland-Altman method (18), which investigates the agreement between two methods, which are supposed to measure the same. When the difference in outcome between the two methods is greater than 2 SD of the difference, one may conclude that one of the methods yields false results. We analyzed our data after excluding participants who had a difference between their measured 24-h creatinine clearance and the modified Cockcroft-Gault creatinine clearance of more than 2 SD according to the Bland-Altman method (18,19). In this article, we present only the measured 24-h creatinine clearance.

Perinatal data were registered prospectively at birth. Birth weights were obtained from obstetric records. Gestational age was reported by the obstetrician at time of birth and was calculated as the number of completed weeks of pregnancy, based on the last menstrual period.

Blood samples were taken to measure fasting glucose levels by using the hexokinase method (Olympus AU600, Tokyo, Japan) and creatinine levels using the Jaffé kinetic method (Olympus AU600). Anthropometric measurements were performed by two trained researchers according to standardized procedures. The intraclass correlation for interobserver reliability reached 0.93 to 0.99. Participants were measured barefoot and lightly clothed. Standing height was measured with a Harpenden fixed stadiometer, and body weight was measured on a balance scale (SECA, Hamburg, Germany) to the nearest 0.1 cm and 0.1 kg, respectively. Body mass index (BMI: kg/m\(^2\)), as a measure of overall body composition, was calculated as body weight divided by the square of height.

After 5 min of supine rest, BP was measured on the right arm in triplicate in supine position by sphygmomanometry and auscultation (Korotkoff phases I and V) by one of four trained investigators. The reported conventional BP is the average of three measurements.

The twins answered an extended and standardized questionnaire that included health, medication use, and tobacco use. The participants were divided into three categories according to smoking status: Non-smokers (never smoked or former light smokers, who stopped >1 yr ago), current smokers (current smokers or former heavy smokers who stopped <1 yr ago), and former smokers (nonsmokers who stopped heavy smoking >1 yr ago).

Statistical Analyses

All statistical analyses were performed with SAS version 8.2 software (SAS Institute Inc., Cary, NC). All analyses were done both unadjusted and adjusted for the following covariates: Age, gestational age, zygos- ity, gender, BMI, systolic and diastolic BP, glucose level, and smoking status. As the BMI had a skewed distribution, this was log-transformed to ensure normality. Eight people were on antihypertensive medication. Correcting for this last parameter did not change the results.

All statistical tests were two sided; \( P < 0.05 \) was considered significant. Twins were considered either as individuals or as members of twin pairs, depending on the analyses.

Twins as Individuals. All individuals were divided into two groups according to their birth weight: an LBW group \((<2500 \text{ g}; n = 304)\) and an HBW group \((\geq 2500 \text{ g}; n = 349)\). To compare the two groups, we used an F test followed by a \( t \) test for dichotomous analysis and a \( \chi^2 \) analysis for frequencies. A multivariate analysis (proc mixed) was performed to evaluate the relationship between birth weight and creatinine clearance taking into account clustering of the twin pairs and adjusting for covariates.

Pairwise Analysis.

Individual Feto-placental Influences and Genetic Influences. To distinguish between individual feto-placental and genetic influences, we analyzed our data for monozygotic twins and dizygotic twins separately. For this purpose, we used the Pearson correlation coefficient between the intrapair birth weight difference (difference between two members of a pair) and the intrapair creatinine clearance difference.

Maternal Factors. Upper case abbreviations refer to the pair, whereas lower case abbreviations refer to the individual. The same-gender twin pairs were divided into three subgroups: Pairs of whom both members had LBW (concordant low [L] birth weight [L pairs]; \( n = 85 \)), pairs of whom one member had LBW and the other had HBW (discordant [D] birth weight [D pairs]; \( n = 54 \)), and pairs of whom both members had HBW (concordant high [H] birth weight [H pairs]; \( n = 94 \)). In each birth weight group, we distinguished the lightest (coded as l) and heaviest (coded as h) twin member, resulting in six subgroups: The lightest (L) and heaviest (H) of L pairs, the lightest (D) and heaviest (Dh) of D pairs, and the lightest (Hl) and heaviest (Hhl) of H pairs. To analyze maternal influences, we used ANOVA to compare the lightest with the heaviest twin member of a pair (Ll versus Lh, Dl versus Dh, and Hl versus Hhl). Furthermore, we compared the creatinine clearance of the three different birth weight groups (L, D, and H pairs) with each other.
Results
The characteristics of the 653 twins are shown in Table 1. The twins had a mean age of 25.6 yr (SD 4.7). Their mean birth weight was 2530 g (SD 475 g), which is within the normal range for twins. The 24-h creatinine clearance, BP, and blood glucose levels were also within their normal range. None of the twins had diabetes, and eight people were on antihypertensive medication.

The difference in mean birth weight between the twins with HBW and with LBW was 747 g ($P < 0.0001$). Gestational age was also significantly different between the two birth weight groups (35.7 wk LBW versus 38.4 wk HBW). There were significantly more male participants in the HBW group as compared with the LBW group (43% LBW versus 53% HBW). Furthermore, there were more dizygotic twins in the HBW group as compared with the LBW group (35% LBW versus 44% HBW). Twins in the LBW group were significantly shorter (170 cm LBW versus 173 cm HBW) and lighter (63 kg LBW versus 66 kg HBW) than twins in the HBW group, whereas there were no differences in BMI between the two groups.

There was a difference of 5 ml/min in creatinine clearance between the two groups ($P = 0.05$), but the difference was not significant after correction for body surface area (BSA; Table 1).

Twins as Individuals
Multivariate analysis with birth weight as a dichotomous variable showed that the group of twins with LBW had a significantly lower mean creatinine clearance as compared with the group of twins with HBW (4 ml/min; $P = 0.04$; Figure 1). However, because dichotomous analysis may render an outcome more favorable than actually is true, we also analyzed our data in a multivariate analysis with both birth weight and creatinine clearance as continuous variables. These data showed that per kilogram decrease in birth weight, there was a 6-ml/min decrease in creatinine clearance ($P = 0.005$).

Pairwise Analysis
As a next step, we evaluated the influence of individual fetoplacental, genetic, and maternal factors.

Table 1. Characteristics of the study population, according to low or high birth weight$^a$

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall  ($n = 653$)</th>
<th>LBW ($n = 304$)</th>
<th>HBW ($n = 349$)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine clearance (ml/min; mean ± SD)</td>
<td>96 ± 28</td>
<td>93 ± 28</td>
<td>98 ± 27</td>
<td>0.05</td>
</tr>
<tr>
<td>Creatinine clearance corrected for BSA (ml/min per 1.73 m²; mean ± SD)</td>
<td>93 ± 23</td>
<td>93 ± 24</td>
<td>94 ± 22</td>
<td>0.51</td>
</tr>
<tr>
<td>Birth weight (g; mean ± SD)</td>
<td>2530 ± 475</td>
<td>2131 ± 270</td>
<td>2878 ± 314</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gestational age (wk; mean ± SD)</td>
<td>37.1 ± 2.5</td>
<td>35.7 ± 2.4</td>
<td>38.4 ± 1.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age (yr; mean ± SD)</td>
<td>25.6 ± 4.7</td>
<td>25.7 ± 4.6</td>
<td>25.5 ± 4.8</td>
<td>0.48</td>
</tr>
<tr>
<td>Body mass index (kg/m²; mean ± SD)</td>
<td>21.9 ± 2.9</td>
<td>21.9 ± 3.1</td>
<td>21.9 ± 2.7</td>
<td>0.96</td>
</tr>
<tr>
<td>BMI &gt; 30 (%)</td>
<td>10 (2%)</td>
<td>8 (3%)</td>
<td>2 (1%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Height (cm; mean ± SD)</td>
<td>171.6 ± 8.7</td>
<td>169.8 ± 8.5</td>
<td>173.2 ± 8.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Body weight (kg; mean ± SD)</td>
<td>64.6 ± 10.3</td>
<td>63.2 ± 10.3</td>
<td>65.9 ± 10.3</td>
<td>0.0006</td>
</tr>
<tr>
<td>Glucose (mmol/L; mean ± SD)</td>
<td>4.7 ± 0.4</td>
<td>4.7 ± 0.4</td>
<td>4.7 ± 0.4</td>
<td>0.60</td>
</tr>
<tr>
<td>Systolic BP (mmHg; mean ± SD)</td>
<td>124 ± 12</td>
<td>125 ± 12</td>
<td>124 ± 12</td>
<td>0.22</td>
</tr>
<tr>
<td>Diastolic BP (mmHg; mean ± SD)</td>
<td>68 ± 10</td>
<td>67 ± 10</td>
<td>67 ± 10</td>
<td>0.61</td>
</tr>
<tr>
<td>Male (n [%])</td>
<td>318 (49%)</td>
<td>132 (43%)</td>
<td>186 (53%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Current smoker (n [%])</td>
<td>200 (31%)</td>
<td>94 (31%)</td>
<td>106 (30%)</td>
<td>0.50</td>
</tr>
<tr>
<td>Former smoker (n [%])</td>
<td>47 (7%)</td>
<td>18 (6%)</td>
<td>29 (8%)</td>
<td>0.50</td>
</tr>
<tr>
<td>Nonsmoker (n [%])</td>
<td>406 (62%)</td>
<td>192 (63%)</td>
<td>214 (61%)</td>
<td>0.50</td>
</tr>
<tr>
<td>Monozygotic twin (n [%])</td>
<td>394 (60%)</td>
<td>199 (65%)</td>
<td>195 (55%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Dizygotic twin (n [%])</td>
<td>259 (40%)</td>
<td>105 (35%)</td>
<td>154 (44%)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

$^a$LBW, low birth weight (<2500 g); HBW, high birth weight (≥2500 g); BSA, body surface area.
Individual Fetoplacental Influences and Genetic Influences. The larger the intrapair difference in birth weight, the larger the intrapair difference in adjusted creatinine clearance became, resulting in a positive correlation for both monozygotic and dizygotic twins ($r = 0.35$, $P < 0.0001$; $r = 0.43$, $P < 0.0001$, respectively). This suggests that fetoplacental factors may influence the relation between LBW and renal function. It is interesting that there was no difference between monozygotic twins and dizygotic twins ($P = 0.11$; Table 2). This may suggest that possible genetic factors that influence the relation between LBW and renal function were too small to be detected.

Maternal Influences. The lighter members had a lower creatinine clearance as compared with the heavier members for each of the three (L, D, and H) groups. Furthermore, twins discordant for LBW (L pairs) had a lower creatinine clearance as compared with twins discordant for HBW (H pairs). These results showed a trend and were NS (Figure 2). This suggests that the effect of maternal factors may be too small to play a significant role in the relationship between LBW and renal function loss at adult age.

Discussion

Our findings support the hypothesis that LBW, as a marker of the intrauterine environment, may be associated with a lower creatinine clearance at adult age and that fetoplacental factors are more important than genetic and maternal factors. We used twins as a model to test the genetic, maternal, and fetoplacental influences. To our knowledge, no other studies have used this approach for renal function. Each twin has a unique fetoplacental environment. By comparing the heavier with the lighter twin member of monozygotic twin pairs, the influence of birth weight on renal function can be estimated while potential confounding maternal characteristics and genetic factors are controlled for. As monozygotic twins are genetically identical, the association between the intrapair difference in birth weight and intrapair difference in creatinine clearance at adult age must be due to environmental differences, including fetoplacental influences and specifically excluding the influence of genetic factors. Alternatively, when the association is absent or less pronounced in monozygotic twins but present or more pronounced in dizygotic twins, this is more likely related to genetic influences.

We found a significant positive correlation between the intrapair difference in birth weight and the intrapair difference in creatinine clearance, in both monozygotic and dizygotic twins. Therefore, we suggest that there is evidence for an influence of the fetoplacental environment. However, there was no difference in the relationship between birth weight and creatinine clearance between monozygotic and dizygotic twins, suggesting a less pronounced role of genetics.

Twin members share the same maternal environment irrespective of their zygosity; therefore, the influence of maternal factors, such as nutrition, hormones, nicotine, and drugs, will be shared by both twin members. By comparing the creatinine clearance of pairs of whom both members had LBW with pairs of whom both members had HBW, the influence of the maternal environment can be determined (14,20). Because there was only a trend but no significant differences in creatinine clearance between pairs discordant for LBW and pairs discordant for HBW, the maternal environment seems to play a role of minor importance.

Which factors that operate in utero seem to contribute to the association between birth weight and renal function? Fetal renal growth is at maximum between 26 and 34 wk, and nephrogenesis is completed by 32 to 34 wk (21). A nephron deficit at birth therefore will persist throughout life (22,23). Reduced nephron endowment may lead to compensatory hypertrophy and hyperperfusion of remaining nephrons, leading to hyperfiltration. It has been postulated that this hyperfiltration accelerates the further loss of nephrons and ultimately leads to renal failure, especially when associated with other nephropathic factors and aging (11,19,24,25). Indeed, animal studies have shown that restriction of maternal protein intake

Table 2. Pearson correlation coefficient ($r$) between intrapair difference in birth weight and intrapair difference in creatinine clearance

<table>
<thead>
<tr>
<th>Difference in Creatinine Clearance</th>
<th>$r_{MZ}$ (n = 161)</th>
<th>$r_{DZ}$ (n = 98)</th>
<th>$r_{DZ}$ vs $r_{MZ}$</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difference in birth weight</td>
<td>0.35</td>
<td>0.43</td>
<td>0.11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$P$ value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for gestational age, zygosity, gender, age, BMI, glucose, systolic BP, diastolic BP, and smoking. $r_{MZ}$, Pearson correlation coefficient for monozygotic twins; $r_{DZ}$, Pearson correlation coefficient for dizygotic twins.

![Figure 2. Mean creatinine clearance (SEM) according to concordance or discordance in birth weight.](Image)
during gestation is related to reduced number of glomeruli or renal impairment (2–5,7,22,24). Likewise, LBW in humans resulting from intrauterine growth retardation may be associated with a nephron deficit (24,25). In this study, we observed that fetoplacental factors play a role in utero and may influence renal function in adult life. In the present study population, the relationship between birth weight and renal function loss seems less influenced by genetic and maternal factors. We realize that it is difficult to distinguish between fetoplacental and maternal factors, because maternal effects might exert their action through fetoplacental influences. Furthermore, we may not have been able to detect maternal influences, because nutritional deficiencies were too small in this sample of Belgian twins.

The strength of our study is the large sample of twins, the variety of covariates available, and the prospective approach, because the perinatal data were collected at birth. However, there are some limitations that should be mentioned. One could argue that using a single measurement of the creatinine clearance is not valid, because of collection errors or variability. To test the validity of our data, we used the Bland-Altman method (18). A total of 111 (15%) individuals were excluded because they had a >2 SD difference between the mean 24-h creatinine clearance and the creatinine clearance calculated with the modified Cockcroft-Gault formula. This indicates that 85% of our participants probably collected their 24-h urine correctly and that our single creatinine clearance is accurate.

The relationship between LBW and adult cardiovascular and metabolic disease is complex. Glucose level, BP, and the percentage of body fat all may be increased in association with LBW (1,26). However, each of these factors enhances the risk for renal disease. In this context, Nelson et al. (13) quoted the pros and cons of adjusting for such risk factors. He stated that these factors “are intermediate variables in the causal pathway between birth weight and renal disease. Controlling for these variables, therefore, may bias the estimated effect of birth weight. On the other hand, failure to control for the confounding effects of these variables may also bias the results, as they are risk factors for renal disease regardless of birth weight. The extent to which these variables are both confounders and intermediates in this relation is unknown.”

Conventionally, renal hemodynamic variables are normalized for BSA, but this approach is not without dispute (27,28). In particular, adjusting for BSA does not entirely remove the dependence of GFR on BSA (27). Recently, Bosma et al. (29) described that even in nonobese individuals, a higher BMI is associated with a higher GFR. In addition, BMI has been found to be inversely associated with renal function (11). Therefore, it is appropriate to compare GFR data also after normalization for BMI. In the case of the relationship between birth weight and creatinine clearance, it is imaginable and even likely that current body size is an intermediate factor between LBW and impaired creatinine clearance, because twins with LBW were shorter and weighed less than the twins with HBW. As expected, after correcting creatinine clearance for BSA, the influence of birth weight disappeared, especially in heavy newborns. Obesity, however, may act more as a confounding factor than as an intermediate factor. Therefore, in the present study, we analyzed unadjusted data of creatinine clearance as well as after normalization for BMI.

Finally, our population is young and does not yet display overt renal disease. Therefore, the array of creatinine clearance is limited to levels within the normal range. In addition, the participants are too young to have been able to develop some risk factors for renal disease, such as hypertension or type 2 diabetes. One should interpret our data with caution in view of the cross-sectional nature of our study. Although a longitudinal study would have provided more insight into the rate of decline of renal function, cross-sectional studies offer the possibility to examine more risky hypotheses, without the efforts and costs of follow-up studies. Nevertheless, we plan to follow the twins from our study for future changes in renal function to confirm our present observation.

In conclusion, we have shown that LBW is associated with a lower creatinine clearance at adult age in young adult twins. We conclude that individual fetoplacental factors may influence this relationship, whereas maternal and genetic influences seem less important. However, we cannot exclude that common genetic factors might explain some part of the birth weight–creatine clearance relationship.

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References

7. Dodic M, Moritz K, Koukoulas I, Wintour EM: Pro-
grammed hypertension: Kidney, brain or both? Trends Endocrinol Metab 13: 403–408, 2002

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