The purpose of this study is to present curves of estimated placental growth in twins and to evaluate the relative contribution of gestational age, zygosity, chorionicity, fusion of the placentas, sex of the individual and of the twin pair, site of the umbilical cord insertion, birth order, maternal age, and parity. Perinatal data and placental data were obtained from 6315 live-born twin pairs from the East Flanders Prospective Twin Survey. Of 4318 twin pairs, with no missing values, the placental weights of different gestational ages were analyzed using a nonlinear multivariate Gaussian regression. Two groups were distinguished: (1) twins with two separate placentas, and (2) twins with only one placental mass (one placenta in case of monozygotic twins or two fused placentas in case of dichorionic placentas). Overall, placental weight was influenced by gestational age, fusion of the placentas, and parity. In the case of one placental mass, monozygotic dichorionic twins had the lowest weights. If two separate placentas were present, birth order played a role in favor of the first-born twin. For parity and zygosity, the differences were most pronounced between 27 and 29 weeks, whereas the difference for birth order was most pronounced between 33 and 37 weeks. In conclusion, basic physiological characteristics, routinely examined at birth, influence placental weight. Taking these covariates into account allows a better evaluation of the placental weight given a gestational age, as an indicator of growth.

In perinatology the role of the placenta is certainly underestimated. However, the placenta may be of great help in understanding fetal and neonatal morbidity and mortality. There is a positive relationship between birthweight and placental weight (Bleker et al., 1979; Falkner, 1986; Knaus 1949; McKeown & Record, 1953; Pinar et al., 1996). Compared to singletons, twins are born earlier, have lower birthweights and smaller placentas (Bleker et al., 1979). However, little is known about what factors influence placental growth in twin pregnancies.

Since 1953, at least five studies presented the progress of placental weight in multiple gestations (Bleker et al., 1979, 1988; McKeown & Record, 1953; Pinar et al., 1996; Ward, 1985; Table 1). Two of these studies presented a smoothed curve and found that placental weight was not influenced by sex and chorionicity (Bleker et al., 1988; Pinar et al., 1996). The only factor that played a role was parity, with multipara having heavier placentas than primipara (Bleker et al., 1979, 1988). From these studies it is not clear whether the fusion of placentas plays a role in placental weight. So far, no other potential determinants have been taken into account. As a result, it remains unclear what factors influence the curve of placental growth.

The authors set out to study these factors in depth. The data of live-born twins from the East Flanders Prospective Twin Survey (Belgium; EFPTS) were used. This large cohort gives the opportunity to examine the contribution of ‘simple and physiological’ characteristics (covariates) that could influence placental weight: gestational age, zygosity, chorionicity, fusion of the placentas, sex of the individual, sex of the twin pair, site of cord insertion, birth order, maternal age, and parity. We aimed to evaluate the relative contribution of these covariates in a multivariate approach. Two separate analyses were performed: (1) one for twins with two separate
Curves of Placental Weights of Live-Born Twins

placentas, and (2) one for twins with only one placental mass (one placenta in case of monochorionic twins or two fused placentas in case of dichorionic placentas). Within each group the influence of zygosity and chorionicity was evaluated. Finally we present the estimated placental growth curves for live-born twins.

**Methods**

**Placentas**

The study sample consisted of placentas of live-born twin pairs selected from the EFPTS (Loos et al., 1998). The EFPTS is a population-based survey, which has prospectively registered all twins born in the Belgian Province of East Flanders since 1964 and which examines the placenta and fetal membranes at birth.

Between July 1964 and the end of December 2002, the EFPTS registered 6315 twin pairs who met the World Health Organization criteria for live-born infants (birthweight of at least 500 g, or gestational age of at least 22 weeks if birthweight is unknown).

**Measurements**

**Perinatal Data**

Birth date and time, birthweight, parental ages, and parity were obtained from the obstetric records within 24 hours of delivery. Gestational age was reported by the obstetrician and was calculated as the number of completed weeks of pregnancy. The obstetricians and the pediatricians answered a structured questionnaire that provided information on mode of conception, fetal presentation, mode of delivery, birth order, abnormalities of the children, ABO and Rh blood groups, health status of the mother before, during and after delivery, and the status of the children for the period they stayed in the maternity or neonatal unit. The umbilical cords were cut and ligated by birth order. For the purpose of this study, not only was the sex of the individual used, but also the sex of the twin pair (male–male, MM; male–female, MF; female–female, FF). Furthermore, the parity was divided into two groups: (1) primiparity and (2) multiparity. Because of their small number and as done previously (Loos et al., 2005), a gestation of less than 25 weeks was recorded as 25 weeks (13 pairs) and a gestation of more than 42 weeks was recorded as 42 weeks (16 pairs).

**Placental Examination**

A trained midwife examined the placentas within 24 hours of delivery and assessed chorionicity macroscopically following a standardized protocol (Derom et al., 1995). In monozygotic monochorionic (MZ MC) pairs only one placenta is present. In dichorionic (DC) pairs (dizygotic [DZ] as well as MZ DC), the two placentas could be separate, sometimes connected by membranes, or could have fused. For this study we distinguished two groups: (1) one placental

---

**Table 1**

<table>
<thead>
<tr>
<th>Publication</th>
<th>Sample size</th>
<th>Examination</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>1953 McKeown &amp; Record</td>
<td>834 pregnancies = 1668 twins</td>
<td>Formalin &lt; 1 week</td>
<td>Descriptive statistics (on mean weight)</td>
</tr>
<tr>
<td>1979 Bleker et al.</td>
<td>734 twins</td>
<td>Fresh &lt; 36 hour</td>
<td>Regression analysis and analysis of variance</td>
</tr>
<tr>
<td>1985 Ward</td>
<td>40 pregnancies</td>
<td>Fresh &lt; 36 hour</td>
<td>Descriptive statistics (on mean weight)</td>
</tr>
<tr>
<td>1988 Bleker et al.</td>
<td>1460 twins</td>
<td>Fresh &lt; 24 hour</td>
<td>Descriptive statistics (on mean weight)</td>
</tr>
<tr>
<td>1996 Pinar et al.</td>
<td>514 pregnancies</td>
<td>Fresh &lt; 24 hour</td>
<td>Cubic smoothing spline function</td>
</tr>
<tr>
<td>2006 Present study</td>
<td>2809 fused and 2966 separate placentas</td>
<td>Fresh &lt; 24 hour</td>
<td>Nonlinear or multivariate Gaussian regression</td>
</tr>
</tbody>
</table>

Note: Sample sizes are noted as published in the original publication.

Fusion — whether one placenta was present or two separate placentas were present:
Chor = chorionicity. Parity = multiparous versus primiparous.
— = not described, + = significant (p \(\leq 0.05\)), ns = not significant.

https://doi.org/10.1375/twin.9.5.664 Published online by Cambridge University Press
mass present, whether one placenta in the case of MC twins or two fused placentas in the case of DC pairs; and (2) two separate placentas present, whether MZ DC or DZ with two separate placentas.

Fetal membranes were dissected and, after removing the membranes and blood clots, the fresh unfixed placentas were weighed, and their length and thickness measured. According to the site of the umbilical cord insertion, two groups were distinguished: (1) central insertion (central and eccentric insertions), and (2) peripheral insertion (paramarginal or marginal insertions, or insertions on the surrounding or dividing membrane). The total weight of the placental mass was recorded and if two separate placentas were present, the individual placental weight was also noted.

Blood was taken from the umbilical cord if the blood groups of the twins had not yet been determined. An obstetrician examined placentas with obvious or suspected abnormalities.

**Zygosity Determination**

Zygosity was determined through sequential analysis based on sex, fetal membranes, umbilical cord blood groups (ABO, Rh, CcDeEe, MNs, Duffy, Kell), placental alkaline phosphatase (Decorte et al., 1990; Vlietinck, 1986), and, since 1982, DNA fingerprints (Decorte et al., 1990). Opposite-sex twins and same-sex twins with at least a different blood group or, if blood groups were the same, at least two different DNA-markers, were classified as DZ; MC twins were classified as MZ. For all same-sex DC twins with the same genetic markers, a probability of monozygosity

---

**Figure 1**

Flow chart illustrating the number of the placental weights taken into the analysis of twins born between July 1964 and December 2002, who met the WHO criteria.

Note: * Missing values in gestational age (581, and 2 implausible), zygosity and chorionicity (70), maternal age (90), parity (76).
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was calculated using a lod-score method (Vlietinck, 1986). After DNA fingerprinting, a zygosity probability of .999 was reached. Since 1994 no placental examination took place for opposite-sex twins (DZ) due to budgetary reasons. A small piece of placenta (1 cm³) was, however, frozen and sent to the EFPTS tissue bank.

Exclusion

Twin pairs of whom one or both children were stillborn (205 pairs) or suffered from major congenital malformation (120 pairs) were excluded (Loos et al., 2001). Two MC pairs were excluded because they were recorded as being MC and having two separate placentas. Missing values in covariates were noted for 758 pairs. Furthermore, if it was unknown whether one placental mass or two separate placentas were present (840 pairs), or if placental weight was unrealistic for gestational age or missing (six placentas), or if insertion of the umbilical cord was unknown (95 placentas), the placentas were excluded. For details, see Figure 1.

Eventually, if only one placental mass was present, 2809 weights with no missing values were analyzed, and if two separate placentas were present, 2996 weights (of 1509 pairs) with no missing values were analyzed.

Table 2

Characteristics of the Twins Taken Into Analysis

<table>
<thead>
<tr>
<th>Zygosity and chorionicity</th>
<th>One placental mass</th>
<th>Two separate placentas</th>
</tr>
</thead>
<tbody>
<tr>
<td>One placenta (MC) or two fused placentas</td>
<td>2809 pairs (65%)</td>
<td>1516 pairs (35%)</td>
</tr>
<tr>
<td>2809 placental masses</td>
<td>2996 placentas</td>
<td></td>
</tr>
<tr>
<td>Zygosity and chorionicity</td>
<td>MC</td>
<td>MZ DC</td>
</tr>
<tr>
<td>Primiparity</td>
<td>1087 (46%)</td>
<td>317 (11%)</td>
</tr>
<tr>
<td>Multiparity</td>
<td>500 (46%)</td>
<td>149 (47%)</td>
</tr>
<tr>
<td>Male-male pair</td>
<td>587 (54%)*</td>
<td>168 (53%)</td>
</tr>
<tr>
<td>Female-female pair</td>
<td>535 (51%)</td>
<td>146 (46%)</td>
</tr>
<tr>
<td>Male-female pair</td>
<td>552 (49%)</td>
<td>171 (54%)</td>
</tr>
<tr>
<td>Male individuals</td>
<td>526 (37%)*</td>
<td>921 (37%)*</td>
</tr>
<tr>
<td>Female individuals</td>
<td>462</td>
<td></td>
</tr>
<tr>
<td>Central cord insertion</td>
<td>193 (31%)</td>
<td>104 (33%)</td>
</tr>
<tr>
<td>Peripheral cord insertion</td>
<td>226 (21%)</td>
<td>88 (28%)</td>
</tr>
<tr>
<td>Central–peripheral</td>
<td>518 (48%)**</td>
<td>125 (39%)*</td>
</tr>
</tbody>
</table>

Mean (SE)

| Placental weight (grams) | 724 (4.0) | 701 (8.9) | 733 (4.7) | 376 (4.0) | 376 (1.7) |
| Gestational age (weeks) | 36.3 (0.1) | 36.3 (0.2) | 36.5 (0.1) | 36.4 (0.1) | 36.5 (0.05) |
| Maternal age (years) | 27 (0.1) | 27 (0.3) | 29 (0.1) | 27 (0.2) | 29 (0.1) |

Note: MZ = monozygotic, DZ = dizygotic, MC = monochorionic, DC = dichorionic
Percentages are given per cell.
*p < .05, **p < .001

Statistical Analysis

According to zygosity, chorionicity, and number of placentas the twins were divided into the mentioned two groups: (1) one placental mass present (one placenta or two fused) or (2) two separate placentas present. For both groups, the following covariates were considered: sex of the twin pair, site of umbilical cord insertion, maternal age, and parity. If two separate placentas were present, sex of the individual as well as birth order were additionally considered as covariate. The contingency chi-squared test was used for comparisons of categorical data. Analysis of variance (ANOVA) was used for continuous data.

The placental weights of pregnancies of different gestational ages were analyzed using a nonlinear multivariate Gaussian regression. The analyses were done separately for the group with one placental mass and for the group with two separate placentas. If two separate placentas were present, the between-pair variation was modeled through a random effect and the variance was allowed to change linearly with gestational age. The placental growth was best modeled by a logistic growth curve in which the first 2 weeks of pregnancy (the time between the last menstrual period and conception) were constrained to be
The different covariates recorded were additively added in the growth curve as shown in the following equation

\[ b_1 \left/ \left[ 1 + \exp(b_2 + b_3 \times \text{Gestational Age} + B \times X) \right] \right. \]

where \( b_1 \), \( b_2 \), and \( b_3 \) are the coefficients of the logistic growth curve. \( X \) represents possible covariates and their interactions included in the model, whereas \( B \) represents the corresponding coefficients. First, a model containing main effects was built, then any possible interactions among these were added if necessary. As the modeling process is exploratory, the inference criterion used for comparing the models under consideration is their ability to fit the observed data, that is, models are compared directly through their minimized minus log likelihood. When the numbers of parameters in models differ, they are penalized by adding the number of estimated parameters, a form of the Akaike information criterion (AIC; Akaike, 1973; Lindsey & Jones, 1998). Smaller values indicate more preferable models. This criterion allows direct comparisons among models that are not required to be nested. A conservative 95% confidence interval was computed for the estimated mean placental weight, as the model is a nonlinear multivariate Gaussian regression. The analyses were conducted with the SAS version 8.2 computer package and R version 2.0.1 (Ihaka & Gentleman, 1996) with the growth library (Lindsey, 2001). All reported \( p \) values are two-sided and were considered statistically significant when \( p \leq 0.05 \).

**Results**

The characteristics of the 4325 twin pairs are shown in Table 2. Peripheral umbilical cord insertion was less frequent in DZ twins and MZ twins with two separate placentas, than in MC twins and MZ twins with one placental mass (\( p < .001 \)). MZ twins had significantly younger mothers than DZ twins (27 years vs. 29 years, \( p < .0001 \)). The mean gestational age of our study population was 36.4 (SD = 2.7) weeks. There was no difference in gestational age for zygosity, chorionicity, and whether one placental mass or two separate placentas were present.

**Unadjusted Analysis**

The estimated curve of the placental growth if one placental mass was present and if two separate placentas were present, are shown in Figure 2 and 3, respectively. The curves follow the actual placental weights per gestational age, whereas the mean weights in the case of separate placentas deviate more than the mean weights in the case of one placental mass. Placental weight increases up to 42 weeks (801 g in the case of one placental mass and 420 g in the case of two separate placentas) with for both at 28 weeks a maximum increase of 46 g in the case of one placental mass and of 23 g in the case of two separate placentas.

Figure 4 shows the total placental weights per pregnancy. From this figure it is clear that the total placental weight is higher in cases with two separate placentas than in cases with one placental mass. The weights are about the same up to 30 weeks of gestation; after 30 weeks, the total weight in cases with
Curves of Placental Weights of Live-Born Twins

For example, the estimated placental weight in the case of one placental mass at the gestational age of 36 weeks (since conception \([36 - 2 = 34]\) weeks) in the case of primiparity and belonging to a MZ DC twin pair, would be \(837.90/[1 + \exp(5.32 - 0.21 \times 34 + 0.24)] = 695\) g. For multiparity, the weight would be \(837.90/[1 + \exp(5.32 - 0.21 \times 34 - 0.35 + 0.24)] = 732\) g. Note that a negative coefficient corresponds to an increase in weight and vice versa.

A difference in placental growth curve between primi- and multiparity was observed, independent of the fact of whether there were one or two placental masses. Placentas of primipara had lower weights than placentas of multipara. For both primipara and multipara, the maximum difference was at 27 weeks with 78 g for one placental mass and 20.5 for two separate placentas (Figure 5). At 42 weeks of gestation, the difference was decreased to 12 g for one placental mass and to 4 g for separate placentas.

If two separate placentas were present, there was no significant influence of zygosity. In Figure 6, we present the growth curves if one placental mass is present according to zygosity and chorionicity. For twins with one placental mass, the curves for DZ and MZ MC twins were not significantly different, but higher than the curve for MZ DC twins (maximum difference of 48 g in the period 27–29 weeks). The difference between the curves reduces after 29 weeks and at 42 weeks the difference is reduced to 10 g.

If two separate placentas are present, it is possible to detect an effect of birth order, which interacts with

two separate placentas is higher, resulting in a maximum difference at 42 weeks of 39 g.

Table 3 shows the coefficients and standard errors of the parameters of the best fitting models for one placental mass and for two separate placentas as well as the interaction term. Sex or sex of the co-twin, insertion of the umbilical cord, and maternal age did not contribute significantly to the model.

Table 3
Regression Coefficients of Best Fitting Nonlinear Multivariate Gaussian (Logistic) Regression Model for Placental Growth

<table>
<thead>
<tr>
<th>Coefficients of the logistic curve</th>
<th>Model for one placental mass (MC &amp; MZ DC fused &amp; DZ)</th>
<th>Model for two separate placentas (MZ DC &amp; DZ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>b1 = asymptote</td>
<td>Coefficient (B) 837.901 SE 13.53</td>
<td>Coefficient (B) 451.84 SE 12.19</td>
</tr>
<tr>
<td>b2 = growth rate</td>
<td>5.32 SE 0.40</td>
<td>5.32 SE 0.59</td>
</tr>
<tr>
<td>b3 = growth rate depending on gestational age</td>
<td>-0.21 SE 0.015</td>
<td>-0.21 SE 0.02</td>
</tr>
<tr>
<td>Covariates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ DC</td>
<td>0.24 SE 0.07</td>
<td>0</td>
</tr>
<tr>
<td>DZ DC</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MZ MC</td>
<td>0</td>
<td>NE</td>
</tr>
<tr>
<td>Multiparity</td>
<td>-0.35 SE 0.06</td>
<td>-0.18 SE 0.06</td>
</tr>
<tr>
<td>First-born</td>
<td>-0.67 SE 0.30</td>
<td>0.67 SE 0.30</td>
</tr>
<tr>
<td>Interaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First-born*gestational age</td>
<td>---</td>
<td>-0.028 SE 0.010</td>
</tr>
</tbody>
</table>

Note: MZ = monozygotic, DZ = dizygotic, MC = monochorionic, DC = dichorionic, --- = uninformative for the analysis, NE = does not exist

Sex or sex of the co-twin, insertion of the umbilical cord, and maternal age were ns.

Equation: \(b1 / (1 + \exp(b2 + b3 \times \text{Gestational Age} + b4 \times X))\).

b1, b2, and b3 = coefficients, X = covariates and interaction, and B = coefficients of covariates and interaction.
gestational age. At 25 weeks of gestation the difference in placental weight between the first- and second-born twin was around zero, but increased up to 19 g between 33 and 37 weeks in favor of the first-born (Figure 7).
implantation is at greater proximity than in DZ twins, by which MZ DC twins will fuse earlier than DZ twins and therefore placentation development is more seriously hampered in MZ DC twins. In contrast with this speculation is the fact that MC twins have placental weights that are higher than the MZ DC with fused placentas and are equal to DZ twins with fused placentas. This could be due to the presence of vascular anastomoses in MC placentas with a bidirectional blood flow which results in blood supply from the optimal vascular support towards the reduced vascular support (Bermudez et al., 2002).

Explanations for heavier placentas in twins born to multipara are the facts that the uterus is heavier and more ‘experienced’ (Bleker et al., 1988) and that the amount of distension which the uterus will tolerate at a given period of pregnancy is higher in multipara than in primipara (Bleker et al., 1979). Furthermore, pregnancy results in permanent anatomical changes in the spiral arteries that may modify subsequent vascular remodeling in the next pregnancy (Khong et al., 2003).

A possible explanation for the fact that first-born twins have higher placental weights than second-born twins could be found in the blood supply of the uterus, which is mostly by the uterine arteries. The uterine artery reaches the uterus at the junction of the body and cervix. It is possible, due to unequal sharing, that the first-born twin with a low placenta will receive more blood and become larger than the second-born twin.

The EFPTS is the only population-based twin registry that collects information about placentation and zygosity at birth over a long-term period. The strength of the present study is the large sample size, the prospective data collection and the variety of covariates available and the use of a nonlinear multivariate Gaussian regression. However, there are some limitations that should be mentioned: the present study is cross-sectional, while placental weights at birth are used to estimate continuous intrauterine growth. This could also bias the results since premature birth itself is probably related to unphysiological states of variable duration in either mother or fetus (Lubchenco et al., 1963). We agree with Kloosterman and Bleker et al. (Bleker et al., 1977; Kloosterman, 1965) that at higher gestational ages, heavier placentas are recorded, but that this does not necessarily mean that the placenta grows until the end of pregnancy. This could also be due to the fact that pregnancies with large placentas deliver later. The fact that curves converge towards term (see Figure 5 and 6) could represent the difference between pregnancies that deliver earlier (i.e., there is a problem) versus those where there is no problem and deliver later.

Ethnicity of the parents was not always known. However a vast majority of the inhabitants of East Flanders are Caucasians and therefore we assume that our results will not be biased by ethnicity.

At birth the placenta is always routinely examined. Since there is a positive relationship between birth-weight and placental weight, and low placental weight can often be related to adverse perinatal outcome (Bleker et al., 1979; Falkner, 1986; Knaus, 1949; McKeown & Record, 1953; Naeye, 1987; Thomson et al., 1969; Victoria et al., 2001), the results of the present study are relevant to the clinician. As a result of this positive relationship, not only birthweight, but also placental weight could reflect part of the physical condition of the newborn. The significant covariates used in this study are characteristics that (1) are known during gestation (chorionicity and gestational age can accurately be determined by prenatal ultrasonographic scanning, parity can be recorded before birth), or (2) can be determined at birth (birth order, and zygosity and chorionicity after examination of the placenta and the fetal membranes). Furthermore, it is known that these covariates also influence birth-weight, which makes examination of the placenta even more important (Bleker et al., 1979; Liu & Blair, 2002; Loos et al., 2001, 2005). Taking the covariates into account provides a better estimation of whether the placental weight is accurate for the gestational age of the newborn twin, and thus could explain in part the physical condition of the newborn.

In conclusion, we showed that basic physiological characteristics, such as zygosity, chorionicity, fusion of the placentas, birth order, and parity influence more or less the placental weight in twin pregnancies. Taking these covariates into account allows a better evaluation of the placental weight given a gestational age, as an indicator of growth.

Acknowledgments

This study was supported by grants from the Fund of Scientific Research, Belgium (number 3.0269.97, G.0383.03), the Association for Scientific Research in Multiple Births (Belgium), and the Dutch Diabetes Fund. Patrick J. Lindsey and Robert Vlietinck were supported from the CARIM, GROW and CAPHRI Research Institutes of the Maastricht University (Netherlands). Ruth J. F. Loos was supported by a postdoctoral fellowship from the American Heart Association; Southeast affiliate (nr. 0325355B). We thank Ingeborg Berckmoes and Lut De Zeure for their excellent technical assistance.

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