Fetal Leptin Influences Birth Weight in Twins with Discordant Growth

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ABSTRACT

The objective of this study was to determine the plasma leptin concentrations in twin pregnancies in relation to chorionicity and discordant fetal growth. We studied 53 twin pregnancies of which 26 had growth discordance of $\geq 20\%$ and 27 were concordant for growth (discordance of $\leq 10\%$). Paired maternal and fetal blood samples were obtained at birth. Plasma leptin concentrations were measured by RIA. In discordant monochorionic pregnancies, fetal plasma leptin concentrations in the intrauterine growth-restricted twins were lower than the co-twins with normal growth (mean difference, 3 ng/mL; 95% CI, 2.2 to 3.3 ng/mL; $p < 0.001$), whereas no such differences were present between concordant monochorionic twin pairs (mean difference, 0.1 ng/mL; 95% CI, -0.2 to 0.5 ng/mL; NS). Similarly, fetal plasma leptin concentrations in appropriate-for-gestational-age twins were higher than in the intrauterine growth-restricted twins of the discordant dichorionic pregnancies (mean difference, 2.4 ng/mL; 95% CI, 1.8 to 3.1 ng/mL; $p < 0.001$). No such differences were present between the concordant dichorionic twin pairs (mean difference, 0.2 ng/mL; 95% CI, -0.1 to 0.5 ng/mL; NS). Maternal plasma leptin concentrations were comparable among all four groups and were higher than the fetal levels. Fetal

plasma leptin concentrations of the intrauterine growth-restricted twins of discordant monochorionic and dichorionic pregnancies were comparable. There was a positive association between cord plasma leptin concentrations and the birth weight of twin pairs ($y = 0.002x - 0.32$; $r = 0.63$; $p < 0.001$; $n = 106$). A significant positive association was also found between percent differences in birth weight and fetal delta plasma leptin concentrations of the discordant monochorionic and dichorionic twin pairs ($y = 0.057x + 0.93$; $r = 0.60$; $p < 0.001$, $n = 26$). In conclusion, irrespective of chorionicity, plasma leptin concentrations in intrauterine growth-restricted twins were 2-fold lower than their co-twins with normal growth. These differences may be attributed to placental factors. (*Pediatr Res* 49: 667–672, 2001)

Abbreviations:

MC, monochorionic
DC, dichorionic
MZ, monozygotic
DZ, dizygotic
IUGR, intrauterine growth restriction
AGA, appropriate growth for gestational age

Leptin is a 16-kD protein encoded by the *ob* gene and produced by adipocytes (1). It regulates body weight through a negative feedback signal between the adipose tissue and the hypothalamic centers of satiety, thereby causing a decrease in food intake and increases in body temperature and energy expenditure (2). In obese or normal weight children, as in adults, plasma leptin concentrations closely correlate with body weight and percentage body fat (3, 4). Raised plasma leptin concentrations are observed during pregnancy and in fetal life (5, 6). Several recent studies have demonstrated a positive correlation between plasma leptin concentrations in cord blood and body weight at birth (7, 8). The mechanisms by

which maternal and fetal or neonatal weight and fat mass are regulated during pregnancy and in fetal life are poorly understood.

Leptin is synthesized by adipocytes and the human placenta (8, 9). The adipose tissue in the newborn is capable of synthesizing and secreting hormonal factors, which provide feedback loops between fat tissue and endocrine glands, thereby controlling lipolysis and thermogenesis. As the accumulation of fat mass is dramatically reduced in fetuses with IUGR (10), it is not surprising that many investigators have reported markedly lower plasma leptin concentrations in newborn infants with IUGR than in those with normal growth (11–13). However, from these studies it is not known whether this association is caused by the impact of intrauterine malnutrition *per se*, which leads to poor accumulation of fat mass, or whether it is a coincidence between the putative variables that may alter plasma leptin concentrations and reduce weight at birth. It is

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also unclear whether differences in gestational age, maternal height, birth order, or sex may have led to this association.

Recent data suggest that there is diurnal variation in the plasma leptin concentrations with an approximately 50% increase in levels from nadir to peak (14). Furthermore, leptin concentrations are shown to be higher in the spring and summer than in the fall and are higher in infants born before noon (15). Another potentially confounding variable is that of the genetic influence. It has been shown that the presence of a family history of obesity on the paternal side, but not on the maternal side, is associated with elevated cord leptin concentration (15). No previous study has made any attempt to isolate the effects of these potentially confounding variables on plasma leptin concentrations in the fetus with abnormal growth and those with normal growth.

We studied twin pregnancies with growth discordance as a model to evaluate the association between plasma leptin concentrations and fetal growth. This model removed some of the above confounding variables by optimizing for diurnal, maternal, and intrauterine environment. Furthermore, abnormal fetal growth, and, in particular, growth restriction of at least one twin, is associated with a 4- to 6-fold higher incidence of perinatal mortality and morbidity (16). The affected fetuses are susceptible not only to all the risks that the singleton IUGR entails, but in addition to a higher risk of cerebral palsy (16). Although several factors may contribute to growth restriction of one twin, in MC pregnancy this may be predominantly related to placental factors such as unequal sharing of the placental parenchyma or inter-twin transfusion (17–19). In contrast, the causes of growth restriction of one of a DC twin pair might be attributed to poor placentation or fetal genome, with the exclusion of maternal factors. In this study, we made a further attempt to evaluate fetal plasma leptin concentrations in relation to chorionicity to clarify whether placental or fetal factors influence the levels of circulating leptin at birth.

METHODS

Patients. Fifty-three women with twin pregnancies were studied. Chorionicity was established prenatally and was confirmed at birth. The pregnancy was said to be MC in the presence of concordant genitalia, intrafetal membrane thickness < 2.0 mm, and a single placental mass. The diagnosis of DC was made if there were two placental masses, discordant genitalia, twin peak sign, and intrafetal membrane thickness > 2.0 mm (16).

The diagnosis of discordant growth was made when the difference in estimated birth weight was $\geq 20\%$ with absence of polyhydramnios in the larger twin's sac and the smaller twin's abdominal circumference was ≤ 5 th centile with abnormal umbilical artery Doppler waveforms. This constituted the study group.

The twins who had concordant growth ($\leq 10\%$) and normal amniotic fluid volumes in both sacs (amniotic fluid index of ≤ 24 cm) throughout the pregnancy constituted the control group.

In the DC twin pregnancies, there were 17 sets of twins with unlike and seven with like sex pairing. In like sex pairs,

zygosity information was available by DNA analysis in four cases. However, in the remaining three sets of twins, zygosity was assigned by phenotype assessment by the parents (20).

All pregnancies were monitored by serial ultrasound scans for fetal growth and amniotic fluid volume. We excluded those pregnancies that were complicated by chronic twin-twin transfusion syndrome, intrauterine demise of one twin, fetal structural and chromosomal abnormalities, selective feticide, and embryo reduction. Pregnancies with the maternal complications of hypertension, preeclampsia, diabetes, or renal and cardiac disease were also excluded.

Collection of samples. Matched maternal and umbilical venous blood samples were obtained from twin pairs at delivery. Blood samples were collected into tubes containing EDTA and centrifuged, and the plasma was stored at -70°C until batch assay was performed. Maternal samples were not available from four DC and five MC pregnancies. Samples were collected at the Institute of Obstetrics and Gynaecology, Hammersmith and Queen Charlotte's Hospitals ($n = 44$), and St Mary's Hospital, Manchester ($n = 9$). All women gave informed consent for the collection of maternal blood samples, which was approved by the local hospital ethics committee.

Measurement of plasma leptin concentrations. The plasma concentration of leptin was measured by RIA using commercially available kits obtained from Linco Research (St. Louis, MO, U.S.A.). The intra-assay coefficient of variation for leptin was $< 5\%$.

Data analysis. Clinical data are expressed as medians and ranges, whereas leptin concentrations are expressed as mean with 95% CI. For parametric data, the paired t test was used to compare values within twin pairs and the t test between groups. Fisher's exact test was used for blocked comparisons. For nonparametric data, comparisons between groups were performed using the Mann-Whitney test. Correlations between plasma fetal leptin concentrations and birth weight were sought using the Spearman coefficient. Percentage growth discordance was calculated as [(birth weight of larger twin minus birth weight of smaller twin) divided by birth weight of larger twin] times 100.

RESULTS

Clinical details of MC and DC/DZ twins are given in Table 1. In this study, as all DC twin pairs were DZ (17 unlike sex pairs, and seven like sex pairs were also DZ by virtue of DNA analysis), we used the abbreviation DC/DZ. Furthermore, in the DC/DZ group, as fetal plasma leptin concentrations were comparable between like and unlike sex twin pairs, data are grouped together according to percent differences in birth weight.

Leptin concentration

MC twins. The maternal plasma leptin concentrations were comparable between concordant and discordant MC pregnancies (30.4 ± 5 ng/mL versus 21.5 ± 3 ng/mL; NS).

In the discordant growth group, plasma leptin concentrations in the IUGR twins were lower than in the AGA twins (mean difference, 2.7 ng/mL; 95% CI, 2.2 to 3.3 ng/mL; $p < 0.001$)

Table 1. Clinical variables for MC and DC twins with or without discordant growth

Variable	Monochorionic twins			Dichorionic twins		
	Concordant (n = 15)	Discordant (n = 14)	p value	Concordant (n = 12)	Discordant (n = 12)	p value
Gestational age at delivery (wk)	35 (28–38)	32 (28–39)	NS	34 (28–37)	33 (30–38)	NS
Birth weight (g)						
Twin 1/AGA	2534 (1580–3040)	1770 (1240–3140)	NS	2175 (1130–3252)	2240 (1600–2888)	NS
Twin 2/IUGR	2410 (1465–2920)	1170 (650–2440)	<0.01	2050 (1000–2964)	1490 (1000–2308)	<0.001
% Discordance in weight	4 (0.4–9)	26 (20–58)	<0.001	4 (0.3–10)	27 (21–47)	<0.001
Twin pair*						
Unlike sex	None	None		8 / 12	9 / 12	NS
Like sex						
Male/Male	5 / 15	4 / 14	NS	2 / 12	3 / 12	
Female/Female	10 / 15	10 / 14	NS	2 / 12	None	
Umbilical artery Doppler IUGR twin†		AEDF-7 Raised PI 6 Normal 1			AEDF-5 Raised PI 5 Normal 2	
Mode of delivery*						
CS	7 / 15	9 / 14	NS	4 / 12	8 / 12	NS
Vaginal	8 / 15	5 / 14		8 / 12	4 / 12	
Delta cord hemoglobin (g/dL)	3 (1–4)	4 (3–5)	NS	2.1 (1.2–2.9)	2.1 (1.1–3.1)	NS
Delta arterial pH	0.06 (0.02–0.1)	0.18 (0.12–0.24)	<0.01	0.06 (0.01–0.11)	0.15 (0.1–0.2)	<0.05
Delta arterial Po ₂ (mm Hg)	2.5 (0.2–4.6)	12 (9–15)	<0.05	2.5 (1.4–3.7)	19 (16–22.0)	<0.001

Values reported are median (range). CS, cesarean section; AEDFP, absent end-diastolic flow; PI, pulsatility index.

† Umbilical artery Doppler waveform of twin 1 and twin 2 of concordant growth group and AGA twin of discordant growth group were normal. *p value by Fisher's exact test.

and also twin 1 (mean, 4.0 ng/mL; 95% CI, 3.0 to 4.9 ng/mL; $p < 0.001$) and twin 2 (mean, 3.9 ng/mL; 95% CI, 3.0 to 4.7 ng/mL; $p < 0.001$) of the concordant growth groups (Fig. 1). There were no significant differences in the leptin concentrations between twin pairs with concordant growth (mean difference, 0.12 ng/mL; 95% CI, -0.2 to 0.5 ng/mL; NS; Fig. 2) and AGA twins of the discordant group ($p = 0.18$). The delta plasma leptin concentrations in the discordant growth group were higher than in the twins in the concordant group (mean difference, 2.7 ng/mL; 95% CI, 2.2 to 3.2 ng/mL *versus* 0.1 ng/mL; 95% CI, -0.2 to 0.5 ng/mL).

In discordant MC pregnancies, there were five pairs of male and 10 pairs of female twins, and in the concordant group, there were 10 pairs of female and five pairs of male twins. The interpair fetal plasma leptin concentrations in female twins of concordant (mean difference, 0.3 ng/mL; 95% CI, -0.1 to 0.8 ng/mL *versus* 0.3 ng/mL; 95% CI, -0.2 to 0.7 ng/mL; NS) and

discordant growth group (mean difference, 3 ng/mL; 95% CI, 2.3 to 3.3 ng/mL *versus* 2.7 ng/mL; 95% CI, 1.5 to 3.8 ng/mL; NS) were comparable with those of the male sex twins.

DC twins. The maternal plasma leptin concentrations were comparable between discordant and concordant growth twin pairs (21.0 ng/mL; 95% CI, 10.1 to 31.3 ng/mL *versus* 25.0 ng/mL; 95% CI, 13.2 to 36.7 ng/mL; NS).

In the discordant growth group, fetal plasma leptin concentrations in the IUGR twin were lower than in AGA twins (mean difference, 2.4 ng/mL; 95% CI, 1.8 to 3.1 ng/mL; $p < 0.001$) and the twin 1 ($p < 0.001$) and twin 2 ($p < 0.001$) of the control group (Fig. 2). There were no significant differences in the leptin concentrations between concordant twin pairs (mean difference, 0.2 ng/mL; 95% CI, -0.1 to 0.5 ng/mL; $p = 0.50$; Table 2), which were similar to those of the AGA fetus of the discordant growth group ($p = 0.11$). The delta plasma leptin concentrations in the discordant group were higher than in the control group (mean difference, 2.4 ng/mL; 95% CI, 1.8 to 3.1 ng/mL *versus* 0.2 ng/mL; 95% CI, -0.1 to 0.5 ng/mL; $p < 0.001$).

Fig. 1. Fetal plasma leptin concentrations in MC and DC twins with or without growth discordance.

Fig. 2. Comparison of plasma leptin concentrations of MC (hatched bars) and DC (open bars) twin pairs with (A) and without (B) discordant growth.

Table 2. Plasma leptin values for MC and DC twins with or without discordant growth

Variable	Concordant growth		Discordant growth	
	Monochorionic (n = 14)	Dichorionic (n = 12)	Monochorionic (n = 15)	Dichorionic (n = 12)
Twin 1/AGA (ng/mL)	4.1 ± 1.6	3.3 ± 1.9	4.9 ± 1.7	4.3 ± 1.2
Twin 2/IUGR (ng/mL)	3.9 ± 1.6	3.2 ± 2.0	2.2 ± 1.4	1.8 ± 1.6
Delta values (ng/mL)	0.12 ± 0.7	0.2 ± 0.5	2.7 ± 1.0	2.4 ± 1.1
Maternal (ng/mL)	30.4 ± 18.5	21 ± 19.0	21.5 ± 11.2	24.9 ± 20.7

All values are expressed as mean ± SEM. There were no significant differences between sets of MC and DC twins.

In discordant DC pregnancies, there were seven female and five male IUGR twins and six each of male and female AGA twins. In contrast, there were nine female and 15 male twins in the concordant group. In the discordant group, fetal plasma leptin concentrations between female and male IUGR (mean, 1.6 ng/mL; 95% CI, 0.2 to 2.9 ng/mL *versus* 2.3 ng/mL; 95% CI, 1.4 to 3.3 ng/mL; NS) and AGA twins (mean, 3.8 ng/mL; 95% CI, 3.1 to 4.4 ng/mL *versus* 4.8 ng/mL; 95% CI, 3.6 to 5.9 ng/mL; NS) were comparable.

In the concordant growth group, the fetal plasma leptin concentrations were comparable between female and male infants (mean, 0.3 ng/mL; 95% CI, -0.1 to 0.8 ng/mL *versus* 0.3 ng/mL; 95% CI, -0.2 to 0.7 ng/mL; NS).

Comparison between MC and DC twin groups

In the discordant growth group, the delta fetal plasma leptin concentrations were similar between MC and DC twins (mean difference, 2.7 ng/mL; 95% CI, 2.2 to 3.3 ng/mL *versus* 2.4 ng/mL; 95% CI, 1.8 to 3.1 ng/mL; NS). Similarly, in the concordant group, the delta fetal plasma leptin concentrations were similar between MC and DC twin pairs (mean difference, 0.1 ng/mL; 95% CI, -0.2 to 0.5 ng/mL *versus* 0.2 ng/mL; 95% CI, -0.1 to 0.5 ng/mL; NS; Fig. 2).

Correlation between fetal plasma leptin concentrations and birth weight

Irrespective of chorionicity or zygosity, there was a significant positive correlation between the birth weight of all twins and fetal plasma leptin concentrations ($y = 0.002x - 0.32$; $r = 0.63$; $R^2 = 0.39$; $p < 0.001$; $n = 106$). Similarly, a positive association was present between percent discordance in birth weight and delta plasma leptin concentrations in twins with discordant growth ($y = 0.06x + 0.93$; $r = 0.60$; $R^2 = 0.36$; $p < 0.001$, $n = 26$; Fig. 3). There were no associations between

fetal plasma leptin concentrations, gestational age, and maternal plasma leptin concentrations.

DISCUSSION

This study reports for the first time that fetal plasma leptin concentrations differ between discordant twin pairs, whereas no such differences were noted between concordant twin pairs. In discordant twins, the plasma leptin concentrations were 2-fold higher in the larger twin than in the growth-restricted co-twin. This occurs irrespective of placental chorionicity or zygosity, which is therefore suggestive of placental dysfunction rather than fetal genetic factors as the prime cause for variations in plasma leptin concentrations between twin pairs. Our findings are consistent with recent reports of 2- to 3-fold decreases in plasma leptin concentrations in singleton fetuses with growth restriction (21). However, our data are in direct conflict with those of Shekhawat *et al.* (22), who showed no difference in the fetal plasma leptin concentrations between 16 twin pairs. Their data, nevertheless, were not evaluated in relation to chorionicity or growth discordance.

Our data fail to confirm previous observations that fetal plasma leptin concentrations are higher in females than males (8, 12, 23). Although sexual dimorphism is well established in children and adults, this observation is not confirmed by others at birth (11, 21, 24, 25). This discrepant finding is difficult to explain. It is possible, however, that it may be an effect of sample size studied. Alternatively, absence of sex differences may be related to comparable serum concentrations of estrogens and testosterone or s.c. adipocytes between male and female fetuses at birth.

In the literature the effect of maternal plasma leptin concentrations on fetal growth remains conjectural (26). It has been proposed that a positive correlation between cord and maternal blood leptin concentrations suggests that maternal plasma leptin concentrations may regulate fetal growth by modulating the placental transfer of nutrients and, thereby, fat mass and circulating insulin levels. In the twin model, interpair differences in leptin are unlikely to be attributed to maternal plasma leptin concentrations as the influence of maternal leptin would have been similar in twin pairs.

An important observation of this study is that interpair differences in fetal plasma leptin concentrations in discordant MC and DC/DZ twin pairs were similar, and thereby, it refutes the conventional belief that IUGR in MC twins occurs because of transfer of blood and nutrients along the placental vascular anastomoses (27). Recent studies suggest that the arrangement of the shunts in the placenta is such that intertwin transfusion

Fig. 3. A, correlation between fetal plasma leptin concentrations and birth weight of all twins. B, relationship between delta leptin concentrations and percentage differences in birth weight of discordant MC and DC twins.

of blood is not a normal event in pregnancies complicated with discordant growth, and fetal growth restriction appears to be independent of type and number of vascular shunts (17–19). This is not all together surprising as plasma leptin concentrations of the larger twins were similar to the twins with concordant growth and, thereby, suggests that growth restriction in twin pregnancies may be related to placental factors.

The placenta is considered to be an important source of leptin in the fetal circulation (11) because 1) at birth umbilical venous levels of leptin are higher than arterial levels (24, 28); 2) there is a precipitous decline in fetal leptin after birth; and 3) specific leptin mRNA transcripts have been shown to be present in placental tissue in the first and third trimesters (8, 9). Furthermore, numerous studies in singletons have shown that plasma leptin concentrations at birth correlate with placental and fetal weight (13, 24, 25). In this study, no attempt was made to correlate fetal plasma leptin concentrations with the placental weight, because in MC twins accurate determination of the placental mass belonging to each twins is not feasible (27). Therefore, the cause for differing plasma leptin concentrations in the discordant group remains conjectural. We speculate that it could be caused by differences in secretion of leptin from each twin's portion of the placenta as recent evidence suggest that IUGR twins have abnormal placentas in terms of size (17, 19), microvasculature (29), and invasion of spiral arteries by the extravillous trophoblast (30). However, further studies are required to establish an association between placental leptin production and fetal growth.

The functional significance for the 2-fold lower plasma leptin concentrations in the smaller twins is not known. We also found a correlation between fetal plasma leptin concentrations and birth weight. This concurs with the findings from several singleton studies, which report that plasma leptin concentrations at term are directly related to birth weight. A strong association between these two variables has also been reported in diabetic and IUGR infants, with a weak association for AGA fetuses (7, 31–33). Furthermore, the strong positive association between the percentage discordance in birth weight and the delta fetal plasma leptin concentrations suggests that intertwin differences in the growth discordant group may be partly related to disparity in fat mass accumulation or intrauterine fetal growth, and points toward a role for leptin as a regulator of fetal growth (33). There is evidence that decrease in body fat is associated with a decline in peripheral leptin concentrations. It is possible that this association may be caused by common factors that regulate both fetal leptin and body fat. It is proposed that this link may be insulin, as leptin might regulate insulin concentration, which then in turn can regulate both fetal growth and leptin production (34, 35).

We appreciate that the demonstration of a relationship between fetal plasma leptin concentrations and birth weight in twin pairs does not indicate whether factors that influence fetal growth also regulate leptin synthesis and secretion. Notwithstanding this, the overall variance in the data indicates that $\geq 39\%$ of the interpair differences in birth weight in the discordant growth group could be accountable by fetal leptin concentrations, whereas the remaining 60% could be caused by other factors. This is not all together surprising, as the regula-

tion of fetal growth *in utero* is complex, and maternal, placental, and parental genetic variables all may be implicated. Twin studies, by optimizing for the maternal and genetic factors (in MC twins), provide an opportunity to study the role of placental function and fetal hormonal milieu on fetal growth. Recent evidence suggests that fetal growth *in utero* is regulated by GH or IGFs (36, 37). Thus, it is possible that fetal leptin concentration influences intrauterine growth directly as well as through an association with other hormonal systems. This is consistent with evidence from rodents that reduced plasma leptin concentrations and GH levels are normalized by exogenous administration of leptin (38, 39). Moreover, leptin is directly associated with circulating concentrations of IGF-I and IGF-I/IGF binding protein-3 ratios (40). We have also recently shown that in MC twins, IUGR twins had higher IGF binding protein-1 and reduced IGF-II levels than did the AGA co-twins (41). Additional factors, such as impaired placental transfer-mediated fetal undernutrition, may be responsible for the association between fetal growth and leptin. To this end, our recent work also suggests that fetal plasma concentrations of certain essential amino acids in growth-restricted MC twins are markedly reduced (42).

In conclusion, our data suggests that in twins with discordant growth, differences in leptin may be attributed to placental factors rather than maternal or paternal genetic variables. The relationship between leptin and birth weight of twins may be of interest in light of epidemiologic evidence linking birth weight with the development in adulthood of hypertension, obesity, and cardiovascular disease.

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