



# Insulin-Like Growth Factor-1 Levels in Twins and Its Correlation With Discordance

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This study aims to determine whether fetal growth is related to insulin-like growth factor-1 in dichorionic and monozygotic twins and also aims to investigate the correlation of insulin-like growth factor-1 to birthweight discordance in twins. We studied 100 women with twin pregnancies. The correlation tests of 36 discordant twins (15 monozygotic, 21 dichorionic) showed correlation between insulin-like growth factor-1 difference and birthweight discordance (insulin-like growth factor-1 vs. birthweight of first twin,  $r = +0.915$ , at 0.01 level, IGF-1 vs birthweight of second twin  $r = +0.790$ , at 0.01 level). In 49 monozygotic twins, independent of discordance, there was a correlation between birthweight discordance and insulin-like growth factor-1 difference ( $r = .538$ , at the 0.01 level). This correlation was not significant in dichorionic twins,  $r = .144$ ,  $p = .01$ . These data suggest that growth discordances of twins exposed to the same maternal environment may be due to variations in IGF-1, depending upon the genetic similarity.

■ **Keywords:** IGF-1, discordance, twin, fetal growth

Intrauterine growth is regulated by fetal, maternal and environmental factors. Several studies of singleton (Baker et al., 1993) and a few twin (Westwood et al., 2001) pregnancies have shown that various hormones are associated with birthweight. However, singleton studies fail to address the question of whether alterations in fetal nutrition leading to impaired birthweight are due to disturbance in placental transport or in fetal hormone function. Therefore, we planned a study including both monozygotic (MC) and dichorionic (DC) twins with concordant and discordant birthweight. MC twinning is a powerful clinical model because of the identical genetical background, the same maternal nutrition, and only the individual differences for the fetal environment. Therefore, they are an excellent model to study the interaction between genetic and environmental factors and their effects on fetal growth.

The insulin-like growth factors (IGF) are involved in the regulation of growth during pregnancy, early embryonic-fetal and also postnatal development (Baker et al., 1993). IGF-1 is the main factor for fetal growth and development (Westwood et al., 2001). In previous studies, cord blood IGF-1 concentrations correlate with birthweight (Ong et al., 2000; Woods et al., 1996). In contrast, others have shown normal IGF-1 (Osario et al., 1996) and reduced

IGF-2 levels in singleton intrauterine growth retardation fetuses (Giudice et al., 1995; Gluckman et al., 1983). There are only a few studies in twins with discordance or inter-pair birthweight differences about IGFs (Bajoria et al., 2006; Gohlke et al., 2005; Westwood et al., 2001). Growth discordance of twins exposed to same environment may be due to variations in IGFs. But there is a need to better understand the relation between IGF-1 and birthweight and correlation of IGF-1 level and discordance.

In this study, our aim was to determine whether fetal growth is related to IGF-1, in DC and MC twins and investigate correlation of IGF-1 to birthweight discordance in twins.

## Materials and Methods

We studied 100 women with twin pregnancies. Chorionicity was established prenatally by ultrasound scan at 15–19 weeks. Twins with same genitalia, a single placental mass

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and intra-fetal septal thickness of under 2 mm were classed as monochorionic (MC;  $n = 49$ ). Dichorionic placentation was diagnosed in the presence of different genitalia, separated placentas, membrane thickness more than 2 mm (DC;  $n = 51$ ). Chorionicity was also confirmed by histopathological examination of the placenta and membranes. The diagnosis of discordant growth was made when the difference in birthweight was 20% (Canpolat et al., 2006). Twins with differences in birthweight of 10% and normal amniotic fluid volumes in both sacs constituted the concordant group. All pregnancies were monitored by serial ultrasound scans for fetal growth, amniotic fluid volume and umbilical artery Doppler waveforms.

Pregnancies complicated by chronic and acute twin-to-twin transfusion syndrome, death of one or both twins in utero or at birth; fetal aneuploidy; intrauterine growth restriction as well as small for gestational age infants, and structural abnormalities or any congenital anomalies were excluded. Pregnancies under 32 weeks of gestational age were also excluded. After birth, twins were measured on the same scale, the smaller twin being recorded as twin 1 and the larger twin as twin 2 independent of birth order.

#### Collection of Blood Samples and Immunoassays

Umbilical venous cord bloods were obtained at birth from each twin. The samples were centrifuged, and stored at  $-20^{\circ}\text{C}$  until assay. Informed consent was obtained from all women who were recruited for collection of samples as required by the Local Hospital Research Ethics Committee. Plasma IGF-I was determined by radioimmunoassay technique.

#### Data Analysis

Datas are expressed as mean  $\pm$  standard deviations. For parametric data, the paired  $t$  test was used to compare values within twin pairs and the Student's  $t$  test between groups. Percentage growth discordance or  $\Delta$  birthweight was defined as the difference in birthweight expressed as a proportion of the birthweight of the larger twin. 'IGF discordance' ( $\Delta$  IGF-1) was calculated by difference between cord IGF-1 level of second twin and first twin divided by second twin's IGF-1 level.  $\Delta$  birthweight and correlation of  $\Delta$  IGF-1 level was tested by Pearson correlation coefficient.

All data entered to a personal computer within use SPSS® for Windows® version 17.0.

## Results

Of these 100 twins, 49 were MC, and of this MC group, 15 twins were discordant and 34 were concordant. Fifty-one of 100 twins were DC and 21 of these were discordant, while the remaining 30 were concordant twins. All twins were between 32 and 36 weeks of gestation, and the median gestational age was 33.9 weeks (range 32–34). There were no differences between MC and DC group according to birthweight of first twin ( $1693 \pm 340$  g/ $1660 \pm 373$  g,  $p = .479$ ), the second twin ( $1914 \pm 403$  g/ $1912 \pm 385$  g,  $p = .699$ ) and IGF-1 levels of first ( $49.8 \pm 9$  ng/ml/ $48.3 \pm 10$  ng/ml,  $p = .213$ ) and second twins ( $54.7 \pm 11$  ng/ml/ $52.3 \pm 10$  ng/ml,  $p = .934$ ) respectively. In concordant twins there was no statistically significant difference between IGF-1 levels of MC and DC groups. But this difference was significant in the discordant group (Table 1).

When correlation was made in all 100 twins,  $\Delta$  IGF-1 was positively correlated with  $\Delta$  birthweight ( $r = +0.376$ , correlation is significant at 0.01 level). The correlation tests of 36 discordant twins (15 MC, 21 DC), showed a correlation between  $\Delta$  IGF1 and  $\Delta$  birthweights (IGF-1 vs birthweight of first twin,  $r = +0.915$ , at 0.01 level, IGF 1 versus birthweight of second twin  $r = +0.790$ , at 0.001 level). There was no correlation between  $\Delta$  birthweight and  $\Delta$  IGF-1 in concordant twins ( $r = 0.106$ ). In 49 MC twins, independent of discordance, there was a correlation between  $\Delta$  birthweight and  $\Delta$  IGF-1 ( $r = 0.538$ , at the 0.01 level). This correlation was not significant in DC twins,  $r = 0.144$ , when  $p = .01$ . The correlation between birthweight discordance and  $\Delta$  IGF-1 in MC and DC groups are shown in Figure 1.

## Discussion

The results of this study support the hypothesis that the placental growth factors are the major control mechanism of the fetal growth axis, through insulin-like growth factors, especially when fetal genotype and maternal environments are similar. There were significant differences in IGF-I levels in MC twins when being discordant and  $\Delta$

**TABLE 1**

Insulin-Like Growth Factor Levels and Birthweights of Infants for Monochorionic and Dichorionic Twins With or Without Discordance

Birthweight mean $\pm$ SD (range) g, IGF 1 mean $\pm$ SD (range), ng/ml	Concordant $n = 64$			Discordant $n = 36$		
	Twin 1	Twin 2	$p$	Twin 1	Twin 2	$p$
Monochorionic, $n = 49$	1748 $\pm$ 360 (1210–1890) 51,6 $\pm$ 9,5	1812 $\pm$ 350 (1235–2010) 52,8 $\pm$ 10	ns	<b>1567 <math>\pm</math> 255</b> (1270–1870) <b>45,7 <math>\pm</math> 7,6</b>	<b>2146 <math>\pm</math> 413</b> (1830–2520) <b>58,9 <math>\pm</math> 12</b>	0.02
Dichorionic, $n = 51$	1786 $\pm$ 376 (1255–1990) 51,4 $\pm$ 10	1848 $\pm$ 360 (1240–2120) 52 $\pm$ 12	ns	1480 $\pm$ 293 (1220–1870) 44 $\pm$ 9	2005 $\pm$ 370 (1720–2690) 48 $\pm$ 8	0.04

Note:  $P$  values are for IGF-1 levels, bold values present discordant monochorionic ( $n = 15$ ), and italic values are for discordant dichorionic ( $n = 21$ ) twins.

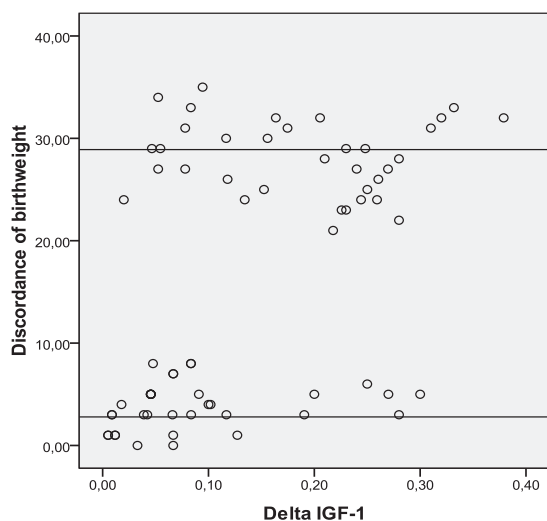


Figure 1A.

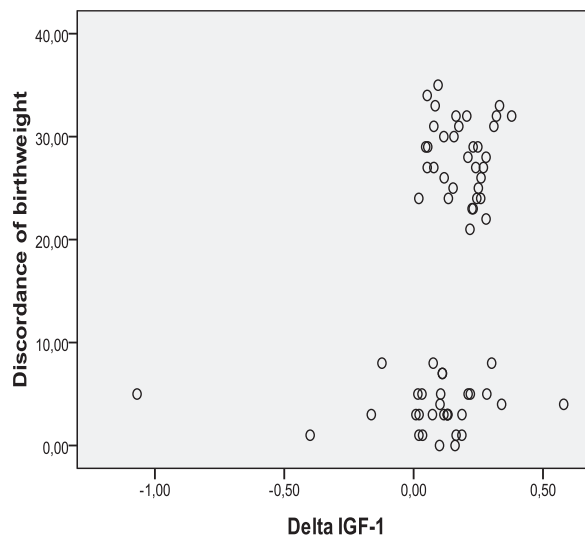


Figure 1B.

**FIGURE 1A AND 1B**

The scatter of correlation between discordance of birthweight and  $\Delta$  IGF-1 in monozygotic (A) and dizygotic (B) twins. As shown in the Figure 1A there is a linear relation in monozygotic group.

birthweight found to be correlated with  $\Delta$  IGF-1 in all twin pairs. This correlation was significant in MC group, no such difference was present between the concordant twin pairs. This correlation in circulating IGF-1 in the fetus by weight, may have a profound impact on fetal growth. These observations therefore suggest that fetal growth is a reflection of the functional status of various pathways by genetic baseline and related to IGF-1.

In our study, the intra-pair IGF-1 concentrations correlated in discordant MC and DC twins, thereby supporting evidence from previous concordant twin studies that endogenous circulating IGF-1 concentrations are genetically determined (Bajoria et al., 2006; Harrela et al., 1996; Kao et al., 1994; Verhaeghe et al., 1996). Studies in mice suggest that genetically determined IGF-1 gene expression regulates growth in the fetus (Gluckman et al., 1992). Accordingly, the comparable circulating IGF-1 concentration in MC twin pairs with discordant birthweight is in direct variance with our data from discordant DC twin pregnancies. In DC discordant twins who are dizygotic with a difference in genetic make-up, there was a slight difference for IGF-1 concentrations (Table 1,  $p = .04$ ). In contrast, the discordant MC twins had significantly different IGF-1 concentrations, which were lower in the smaller twin. Furthermore, birthweights also correlated with IGF-1 concentrations, thereby supporting the data of singleton pregnancies (Baker et al., 1993).

In singleton small for gestational age babies the reported abnormalities in the IGF axis may reflect maternal maladaptation to pregnancy. Maternal nutrition, hypertension, and diabetes are known to alter the maternal IGF axis, such that placental nutrient transfer and

ultimately the fetal IGF axis and prenatal growth are reduced (Verhaeghe et al., 1996). However, maternal environment is obviously not the explanation for growth restriction in only one infant of a twin pair, suggesting that placental factors may underlie the findings of our study. Excluding maternal diseases and comparing MC and DC twins gave us the opportunity to observe the factor affecting birthweight in twins. We found a strong correlation of IGF-1 levels and birthweight among the group of discordant twins, especially in MC twins. This data demonstrates two major points: first, IGF-1 is important in the regulation of fetal growth, and the second we found a strong intertwin correlation for IGF-1 MC twin pairs, confirming the underlying genetic influence on the IGF-1 level (Verhaeghe et al., 1996). But also there is a statistically significant difference between MC and DC discordant twins (Twin 2), this data supports the non-genetic theory and only suggests that IGF correlates with birthweight independently.

In conclusion, our data confirm the importance of IGF-1 for fetal development, although IGF-1 levels were significantly higher in larger twin, compared with the discordant smaller co-twin, whereas these levels were similar in the concordant twin pairs. We also observed that the difference between IGF-1 levels in twin pairs are correlated with birthweight discordance. Furthermore, this demonstrates a possible contribution of genetic baseline for fetal growth through IGF-1. However, specific and different growth hormones should be investigated in further studies to observe the effects and role of other growth factors to show genetic impact on growth.

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