Journal of the Formosan Medical Association (2015) 114, 359–362

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.jfma-online.com

ORIGINAL ARTICLE

Cord blood level of insulin-like growth factor-1 and IGF binding protein-3 in monochorionic twins

Ru-Jeng Teng [a,*], **Tzong-Jin Wu** [a], **Fon-Jou Hsieh** [b]

[a] *Division of Neonatology, Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI 53226, USA*

[b] *Department of Obstetrics and Gynecology, National Taiwan University, Taipei, Taiwan*

Received 19 August 2012; received in revised form 27 November 2012; accepted 26 December 2012

KEYWORDS
insulin-like growth factor (IGF);
insulin-like growth factor binding protein (IGFBP);
intrauterine growth;
monochorionic twin

Background/Purpose: Insulin-like growth factors (IGFs) and their binding proteins (IGFBPs) are known to modulate fetal growth but their role in intrauterine growth of monochorionic twins (MCT) has not been studied.

Methods: Cord venous blood was collected directly after birth. IGF-1 and IGFBP-3 in the cord venous blood were quantified by radioimmunoassay. Birth weights (BWs) were obtained electronically. Placentas were examined for chorionicity.

Results: Cord blood was collected in 37 pairs of MCT (15 pairs were males). BWs ranged from 564 to 3240 g, and gestational ages (GAs) were between 24 weeks and 39 weeks. There was a correlation between BW and cord venous blood IGFBP-3 concentration ($r = 0.28$, $p = 0.015$), but not between BW and cord venous blood IGF-1 level. There was no difference in IGF-1 between the heavier twins (30.8 ± 61.8 ng/mL) and lighter twins (33.2 ± 63.7 ng/mL), but a trend ($p = 0.096$) of higher IGFBP-3 level was demonstrated in heavier twins (3.14 ± 1.23 μg/mL) than in lighter twins (2.71 ± 1.19 μg/mL). The IGFBP-3 levels were higher ($p = 0.042$) in female twins (3.20 ± 1.33 μg/mL) than in male twins (2.64 ± 1.04 μg/mL). The IGF-1 level of the heavier twins correlated significantly to their lighter co-twin ($r = 0.73$, $p < 0.001$).

Conclusion: Our data showed that cord venous blood IGF-1 level might be controlled mainly by genetic factors. IGFBP-3 might play an important role in fetal growth.

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

* Corresponding author. Division of Neonatology, Department of Pediatrics, Medical College of Wisconsin, C410, 999N 92nd Street, Milwaukee, WI 53226, USA.

E-mail address: rteng@mcw.edu (R.-J. Teng).

0929-6646/$ - see front matter
http://dx.doi.org/10.1016/j.jfma.2012.12.014

Introduction

Insulin-like growth factors (IGFs) are a family of polypeptides with growth promoting and insulin-like activities, separated into two main classes, IGF-1 and IGF-2, by their molecular weight and biological properties.[1] Intrauterine fetal growth is believed to be controlled by both the IGF axis and maternal factors.[2,3] The actions of the IGFs are modulated by insulin-like growth factor binding proteins (IGFBPs).[4] Six binding proteins, IGFPB-1 to IGFBP-6, have been characterized to date,[5,6] of which IGFBP-3 is the most important in human blood.[6] There were several studies demonstrating that serum IGF-1 levels correlated positively with fetal weight, length, and placental weight.[7–9] Because of the wide variation of IGF-1 in cord blood, and complex interplay among IGFs and IGFBPs, contradictory results were seen from previous studies.[10] Study in full-term newborn infants demonstrated a good correlation between IGFBP-3 level and birth weight (BW).[11]In an attempt to confirm the relationship between cord IGF-1 and IGFBP-3 levels and fetal growth, we selected monochorionic twins as our study subjects due to their identical genetic makeup.

Subjects and methods

A total of 37 pairs of monochorionic twins, delivered in the National Taiwan University Hospital, were enrolled prospectively in our study. Informed consent was waived by the Institutional Review Board because cord blood was viewed as medical waste as long as patients' identifiers were not reported. Cord venous blood was collected and centrifuged at 3000 rpm for 15 minutes and plasma was stored at -80°C until analysis. Monochorionicity was confirmed by placental inspection and milk injection. BW was measured to the nearest gram using electronic scales.

The IGF-1and IGFBP-3 levels were quantified by radioimmunoassay kits (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) in triplicate. An acid—ethanol extraction method was used for sample preparation before the IGF-1 radioimmunoassay.[12] All reagents required for the assay were prepared according to the manufacturers' instructions. The sensitivity was 0.3 ng/mL with intra-assay variation below 3.0% and inter-assay variation below 8.4%. In the IGFBP-3 assay, the sensitivity was 0.12 μg/mL with the intra-assay variation below 8.0% and the inter-assay variation below 6.3%.

The paired Wilcoxon test was used to compare the IGF-1 and IGFBP-3 levels between heavy and light twins for their skewed distributions. The Mann-Whitney *U* test was used for other comparisons. To compare the proportions of small for gestational age (GA), the Chi-squared test was used. A p value < 0.05 was considered statistically significant.

Results

There were 15 pairs of male twins and 22 pairs of female twins enrolled. The GAs ranged from 24 weeks to 39 weeks. The BWs ranged from 600 g to 3098 g. Six of the 37 heavy twins were small for GA ($<10^{th}$ percentile of corresponding GA)[13] as compared to 17 of 37 of their light co-twins [Chi-squared test 7.63, odds ratio (OR) 0.228, 95% confidence interval (CI) 0.077—0.675, $p < 0.01$]. The GA was similar between male twins (35 ± 3 weeks) and female twins (35 ± 2 weeks). There was no difference in BWs between male twins (2161 ± 514 g) and female twins (2198 ± 480 g). IGF-1 levels were between 11.25 ng/mL and 402.75 ng/mL, and IGFBP-3 levels were between 0.24 μg/mL and 6.30 μg/mL. No difference ($p > 0.5$) was detected between male and female twins in respect of body length and head circumference in our subjects.

Significant positive correlation was observed between BW and IGFBP-3 concentration ($r = 0.28$, $p = 0.015$) (Fig. 1) but such correlation was not observed between BW and IGF-1. We also failed to detect any correlation between birth length and IGF-1 level ($p > 0.5$). There was no difference in cord venous blood IGF-1 between heavy twins (30.8 ± 61.8 ng/mL) and light twins (33.2 ± 63.7 ng/mL) (Fig. 2), but a trend ($p = 0.096$) towards higher IGFBP3 levels could be demonstrated in heavy twins (3.14 ± 1.23 μg/mL) compared to light twins (2.71 ± 1.19 μg/mL) (Fig. 3). The IGFBP3 levels were significantly higher ($p = 0.042$) in female twins (3.20 ± 1.33 μg/mL) than in male twins (2.64 ± 1.04 μg/mL). The IGF-1 level of the heavy twins correlated significantly with their co-twins ($r = 0.75$, $p < 0.001$), but the IGFBP3 level did not ($p = 0.24$). After controlling for BW, sex, birth length, head circumference, and GA, there were no differences for IGF-1 ($F = 1.6$, $p = 0.21$) and IGFBP-3 ($F = 0.002$, $p = 0.96$) between small-for-GA and appropriate-for-GA twins.

Discussion

Two IGFs have been studied to date in human subjects, IGF-1 and IGF-2, and the cord blood levels have been shown to correlate with gestational age[14] or birth weight[7,15] of the newborn infants. Low IGFs have been shown in small-for-GA infants.[16] In term infants, the cord levels of IGF-1 and IGFBP3 significantly correlate with BW, Ponderal index, and placental weight.[17] Owing to a variety of bioassay

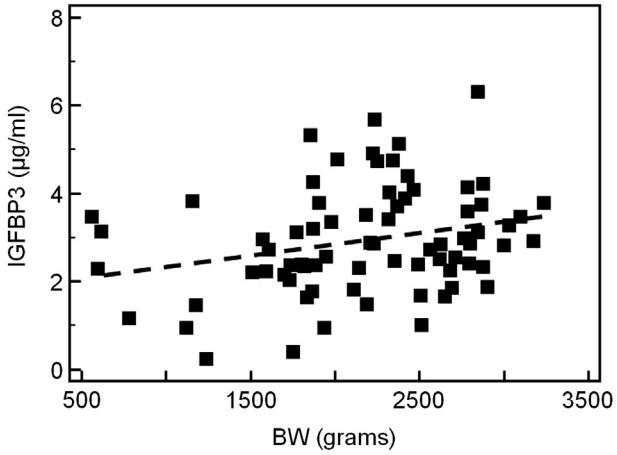

Figure 1 The relationship between cord venous blood insulin-like growth factor binding protein-3 (IGFBP-3) and birth weight (BW) ($r = 0.28$, $p = 0.015$).

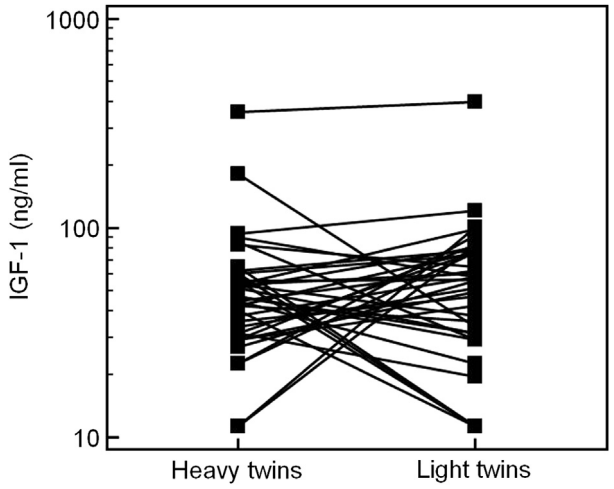

Figure 2 The distribution of cord venous blood insulin-like growth factor-1 (IGF-1) levels.

techniques, and differences in the study subjects, the results have not been conclusive to date.[9] Intrauterine growth retarded infants have significantly lower levels of IGF-1[16,18] and IGFBP-3,[19] but conflicting results have been reported.[9]

The actions of the IGFs are modulated by the IGFBPs.[4] IGFBP-3 is the most important one: it accounts for the majority of IGF binding, and it serves as the reservoir of IGFs.[6] Decreased levels of IGFBP-3 have been shown in the cord blood of intrauterine growth retarded infants.[20]

Monochorionic twins, with identical genetic makeup, are useful to study the relationship between IGFs, IGFBPs, and fetal growth. It has been shown that monozygotic twin children have high within-pair correlation of IGF-1.[21] A similar result has also been shown in adult twins.[21] The results of these studies suggest a genetic contribution to the inter-individual variation of the levels of IGF-1 postnatally. Although mutations in genes controlling IGF, IGFPB, and IGF receptors have been reported to be associated with intrauterine growth retardation, the results obtained from our study subjects, monochorionic twins, may eliminate most of these concerns. We demonstrated the within-pair correlation of the cord venous levels of IGF-1 in monochorionic twins. Our model removed the environmental effects on IGF-1 and IGFBP-3 and further strengthens the genetic contribution of the IGF axis in fetal growth. From our results, we believe that the correlation between BW and cord level of IGF-1 in previous reports may be a secondary effect. It is the environmental factors that determined the BW rather than IGF-1 level. Judging from the high heritability of IGF-1in a recent twin study, we believe that IGF-1 levels are determined mainly by genetic attributes.[22,23]

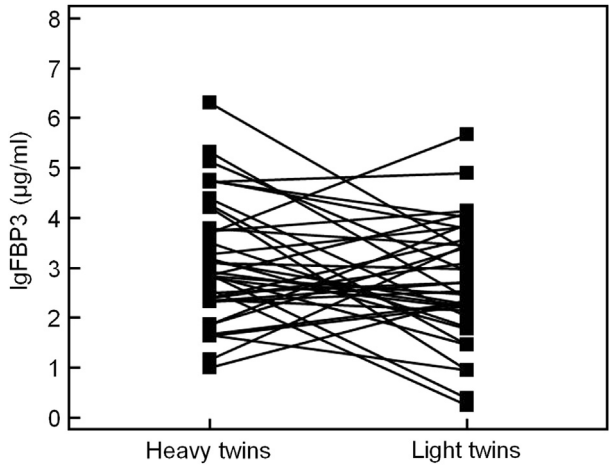

Figure 3 The distribution of cord venous blood insulin-like growth factor binding protein-3 (IGFBP3) levels.

Our data show that the cord venous IGFBP-3 level is higher in females than in males, which is similar to twin studies performed in adults.[23] However, we failed to show significant intra-twin correlation in IGFBP-3 levels. The significance of higher IGFBP-3 in female infants is unclear, because our results showed that IGFBP-3 levels were correlated with BW but there was no weight difference between male twins and female twins. Further studies may be required to clarify this issue.

In conclusion, our data suggests that the IGF-1 level in cord blood is more genetically determined, whereas IGFBP3 may play an important role in determining fetal growth. Due to the low prevalence of monochorionicity, our data fail to answer several important issues. Future collaboration among major centers may provide more information in this regard.

Acknowledgments

This study was supported by a grant (NSC 85-2331-B-002-328b to RJT) from the National Science Council, Republic of China. We are indebted to Dr Elaine St. John, Dr Sidhartha Tan, and Dr Ambalavanan Namasivayam for their help in editing the manuscript.

References

1. Setia S, Sridhar MG. Changes in GH/IGF-1 axis in intrauterine growth retardation: consequences of fetal programming? *Horm Metab Res* 2009;**41**:791—8.
2. Gicquel C, Le Bouc Y. Hormonal regulation of fetal growth. *Horm Res* 2006;**65**:28—33.
3. Randhawa R, Cohen P. The role of the insulin-like growth factor system in prenatal growth. *Mol Genet Metab* 2005;**86**:84—90.
4. Kobayashi S, Clemmons DR, Ventkatachalam MA. Colocalization of insulin-like growth factor-binding protein with insulin-like growth factor-1. *Am J Physiol* 1991;**261**:F22—8.
5. Holly JM, Perks CM. Insulin-like growth factor physiology: what we have learned from human studies. *Endocrinol Metab Clin North Am* 2012;**41**:249—63.
6. Baxter RC. Insulin-like growth factor binding proteins in the human circulation: a review. *Horm Res* 1994;**42**:140—4.
7. Bang P, Westgren M, Schwander J, Blum WF, Rosenfeld RG, Stangenberg M. Ontogeny of insulin-like growth factor-binding protein-1, -2, and -3: quantitative measurements by radioimmunoassay in human fetal serum. *Pediatr Res* 1994;**36**:528—36.
8. Davidson S, Hod M, Merlob P, Shtaif B. Leptin, insulin, insulin-like growth factors and their binding proteins in cord serum:

insight into fetal growth and discordance. *Clin Endocrinol* 2006;**65**:589—92.
9. Wang HS, Lee JD, Soong YK. Effects of labor on serum levels of insulin and insulin-like growth factor-binding proteins at the time of delivery. *Acta Obstet Gynecol Scand* 1995;**74**: 186—93.
10. Holmes R, Montemagno R, Jones J, Preece M, Rodeck C, Soothill P. Fetal and maternal plasma insulin-like growth factors and binding proteins in pregnancies with appropriate or retarded fetal growth. *Early Hum Dev* 1997;**49**:7—17.
11. Osorio M, Torres J, Moya F, Pezzullo J, Salafia C, Baxter R, et al. Insulin-like growth factors (IGFs) and IGF binding proteins-1, -2, and -3 in newborn serum: relationships to fetoplacental growth at term. *Early Hum Dev* 1996;**46**:15—26.
12. Daughaday WH, Mariz IK, Blethen SL. Inhibition of access of basic somatomedin to membrane receptors and immunoblotting sites. A comparison of radioreceptor and radioimmunological somatomedin in native and acid-ethanol extracted serum. *J Clin Endocrinol Metab* 1980;**51**:781—8.
13. Hsieh TT, Hsu JJ, Chen CJ, Chiu TH, Liou JD, Hsieh CC, et al. Analysis of birth weight and gestational age in Taiwan. *J Formosan Med Assoc* 1991;**90**:382—7.
14. Leger J, Oury JF, Noel M, Baron S, Benali K, Blot P, et al. Growth factors and intrauterine growth retardation. I. Serum growth hormone, insulin-like growth factor (IGF) -I, IGF-II, and IGF-binding protein 3 levels in normally grown and growth-retarded human fetuses during the second half of gestation. *Pediatr Res* 1996;**40**:94—100.
15. Ashton IK, Zapf J, Einschenk I, MacKenzie IZ. Insulin-like growth factors (IGF) 1 and 2 in human foetal plasma and relationship to gestational age and foetal size during midpregnancy. *Acta Endocrinol* 1985;**110**:558—63.
16. Randhara RS. The insulin-like growth factor system and fetal growth restriction. *Pediatr Endocrinol Rev* 2008;**6**:235—40.
17. Fant M, Salafia C, Baxter RC, Schwander J, Vogel C, Pezzullo J, et al. Circulating levels of IGFs and IGF binding proteins in human cord serum: relationships to intrauterine growth. *Regul Pept* 1993;**40**:29—39.
18. Lassarre C, Hardouin S, Daffos F, Forestier F, Frankenne F, Binoux M. Serum insulin-like growth factor and insulin-like growth factor binding proteins in the human fetus. Relationships with growth in normal subjects and in subjects with intrauterine growth retardation. *Pediatr Res* 1991;**29**:219—25.
19. Leger J, Noel M, Limal JM, Czernichow P. Growth factors and intrauterine growth retardation. II. Serum growth hormone, insulin-like growth factor (IGF) I, and IGF-binding protein 3 levels in children with intrauterine growth retardation compared with normal control subjects: prospective study from birth to two years of age. *Pediatr Res* 1996;**40**:101—7.
20. Giudice LC, de Zegher F, Gargosky SE, Dsupin BA, de las Fuentes L, Crystal RA, et al. Insulin-like growth factors and their binding proteins in the term and preterm human fetus and neonate with normal and extremes of intrauterine growth. *J Clin Endocrinol Metab* 1995;**80**:1548—55.
21. Kao PC, Matheny Jr AP, Lang CA. Insulin-like growth factor-I comparisons in healthy twin children. *J Clin Endocrinol Metab* 1994;**78**:310—2.
22. Li HJ, Ji CY, Wang W, Hu YH. A twin study for serum leptin, soluble leptin receptor, and free insulin-like growth factor-I in pubertal females. *J Clin Endocrinol Metab* 2005;**90**:3659—64.
23. Harrela M, Koistinen H, Kaprio J, Lehtovirta M, Tuomilehto J, Eriksson J, et al. Genetic and environmental components of interindividual variation in circulating levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-3. *J Clin Invest* 1996;**98**:2612—5.