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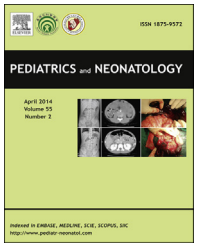

ORIGINAL ARTICLE

Association of Umbilical Cord Plasma Acid-labile Subunit of the Insulin-like Growth Factor Ternary Complex with Anthropometry in Term Newborns

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acid-labile subunit;
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Background: Birth size can affect neonatal morbidity and mortality. The insulin-like growth factor (IGF) system is the most important endocrine factor influencing fetal growth. In the circulation, IGFs (mostly IGF-I) are bound to IGF-binding protein 3 (IGFBP-3) and an acid-labile subunit (ALS) to form a ternary complex. The ALS protects IGFs from decay and facilitates their endocrine activity. However, the function of ALS in fetal growth has not yet been fully determined.

Methods: Venous umbilical plasma samples were obtained from 98 term neonates and analyzed using enzyme-linked immunosorbent assays. The ALS, IGF-I, and IGFBP-3 umbilical cord plasma levels were analyzed for their association with anthropometric measurements of the neonates.

Results: The ALS, IGF-I, and IGFBP-3 cord plasma levels were positively correlated with birth weight ($r = 0.42$, $p < 0.001$; $r = 0.43$, $p < 0.001$; and $r = 0.27$, $p < 0.01$, respectively) and placental weight ($r = 0.37$, $p < 0.001$; $r = 0.31$, $p < 0.01$; and $r = 0.30$, $p < 0.01$, respectively). In addition, the ALS cord plasma levels were also positively correlated with head circumference ($r = 0.29$, $p < 0.01$). Multiple linear regression analyses showed that both ALS and IGF-I cord plasma levels were independent predictive variables for birth weight

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($p < 0.01$ and $p < 0.005$, respectively). The ALS cord plasma levels were the only independent predictive variables, however, for head circumference and placental weight ($p < 0.01$ and $p < 0.05$, respectively).
Conclusion: The ALS umbilical cord plasma levels are one important factor, in addition to IGF-I, in the IGF system for predicting birth anthropometry, at least for near-term gestation. Our results suggest that the influence of ALS on the IGF system may develop prior to birth and affect fetal growth.

1. Introduction

Birth size can affect neonatal morbidity and mortality. Large-for-gestational age neonates have a higher risk of perinatal asphyxia and birth trauma, such as clavicular fracture and brachial plexus injury.[1] Small-for-gestational age infants also have a higher risk of hypoglycemia, polycythemia, asphyxia, and abnormal neurologic symptoms in the neonatal period as well as a higher risk of long-term developmental abnormalities.[2] Moreover, birth size is even suggested to be associated with long-term adult problems, such as the amount of food intake, cardiovascular disease, and type 2 diabetes mellitus.[3,4]

The insulin-like growth factor (IGF) system is the most important endocrine factor influencing fetal growth.[5] In IGF-I or IGF-II knockout mice, the birth weights were around 60% of those of their wild-type littermates.[6] In humans, the *IGF-I* gene homozygous partial deletion caused severe intrauterine growth retardation and mutation in the promoter region of the *IGF-I* gene, which led to low IGF-I levels and low birth weight.[7,8] Studies on umbilical cord blood have also reported that IGF-I, but not IGF-II or IGF-binding protein 3 (IGFBP-3), levels are positive predictors of birth size.[9,10]

In the circulation, most of the IGF-I is bound to IGFBPs, mostly IGFBP-3, to form a binary complex, and an 85-kDa acid-labile subunit (ALS) to form a 150-kDa IGF-I—IGFBP-3—ALS ternary complex.[11] The ALS is important for stabilizing the ternary complex in the circulation.[12] It protects IGFs from decay and facilitates their endocrine activity. Free IGF-I has a half-life of 10—12 minutes, which is extended to 12—15 hours when forming a ternary complex.[13]

In a mouse model with an inactivated ALS gene (*Igfals*), mice with two null alleles ($ALS^{-/-}$) were significantly lighter than wild-type mice by 3 weeks after birth and were 13—20% lighter by 9—10 weeks after birth.[14,15] Mice with a single null allele ($ALS^{+/-}$) were only 4% lighter than wild-type mice by 10 weeks after birth. However, in both the $ALS^{-/-}$ and $ALS^{+/-}$ mice, birth weights were not significantly different from the wild type. In humans, postnatal short stature or growth failure has been reported with ALS gene mutations.[16,17] However, it is usually believed that ALS is not important for regulating fetal growth because birth weight and length are normal in most of the reported patients with an ALS gene mutation.[18,19]

In fetal circulation, ALS umbilical cord blood levels increase significantly after 25—30 weeks of gestation.[20] Previously, only one study, on a group of 81 preterm and term neonates, reported that ALS umbilical cord blood levels are one positive predictor of birth length.[21] To further explore the function of ALS in fetal growth, we measured components of the IGF-I—IGFBP-3—ALS ternary complex in umbilical cord plasma levels of term neonates to investigate their associations with birth anthropometry.

2. Materials and Methods

2.1. Newborns and plasma samples

Ninety-eight venous umbilical cord plasma samples (45 male; 53 female) were obtained from term newborns at the Chi Mei Hospital (Tainan, Taiwan) between July 2005 and January 2006. Newborns with major congenital anomalies, multiple gestation, congenital heart diseases, suspected congenital infection, and renal diseases were excluded from the study. The sample was collected using a Vacutainer tube (Becton Dickinson, Franklin Lakes, NJ, USA) containing disodium ethylenediaminetetraacetic acid and stored at 4°C immediately after the newborn had been delivered. Within 24 hours, all samples were centrifuged at 2500*g* for 15 minutes to obtain umbilical cord plasma, which was stored at −80°C until further analysis. The clinical records of the neonates were reviewed to obtain information about their gestational age, birth weight, birth length, head circumference, and sex. Their Ponderal index was calculated as 100 times birth weight in gram divided by the cube of birth length in centimeter. Gestational age at birth was calculated from the 1st day of the last menstrual period of the mother. The placental weights were obtained from information recorded by the Obstetrics Department. Term neonates were defined as having 37 or more weeks of gestation. Ethical approval was obtained from the Institutional Review Board of the Chi Mei Hospital. Consent forms were signed by the participating mothers.

2.2. Assays

The levels of IGF-I, IGFBP-3, and ALS were measured using enzyme-linked immunosorbent assays (Diagnostic Systems Laboratories, Webster, TX, USA). All samples were run in triplicate. The detection limits of the assays for IGF-I, IGFBP-3, and ALS were 0.01 ng/mL, 0.04 ng/mL, and 0.07 μg/mL, respectively. The intra-assay coefficients of variation (CV) for IGF-I, IGFBP-3, and ALS were less than 9%,

10%, and 8%, respectively. The interassay CVs for IGF-I, IGFBP-3, and ALS were less than 7%, 12%, and 9%, respectively.

2.3. Statistics

All data were statistically analyzed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Pearson correlations were used to examine the associations between umbilical plasma levels of the study hormones and birth weights, birth lengths, head circumferences, and the Ponderal index of the newborns and placental weights of the mothers. Multiple linear regressions were used to examine the effect of the hormone of interest on these anthropometric data of the newborns. Significance was set at $p < 0.05$.

4. Results

The anthropometric data, placental weights, and cord plasma hormone levels of the participating newborns are provided in Tables 1 and 2. There were no significant sex differences in umbilical cord plasma levels of the study hormones. The weights of the neonates participated in this study were plotted on the nationwide singleton birthweight percentile curves for Taiwan (Figure 1).[22]

4.1. Correlations between anthropometric data and study hormones of the newborns

To explore the relationship between ALS, IGF-I, and IGFBP-3 cord plasma levels and birth anthropometry, correlation coefficients (r) were calculated (Table 3). The birth weight, birth length, and head circumference were all highly positively correlated with one another. The placental weights were positively correlated with the birth weights and birth lengths. Moreover, the birth weights were positively correlated with ALS, IGF-I, and IGFBP-3 cord plasma levels ($r = 0.42$, $p < 0.001$; $r = 0.43$, $p < 0.001$; $r = 0.27$, $p < 0.01$, respectively). The relationships between birth heights and ALS, IGF-I, and IGFBP-3 cord plasma levels were not significant; however, the correlation coefficient between ALS and birth heights was marginally significant 0.19 ($p = 0.069$). The head circumferences of the newborns were positively correlated with ALS cord plasma levels ($r = 0.29$, $p < 0.01$), but not correlated with IGF-I and IGFBP-3 levels. The Ponderal index was positively correlated with ALS and IGF-I cord plasma levels ($r = 0.23$, $p < 0.05$ and $r = 0.25$, $p < 0.05$, respectively). Placental weights were positively correlated with ALS, IGF-I, and IGFBP-3 cord plasma levels ($r = 0.37$, $p < 0.001$; $r = 0.31$, $p < 0.01$; and $r = 0.30$, $p < 0.01$, respectively).

4.2. Predictors of birth weight, birth length, head circumference, and placental weight

Results of multiple linear regression analyses using birth anthropometry and placental weights as dependent variable are provided in Table 4. Both ALS and IGF-I cord plasma levels were independent predictive variables for birth weights ($p < 0.01$ and $p < 0.005$, respectively), which together explained 24.3% of the variance in birth weight. In addition, ALS cord plasma levels were the only independent predictive variables for the head circumferences and placental weights ($p < 0.01$ and $p < 0.05$, respectively), explaining 6.2% and 14.6% of the variance, respectively. None of the levels of the study hormones were independent predictive variables for birth length or the Ponderal index.

5. Discussion

We found positive associations between the components of the IGF ternary complex in cord plasma levels and birth anthropometry in a group of singleton Taiwanese infants born at full term. Regression analyses indicated that both ALS and IGF-I cord plasma levels were independent predictors for birth weight, and that the ALS cord plasma level was also an independent predictor for head circumference and placental weight.

It is unclear whether ALS affects prenatal growth.[23] The birth weights of $ALS^{-/-}$ and $ALS^{+/-}$ mice are not different from those of the wild type,[14] and most patients with homozygous or heterozygous ALS gene mutations have normal birth weights and heights.[18,19] Our results, however, showed that ALS was a positive independent predictor of birth weight, head circumference, and placental weight. Compatible with our results is that there is a trend for patients of short stature with the *IGFALS* gene mutation to have a low birth weight, according to the small number of case reports published on patients whose birth weights were available. Their mean birth weight is −1.0 standard deviation score (range: −2.23 to −0.08).[24] In human fetal circulation, ALS umbilical cord blood levels increase significantly after 25–30 weeks of gestation.[20] Our data further indicated that IGF-I, IGFBP-3, and ALS plasma levels in the umbilical cord were highly correlated to one another at term gestation. This suggests that ALS may act well

Table 1 Anthropometry of the newborns and their placental weights.

	All births ($n = 98$)	Male ($n = 45$)	Female ($n = 53$)
Gestational age (wk)	39.5 (37.1–41.4)	39.3 (37.4–41.4)	39.6 (37.1–41.3)
Birth weight (g)	3160 (2300–4038)	3200 (2348–4038)	3120 (2300–3914)
Birth length (cm)	50.7 (45.0–58.0)	51.1 (45.0–58.0)	50.3 (46.0–56.0)
Head circumference (cm)	33.8 (31.0–36.0)	34.0 (32.0–36.0)	33.6 (31.0–35.0)
Ponderal index (g/cm^3)	2.44 (1.95–3.80)	2.41 (2.02–2.97)	2.46 (1.95–3.80)
Placental weight (g)	666 (450–1100)	680 (450–1000)	653 (470–1100)

Data are presented as means with range in parentheses.

Table 2 Cord plasma levels of the study hormones.

	All births (n = 98)	Male (n = 45)	Female (n = 53)
ALS (μg/mL)	3.01 (0.29–6.41)	2.78 (0.73–5.94)	3.19 (0.29–6.41)
IGF-I (ng/mL)	91.0 (30.7–190.5)	86.8 (33.9–171.2)	96.4 (41.2–190.1)
IGFBP-3 (ng/mL)	1524.4 (986.7–2182.5)	1467.1 (1054.2–2182.5)	1576.0 (1075.2–2140.3)

Data are presented as means with range in parentheses.
ALS = acid-labile subunit; IGF-I = insulin-like growth factor-I; IGFBP-3 = IGF-binding protein 3.

before birth to prolong IGF-I half-life, thereby affecting birth size. The differential effect of the *IGFALS* gene null mutation on birth weights in humans and mice may reflect the difference in the timing of the maturation of the IGF endocrine system.

Of all the predictor variables, only ALS significantly predicted three of the four parameters associated with birth size, namely, birth weight, head circumference, and placental weight, but not birth length. This is compatible with the observations of a previous study which reported that patients of short stature who have the *IGFALS* gene mutation had normal birth lengths.[24] In contrast to our results, Lo et al reported that in a mixed population of 81 preterm and term neonates, ALS umbilical levels were independent predictors for birth length.[21] The difference may be due to their sample size or the effect of ALS on different gestational age groups.

The Ponderal index is an indicator of soft-tissue mass relative to skeletal development.[25] In our study, regression analysis indicated that ALS levels were positive predictors for birth weights but not birth lengths. The Ponderal index was calculated as 100 times birth weight in gram divided by the cube of birth length in centimeter. Therefore, it is conceivable that ALS levels were not a predictor for the Ponderal index in regression analysis because of the cubic effect of birth length. This suggests that the primary influence of ALS on birth anthropometry is on birth weight.

Our study has some limitations. Hormone measurements were obtained from term neonates at birth. Therefore, our data cannot reflect the effect of these hormones on fetal growth at different gestational ages. In addition, our study is an association study, not a cause–effect study, which is usually a limitation in human studies.

It is known that hormones act through autocrine, paracrine, or endocrine functions. The autocrine/paracrine IGF system is generally thought to be of primary importance in fetal growth.[26] Our samples were obtained from cord blood and can represent at most the endocrine aspect of these study hormones. Therefore, we could not detect the differential effects of ALS, IGF-I, and IGFBP-3 autocrine/

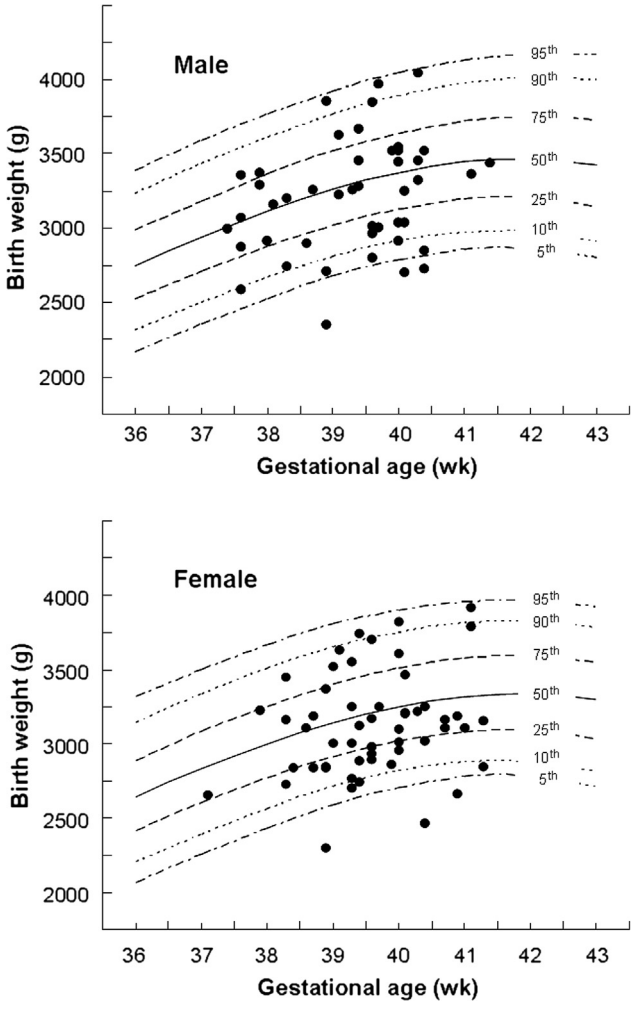

Figure 1 The birth weights of the neonates that participated in this study are plotted on nationwide singleton birth-weight percentile curves for Taiwan.

Table 3 Correlation coefficients (r) between anthropometric data and the study hormones in cord plasma.

	BW	BL	HC	PI	PW	ALS	IGF-I
BL	0.62*	—	—	—	—	—	—
HC	0.63*	0.43*	—	—	—	—	—
PI	0.35*	−0.52*	0.17	—	—	—	—
PW	0.54*	0.29**	0.20	0.24***	—	—	—
ALS	0.42*	0.19	0.29**	0.23***	0.37*	—	—
IGF-I	0.43*	0.17	0.11	0.25***	0.31**	0.47*	—
IGFBP-3	0.27**	0.11	0.07	0.16	0.30**	0.40*	0.55*

*$p < 0.001$.
**$p < 0.01$.
***$p < 0.05$.
ALS = acid-labile subunit; BW = birth weight; BL = birth length; HC = head circumference; IGF-I = insulin-like growth factor-I; IGFBP-3 = IGF-binding protein 3; PI = Ponderal index; PW = placental weight.

Table 4 Multiple linear regression analyses of the study hormones and size at birth.

Covariate	β (95% CI)	Adjusted r^2 (%)	p
Birth weight (g)		24.3	
ALS (μg/mL)	64.6 (18.5—110.8)		<0.01
IGF-I (ng/mL)	3.99 (1.51—6.46)		<0.005
IGFBP-3 (ng/mL)	−0.12 (−0.40 to 0.16)		0.41
Birth length (cm)		2.3	
ALS (μg/mL)	0.21 (−0.09 to 0.51)		0.164
IGF-I (ng/mL)	0.01 (−0.01 to 0.03)		0.221
IGFBP-3 (ng/mL)	0.000 (−0.003 to 0.001)		0.298
Head circumference (cm)		6.2	
ALS (μg/mL)	0.203 (0.05—0.36)		<0.01
IGF-I (ng/mL)	0.002 (−0.006 to 0.01)		0.67
IGFBP-3 (ng/mL)	0.000 (−0.001 to 0.001)		0.41
Ponderal index (g/cm^3)		5.6	
ALS (μg/mL)	0.023 (−0.018 to 0.06)		0.27
IGF-I (ng/mL)	0.002 (0.000 to 0.004)		0.15
IGFBP-3 (ng/mL)	0.00 (0.00 to 0.00)		0.76
Placental weight (g)		14.6	
ALS (μg/mL)	22.8 (3.78—41.86)		<0.05
IGF-I (ng/mL)	0.69 (−0.36 to 1.74)		0.19
IGFBP-3 (ng/mL)	0.04 (−0.07 to 0.16)		0.47

ALS = acid-labile subunit; CI = confidence interval; IGF-I = insulin-like growth factor-I; IGFBP-3 = IGF-binding protein 3.

paracrine function on fetal growth. Indeed, it has been reported that, in addition to the liver, local ALS gene expression is also found in developing bone, kidney, thymus, lactating mammary gland, and lung tissue.[27,28] Moreover, ALS may have its own functions independent of the IGF system. Indeed, despite similar degrees of reduction in circulating IGF-I, liver-specific IGF-I knockout mice and ALS knockout mice have different insulin sensitivity, growth hormone levels, and fat metabolism.[29—31] Therefore, the differential roles of ALS and IGF-I on fetal growth warrant further investigation.

In conclusion, we have found that ALS, in addition to IGF-I, is a significant positive predictor for birth weight and head circumference. The endocrine IGF system may start to mature during the second or third trimester, when ALS excretion increases and forms ternary complexes with IGF-I and IGFBP-3 to prolong the action of IGF-I and affect fetal growth.

Conflicts of Interest

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

Acknowledgments

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