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ORIGINAL ARTICLE

Association of Cord Plasma Leptin With Birth Size in Term Newborns

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Background: Leptin is secreted from adipose tissue and plays an important role in obesity. Recent studies have shown that the relationship between leptin and body fat mass may have ethnic differences. The purpose of our study was to investigate the relationship between venous umbilical cord plasma leptin and anthropometric markers in term healthy Taiwanese newborns.
Methods: Umbilical venous plasma samples were obtained from 98 term neonates (48 males and 50 females) and leptin levels were analyzed by enzyme-linked immunosorbent assay.
Results: Umbilical cord plasma levels of leptin were significantly higher in the female neonates than in males ($p<0.001$). The large-for-gestational age and appropriate-for-gestational age newborns had significantly higher leptin cord plasma levels than the small-for-gestational age newborns ($p<0.01$ and $p<0.05$, respectively). In both male and female neonates, umbilical leptin levels showed significant positive correlations with birth weight and birth length. Multiple linear regression analysis revealed that birth weight was the only significant predictor of umbilical cord plasma leptin levels in both male and female neonates. However, the slopes of the regressions between leptin and birth weight in male and female neonates were not different.
Conclusion: In Taiwanese healthy term neonates, leptin umbilical cord plasma levels are associated with sex and birth weight of the neonate. The relationship between leptin and birth weight may differ among different ethnic groups. These findings imply that the relationship between leptin and body fat mass may develop early in life.

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1. Introduction

Leptin is a 16-kD hormone mainly secreted from adipocytes.[1] The physiological role of leptin in humans is to inform the hypothalamus of the amounts of adipose tissue in the body and to regulate food intake and energy expenditure to maintain appropriate body fat stores.[2,3] Therefore, leptin may play an important role in the mechanism of obesity.

Leptin has been reported to be positively associated with intrauterine fetal growth, birth weight,[4,5] and total body fat content of neonates.[6,7] Moreover, it has been postulated that the leptin:fat ratio may contribute to leptin feedback regulation at the hypothalamic level and that a set point may develop early in life.[8–10] Therefore, the tendency to be obese may be at least partly established during the intrauterine period.

Ethnic difference of fat distribution has been reported in prepubertal children.[11] Moreover, it has been found that there are gender and ethnic differences in the association of leptin with anthropometric markers at birth.[12] In Taiwan, there has been a previous report on the relationship between leptin and anthropometric markers at birth. However, it did not focus on term healthy newborns, and the sample size was small (28 healthy term neonates).[13] The purpose of our study was to investigate the relationship between venous umbilical cord plasma leptin and anthropometric markers at birth in a group of Taiwanese term healthy newborns. We also analyzed the gender differences between leptin levels and the anthropometric markers at birth.

2. Materials and Methods

2.1. Subjects and sample collection

Umbilical venous cord plasma samples were obtained from neonates born at term gestation at Chi Mei Foundation Hospital in Tainan, Taiwan, from July 2005 to February 2006. A total of 98 samples were obtained during this period (48 males and 50 females). Multiple birth newborns, or newborns with major congenital anomalies, congenital heart diseases, suspected congenital infection or renal diseases were excluded from the study.

The sample was first collected with a tube containing ethylenediamine tetraacetate and stored at 4°C immediately after delivery of the neonate. All samples were then centrifuged at 3000 rpm for 15 minutes within 24 hours to obtain umbilical cord plasma. The plasma was then stored at −80°C until analysis.

Clinical records of newborns were reviewed for information relating to neonatal birth weight, body length and head circumference, gestational age, and sex. Gestational age at birth was calculated from the first day of the last menstrual period of the mother. Term neonates were defined as more than or equal to 37 weeks of gestation. Ponderal index (PI) was calculated as [birth weight (g) × 100] / birth length $(cm)^3$. Body mass index (BMI) was calculated as birth weight (kg) / birth length $(m)^2$. For comparison of the leptin levels, newborns were stratified into three groups: (1) SGA (small-for-gestational age; birth weight below the 10th percentile for the gestational age); (2) AGA (appropriate-for-gestational age; birth weight between the 10th and 90th percentile for the gestational age); and (3) LGA (large-for-gestational age; birth weight beyond the 90th percentile for the gestational age). Ethical approval was obtained from the Institutional Review Board of Chi Mei Foundation Hospital. Consent forms were signed by the mothers of the participating neonates.

2.2. Assays

Commercially available enzyme-linked immunosorbent assay (Diagnostic Systems Laboratories, Inc., Webster, TX, USA) kits were used to measure the cord plasma levels of leptin. All samples were run in triplicate, using the protocols provided by the producer. The detection limits of the assays for leptin were 0.05 ng/mL. The intra-assay coefficients of variation (CV) for leptin were less than 4.4%. The interassay CVs for leptin were less than 4.9%.

2.3. Statistics

Statistical analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). Analysis of variance was used to compare the differences in leptin levels among SGA, AGA, and LGA infants. Pearson's correlations were used to examine the relationships between leptin levels and anthropometric data. Multiple linear regressions were used to examine the effect of anthropometric data on leptin levels. A value of $p < 0.05$ was considered statistically significant.

3. Results

Table 1 shows the characteristics and leptin umbilical cord plasma levels of the neonates under study. Umbilical cord plasma levels of leptin were significantly higher in the female neonates than in males ($p < 0.001$).

Umbilical cord plasma levels of leptin among SGA, AGA, and LGA newborns were compared and are shown in Figure 1. The LGA and AGA newborns had significantly higher leptin cord plasma levels

Table 1 Characteristics and leptin umbilical cord plasma levels of the neonates*

	All births (n=98)	Male (n=48)	Female (n=50)
Gestational age (wk)	39.3 (1.2)	39.1 (1.3)	39.5 (1.0)
Birth weight (g)	3137 (360)	3196 (385)	3090 (339)
Birth length (cm)	50.6 (2.3)	51.0 (2.4)	50.2 (2.0)
Head circumference (cm)	33.3 (1.1)	33.9 (1.1)	33.6 (1.0)
Ponderal index (g/cm^3)	2.4 (0.3)	2.4 (0.2)	2.5 (0.3)
Body mass index (kg/m^2)	12.2 (1.1)	12.3 (1.0)	12.3 (1.2)
Leptin (ng/mL)	15.7 (10.9)	11.5 (8.3)	19.6 (11.5)†

*Values are expressed as mean (standard deviation); †$p < 0.001$, compared with male group.

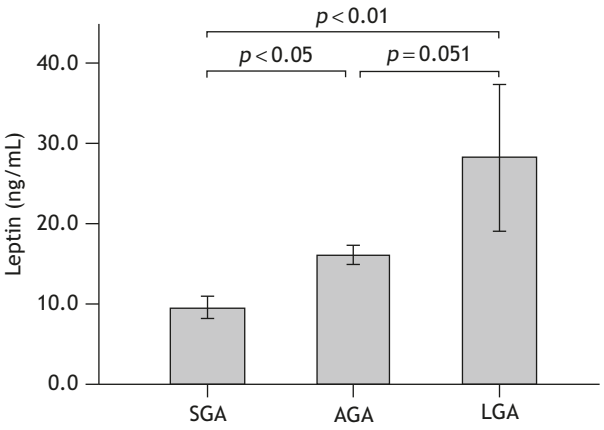

Figure 1 Comparison of leptin umbilical cord plasma levels among SGA (n=12), AGA (n=83), and LGA (n=3) newborns. SGA = small-for-gestational age; AGA = appropriate-for-gestational age; LGA = large-for-gestational age.

than the SGA newborns ($p<0.01$ and $p<0.05$, respectively). The LGA neonates also had higher umbilical cord plasma levels of leptin (mean, 28.2 ng/mL) than AGA neonates (mean, 16.0 ng/mL), but not to a significant level ($p=0.051$).

To examine the relationship between leptin levels and anthropometric markers of the neonates, scatter plots were drawn (Figure 2). The plots were stratified into male and female groups due to the significant difference in leptin levels. In male neonates, umbilical leptin levels ($\log_{10}$ transformed) showed significant positive correlations with birth weight, birth length, head circumference, and BMI, whereas PI was not significantly correlated. In female neonates, umbilical leptin levels ($\log_{10}$ transformed) showed significant positive correlations with birth weight and birth length, whereas head circumference; PI and BMI were not significantly correlated. There were no significant difference in slopes of the regression between leptin cord plasma levels and birth weight between male and female neonates.

Stepwise multiple linear regression analysis revealed that birth weight was the only significant predictor of leptin levels in both male and female neonates, accounting for 19% and 23% of umbilical cord plasma leptin variability in the male and female neonates, respectively (Table 2).

4. Discussion

In this study, we demonstrated that in a group of singleton Taiwanese term neonates, female neonates had higher umbilical cord plasma levels of leptin than the male neonates. LGA and AGA neonates had higher leptin umbilical cord plasma levels than the SGA neonates. Multiple regression analysis revealed that birth weight was the most important predictive variable for umbilical cord plasma leptin levels in both male and female neonates at term gestation.

Our data revealed that female neonates had significantly higher levels of umbilical cord plasma leptin than males. This is compatible with previous reports.[14,15] Higher leptin levels in female neonates may suggest that female neonates have a higher total body fat content compared with males. However, it has been reported that there is no difference in the fat content and distribution between male and female human fetuses.[16] In an animal model, it has been reported that, at any given total body fat content, female mice had higher serum leptin levels than males.[17] Given that there were no significant differences in birth weight, PI, and BMI between our male and female neonates, different levels of leptin between male and female neonates may imply a difference in genetic background and hormonal regulation (instead of fat content). Indeed, it has been reported that cerebrospinal fluid (CSF) levels of leptin in girls are only slightly higher than those of boys; therefore, the CSF/plasma leptin ratios in girls are much lower.[18] This implies that, for males and females, the transport of leptin across the blood-brain barrier may differ for a given peripheral leptin level, and the hypothalamus may sense the body fat store differently. However, whether

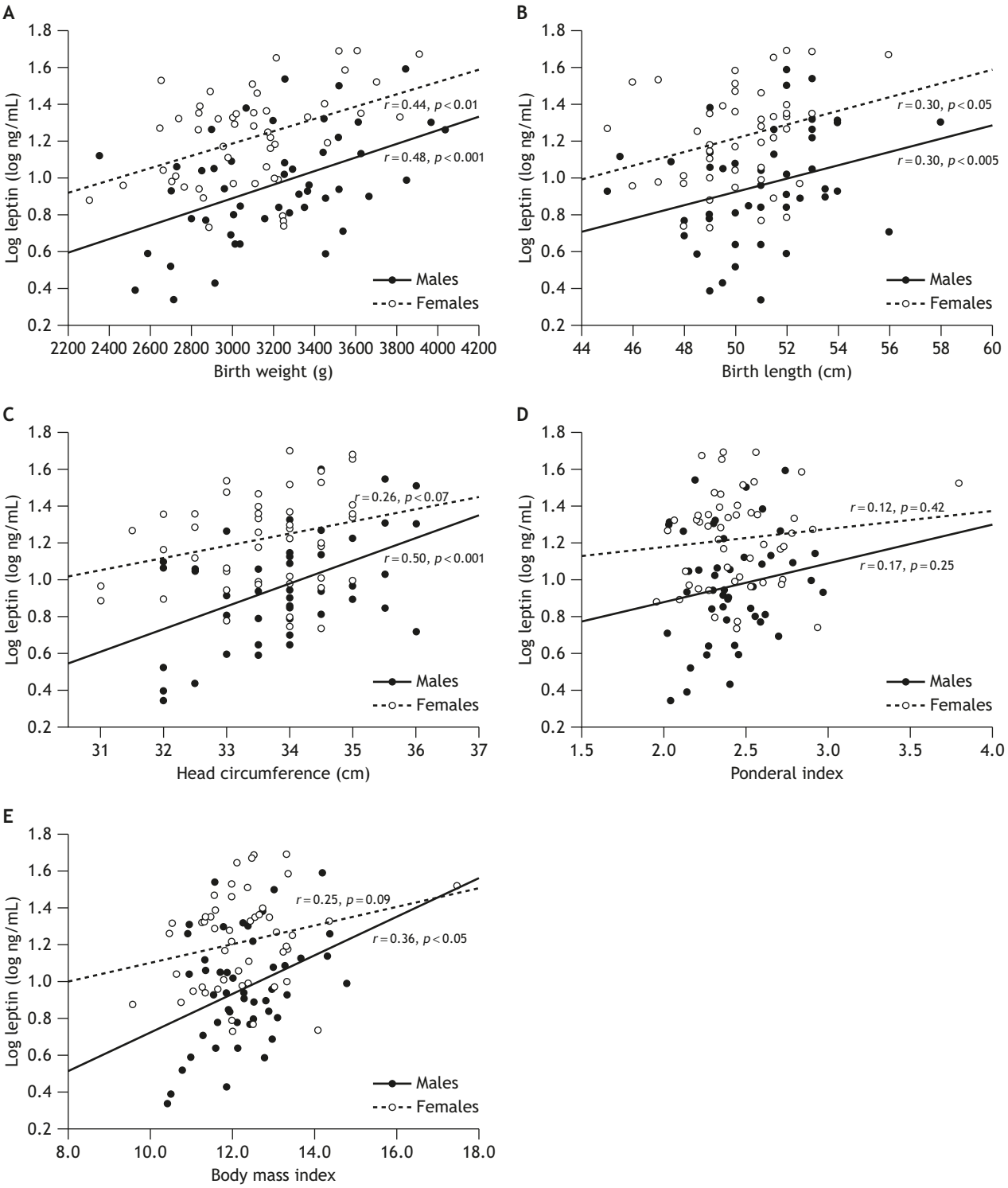

Figure 2 Correlations between $\log_{10}$ leptin umbilical cord plasma levels and anthropometrics of the neonates.

the same situation applies to neonates requires further investigation.

Leptin cord plasma levels were significantly different among LGA, AGA, and SGA newborns, being highest in the LGA infants. Our results are subject to a limited sample size of SGA ($n=12$) and LGA ($n=3$) neonates. However, these results are compatible with those of previous reports.[4,5] Umbilical cord plasma leptin could potentially be secreted from fetal adipocytes or from placenta. It has been reported that fetal adipose tissue is the major source of circulating fetal leptin.[19,20] Adipose tissue contributes 2% of birth body weight in SGA infants, 13% in AGA infants,[21] and up to 30%

Table 2 Multiple linear regression analyses of umbilical cord plasma leptin levels and anthropometrics of the neonates

Dependent variable: leptin	Adjusted r^2 (%)		p	
	Male	Female	Male	Female
Birth weight	19	23	<0.005	<0.001
Birth length			0.64	0.51
Head circumference			0.25	0.84
Ponderal index			0.66	0.50
Body mass index			0.69	0.48

in LGA infants.[22] The higher plasma leptin concentrations in LGA infants may be because there is a higher quantity of leptin secreted from each adipocyte or more adipocytes in LGA infants. It has been reported that both body fat mass and fat cell weight were higher in LGA infants and lower in SGA infants.[23] It has been proposed that in the fetus, leptin may be signaling the fat stores to brain and other tissues, as in adult life.[24]

In male neonates, umbilical leptin levels were positively associated with birth weight, birth length, head circumference, and BMI. In female neonates, umbilical leptin levels were positively associated with birth weight and birth length. However, upon multiple linear regression analysis, birth weight was the only significant predictor (among neonatal anthropometrics) of leptin levels in both male and female neonates. Due to the positive correlation between leptin levels and birth weight, leptin has been suggested to act as a growth factor.[25,26] However, neonates with congenital leptin deficiency were born of normal birth weight in both humans and mouse models.[27,28] Therefore, the evidence for leptin as a causative factor in fetal growth is still lacking. Rather, body weight and calf circumference have been reported to be two of the best anthropometric variables for predicting total body fat measured by total-body electrical conductivity in infants.[29] Since leptin is secreted from adipose tissue, the strong relationship between leptin levels in umbilical cord plasma and birth weight in our data may reflect the relationship between leptin and total fat mass of the neonates.

The regression slope between leptin and birth weight represents the change in leptin for a given change of birth weight. Since leptin is secreted from adipose tissue and birth weight is one of the best predictors of body fat mass, difference in regression slope may represent different relationships between leptin and fat mass. Our data revealed no sex difference in regression slope between leptin and birth weight, a finding in contrast with one report on Caucasian neonates.[12] It has been reported adult Asians have lower BMI than adult Caucasians[30] and, for a given BMI, adult Caucasians have a lower percentage body fat than adult Chinese.[31] Together, these data imply that the relationship between leptin and body fat mass may be different in different ethnic groups. Although the cause of the difference in newborns is still unclear, it may be of genetic or environmental origin. Compatible with this concept are recent reports examining the association between obesity and leptin gene polymorphisms.[32–34]

In conclusion, in Taiwanese healthy term neonates, leptin umbilical cord plasma levels are associated with sex and birth weight of the neonate. The relationship between leptin and birth weight may differ among different ethnic groups. These findings imply that the relationship between leptin and body fat mass may develop early in life and contribute to obesity later in life. This mechanism warrants further investigation.

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