



Leptin as a marker of sexual dimorphism in newborn infants

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Abstract

Objective: To determine cord blood leptin levels in newborns appropriate for gestational age, according to gender, birth weight, birth height and ponderal index.

Methods: A cross-sectional study was carried out with 132 term newborns appropriate for gestational age (68 females, 64 males), gestational age between 35-42 weeks. Data were collected through interviews with the mothers at the maternity, anthropometrical study of the newborns, and cord blood estradiol, testosterone and leptin assays obtained immediately after birth.

Results: The levels of leptin were significantly higher in females than in males (8.34 ± 0.65 ng/ml versus 6.06 ± 0.71 ng/ml; $p = 0.000$). The concentrations of estradiol and testosterone did not differ between males and females. Leptin levels were positively correlated with gestational age ($r = 0.394$, $p < 0.01$), birth weight ($r = 0.466$, $p < 0.01$), birth length ($r = 0.335$, $p < 0.01$) and ponderal index ($r = 0.326$, $p < 0.01$).

Conclusions: Leptin concentration in the umbilical cord is positively correlated with gestational age, birth weight, birth height, and ponderal index, suggesting its participation in the neonatal growth process. In addition, a gender difference with higher levels of leptin in females neonates was observed, suggesting that the sexual dimorphism in relation to body composition already exists in newborns.

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Introduction

Leptin (from the Greek word leptos = thin), a 16 kDa protein synthesized by adipocytes, has been recently discovered by the genetic studies conducted by Zhang et al.¹ Since then, several studies have been conducted to elucidate the role of leptin in human physiology.

Murine studies have revealed that leptin plays an important role in neuroendocrine function. Mutation in the *ob/ob* gene, which encodes leptin in female rats, causes infertility, absence of pubertal development, obesity, and insulin resistance.¹

In human beings, leptin has been correlated with body fat mass and energy balance, and varies according to gender and pubertal development.^{2,3}

In 1997, it was discovered that leptin is also produced by the placenta and the fetus.^{4,5} This resulted in studies that investigate the relationship of leptin with fetal growth and also as an indicator of energy stores in newborns by the determination of its cord blood concentrations. In Brazil, there is a paucity of studies that assess cord blood leptin concentrations and their variation according to gender.

The aim of the present study is to assess cord blood leptin levels in appropriate-for-age newborns, considering their gender, weight, length, and ponderal index at birth.

Material and methods

A cross-sectional epidemiological study was carried out to assess 132 appropriate-for-age newborns (68 females and 64 males), at 35-42 weeks' gestation, born at the Hospital Regional de Sorocaba, between January 2001 and February 2002. Only those infants born during the day (between 7 a.m. and 4 p.m.) were included in the study, since some studies indicate that leptin levels vary during the night.⁶

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The sample size was calculated based on Koistinen *et al.*,⁴ and 96 was considered to be the minimal number of samples for the present study. Values of α and β were established as 0.05 and 0.10, respectively.

Newborns were selected according to the following criteria: gestational age between 35-42 weeks, appropriate for gestational age, preterm babies whose mothers did not use corticoids, Apgar score at 1 minute equal to or greater than 7, absence of congenital malformations, singleton pregnancy, absence of maternal diseases such as hypertension, diabetes mellitus, thyroid disorders or hypercholesterolemia.

All babies whose mothers smoked or drank during pregnancy were excluded from the study.

The study was conducted in a stepwise fashion:

First, a form with maternal data was filled out, umbilical cord blood samples were collected, and the newborns were evaluated.

Secondly, laboratory measurements were performed.

The first step consisted in collecting clinical data about the gestational period using a previously tested questionnaire. The following information about the newborns was also obtained: birth length and birthweight, ponderal index, gender, Apgar score, gestational age and placental weight. Newborns were weighed on a portable microelectronic scale and measured using an appropriate anthropometer. Two measurements were made and compared, and if they differed, their mean was used. Ponderal index corresponded to 100 times the weight in grams divided by the cube of the length in centimeters. Gestational age was calculated according to the date of last menstruation and confirmed by ultrasonography.

Newborns were classified as to their weight-for-age percentiles based on the curve devised by Alexander *et al.*,⁷ after that, only appropriate-for-age newborns were selected for the study.

The second step consisted of laboratory measurements. Leptin was obtained from umbilical cord venous blood immediately after birth, and no contamination with maternal blood occurred. The collected blood was centrifuged and frozen for simultaneous analysis. Cases in which placental abruption was observed were not included in the study. Leptin was measured by radioimmunoassay (Linco Research, St. Charles, MO). The minimum detectable leptin concentration was of 0.5 ng/ml, with intra and interassay coefficients of variation lower than 3 and 6%, respectively. Estradiol and testosterone levels were measured using the radioimmunoassay technique (Diagnostic Systems Laboratories, Texas, USA). The minimum detectable concentration of estradiol was of 0.01 ng/ml, whereas that of testosterone was of 0.08 ng/ml. The intra and interassay coefficients of variation for estradiol and testosterone were lower than 5%.

The present study was based on the current review of the Declaration of Helsinki and on Brazilian Resolution 196 of October 10, 1996, and approved by the local Ethics Committee.

Means and their respective standard errors or medians and variance were calculated for the analysis of results. On top of that, the differences were analyzed by nonparametric Mann-Whitney test and the chi-square test was used for categorical variables. The Spearman rank correlation coefficient was also used and a p value of < 0.05 was adopted. The SPSS version 7.5 was used for the statistical analysis.

Results

The clinical characteristics of the newborns are summarized in Table 1. The mean gestational age was of 38.58 weeks, mean weight of 3,053 g, mean length of 47.78 cm, mean ponderal index of 2.79 and placental weight of 539.96 g. There were 10 female preterm babies

Table 1 - Characteristics of newborns and levels of estradiol, testosterone and leptin according to sex

Sex	Male (n = 64)	Female (n = 68)	Total (n = 132)
Gestational age (weeks)	38.51±0.23	38.64±0.22	38.58±0.16
Birth weight (g)	3,074±50.45	3,033±51.77	3,053±36.09
Length (cm)	47.95±0.24	47.61±0.30	47.78±0.19
Ponderal index	2.78±0.03	2.80±0.03	2.79±0.02
Placental weight (g)	546.25±13.35	534.04±12.51	539.96±9.12
Estradiol (ng/ml)	9.17±0.50	9.53±0.45	9.48±0.29
Testosterone (ng/ml)	3.45±0.24	4.23±0.38	4.12±0.22
Leptin (ng/ml)	6.06±0.71	8.34±0.65*	7.23±0.49

Mean and standard error of mean.

* p = 0.000 versus male.

and 12 male preterm babies ($p = 0.728$). No significant gender difference was noted.

Apgar scores at 1 and 5 minutes were not statistically different between male and female newborns. The median Apgar score at 1 minute for both male and female newborns was 8 (range of 7-9) while the Apgar score at 5 minutes was 9 (range of 9-10).

Female newborns have significantly higher leptin levels ($p = 0.000$) than male newborns (8.34 ± 0.65 ng/ml versus 6.06 ± 0.71 ng/ml). The serum concentrations of estradiol and testosterone did not vary between male and female newborns (Table 1).

Umbilical cord leptin was correlated with neonatal anthropometric parameters (Table 2). Gestational age and birthweight were moderately associated with serum leptin ($p < 0.01$). Umbilical cord leptin showed a lower correlation with length and ponderal index ($p < 0.05$).

Table 2 - Correlation between umbilical cord leptin and neonatal anthropometric parameters

Newborns	Total (n = 132)
Leptin <i>versus</i> gestational age	0.394*
Leptin <i>versus</i> birth weight	0.466*
Leptin <i>versus</i> birth length	0.335*
Leptin <i>versus</i> ponderal index	0.326*
Leptin <i>versus</i> placental weight	0.103

Spearman's correlation coefficient * $p < 0.01$.

Discussion

This study revealed that cord blood leptin concentrations were correlated with neonatal anthropometric parameters and that female newborns had higher levels of leptin than the male ones. Since the gender distribution of newborns was virtually the same in the present study and there were no differences in terms of ponderal index, weight, length, and sex hormones between male and female newborns, it is suggested that this discrepancy in body composition may be due to the increased deposition of subcutaneous fat in female newborns.

Fetal growth is characterized by an initial phase of organization and tissue differentiation, combined with intense cell proliferation. Genetic factors, transplacental supply of oxygen and substrates, in addition to the endocrine and paracrine activity of hormones produced by the fetus, placenta or mother, are the main regulators of fetal growth.⁸

Fetal growth is controlled by hormones found in the placenta or in the fetus. Regulation may be indirect, by

controlling placental growth and blood flow, or direct, by transferring placental hormone to the fetus. These hormones include: fetal GH, insulin, somatomedin, thyroid hormones and sex steroids.⁹ Recent studies have suggested that leptin may also be involved in this process.¹⁰⁻¹¹

Leptin levels can be measured in the amniotic fluid or in the umbilical cord after the 25th week of gestation.⁵ Amniotic fluid leptin reflects the maternal environment, whereas umbilical cord leptin is derived from the placenta or from fetal tissue. Most studies published so far have unanimously concluded that maternal blood leptin is only correlated with maternal adiposity.¹²⁻¹⁴

The presence of leptin in human umbilical cord blood has been shown in several studies. The present study showed a positive correlation between umbilical cord leptin and gestational age, weight, length and ponderal index in newborns. This finding is similar to the ones observed in previous studies, suggesting that the production of leptin in umbilical cord is associated with the development of adipose tissue by the fetus. Koistinen *et al.*⁴ found a positive association between fetal growth and cord blood leptin concentration. Matsuda *et al.*¹⁵ conducted a study with 82 newborns, with 36-42 weeks' gestation and birthweights between 2,306 and 4,128 g, and found a positive association between birthweight and cord blood leptin concentrations. Helland¹⁶ also found the same association in a study published in 1997.

Controversies exist in the world literature over a possible correlation between cord blood leptin and placental weight: some studies show this association¹⁷⁻¹⁸ while others do not.¹⁹⁻²⁰ In the present study, no correlation was found between leptin and placental weight.

Recently, new studies have been published confirming the importance of leptin to fetal growth. A study published in 2000 showed that cord blood leptin is negatively correlated with ICTP (carboxyterminal telopeptide of type I collagen), which is a marker for bone resorption, suggesting that leptin probably reduces bone resorption, consequently increasing bone mass.²¹ Christou *et al.*²² observed that birthweight shows an independent association between IGF-I levels and cord blood leptin concentrations, suggesting that leptin is involved in yet unknown mechanisms of fetal growth regulation.

Although the different gender-related leptin concentrations found in adults have been well documented, there is a paucity of studies with infants and newborns.²³⁻²⁴ The present study showed that female newborns had higher serum leptin levels than their male counterparts. We noted this difference when comparing male newborns with the female ones using the same characteristics regarding weight, length and ponderal index. A similar result was found in an English cohort study with 197 newborns, published in 1999,²⁵ which showed that female newborns have higher levels of leptin than male ones regardless of their size at birth. This suggests that sexual dimorphism in terms of body composition may be already present in newborns.

The mechanisms implicated in the gender-related variation of leptin concentrations in newborns are still unclear. The concentration of sex hormones in the umbilical cord in both male and female newborns was similar in the present study. Therefore, sex hormones in the umbilical cord should not interfere with gender-related variation of leptin levels. This study suggests that the differences in subcutaneous fat deposition in terms of gender may explain the discrepant leptin concentrations between male and female newborns. A previous study²⁶ revealed that ponderal index is not a good marker for fat deposition in newborns, and that skinfolds in females are thicker than in males. Future studies using bioimpedance may be able to explain the reason for such differences and their associations with neonatal growth.

Our conclusion is that there is a positive correlation between cord blood leptin and gestational age, weight, and ponderal index in newborns, suggesting an association with neonatal growth. Female newborns have higher serum levels of leptin than male ones, indicating that sexual dimorphism in terms of body composition is already present in newborns.

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