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Effect of Birth Weight and Maternal Smoking on Cord Blood Leptin Concentrations of Full-Term and Preterm Newborns*

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ABSTRACT

Prematurity, maternal smoking, and low birth weight each result in neuroendocrine dysfunction and increased perinatal morbidity and mortality. Leptin, an adipocyte-secreted protein, has provided the first physiological link to the regulatory system controlling starvation-induced neuroendocrine changes in rodents. This study investigated whether leptin concentrations were detectable in cord blood of newborns, and assessed the effect of birth weight, prematurity, and maternal smoking on cord blood leptin concentrations. Fifty consecutively enrolled full-term and 12 preterm newborns born to mothers who smoked during pregnancy were compared to 50 full-term and 12 preterm newborns born to parents who were nonsmokers. RIA for leptin was performed using cord blood samples collected immediately after birth. Leptin concentrations were detectable in newborns and correlated positively with obesity (full-term, $r = 0.30$, $P < 0.01$; preterm, $r = 0.47$, $P < 0.05$). Maternal smoking during pregnancy was associated with decreased leptin concentrations in the cord blood of both full-term and preterm newborns. This effect was independent of obesity (full-term newborns: 5.25 ± 2.48 *vs.* 4.21 ± 2.71 ng/ml, $P = 0.01$) and was more pronounced in premature newborns (5.67 ± 3.6 *vs.* 2.46 ± 2.03, $P = 0.02$), and its magnitude in full-term newborns was directly related to the reported number of cigarettes the mothers of the full-term newborns smoked per day ($r = -0.438$, $P < 0.001$). Thus, low birth weight and maternal smoking are both associated with decreased leptin concentrations, and these effects are more pronounced in premature newborns. Future studies will be needed to determine whether administration of leptin might reverse the neuroendocrine dysfunction caused by maternal smoking. (*J Clin Endocrinol Metab* **82:** 2856–2861, 1997)

LOW BIRTH weight remains the most important determinant of perinatal mortality and impaired development worldwide (1–3). Birth weight is adversely affected by prematurity (1, 4) and maternal smoking during pregnancy (1, 5–7). Maternal smoking has been incriminated in several neurological and endocrine abnormalities observed in newborns born to smoking mothers (8–15). Although the underlying pathophysiological mechanism remains to be elucidated, it has been suggested that these adverse consequences of smoking on birth weight and neuroendocrine function may result from the smoking-induced compromise of the uteroplacental (13, 16) and fetal blood flow (13, 17, 18), which impairs oxygen, nutrient, and energy delivery to the fetus. Alternatively, it is also possible that all or some of the above adverse effects may result from a direct effect of smoking either on fetal tissues (13, 19) or a tissue-derived factor that mediates these deleterious effects of maternal smoking.

Important insights into the regulation of body weight and the pathophysiology of food deprivation-induced neuroendocrine changes have been provided by the discovery of leptin. Leptin, the product of the ob gene (20), is an adipocyte-secreted protein (21) whose circulating levels signal the status of energy stores to the brain (22, 23). Accumulating evidence suggests that leptin plays a key role in a feedback loop that maintains energy homeostasis (23). Leptin concentrations decrease in response to food deprivation in both rodents (24) and adult humans (25, 26). Moreover, normalization of circulating leptin levels by exogenous administration of the hormone restores the food deprivation-induced changes in the pituitary-adrenal, thyroid, and reproductive axes of fasting rodents, suggesting that leptin plays an important role in mediating the neuroendocrine response to starvation (24).

Because previous papers have focused on rodents (22–24) and adult humans (25, 26), little is known about leptin levels in fetuses and newborns (27, 28). More specifically, it remains unknown whether prematurity or maternal smoking can influence circulating leptin concentrations. In view of the postulated role for leptin as the factor mediating the neuroendocrine response to food deprivation, we determined whether leptin is detectable in the cord blood of normal

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newborns, and whether maternal smoking or prematurity decreases leptin concentrations.

Methods

Subjects

This hospital-based study was carried out in the Maternity Unit of the Paras General University Hospital, a hospital providing tertiary care to the entire Southwestern Greece. Over a 6-month period, 50 consecutively enrolled full-term and 12 preterm newborns delivered by mothers who smoked during pregnancy were studied. Additionally, 50 full-term and 12 preterm newborns whose parents were nonsmokers were used as control subjects. The full-term control neonates were obtained randomly, following the delivery of a neonate by a smoking mother, provided that they fulfilled the entry criteria of the study. The preterm control neonates were selected only by gestational age to match with preterm newborns of the study group. Mothers responded to an interviewer-administered questionnaire indicating whether they were smoking during pregnancy or not, and if so, the daily number of cigarettes smoked. Mothers who discontinued smoking during pregnancy were excluded from the study. Mothers who admitted smoking during pregnancy reported a cigarette consumption of 2–50 cigarettes per day. The mothers of study infants did not have preeclampsia, diabetes, or hypertension and denied the use of any illegal substance during pregnancy. Subjects with complications during pregnancy or labor were excluded from the study. With respect to maternal age, there was no statistically significant difference among mothers in the study groups.

All neonates were appropriate in size for gestational age and were born with normal labor after an uneventful pregnancy. No medications were used during labor, and the amniotic fluid was clear in all pregnancies. All neonates had a healthy appearance, no signs of fetal distress, no intrauterine growth retardation, and Apgar scores of 7 or more at 1 and 5 min.

Newborns' gestational age was determined according to Ballard's scoring system. Neonates with a more than 2 weeks discrepancy among the gestational age as assessed by neurological, physical, and ultrasonographic criteria, or as estimated based on menstrual history, were excluded from the study, as previously described (13).

The study was approved by the ethics committee of the University Hospital, and blood was obtained with informed consent of the parents.

Measurements

Body weight and length were recorded and ponderal index was calculated as the ratio of the birth weight in grams $\times$ 100 to the cube root of the length in centimeters. Umbilical mixed arterial-venous blood was collected and centrifuged immediately after delivery. The serum was frozen at -70 C until packed in dry ice and shipped to Boston for leptin determination.

RIA for leptin was performed as described earlier (29, 30). All assays were run in duplicate, and the two measurements were averaged for statistical analysis.

Statistical analysis

Data are presented as the mean and SD. We used Student's *t* test to compare demographic and anthropometric measurements between full-term *vs.* preterm newborns, and between neonates born to smoking *vs.* nonsmoking parents. The Kolmogorov Smirnov test was applied to test the normality of the leptin levels. Because serum leptin concentrations were not normally distributed, we used the Mann-Whitney test to compare serum leptin levels in the above groups. Correlations between leptin and the continuous variables of this study were assessed using the Spearman rank test. Results were verified after logarithmic transformation of leptin values. Also, after logarithmic transformation, the general factorial analysis of covariance (ANCOVA) model was used to evaluate the potential effects of gender (male *vs.* female), maturity group (preterm *vs.* full-term), obesity (birth weight greater than median *vs.* birth weight equal or less than median), and maternal smoking status (yes, no). No adjustments were made for multiple comparisons. All analyses were two tailed and performed with the Fastat program for Macintosh computer, version 2 (Systat, Inc., Evanston, IL).

Results

Total sample data

Table 1 presents demographic and anthropometric data from the sample of full-term and preterm newborns. All newborns had detectable leptin concentrations. The mean $\pm$ (SD) serum leptin concentrations in the sample of the 100 full-term and the 24 preterm newborns of both the smoking and the nonsmoking mothers were 4.73 $\pm$ 2.63 and 4.07 $\pm$ 3.31 ng/mL, respectively. In the full-term newborns, the range was from 0.79 to 16.65 ng/mL, and in the preterm newborns, the range was was from 0.45 to 11.94 ng/mL. The difference between the two groups was not significant (U = 1408, $P = 0.112$).

Leptin concentrations in full-term and preterm newborns: effect of maternal smoking

Leptin concentrations in cord blood of both full-term (4.21 $\pm$ 2.71 ng/mL) and preterm newborns (2.46 $\pm$ 2.03 ng/mL) born to smoking mothers were significantly decreased in comparison with leptin concentrations in cord blood of newborns born to nonsmoking mothers (5.25 $\pm$ 2.48 ng/mL and 5.67 $\pm$ 3.62 ng/mL, $P = 0.011$ and $P = 0.021$, respectively) (Fig. 1).

To investigate the potential independent effect of maternal smoking on leptin concentrations in the two groups of neonates (preterm and full-term) a 2 (group) $\times$ 2 (smoking) ANCOVA was performed. A highly significant main effect was found for both smoking status (F = 11.672, $P = 0.001$) and for group (F = 6.473, $P = 0.012$).

Comparison of the leptin concentrations between full-term and preterm neonates in relationship to the smoking status showed that, in the group of the neonates of the nonsmoking mothers, the preterm newborns had leptin concentrations similar to those of full-term newborns. However, among the newborns whose mothers smoked during pregnancy, the preterm neonates had leptin concentrations significantly lower than the full-term neonates (U = 429, $P = 0.014$).

Finally, in the full-term neonates born to mothers who smoked during pregnancy, there was a significant negative correlation between leptin concentrations in the cord blood and the number of cigarettes smoked per day ($r = -0.438$, $P < 0.001$) (Figs. 2, 3).

Effect of obesity on leptin concentrations in newborns

Body weight comparison. Leptin concentrations were significantly correlated with body weight in the combined group

TABLE 1. Demographic and anthropometric variables in full-term and preterm neonates

Variable (mean $\pm$ SD)	Full-term (n = 100)	Preterm (n = 24)
Gestational age (wks)	39.0 $\pm$ 1.0	35.4 $\pm$ 1.7
Gender (Males)	51 (51%)	10 (41.6%)
Birth weight (g)	3312 $\pm$ 350	2599 $\pm$ 550
Length (cm)	50.9 $\pm$ 2.0	46.2 $\pm$ 3.1
Ponderal index	2.52 $\pm$ 0.3	2.60 $\pm$ 0.2
Blood pressure (mmHg)		
Systolic	61.7 $\pm$ 9	53.9 $\pm$ 5.6
Diastolic	41.2 $\pm$ 8.3	36.6 $\pm$ 3.8

of full-term and preterm neonates (r = 0.332, $P < 0.001$). In the full-term neonates there was a significant positive correlation of leptin concentrations with both birth weight (r = 0.302, $P < 0.01$) and length (r = 0.581, $P < 0.001$). Also, there was a significant positive correlation with both birth weight (r = 0.467, $P < 0.05$) and length (r = 0.504, $P < 0.05$) in the group of preterm neonates.

To investigate whether the effect of obesity on leptin concentrations is independent of the group of neonates (full-term *vs.* preterm) born to nonsmoking mothers, a 2 (group) × 2 (obesity) ANCOVA was performed. A significant main effect of obesity (F = 26.469, $P < 0.0001$), and a significant, albeit weaker, independent effect of group (full-term *vs.* preterm) (F = 4.987, $P = 0.049$) was detected. Conversely, in the neonates whose mothers smoked during pregnancy, no significant effect of either obesity or group was observed.

Obesity and smoking. Birth weight, length, head circumference, and ponderal index were lower in the full-term neonates of smoking compared with nonsmoking mothers ($t = 5.118$, $P < 0.000$; $t = 2.407$, $P = 0.018$; $t = 4.639$, $P < 0.000$; and $t = 1.837$, $P = 0.06$, respectively) (Fig. 1). In contrast, although the difference in the above somatometric parameters was of similar magnitude in the preterm newborns of mothers who smoked and those who did not smoke during pregnancy, the difference did not achieve statistical significance, probably because of the small number (Tables 2 and 3). Thus, to evaluate whether the effect of smoking on leptin was independent of birth weight/obesity, we performed a 2 (obesity) × 2 (smoking) ANCOVA. Smoking had an effect on leptin concentrations that was independent of obesity in both preterm (F = 5.142, $P = 0.034$) and full-term newborns (F = 3.214, $P = 0.076$).

Positive correlations between leptin concentrations and birth weight were observed when the neonates born to mothers who were nonsmokers were considered separately both in the full-term (r = 0.434, $P < 0.01$) (Fig. 2A) and in the preterm group (r = 0.620, $P < 0.05$). In contrast to the neonates of the nonsmoking mothers, in newborns born to mothers who smoked during pregnancy, no significant correlation was found between leptin concentrations and birth weight (in full-term, r = 0.134, $P > 0.1$ (Fig. 2B); in preterm, r = −0.035, $P > 0.1$) (Fig. 3).

Effect of gender on leptin concentrations in newborns

Leptin concentrations in the male and female full-term newborns of the nonsmoking mothers were 5.73 ± 1.95 and 4.68 ± 2.82 ng/mL, respectively (U = 372, $P = 0.081$). In the full-term newborns of the mothers who smoked during pregnancy, the corresponding values were 3.67 ± 1.94 and 4.78 ± 3.28 ng/mL (U = 243, $P = 0.280$). Additionally, no significant difference between the sexes was detected in the preterm newborns of both smoking and nonsmoking mothers.

To investigate the potential interaction between gender and leptin concentrations in full-term *vs.* preterm neonates of

TABLE 2. Demographic and anthropometric variables in full-term and preterm neonates of nonsmoking mothers

Variable (mean ± SD)	Full-term (n = 50)	Preterm (n = 12)
Gestational age (wks)	39.0 ± 1.0	35.5 ± 1.6
Birth weight (g)	3498 ± 415	2735 ± 512
Length (cm)	51.4 ± 1.6	50.0 ± 2.2
Head circumference (cm)	35.3 ± 1.0	32.8 ± 1.4
Ponderal index	2.58 ± 0.20	2.63 ± 0.20

TABLE 3. Demographic and anthropometric variables in full-term and preterm neonates of mothers who smoked during pregnancy

Variable (mean ± SD)	Full-term (n = 50)	Preterm (n = 12)
Gestational age (wks)	39.0 ± 1.2	35.2 ± 1.9
Birth weight (g)	3151 ± 242	2463 ± 575
Length (cm)	50.4 ± 2.2	45.4 ± 3.6
Head circumference (cm)	33.4 ± 1.3	31.4 ± 1.6
Ponderal index	2.46 ± 0.30	2.50 ± 0.20
Number of cigarettes/day	14.5 ± 7.6	15.1 ± 14.7

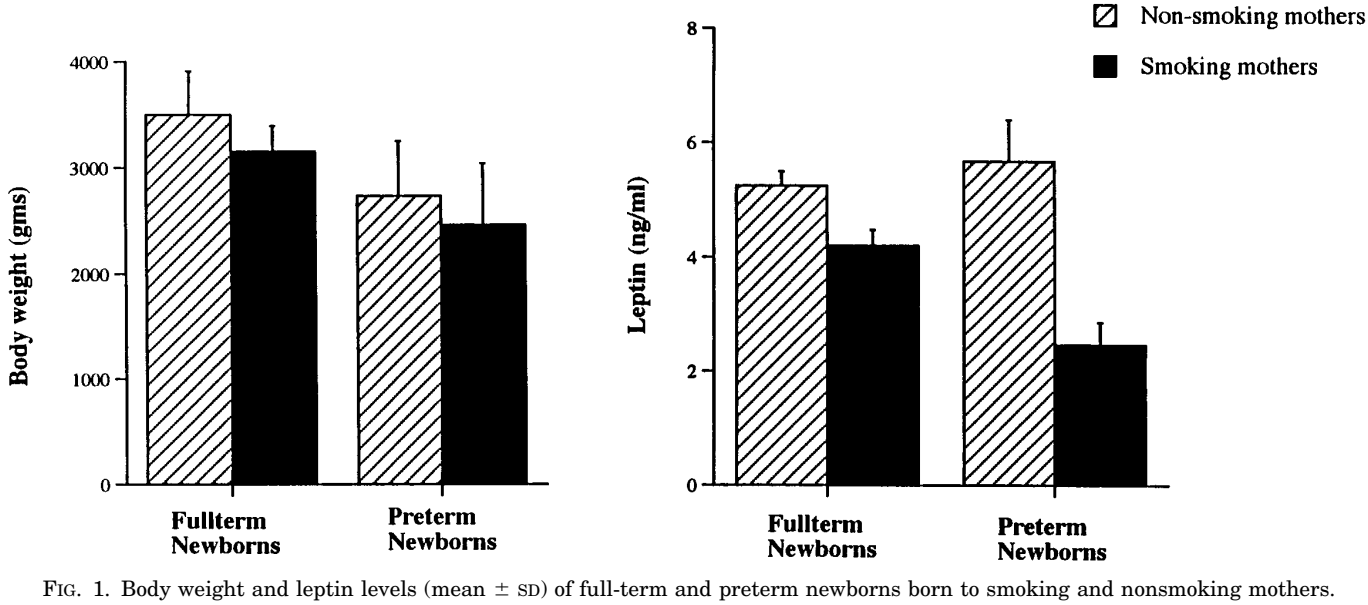

FIG. 1. Body weight and leptin levels (mean ± SD) of full-term and preterm newborns born to smoking and nonsmoking mothers.

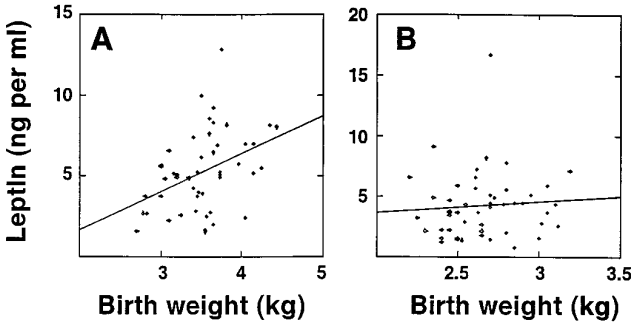

FIG. 2. Correlation between cord blood leptin concentrations and birth weight. A, Fifty newborns of nonsmoking parents. B, Fifty full-term newborns of mothers who smoked during pregnancy.

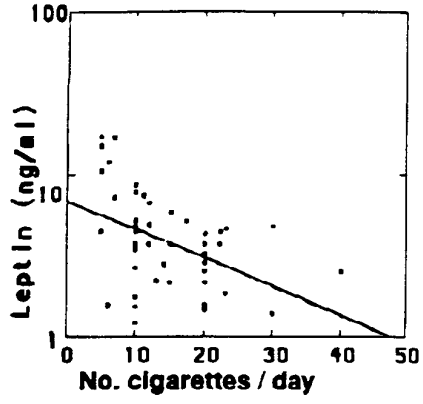

FIG. 3. Correlation between cord blood leptin concentrations and number of cigarettes smoked per day.

nonsmoking mothers, a 2 (group) × 2 (gender) ANCOVA was performed. A main effect was significant for group ($F = 24.010$, $P < 0.000$), but not for gender ($F = 2.731$, $P = 0.104$). No interactions were found. A similar ANCOVA on neonates of mothers who smoked during pregnancy showed no significant main effects or interactions between the two groups.

Maternal obesity

Maternal body mass index at baseline (before pregnancy) was similar in both the full-term (smokers: 24.03 ± 3.65 *vs.* nonsmokers: 24.57 ± 5.27) and preterm (20.89 ± 1.43 *vs.* 20.42 ± 2.49) groups of newborns. Similarly, body mass index when the blood was drawn (after pregnancy) was not significantly different in the study groups (28.81 ± 3.44 *vs.* 30.61 ± 5.68 and 24.61 ± 5.68 *vs.* 25.44 ± 4.05, respectively).

Discussion

This study demonstrates that leptin is detectable in the serum of newborns, as has been recently reported (27, 28). Leptin concentrations in mixed arterial-venous cord blood of newborns are comparable with the concentrations found in normal adults (29–30) and older children (31) and are strongly correlated with birth weight and ponderal index, the indirect measures of adiposity used in the present study and most previous studies (32). These data are consistent with previous observations suggesting that adiposity in humans is associated with increasing leptin production (32) which, in turn, signals the state of adipose stores to the brain and regulates energy homeostasis (22, 23, 33–37). The present study demonstrates clearly that the components of the system are operative even during the first day of life, not only in full-term (27, 28), but also in preterm neonates.

Prematurity and maternal smoking during pregnancy are both associated with decreased birth weight (1–7), impaired neuroendocrine function of the newborn (8–15), and increased morbidity and mortality (1, 2, 5, 8–15). More specifically, maternal smoking has been linked to increased PRL, GH, and growth factor levels at birth (13), as well as to growth retardation (7) and behavioral abnormalities later in life (8, 11). We and others have recently shown that food deprivation and decreased weight/body mass is associated with decreased circulating concentrations of the adipocyte-secreted hormone leptin (23–26), and that the fall in leptin concentrations mediates the neuroendocrine response to food deprivation in rodents (24). This study demonstrates that leptin concentrations were significantly lower in both full-term and preterm newborns born to mothers who had been smoking during pregnancy, than in newborns who were born to nonsmoking parents, independent of adiposity. Importantly, our data reveal a significant dose-response relationship between the reported number of cigarettes smoked during pregnancy and leptin concentrations in the term newborns' cord blood, providing further support to the notion that the association between maternal smoking and newborns' leptin concentrations is causal. Another finding of this study is that leptin concentrations were similar in preterm and term newborns born to nonsmoking parents. However, the preterm newborns of mothers who smoked during pregnancy had leptin levels almost half of those measured in term newborns born to mothers who were smokers. Thus, although smoking significantly decreases leptin concentrations in both groups of newborns, it appears that the effect of smoking to lower leptin concentrations may be more pronounced early in the third trimester of pregnancy than at term.

How could maternal smoking decrease leptin concentrations? It is well recognized that smoking may affect energy delivery and, consequently, the newborns' energy stores (4), either directly through the toxic effect of substances present in tobacco smoke (13), or indirectly, by compromising the uteroplacental and fetal blood flow (13, 16–18). Because it is also well recognized that maternal smoking has a direct effect on reducing birth weight (1, 4), decreased circulating leptin concentrations would be expected on the basis of decreased energy stores in adipose tissue alone. However, the effect of maternal smoking on newborns' leptin concentrations in this study persists after controlling for obesity, indicating that the effect of maternal smoking to reduce leptin concentrations is mediated by other factor(s) in addition to decreased adiposity. Smoking has previously been shown to increase plasma catecholamines and free fatty acid concentrations (38, 39). Catecholamines, in turn, increase lipolysis in humans (39) and have been shown to decrease leptin concentrations *in vitro* and *in vivo* in rodents (40) through a cAMP-dependent mechanism (41). Thus, maternal smoking, by compromising the uteroplacental and fetal blood flow and reducing the newborn's energy stores in adipose tissue, and/or indirectly

through its effect on catecholamines, decreases leptin concentrations in the circulation. Several mechanisms, including socioeconomic and nutritional factors, could account for the low birth weight and smoking-associated neuroendocrine dysfunction in newborns. Whether the decreased leptin concentrations in response to low birth weight and smoking mediate their effect on neuroendocrine dysfunction remains to be fully elucidated by future studies.

Another aspect of this study is the lack of sexual dimorphism of leptin concentrations in newborns. Data from neonates in this study do not confirm previous observations in adults and older children that show females to have higher leptin concentrations than males (31, 42). It has previously been suggested that circulating androgens in men may depress circulating leptin levels, whereas serum estrogens and/or progesterone may increase circulating leptin concentrations (42). However, during the first day of life, male newborns have significantly higher levels of circulating testosterone and dihydrotestosterone than females (43), and female newborns have androgen levels similar to those of adult females (43). Thus, our data suggest that short-term exposure to relatively higher androgen levels in male newborns does not up-regulate their leptin concentrations. Additionally, leptin appears to be expressed predominantly by subcutaneous adipocytes, particularly in women, suggesting that this may represent a reason for the observed sexual dimorphism of leptin concentrations (44). Lack of differences with respect to body fat distribution may underlie the lack of sexual dimorphism of leptin concentrations in newborns.

The present study has certain potential limitations. First, circulating leptin concentrations at birth may simply reflect maternal leptin concentrations and not the endogenous production of the newborns per se. Whether leptin can cross the placenta has not yet been studied. Although no direct measurements of leptin concentrations in sera of mothers were performed, the fact that maternal body mass indices did not differ significantly in this study suggests that maternal leptin concentrations could not account for the observed differences. Also, the finding of a significant positive correlation between the leptin concentrations in the cord blood and the birth weight of neonates of nonsmoking mothers supports a fetal origin of the circulating leptin at birth. Second, our findings might have been confounded by a misclassification of mothers with respect to their smoking status, which was ascertained using an interviewer-administered questionnaire. Although recall bias regarding smoking during pregnancy is unlikely to occur, it remains possible that because of the widely prevailing idea that smoking may be the cause of potential adverse effects on the fetus, reporting bias could have occurred (45). Any resultant misclassification would be expected to be unrelated to leptin measurements, however. Thus, nondifferential misclassification would have resulted in an underestimation of the demonstrated effect of smoking on circulating leptin concentrations and attenuation of the corresponding effect estimates and levels of statistical significance but would not have altered the direction of the associations observed in this study (46). Use of relevant laboratory measurements cannot separate with certainty smokers from nonsmokers (47). Also it has been shown that even under controlled conditions of cigarette smoking by pregnant women, there is no correlation between the number of cigarettes consumed per day and the maternal levels of carboxyhemoglobin and nicotine (48). Similarly, there is a poor correlation between the carboxyhemoglobin, cotinine, and thiocyanate concentrations in the smokers' blood and the daily cigarette consumption (49, 50).

In summary, our data indicate that leptin concentrations are correlated with birth weight and length, and that maternal smoking during pregnancy is associated with decreased leptin concentrations in the cord blood of newborns, raising the possibility that low concentrations of leptin may be one of the factors that mediate the neuroendocrine dysfunction of neonates born to smoking mothers.

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