

Original articles – Fetus

Increased cortisol concentrations in the cord blood of newborns whose mothers smoked during pregnancy

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Abstract

Aim: To investigate the effect of tobacco smoke on cord blood cortisol concentrations.

Methods: Cortisol concentrations were measured in cord blood from 211 term newborns of smoking and 211 term newborns of nonsmoking mothers; 48 and 36 newborns were delivered by cesarean section, respectively. In 16 cases, in addition to cord blood, maternal venous blood was obtained at delivery.

Results: The median cord blood cortisol concentration in neonates of the smoking and the nonsmoking mothers was 23 and 13 $\mu\text{g/dL}$, respectively ($P < 0.0001$). Cortisol concentrations were greater in the newborns whose mothers smoked, when compared to corresponding controls, whether they were delivered vaginally or by cesarean section. In the newborns delivered by cesarean section, there was a positive correlation between number of cigarettes smoked/day and cortisol concentrations, as well as a negative correlation between cortisol concentrations and neonatal length. There was no significant correlation between cortisol concentrations and birth weight or head circumference. Cortisol concentrations in the cord blood of neonates whose mothers were smokers and nonsmokers were by 29% and 45% lower from those measured in their mothers, respectively.

Conclusions: Although a causal relationship between maternal smoking and high cortisol concentrations in cord blood was not established, the findings are in accordance with previous reports indicating elevated stress-hormones in newborns whose mothers smoked during pregnancy.

Keywords: Cigarette smoking; cord blood; cortisol; fetal stress; tobacco smoke.

Introduction

There are several reports indicating that smoking during pregnancy causes disturbances of the endocrine status of the newborn, such as increased concentrations of prolactin, growth hormone and insulin-like growth factor-I [5], increased concentrations of erythropoietin [3, 18], and reduced concentrations of leptin [13]. The endocrine dysfunction in newborns of mothers who smoked during pregnancy could result either from direct or indirect toxic effect of substances present in the tobacco smoke, or by the cigarette smoke induced compromise of the uteroplacental blood flow, that has been shown to occur in fetuses when pregnant women smoke [1, 10, 12, 17].

It is unknown whether or not the levels of cortisol in the cord blood of newborns, whose mothers smoked during pregnancy, are disturbed. The high concentrations of the “stress” hormones prolactin and growth hormone, found in the cord blood of such newborns suggest that these fetuses are in a state of stress [5], possibly caused by the placental circulatory abnormalities induced by tobacco smoke [1, 10, 12, 17]. This is supported by reports showing that some fetuses of smoking mothers are in a state of chronic hypoxia [3, 18] and that neonates of mothers who smoked in pregnancy have increased blood pressure [2, 4]. Similarly, the finding that neonates of mothers who smoked during pregnancy are at a decreased risk for neonatal respiratory distress syndrome is interpreted as a result of chronic stress in the fetus caused by smoking-related acceleration of fetal pulmonary maturation [7, 11]. The more advanced lung maturation in the smoke-exposed fetuses is evidenced by increased lecithin-sphingomyelin ratios, as well as saturated phosphatidylcholine and cortisol levels in the amniotic fluid [11]. These observations would support the hypothesis of increased cortisol concentrations as a result of fetal stress in the blood of fetuses whose mothers smoked during pregnancy.

In order to investigate whether maternal smoking during pregnancy affects the cortisol concentrations in the fetal blood, we measured the levels of this hormone in: (a) cord blood of newborns whose mothers smoked or did not smoke during pregnancy; (b) maternal venous blood at the time of delivery; and (c) venous blood of neonates of smoking and nonsmoking mothers at 24, 48 and 72 h of life. The cord blood cortisol levels were correlated to the number of cigarettes smoked per day and to growth parameters of the neonates that are known to be affected by maternal smoking.

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Table 1 Maternal demographic data, gestational age, and anthropometric parameters in 211 newborns each of mothers who smoked and did not smoke during pregnancy.

Parameter	Smoking	Nonsmoking	Significance	
			t or z	P
Maternal				
Age (years)*	25.6±3.2	26.4±4.1	2.65	0.043
Parity†	2 (0–9)	2 (0–6)	1.03	0.303
Gestational age (weeks)*	39.2±1.2	39.7±0.9	1.69	0.086
Neonatal				
Birth weight (g)*	3130±334	3236±320	3.33	0.001
Length (cm)*	49.2±2.1	50.3±2.4	3.77	≤0.0001
Head circumference (cm)*	33.9±1.0	34.2±1.0	1.76	0.079

*Mean±SD, †Median (range).

Table 2 Cord blood cortisol concentrations in neonates of smoking and nonsmoking mothers delivered vaginally or by cesarean section.

Mode of delivery	Non smoking		Smoking			
			1–9 cig/d		≥10 cig/d	
	n	Cortisol*	n	Cortisol*	n	Cortisol*
Vaginal	175	15 (1–80)	73	25 (3–80)	90	22 (1–75)
Cesarean section	36	10 (2–45)	19	10 (2–80)	29	32 (9–64)

*Median (range), µg/dL, cig/d=cigarettes per day.

Methods

Subjects

We studied 211 term newborns of mothers who smoked during pregnancy (study neonates) and 211 term newborns of mothers who were nonsmokers (controls). The study neonates were enrolled consecutively provided that they fulfilled the criteria of the study and the parents agreed to participate. The control neonates followed the delivery of a neonate whose mother smoked, provided that they also fulfilled the criteria and the parents agreed to enroll in the study. In the groups of the smoking and the nonsmoking mothers 48 and 36 newborns were delivered by elective cesarean section, respectively, because of previous low-transverse cesarean birth, placenta previa, breech presentation, and advanced maternal age. In all cases the cesarean sections were performed before the onset of labor. The remaining neonates were born vaginally. In a limited number of random cases, provided that they agreed to participate, the cortisol concentrations were measured in the maternal venous blood at delivery and the cord blood, as well as the cortisol concentrations were followed in the neonatal blood during the first three days of life. Specifically, from seven cases of mothers who smoked during pregnancy, ten or more cigarettes per day, and from nine cases of nonsmoking mothers, maternal venous blood was obtained in addition to cord blood at the time of delivery. Also, from 15 neonates of mothers who smoked ten or more cigarettes per day and from eight control neonates, venous blood was obtained at 24, 48 and 72 h after delivery in addition to cord blood.

Neonates admitted to the study were singleton, had clear amniotic fluid, an Apgar score ≥7 at 1 and 5 min, and no signs of distress or evident congenital anomalies. They were healthy during the first three days of life. The mothers of both study and

control neonates did not have preeclampsia, diabetes or hypertension, and they did not receive steroids.

Mothers were interviewed and replied to a questionnaire about their smoking status during pregnancy, and if they were smokers, about the number of cigarettes smoked per day. Neonates whose mothers discontinued smoking during pregnancy were excluded from the study. Mothers denied the use of any illicit substances during pregnancy.

The gestational age was determined according to Ballard's scoring system. Neonates with a >2 week's discrepancy for gestational age, as determined by physical, neurological and ultrasonographic criteria, or as estimated according to the menstrual history were excluded.

The study was approved by the Ethics Committee of the University Hospital and blood was obtained with informed consent of the parents.

Measurements

Umbilical mixed arterial-venous blood was collected and centrifuged immediately after delivery. Serum was stored at –70°C until assayed.

Cortisol was determined by using a radioimmunoassay (Radim SpA, Rome, Italy). The coefficient of inter-assay variation was 6.0%, and of intra-assay variation 4.8%.

Statistical analysis

Because cortisol concentrations in cord blood of newborns whose mothers smoked during pregnancy was unknown, initially, for the determination of the sample size, we measured the hormone concentrations in 50 cases of study and 50 cases of control neonates. Using these data it was calculated that the

required sample size, for statistical power 0.9 and significance level 0.05, was 80 cases in each group.

Values are expressed as mean \pm SD or median with range, as appropriate. The anthropometric parameters of the study and the control neonates were compared with the *t*-test. The concentrations of cortisol were compared with the Mann-Whitney *U*-test or the Wilcoxon signed ranks test, as required. For the comparison of more than two samples of nonparametric data the Kruskal-Wallis test was applied, followed by the Dunn's multiple comparisons test. The cortisol concentrations in the cord blood were correlated with the number of cigarettes smoked per day and the anthropometric parameters of the neonates by Spearman's Correlation. Normality was tested by the Kolmogorov-Smirnov method. Statistical significance was set at $P < 0.05$. For statistics, the statistical package SPSS for Windows (version 13) was used, except for the Dunn's post hoc comparisons for which the Prism statistical software (Prism 3.02 for Windows) was applied.

Results

The median number of cigarettes smoked per day during pregnancy by the smoking mothers was ten, with a range from 1 to 70. The median (range) Apgar score at 1 and 5 min in the neonates of the mothers who smoked during pregnancy was 9 (7–10) and 9 (8–10), respectively; in the control neonates it was 9 (8–10) and 10 (9–10), respectively.

The birth weight and neonatal length were significantly lower in the newborns of the mothers who smoked during pregnancy than in the neonates of the mothers who were nonsmokers. The head circumference in the study neonates was smaller than in the control neonates, but the difference did not reach significance (Table 1).

The median cortisol concentration in the cord blood from the newborns whose mothers smoked during pregnancy was 23 $\mu\text{g/dL}$ (range 1 to 80), whereas in those whose mothers did not smoke it was 13 $\mu\text{g/dL}$ (range 0.5 to 80); the difference is significant ($z = 4.69$, $P < 0.0001$). The median cortisol concentration in the neonates of the smoking and the nonsmoking mothers born vaginally was 24 $\mu\text{g/dL}$ (range 1 to 80) and 15 $\mu\text{g/dL}$ (range 1 to 80), respectively ($z = 3.68$, $P < 0.0001$); in those delivered by cesarean section it was 19 $\mu\text{g/dL}$ (range 2 to 80) and 10 $\mu\text{g/dL}$ (2–45), respectively ($z = 3.39$, $P = 0.001$). In the neonates delivered vaginally by mothers who were nonsmokers, the cortisol concentrations were significantly greater than in those delivered by cesarean section ($z = 2.56$, $P = 0.01$), whereas in the group of the neonates whose mothers smoked, the difference between the two groups was insignificant ($z = 0.52$, $P = 0.61$). The power of the analysis performed for the total sample of the 422 study and control neonates was one, for the neonates delivered by cesarean section it was 0.96, and for those delivered vaginally it was 0.82.

There was a significant positive correlation between the number of cigarettes smoked per day and cord blood

cortisol concentrations in the neonates delivered by cesarean section ($n = 48$, $r = 0.340$, $P = 0.018$); there was no significant correlation between the above parameters in newborns delivered vaginally. The median cord blood cortisol concentration in the newborns delivered by cesarean section, whose mothers smoked 1–9 cigarettes per day was 10 $\mu\text{g/dL}$ (range 2 to 80) and in those whose mothers smoked ten or more cigarettes per day it was 32 $\mu\text{g/dL}$ (range 9 to 64). The Kruskal-Wallis test showed a significant difference among the above groups of neonates and the control neonates delivered by cesarean section ($\chi^2 = 23.42$, $P < 0.0001$). Dunn's post hoc multiple comparisons showed that cortisol concentrations in cord blood of the newborns whose mothers smoked ten or more cigarettes per day were significantly greater than in those whose mothers smoked 1–9 cigarettes per day ($P < 0.01$) or in control subjects ($P < 0.001$). There was insignificant difference between the neonates of mothers who smoked 1–9 cigarettes per day and those whose mothers did not smoke ($P > 0.05$). In the neonates delivered vaginally, the median cortisol concentration in those whose mothers smoked 1–9 and ten or more cigarettes per day was 25 $\mu\text{g/dL}$ (range 3 to 80) and 22 $\mu\text{g/dL}$ (range 1 to 76), respectively ($z = 0.95$, $P = 0.34$). Table 2 lists the median cortisol concentrations in the cord blood of the neonates of mothers who smoked and did not smoke, delivered vaginally or by cesarean section.

A significant negative correlation was observed between cord blood cortisol concentrations and length of the neonates delivered by cesarean section by smoking mothers ($n = 48$, $r = -0.284$, $P = 0.05$), but there was no such correlation in neonates delivered vaginally. In the neonates delivered by the smoking mothers both with cesarean section or vaginally, there was no significant correlation between cord blood cortisol concentrations and birth weight or head circumference.

The relationship between cortisol concentrations in the venous blood of the mothers who smoked and did not smoke during pregnancy, and in the cord blood of their newborns is illustrated in Figure 1. In general, the median concentrations of the hormone were lower in the cord blood of the neonates than in the venous blood of their mothers, but in three neonates of smoking and one neonate of a nonsmoking mother the cortisol concentrations were higher.

The cortisol concentrations measured in cord blood and venous blood obtained at 24, 48 and 72 h from the *in utero* exposed and unexposed neonates to tobacco smoke is illustrated in Figure 2. Compared to cord blood, the cortisol concentration in the venous blood at 24 h was significantly lower in both the study ($z = 3.41$, $P = 0.001$) and control ($z = 2.55$, $P = 0.01$) neonates. Also, significant difference was observed in cord blood cortisol concentrations between the neonates of smoking and nonsmoking mothers ($z = 2.6$, $P = 0.01$); thereafter the difference between the neonates of smoking and nonsmoking mothers was insignificant, although the cortisol concentrations tend to be higher in control neonates.

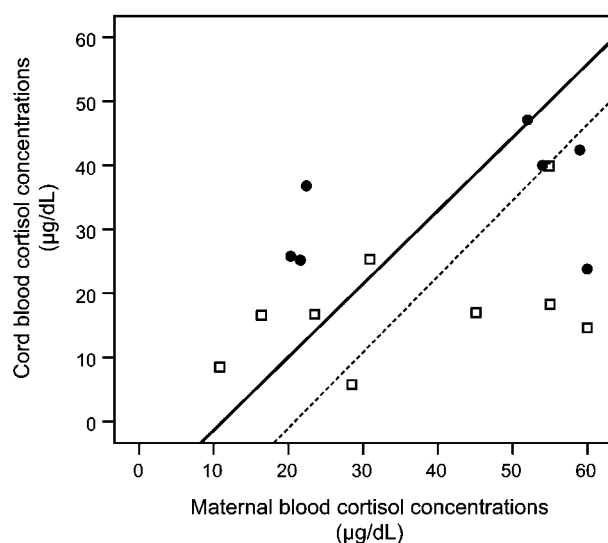


Figure 1 Scattergram and lines of best fit of the relationship between neonatal cord blood and maternal venous blood cortisol concentrations of mothers who smoked and did not smoke during pregnancy. (●, —) smoking, (□, ----) non smoking.

Discussion

The data show that the median cortisol concentrations in mixed venous and arterial cord blood of newborns whose mothers smoked during pregnancy are greater than in newborns of nonsmoking mothers. This was observed irrespective of the mode of delivery, though in those delivered vaginally the hormone concentrations were

greater. The higher levels of cortisol in cord blood of newborns following vaginal delivery as compared to those delivered by cesarean section were reported earlier [8, 19] and appear to be caused by stressful events for the fetus that occur during labor. Although the observed significant positive correlation between number of cigarettes smoked per day and cortisol concentrations in the neonates delivered by cesarean section does not show a causal relationship between maternal smoking and fetal cortisol levels, it indicates that the increased cortisol concentrations are related to tobacco smoke. The absence of a similar correlation in the neonates delivered vaginally, may be due to the contribution of stressful intrapartum events for the fetus, that partially obliterate the fetal stress response caused by tobacco smoke. Also, the data from neonates delivered by cesarean section, but not vaginally, suggest the presence of a threshold at about ten cigarettes per day, beyond which the cortisol concentration starts to increase in fetal blood. Similarly, our group reported the presence of a threshold in the amount of the tobacco smoke consumed by the pregnant women beyond which intrauterine growth retardation in body weight, length and head circumference ensues, which is close to ten cigarettes per day [9].

Of the anthropometric parameters studied, birth weight and length were significantly lower in neonates of smoking mothers, whereas the head circumference was smaller but the difference did not reach significance. It should be mentioned that in these analyses the total number of neonates whose mothers smoked one or more cigarettes per day was included. It is uncertain whether or not the negative correlation between cortisol concentration and length that was observed in neonates delivered by cesarean section, results from an effect of cortisol *per se* on fetal growth or both are independent results of the effect of the tobacco smoke. The absence of a correlation between cord blood cortisol concentrations and birth weight or head circumference might indicate a direct relationship between cortisol concentrations and fetal length. In this study, umbilical mixed arterial-venous blood was used because very similar cortisol concentrations have been found in the umbilical artery and the umbilical vein [8].

An issue that should be clarified is whether or not the higher fetal cortisol concentrations, when the mothers smoked in pregnancy, are of maternal origin. The finding of this study that the median cortisol concentration in the cord blood of the study neonates was lower by 29% from that measured in the venous blood of their mothers, whereas in the cord blood of the control neonates it was lower by 45%, suggests that the 2.2-fold greater concentration of this hormone in the cord blood of the neonates whose mothers smoked did not merely result from placental crossing of cortisol synthesized by the mother. This is reflected by the observation that the cortisol concentrations in the cord blood of the study newborns were significantly greater than in control newborns, whereas

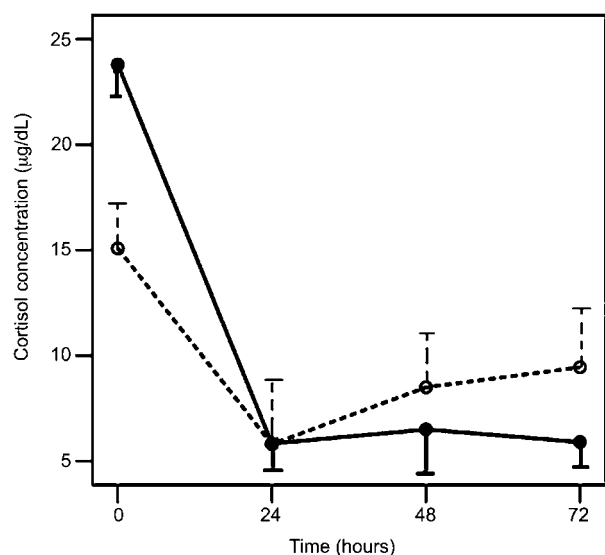


Figure 2 Median \pm SE cortisol concentrations in cord blood (0 time) and venous blood obtained at 24, 48 and 72 h after delivery from 15 neonates of mothers who smoked (●—) and eight neonates of mothers who did not smoke (○---) during pregnancy.

there was insignificant difference between the concentrations of this hormone in the venous blood of the mothers who smoked and those who did not smoke. Also, the observation in three neonates of smoking and one neonate of nonsmoking mother showing higher cord blood cortisol concentrations compared to those measured in venous blood of their mothers supports the notion that the higher concentrations of this hormone in the newborns' cord blood, whose mothers smoked, are of fetal origin. However, these results should be interpreted with caution because the number of cases in which both the maternal and cord blood cortisol concentrations were studied is small. Further support for the endogenous secretion of the greatest part of fetal cortisol is provided by the report indicating that about 85% of the maternal cortisol crossing the placenta is metabolized to inactive cortisone [15]. This is in line with the increased cord blood cortisol concentrations found in stressful deliveries, that were attributed to a stress response of the fetus *per se* and not to cortisol of maternal origin [8].

Because cortisol constitutes a major component of the hormonal stress response, the findings of this study indicate that tobacco smoke induces fetal stress suggested by the elevated cortisol concentrations in cord blood of newborns whose mothers smoked during pregnancy. The apparent cortisol response to fetal stress occurs when pregnant women smoke approximately ten or more cigarettes per day, a tobacco smoke dose previously reported [9] to affect intrauterine growth. Since animal studies have shown that fetal or neonatal stress can have long-term effects on the hypothalamic-pituitary-adrenal axis [6, 16], it remains unknown whether or not children exposed *in utero* to tobacco smoke might demonstrate malfunction of the axis later in life. This issue appears to be supported by the observation that cortisol concentrations in the neonates whose mothers smoked tended to be lower on the third day of life, though the difference from control neonates did not reach significance. Further investigation on this matter is warranted.

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