The influence of active and passive smoking during pregnancy on umbilical cord blood levels of vitamins A and E and neonatal anthropometric indices

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Abstract
Smoking during pregnancy has been shown to be detrimental for the developing fetus. The effects of active and passive maternal smoking on umbilical cord serum levels of vitamin A and vitamin E were examined. Secondary measures included anthropometric parameters in the newborn. Maternal and umbilical cord serum levels of vitamins A and E were measured at delivery. The mothers were assigned to three groups: non-smoking (n 12); passive smoking (n 13); active smoking (n 18). Based on multivariate linear regressions, active smoking during pregnancy was associated with increased umbilical cord serum levels of vitamin A and vitamin E. While enhanced circulating levels of vitamin A in cord blood were also found in non-smoking mothers exposed to tobacco smoke during pregnancy, those of vitamin E were not influenced. Further, an inverse association between smoking behaviour during pregnancy and birth length was observed, with shortest length in active smokers followed by passive smoking mothers. Active and passive maternal smoking behaviour during pregnancy increases the fetal demand for antioxidant compounds in order to counteract the oxidative burden by cigarette smoke. Against this background, the observed increase in umbilical cord serum levels of vitamins A and E may subserve antioxidative processes in response to tobacco smoke-induced oxidative stress. This would reduce the availability of vitamins A and E for fetal maturation, which is critical inasmuch as both vitamins are indispensable for the developing fetus. However, due to the cross-sectional nature of our observation, this line of reasoning definitely requires validation in cause–effect experiments in the future.

Key words: Pregnancy: Maternal smoking: Antioxidant vitamins: Birth length

Maternal smoking during pregnancy has been shown to be harmful for the developing fetus¹–⁵. A striking feature of cigarette smoking is that the body is polluted with reactive oxygen species. These compounds have been shown to produce DNA damage⁶, which may contribute to the detrimental effects of maternal smoking on fetal development. Previous studies have observed that umbilical cord blood levels of both retinol (hereafter named vitamin A) and α-tocopherol (hereafter named vitamin E) are decreased in children who were born to mothers who smoked during pregnancy⁷. This is detrimental inasmuch as both vitamins are important players in the human antioxidative system, e.g. they can scavenge reactive oxygen species⁸. Further, these nutrients are hypothesised to be indispensable for fetal growth and development⁹,¹⁰. However, the present evidence whether passive smoking during pregnancy also exerts detrimental effects on umbilical cord blood levels of these vitamins, as it has been reported for active maternal smoking, is insufficient¹¹. Thus, we collected umbilical cord blood of full-term offspring at the time of birth in order to examine the influence of active and passive smoking during pregnancy on respective levels of vitamins A and E. Importantly, only females with pregnancy

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due dates in the winter were included in order to reduce the likelihood of measurement bias produced by seasonal changes in maternal vitamin status. Secondary measures included anthropometric parameters of the newborn as well as blood samples from the mother.

Methods

Subjects

Demographic and clinical characteristics are presented in Table 1. A total of forty-three pregnant Russian females aged between 19 and 34 years agreed to participate in the study and were assigned to three groups: active smokers; passive smokers; non-smokers. The inclusion criteria were defined as follows: term at $37$ weeks; delivery in the winter season; singleton pregnancy; vaginal delivery without any instrumental support. Pregnancy-related illness (e.g. pre-eclampsia) or other chronic illness of pregnant females that could have an impact on the overall nutritional status, severe diseases of newborns, severe complication of labour, and/or alcohol or drug dependence served as exclusion criteria. All subjects lived in an urban area and were of similar socio-economic status. Furthermore, the number of pregnancies did not differ among the groups.

Smoking habits were assessed by standardised questionnaires. The active smoker group included females who reported smoking during pregnancy (on average, they stated that they had smoked more than ten cigarettes/d). Passive smokers were defined as females who reported having been exposed to tobacco smoke at home during pregnancy (i.e. smoking spouses or relatives), but who had never smoked themselves. Non-smokers were defined as those women who both had never smoked during pregnancy and had no exposure to environmental tobacco smoke during pregnancy. All subjects gave written informed consent to the study that conformed to the Declaration of Helsinki and was approved by the Ethical Committee of the Northern State Medical University, Archangelsk.

Biochemical analysis

Maternal blood was collected by venepuncture immediately after the delivery of the newborn. Blood of the newborn was obtained postpartum from the placental end of the cord. Maternal and umbilical blood samples were handled identically. Blood and umbilical blood samples were obtained by the fluorometric method (Fluorat-02; Lumex), as described previously(9). Standard solutions containing either $\alpha$-tocopherol acetate or retinol acetate (Sigma) were used to obtain the respective standard curves. In brief, $500$ ml of serum were added, and the sample was centrifuged for 10 min at 15000 $g$. Serum vitamin E concentrations were measured by the fluorometric method using standard solutions containing either reduced vitamin E or vitamin E acetate (Sigma). Serum and umbilical blood samples were stored at $-20^\circ$C. Serum vitamin A concentrations were measured by the fluorometric method (Fluorat-02; Lumex), as described previously(9). Standard solutions containing either reduced vitamin A or vitamin A acetate (Sigma) were used to obtain the respective standard curves. In brief, $0.5$ ml of serum were added, and the sample was centrifuged for 10 min at 15000 $g$. During the entire procedure, the tubes were protected against light exposure. The recovery was over 95%, and the CV in both cases was less than 5%.

Table 1. Maternal and neonatal characteristics (Mean values with their standard errors)

<table>
<thead>
<tr>
<th></th>
<th>Non-smokers ($n=12$)</th>
<th>Passive smokers ($n=13$)</th>
<th>Active smokers ($n=18$)</th>
<th>Active v. non-smokers $\beta$, $P^*$</th>
<th>Passive v. non-smokers $\beta$, $P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26.4 1.5</td>
<td>26.1 1.0</td>
<td>24.3 0.9</td>
<td>NS†</td>
<td>NS†</td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>2.3 0.5</td>
<td>1.8 0.2</td>
<td>1.8 0.3</td>
<td>NS†</td>
<td>NS†</td>
</tr>
<tr>
<td>Duration of labour (min)</td>
<td>567.5 54.4</td>
<td>570.4 47.8</td>
<td>570.4 47.8</td>
<td>NS†</td>
<td>NS†</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>39.8 0.3</td>
<td>40.2 0.3</td>
<td>39.6 0.4</td>
<td>NS‡</td>
<td>NS‡</td>
</tr>
<tr>
<td>Maternal serum level of vitamin A ($\mu$mol/l)</td>
<td>0.57 0.03</td>
<td>0.67 0.03</td>
<td>0.68 0.04</td>
<td>NS†</td>
<td>NS†</td>
</tr>
<tr>
<td>Cord serum level of vitamin A ($\mu$mol/l)</td>
<td>0.41 0.03</td>
<td>0.61 0.07</td>
<td>0.73 0.07</td>
<td>0.506, 0.006§</td>
<td>0.383, 0.039§</td>
</tr>
<tr>
<td>Maternal serum level of vitamin E ($\mu$mol/l)</td>
<td>20.35 4.76</td>
<td>21.71 5.49</td>
<td>12.08 2.09</td>
<td>NS†</td>
<td>NS†</td>
</tr>
<tr>
<td>Cord serum level of vitamin E ($\mu$mol/l)</td>
<td>8.49 2.68</td>
<td>11.81 2.02</td>
<td>15.64 2.21</td>
<td>0.506, 0.006§</td>
<td>0.383, 0.039§</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>35.18 90</td>
<td>35.42 72</td>
<td>33.73 135</td>
<td>-0.267, 0.030</td>
<td></td>
</tr>
<tr>
<td>Neonatal birth length (cm)</td>
<td>52.3 0.4</td>
<td>51.5 0.3</td>
<td>50.8 0.5</td>
<td>NS‖</td>
<td>NS‖</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>35.0 0.3</td>
<td>35.0 0.3</td>
<td>34.6 0.3</td>
<td>NS‖</td>
<td>NS‖</td>
</tr>
<tr>
<td>Chest circumference (cm)</td>
<td>34.1 0.3</td>
<td>34.5 0.3</td>
<td>33.9 0.5</td>
<td>NS‖</td>
<td>NS‖</td>
</tr>
<tr>
<td>Boys/girls ($n$)</td>
<td>6/6</td>
<td>5/8</td>
<td>8/10</td>
<td>NS†</td>
<td>NS†</td>
</tr>
</tbody>
</table>

* Overall, a $P$ value less than 0.05 was considered significant [i.e. NS = $P$ > 0.05].
† Specified by univariate ANOVA or $\chi^2$ test.
‡ Multivariate linear regression analyses were adjusted for age and number of pregnancies.
§ Multivariate linear regression analyses were adjusted for mother's age, maternal vitamin levels, sex of the newborn and birth weight.
|| Multivariate linear regression analyses were adjusted for mother's age and sex of the newborn.

Due to the nature of the study, all analysis included anthropometric parameters of the newborn as well as at blood samples from the mother.
The fluorometric determination of circulating retinol concentration was conducted at an emission wavelength of 460 nm with an excitation wavelength of 336 nm. Serum α-tocopherol concentration was measured at 320 nm with excitation at 292 nm. All samples were analysed in duplicate.

**Paediatric anthropometric measurements**

The weight, crown–heel length, and head and chest circumferences were measured at the day of delivery by trained nurses.

**Statistical analysis**

Data were analysed using multivariate linear regression models. Normal distribution of all variables was confirmed by the Kolmogorov–Smirnov test. Because of a skewed distribution, both cord and maternal serum levels of vitamins A and E were log-transformed in order to reach normality (Kolmogorov–Smirnov after log-transformation; \( P > 0.70 \) for all comparisons). Covariates of no interest were as follows, if not otherwise stated: mother's age; maternal serum vitamin levels; sex of the newborn; birth weight. Statistical analysis was performed using SPSS (version 19; SPSS, Inc.). A two-sided \( P \) value less than 0.05 was considered significant.

**Results**

**Maternal smoking behaviour is linked to umbilical cord serum vitamin levels and neonatal length**

Multivariate regression analyses revealed an overall positive association between maternal smoking status and umbilical cord serum levels of vitamin A (\( P < 0.005 \)). Subsequent regression analyses revealed that active smoking, compared with non-smoking, was linked to significantly higher cord serum concentrations of both vitamins A and E (Table 1 and Fig. 1). A similar pattern was found when comparing cord serum vitamin A concentrations of passive smokers with those of non-smokers. However, these groups did not significantly differ in terms of cord serum levels of vitamin E (Table 1 and Fig. 1). There was no association between smoking status and serum concentrations of vitamins A and E in the mothers (\( P > 0.104 \) for all comparisons).

Further multivariate linear regression analyses showed that maternal smoking status was inversely linked to birth length (Table 1), with the shortest length in non-smokers followed by passive smoking mothers. Birth weight, head circumference and chest circumference were not significantly associated with smoking status (\( P > 0.24 \) for all comparisons).

**Discussion**

In the present cross-sectional study, we demonstrate that maternal smoking during pregnancy is linked to increased umbilical cord serum concentrations of the antioxidant vitamins A and E at birth. In addition to this observation, enhanced circulating levels of vitamin A in cord blood were also found in non-smoking mothers exposed to tobacco smoke during pregnancy, while those of vitamin E were not influenced. Further, active and passive smoking during pregnancy has been found to be associated with a slight reduction in the crown–heel length of the newborns, when compared with those of non-smoking mothers. This indicates that not only active but also passive smoking during pregnancy may yield developmental deficits of the offspring.

The present study revealed that maternal smoking during pregnancy is linked to elevated umbilical cord serum levels of vitamins A and E, whereas a previous study produced opposing results\(^5\). Other researchers, in turn, did not observe any association between maternal smoking during pregnancy and umbilical cord serum levels of these vitamins at birth\(^6,10\). There are some methodological differences that may account, in part, for the discrepant results between these studies and
the present study. This includes the consideration in our regression model of a variety of potential confounders which these other studies did not account for\(^{(1,5,10)}\). For instance, cord blood retinol and \(\alpha\)-tocopherol concentrations have been found to be associated with neonatal birth weight\(^{(7,11)}\). Further, the present study only included females with pregnancy due dates in the winter in order to reduce the likelihood of measurement bias produced by seasonal changes in maternal vitamin status\(^{(12)}\). The maternal serum concentrations of vitamins measured here were relatively low, compared with those found in previous studies\(^{(5)}\). Thus, it might be that the increase in cord serum concentrations of vitamins A and E observed in our smoking groups may be related to the low maternal antioxidant vitamin status.

During pregnancy, the production of reactive oxygen species is increased, probably due to an increased lipid peroxidation\(^{(13,14)}\). Since maternal smoking causes additional oxidative burden, it may be that the observed increase in umbilical cord serum vitamin concentrations in those who smoked and those who were exposed to tobacco smoke during pregnancy represents a compensatory mechanism to maintain the fetal antioxidative capacity. As a result, it might be hypothesised that both vitamins are less available for fetal maturation. However, due to the cross-sectional nature of our observation, as well as the fact that differences in maternal vitamin levels among the smoking and non-smoking groups were quite variable and did not reach statistical significance, this line of reasoning definitely requires validation in a longitudinal study. One might speculate that the increase in cord serum vitamin concentrations might be due to the drop in maternal vitamin levels observed in smoking women in comparison with non-smoking women, although this would have to be interpreted with caution due to the cross-sectional nature of the study. This line of reasoning definitely requires validation in a longitudinal study. Additionally, we should note that other studies have observed detrimental effects of maternal smoking on birth outcomes\(^{(8,9,11)}\) and in utero hormone deficiency in the newborn. However, our study did not account for other factors such as smoking duration, smoking intensity, or smoking cessation.

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References


