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RESEARCH ARTICLE

# Heart rate variability in preterm infants and maternal smoking during pregnancy

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**Abstract**
*Objective* Tobacco smoke exposure increases the risk of premature birth and of dying of sudden infant death syndrome (SIDS). Prematurity significantly increases the risk of dying of SIDS, but mechanisms underlying this epidemiological finding are unclear. The cumulated effect of both prematurity and prenatal exposure to nicotine on autonomic heart rate control has not been studied.
*Methods* Using coarse-graining spectral analysis, we compared heart rate variability (HRV) indices of preterm newborns at 33–34 weeks post-conceptional age from smoking ($n = 19$) and non-smoking ($n = 21$) mothers. Assessment of tobacco exposure relied on maternal reports and newborns cotinine analysis. We observed how indicators of HRV depended on gestational age at birth.
*Results* At 33–34 weeks postconceptional age, the newborns from smoking mothers had lower HRV low frequency power normalised to the total spectral power (LF/TP) than the control group (median values: 8% vs. 15% respectively, $p < 0.02$). In the non-smoking group, RR-interval values and total HRV power were correlated with gestational age at birth, with a shorter RR and a lower total HRV power in lesser gestational ages ($\rho = 0.67$, $p = 0.03$, $\rho = 0.71$, $p = 0.003$ respectively). This correlation was not observed for RR values in the group with smoking mothers.

**Keywords** Cigarette smoking · Premature infant · Heart rate variability · Autonomic nervous system · Sudden infant death syndrome

**Abbreviations**

| | |
|---|---|
| GA | Gestational age at birth |
| PCA | Postconceptional age |
| HRV | Heart rate variability |
| LF | Low frequency band of HRV (0–0.2 Hz) |
| HF | High frequency band of HRV (0.2–2 Hz) |
| TP | Total spectral power of HRV |
| HF/TP | HF power normalised to the total spectral power |
| LF/TP | LF power normalised to the total spectral power |
| SIDS | Sudden infant death syndrome |

## Introduction

Since the reduction in the incidence of the prone sleeping position, maternal cigarette smoking has become the

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strongest modifiable risk factor for Sudden Infant Death Syndrome (SIDS) [36]. Maternal smoking may also be a confounding risk factor for SIDS owing to its association with other major risk factors such as low birth-weight, prematurity and/or intra-uterine growth restriction. The relative risk of SIDS incidence is 4–6 times higher in infants born prematurely. The relative risk increases as gestational age decreases [22]. The association between SIDS incidence and premature birth could be largely explained by the high maternal smoking prevalence in this population [32].

Various mechanisms have been postulated to explain the increased risk of SIDS associated with maternal smoking including impairment of autonomic functions [18]. Infants who have succumbed to SIDS have altered heart rate variability (HRV) with reduced heart rate variation and lower high-frequency spectral power of HRV (HF) than control infants [12, 31]. The mid-to-late period of the gestation is a developmental period during which the brainstem tegmentum is likely to be most vulnerable to the harmful effects of nicotine brought by maternal cigarette smoke [19]. A shorter RR-interval and a lower power of HRV have been described in preterm newborns reaching term compared with full-term newborns [8, 27]. Other works have suggested an impairment of cardiovascular autonomic maturation during pregnancy in infants from smoking mothers [1, 11].

Since the cumulated effect of both prenatal exposure to nicotine and premature birth on autonomic heart rate control has not been studied, we used spectral analysis of HRV, a non-invasive assessment of autonomic control of heart activity, to analyse the effect of maternal tobacco smoking during pregnancy in preterm newborns at 33–34 weeks postconceptional age.

## Methods

### Patient selection

We prospectively studied over an 8-month period, 40 healthy preterm infants from the Special Care Nursery at St. Jacques University Hospital (Besançon, France). Infants were considered for inclusion when they reached 33–34 weeks postconceptional age (PCA), whatever their gestational age at birth (GA). The rationale for the choice of this age was to avoid the immediate unstable postnatal period in the most premature newborns. In addition, since no HRV difference has been found in full-term infants from smoking versus non-smoking mothers [1], one could hypothesise that certain compensatory mechanisms provide adaptation against tobacco intoxication. Such adaptation has been demonstrated in animals [6]. In order to avoid these possible compensatory mechanisms before they occur, we choose to record our population when they reached 33–34 weeks of PCA. Infants were excluded if they had any congenital anomalies, central nervous system lesions, or if they required any respiratory support at the time of the study.

### Ethics

The study was approved by the Ethical committee of our University Hospital. Informed consent was obtained from the parents.

### Urinary cotinine analysis and maternal reporting of smoking

Mothers were asked to complete a questionnaire on their smoking habits detailing the number of cigarettes smoked during pregnancy. Samples of infant urine were collected at birth using sterile paediatric urine collection bags. Samples were frozen immediately and stored at −20°C until analysis. Nicotine has a relatively short serum half life (approximately 2 h) and is excreted as cotinine (and 3-hydroxycotinine) in the urine. We analysed urinary cotinine as an indicator of maternal smoking [16]. Urinary cotinine was assessed using a high-performance liquid chromatographic determination, performed in the Pharmacology Department of our University Hospital [2]. Infants of women who reported having smoked at least 1 cigarette per day or more throughout the first two trimesters of pregnancy were placed in the smoking-mother group. The remaining infants were placed in the control group, except in case of a positive cotinine urine sample, which resulted in exclusion from the study.

### Recordings

All of the included infants were submitted to a single recording, at 33–34 weeks of PCA. Recordings were performed in the Special Care Nursery. Incubator temperature was regulated to maintain a core temperature of 37°C. Infants were placed in supine position, propped by a blanket roll, and loosely wrapped by a single blanket. Incubators were covered by a cloth to protect the preterm infant from ambient light in accordance with the caring program of neonatal development. ECG electrodes were placed in a lead II position on the chest wall. The respiratory signal derived from thoracic impedance monitoring was visually controlled during the recording to insure the absence of apnoea. The infants were recorded for a daytime nap after feeding. Recording of the ECG signal was performed when the child was asleep and quiet, i.e. without any body or eye movement (visual control). The length of

the recording depended on the time necessary to obtain at least 2 min of stationary ECG signal as surveyed by visual inspection.

### HRV measurements

Recordings were conducted with a signal acquisition unit (Biopac Systems, Inc., Santa Barbara, CA, USA), which uses conventional electrocardiographic monitoring techniques at a sampling rate of 1,000 Hz. The ECG data were then digitised and stored on the hard disk of a laptop computer. The intervals between the successive R waves were measured with AcqKnowledge® software for signal analysis (Biopac Systems, Inc., Santa Barbara, CA, USA). For each subject, the power spectra of HRV were generated from artefact-free and stationary recording segments of 256 successive RR-intervals, selected by visual inspection. This choice was blind of subject characteristics. Undesirable beats accounted for <1% in each recording segment or subject. Using coarse-graining spectral analysis (CGSA) [38], the RR-interval series were analysed to determine the power spectrum of HRV for each infant. CGSA has shown great sensitivity for the analysis of the HRV spectrum during exercise and is more consistent for short RR-interval series than conventional HRV analysis [38, 39]. In newborns and particularly preterm newborns, sympathetic influence on HRV modulation is predominant, leading to a HRV spectrum that is closer to that of an exercising adult than an adult at rest [8, 27]. Moreover, in premature newborns, long-term stationary ECG recording is difficult. Even in quiet sleep, premature newborns show frequent muscular bursts with concomitant heart rate accelerations. CGSA therefore appeared to be a good technique for HRV spectral analysis in our population.

The power spectrum was then calculated and the analysis of results focused on the powers in the domains of low frequency (LF: 0–0.2 Hz) and high frequency (HF: 0.2–2 Hz) [9, 35]. The total power of HRV (TP) contains the non-harmonic (or fractal) power of HRV and the harmonic (sinusoidal like) power of HRV. In the CGSA method, the non-harmonic variability of RR-interval series is subtracted before performing Fast Fourrier Transform (FFT) decomposition of signal. Non-harmonic variability is therefore not included in our frequency of interest. Also, since short-term RR-interval series were used, a very low frequency variability of heart rate could be considered as negligible and subtracted with non-harmonic variability. We used 0.0 Hz to 0.2 Hz to define LF range. The limits of HF variability components were made higher than the usual upper limit to take into account the high respiratory rate of infants [9]. With Yamamoto and Hughson CGSA software, the upper limit is in fact half the lower heart rate of each series (for example 1.17 Hz for 140/mn) [38]. The components of spectral power were considered separately and their normalised indicators were calculated. HF/TP was used as an indicator of vagal cardiac control. LF/TP reflects cardiac sympathetic modulation and also contains some parasympathetic modulation. LF/HF is usually considered as an indicator of the sympathetic modulation of heart rate and as a marker of sympatho-vagal balance [35, 38].

### Statistical analysis

Median was calculated for demographic data as well as for RR-interval duration, TP, Harmonic Power (HF and LF powers), HF/TP, LF/TP and LF/HF ratios.

Maternal and newborn characteristics were compared according to mother smoking status (non-smoking or smoking mother). The Mann–Whitney *U* test for quantitative continuous variables and a Chi-Square test for qualitative variables were used. The values of HRV measurements from ECG recorded at 33–34 weeks of PCA were compared between infants from non-smoking mothers and from smoking mothers with the Mann–Whitney *U* test.

Finally, in each group, a Spearman's rank correlation was performed to assess the impact of GA on the values of these HRV measurements. GA also represents an indicator of ex-uterus maturation time before reaching 33–34 weeks of PCA.

All statistical analyses were conducted using StatView® software (Abacus Concepts, Inc., Berkeley, CA, USA). Differences were considered to be statistically significant when the $p$ value was lower than 0.05.

## Results

Forty-one patients were initially enrolled in this study, including 22 infants of non-smoking mothers and 19 infants of smoking mothers. Only one inconsistency was found with a positive cotinine urine sample in the newborn of a woman who said she did not smoke, and this patient was excluded from the study.

As shown in Table 1, both the birth weight and GA were similar in infants of non-smoking and smoking mothers. There were also no significant differences in PCA and weight at the time of the study, male to female ratio, Apgar scores, incidence of caesarean birth, twin pregnancy, previa placenta, preterm labour, premature rupture of membranes, or treatment by supplemental oxygen, caffeine and anti-reflux therapy. However, when the mothers smoked during pregnancy, there was a less frequent incidence of maternal preeclampsia and less corticosteroid therapy (betamethasone) than in non-smoking mothers. There was also more frequent paternal smoking when mothers smoked.

**Table 1** Characteristics of newborn group whose mothers smoke and newborn control group

| | Infants from non-smoking mothers ($n$ = 21) | Infants from smoking mothers ($n$ = 19) |
|---|---|---|
| Gestational age at birth (week) | 32.4 (25.6–34) | 31.7 (26–34.6) |
| Birth weight (g) | 1570 (870–2370) | 1470 (700–2380) |
| Post-natal age (weeks) | 1.6 (0.3–9) | 2.1 (0.3–8.7) |
| Weight at time of study (g) | 1560 (1210–2170) | 1580 (920–2210) |
| Female/male | 7/14 | 10/9 |
| APGAR (5 min) | 10 (4–10) | 10 (6–10) |
| Caesarean birth (%) | 71.4 | 57.9 |
| Twin pregnancy (%) | 19 | 42.1 |
| Preeclampsia (%) | 19 | 0 * |
| Previa placenta (%) | 4.8 | 5.3 |
| Preterm labour (%) | 28.6 | 31.6 |
| Premature rupture of membranes (%) | 19 | 42.1 |
| Antenatal glucocorticoids (%) | 100 | 73.7 * |
| Infant with supplemental oxygen (%) | 9.5 | 10.5 |
| Caffeine citrate therapy (%) | 71.4 | 79 |
| Anti-reflux therapy (%) | 23.8 | 5.3 |
| Paternal smoking (%) | 33.3 | 89.5 * |

The figures represent median and, between brackets, range values; absolute values; percentage values

* Statistically significant difference ($p < 0.05$) between groups

At 33–34 weeks of PCA, the LF/TP ratio was significantly lower in the smoking-mother group than in control infants (median and interquartile range values: 8% [4–12] vs. 15% [9–21] respectively, $p < 0.02$; Table 2). No significant differences were observed in median RR-interval duration between the two groups.

In the control group, the duration of RR-intervals and the TP measured at 33–34 weeks of PCA were both significantly correlated with GA (respectively $\rho = 0.67$, $p = 0.03$ for duration of RR-intervals and $\rho = 0.71$, $p = 0.003$ for TP) (Fig. 1). In the group from smoking mothers, TP, but not RR-intervals, correlated with GA (respectively $\rho = 0.39$, $p = 0.10$ for duration of RR-intervals and $\rho = 0.52$, $p = 0.02$ for TP) (Fig. 1).

## Discussion

This study sought to analyse the cumulated effects of prenatal exposure to nicotine and premature birth on autonomic heart rate control. At 33–34 weeks of PCA, there was a correlation between GA and RR-interval

**Table 2** Spectral power of RR variability

| | Infants from non-smoking mothers | Infants from smoking mothers |
|---|---|---|
| Whole group | | |
| | $n = 21$ | $n = 19$ |
| RR (m s) | 451 (385–477) | 441 (433–467) |
| Total power (m $s^2$) | 23 (15–64) | 28 (16–41) |
| Harmonic power (m $s^2$) | 5 (2–15) | 3 (1–4) |
| HF/TP (%) | 2 (0–4) | 1 (0–5) |
| LF/TP (%) | 15 (9–21) | 8 (4–12) * |
| LF/HF[a] | 8 (1–11) | 5 (1–21) |
| Only children with cotinine analysis | | |
| | $n = 16$ | $n = 14$ |
| RR (m s) | 417 (382–474) | 445 (432–463) |
| Total power (m $s^2$) | 25 (12–63) | 30 (18–41) |
| Harmonic power (m $s^2$) | 5 (2–12) | 3 (1–4) |
| HF/TP (%) | 2 (0–2) | 1 (0–4) |
| LF/TP (%) | 19 (10–25) | 7 (5–10) * |
| LF/HF[a] | 8 (6–17) | 7 (1–19) |

The figures represent median and interquartile range values

* Statistically significant difference ($p < 0.01$) between groups

[a] For infants with HF $\neq$ 0, respectively in infants from non-smoking and smoking mothers: 16/21 and 14/19 in the whole group

11/14 and 12/16 in the children with cotinine analysis group

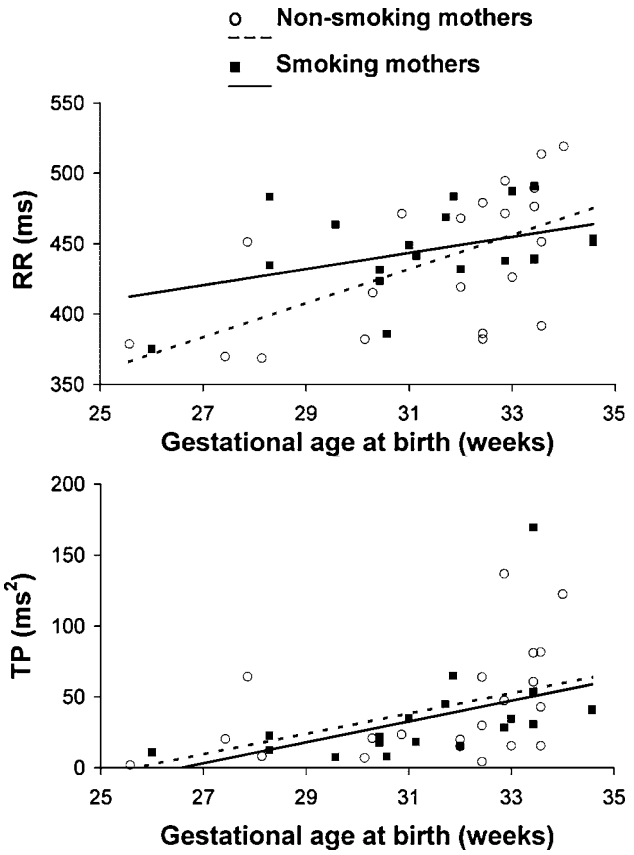


**Fig. 1** Duration of RR-intervals (RR $\rho = 0.67$, $p = 0.03$ for non-smoking mothers; $\rho = 0.39$, $p = 0.10$ for smoking mothers) and total HRV spectral power (TP $\rho = 0.71$, $p = 0.003$ for non-smoking mothers; $\rho = 0.52$, $p = 0.02$ for smoking mothers) at 33–34 week post-conceptional age according to gestational age at birth

duration in infants from non-smoking mothers; the shortest RR-interval duration corresponded to the lowest GA. This correlation was not found when the mother smoked during pregnancy. In addition at 33–34 weeks of PCA, infants from smoking mothers exhibited a significantly lower LF/TP ratio than those from non-smoking mothers.

Spectral analysis of HRV provides indirect non-invasive assessment of autonomic control of heart activity and is validated by international recommendations [35]. In children, substantially less data are available than in adults, and caution should be taken in adapting this tool to this specific population [30]. Our ECG recordings and processing complied with the recommendations of the Task Force with appropriate frequency bands for HRV spectral analysis of newborn infants [9, 35].

In the preterm infants from non-smoking mothers, the duration of RR-interval and the TP measured at 33–34 weeks of PCA were related to GA. A child born at 27 weeks of gestation had a higher heart rate (shorter RR-intervals) and a lower TP than a child born at 31 weeks of gestation and reaching the same PCA. Compared with full-term newborns, shorter RR-intervals and lower spectral powers of HRV have already been reported in preterm newborns reaching term [8, 10, 27]. These results may be related to an altered maturation of the balance between the sympathetic and parasympathetic autonomic influences on heart rate, suggesting a lower parasympathetic activity controlling heart rate than in full-term newborns [8, 27]. These findings suggest that prematurity could represent a risk factor for impaired maturation of autonomic cardiac control with potential long-term effects [17]. It is not known if it is the interruption of relations with the mother or the more external stimulations of the ex-utero life which predominantly contributes to the observed changes in the maturation of cardiac autonomic control for this population.

Tobacco exposure during pregnancy affects the maturation of autonomic cardiac control. Indeed, there was no significant correlation between RR-intervals and GA in infants from smoking mothers (Fig. 1). These infants had also a lower LF/TP ratio than the control preterms (Table 2).

The effects of maternal smoking during pregnancy on cardiorespiratory development and activity before and after birth are poorly understood. Nicotine, the major constituent of cigarette smoke, readily crosses the placenta and has been found in foetal cord serum in concentrations that are generally 15% higher than those in maternal serum [21]. It has been suggested that maternal cigarette smoking could alter infant brain structures leading to changes in cardiorespiratory autonomic control via a complex interplay of foetal hypoxia, absorption of toxins and metabolic changes. Maternal smoking causes foetal hypoxia through at least two mechanisms. Uterine blood flow has been found reduced by 30% to 40%, depending on the level of nicotine exposure, and the lower blood flow reduces in turn the supply of oxygen and nutrients to the growing foetus [21]. Infants of smoking mothers also have high blood levels of carboxyhaemoglobin [3]. The combination of decreased blood flow and hampered oxygen carrying capacity of haemoglobin probably results in foetal hypoxia during uterine life [13, 24, 28]. There is also substantial evidence that the developing foetal brain could also be damaged by the direct toxic effects of nicotine and that the effects of maternal smoking on later neurological outcome may not all be secondary to hypoxia-ischaemia [21]. Nicotine interacts directly with endogenous nicotinic acetylcholine receptors in the brain and can profoundly affect central nervous system activity and development. These [3H]nicotine binding sites are heavily concentrated in the tegmental nuclei of the brain which are involved with cardiopulmonary integration, somatic motor control and arousal [19].

Cohen et al. observed that foetal nicotine exposure led to a desensitisation of some nicotinic acetylcholine receptors

(nAChR) with a consequent decline of catecholamine synthesis [6]. Such impaired catecholamine synthesis after nicotine exposure during pregnancy has been found in other animal studies [5, 6, 37]. In human newborns at delivery, epinephrine and norepinephrine concentrations in umbilical artery cord blood were significantly lower in the offspring of smokers compared with non-smokers [26]. An impaired catecholamine release in newborns of smoking mothers could contribute to a lower LF/TP ratio since the sympathetic nervous system determines a substantial part of this indicator [35].

Other studies have also suggested an impairment of cardiovascular autonomic maturation during pregnancy in infants from smoking mothers. The number of cigarettes smoked was correlated with deeper infant heart rate decline during hyperoxia and smaller heart rate rises during hypoxia and hypercapnia [34]. Browne et al. studied the blood pressure responses to tilting at 2-3 days and 3 months of age in infants from smoking and non-smoking mothers [1]. At 2-3 days, systolic pressure decreased in infants of smokers but remained unchanged in the non-smoking group. At 3 months, systolic pressure remained unchanged in the smoking group but increased in the non-smoking. Compared with non-smokers, 2-month-old infants born to smoking mothers had significantly lower HF powers and HF/TP and higher LF/HF power ratios during active sleep [11]. In the same study, the absence of correlation between the heart rate spectral values and age was also reported in infants from smoking mothers.

In these two studies, tobacco during pregnancy had an impact on the maturation of autonomic cardiac control, but not exactly with the same qualitative effect on HRV measurements as in our work. A first explanation for these differences could be that we did not explore HRV at the same maturation time. We recorded premature infants since other studies involved term newborns and 2-month-old infants. We reported in our Methods section that some compensatory mechanisms may allow physiological adaptation against in uterus tobacco intoxication. Such an adaptation mechanism could explain the observed differences between different age studies. Second, we used CGSA software with a powerful extraction of non-harmonic spectral power before spectral analysis in the frequency band of interest. Such extraction provided a more accurate LF analysis than usual spectral analysis [38] and could explain our original results.

Maternal tobacco exposure during pregnancy may favour SIDS by different mechanisms. Cigarette smoking during pregnancy could induce alter breathing pattern and impair ventilatory response or arousal reaction to hypoxia or hypercapnia [14]. An exaggerated laryngeal reflex could also impair autoresucitation ability after prenatal exposure to maternal tobacco [14]. Moreover, we have seen that infants of smoking mothers have an altered sympatho-vagal modulation of the cardiovascular system [1, 11]. In full-term newborns, the smaller heart rate increase during hypoxia was correlated with the increasing number of cigarettes smoked by the mother [34]. In rats, prenatal nicotine suppressed the tachycardia caused by sinoatrial reactivity to hypoxia and within a few minutes of hypoxia, the heart rate declined rapidly and precipitously [33]. In animals exposed to nicotine in the prenatal period, hypoxia/hypercapnia recruited an excitatory neurotransmission to cardiac vagal neurons in the brainstem that could lead to fatal bradycardia like in SIDS [15, 25]. This response to hypoxia/hypercapnia was not found in animals which had not been exposed to nicotine. The observed impaired modulation of HRV during sleep could be associated with impaired reactivity of heart rate control in autoresucitation situation.

There are several limitations in our study. First, the limited number of infants studied may prevent reaching significance in some statistical assessments. Second, polysomnographic recordings were not performed in this study to establish sleep stages. Sleep was determined by visual control of the infant and cardiorespiratory signal [29]. Third, regarding group allocation, only one inconsistency was encountered with a positive cotinine urine sample for the newborn of a woman who had reported herself as a non-smoker. This patient was not included in the study. The half-life of cotinine is approximately 16 h in blood and 22 h in urine [7]. If the test is performed after the mother stops smoking, for example due to hospitalisation, or if the test is done a long time after birth (difficulty of collecting urine samples from a preterm female newborn), the evidence of nicotinic exposure could be less significant. For these reasons, cotinine value could not be used as a quantitative indicator of foetal tobacco exposure. When the urine test could not be accurately performed, we referred to data from different medical files to ensure the absence of nicotinic exposure. The cotinine urine sample test could be accurately carried out for 31 of these 41 infants (17 of the 22 infants from non-smoking mothers and for 14 of the 19 infants from smoking mothers, with median urinary cotinine/creatine ratios respectively of 0 and 0.26 [0–4.4] μmol/mmol). Our single case of inconsistency amongst the 17 out of the 22 infants from non-smoking mothers is consistent with the published rate of 6% unconfessed maternal smokers [23]. One could argue that without the cotinine test, we cannot ensure that our non-smoking mothers really did not smoke during pregnancy. Therefore we repeated the statistical analysis with only the 31 children with an effective cotinine measure and we found similar differences between groups. There was a significantly lower LF/TP ratio in children from smoking mothers than in those from non-smoking mothers (7% [5–10] vs.

19% [10–25] respectively, $p < 0.01$; Table 2). There was also a correlation between GA and RR-interval duration for children from non-smoking mothers ($\rho = 0.71$, $p < 0.01$) but no correlation for infants from smoking mothers ($\rho = 0.35$, $p = 0.21$).

Fourth, the lower incidence of maternal antenatal glucocorticoid therapy (betamethasone) and preeclampsia in the smoking-mother group was expected [4, 20]. The size of our groups did not make it possible to account for antenatal glucocorticoid therapy or preeclampsia in the statistical analysis. However, when we excluded infants whose mothers did not have prenatal glucocorticoid therapy, the observed difference between groups for LF/TP ratio reached the limit of significance (Fig. 2a). On the other hand, when we excluded infants whose mothers had preeclampsia, we again found a lower LF/TP ratio in the group of smoking mothers (Fig. 2b). Therefore, this clinical status that occurred more frequently in non-smoking mothers probably did not bias our results. Finally, the LF/TP must be interpreted with care. The low-frequency range of heart rate fluctuations depends on both sympathetic and parasympathetic controls [35].

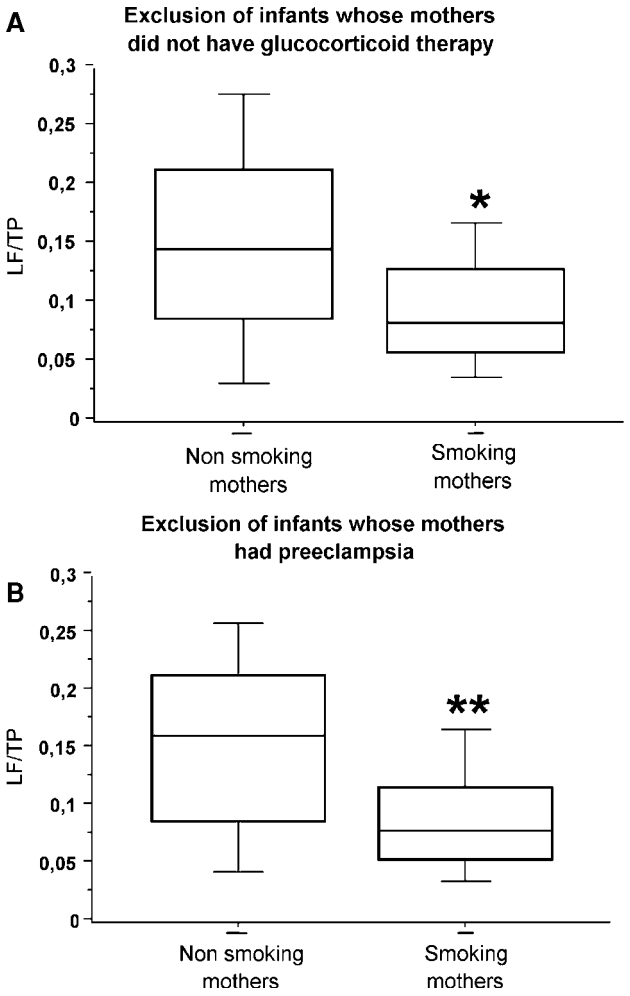


**Fig. 2** LF/TP ratio between groups, when the potential influences of antenatal glucocorticoid therapy (**a**) and preeclampsia (**b**) were excluded. Comparison between infants of smoking versus non-smoking mothers: * $p = 0.05$; ** $p = 0.01$

In conclusion, cigarette smoking during pregnancy and prematurity induced independent changes in autonomic cardiovascular control and maturation. The clinical relevance of this toxicity is a critical avenue to pursue since it could contribute to understanding why the risk of SIDS is markedly increased in preterm infants exposed to tobacco.

These effects can be added to those already reported and offer additional evidence for obstetricians and paediatricians advising mothers to cease smoking during pregnancy.

Together, this further supports the critical nature of evaluating maternal smoking during pregnancy when analysing newborn heart rate variability, particularly in preterm newborns.

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