Cardiovascular Stress Hyperreactivity in Babies of Smokers and in Babies Born Preterm

Gary Cohen, PhD; Silvano Vella, MD; Heather Jeffery, PhD, MPH, FRACP; Hugo Lagercrantz, MD, PhD; Miriam Katz-Salamon, PhD

Background—Being born preterm, small, and to a mother who smokes are common perinatal complications with major public health implications. Evidence suggests that each affects the body’s structure and function in ways that could increase susceptibility to cardiovascular dysfunction later in life. Here, we used 2 routine stress reactivity tests to identify incipient “silent” programming of cardiovascular dysfunction associated with adverse perinatal events.

Methods and Results—We studied 29 control babies born at term to nonsmokers, 18 term-born babies of mothers who smoked throughout pregnancy (mean, 15 cigarettes a day), and 31 babies born preterm to nonsmokers. All infants were compared at the same age after conception (ie, at 40 to 42 weeks), during sleep. We analyzed blood pressure (BP) and heart rate responses to breathing 4% CO2 for 4 minutes or to passive head-up tilt to 60°. BP was measured continuously from a wrist cuff. CO2 exposure raised heart rate and BP in controls by 10%, and tilt increased their BP by 5%. CO2 elicited the expected BP but no heart rate response from preterm infants but a much-greater-than-expected BP and heart rate response from babies of smokers. Tilt elicited a 3- to 4-fold greater rise in BP from preterm and tobacco-exposed babies.

Conclusions—Vascular, cardiac, and blood pressure reactivity is heightened in babies born preterm or to smokers. The findings are consistent with in utero and early postnatal “programming” of human cardiovascular dysfunction by adverse circumstances. This incipient dysfunction may be an early manifestation of processes that lead to other problems or complications later on (eg, higher BP or sudden infant death syndrome). (Circulation. 2008;118:1848-1853.)

Key Words: blood pressure ■ postnatal development ■ nervous system, autonomic ■ pediatrics ■ physiology ■ tobacco

The evidence is compelling that events early in life have an enduring impact on development and health. Being born too early, too small, and to a mother who smokes are 3 such common events with major public health implications. Preterm birth, currently accounting for nearly 8% of all deliveries, is the leading cause of neonatal death and disability and is a risk factor for elevated blood pressure later in life.1–3 Nearly 10% of all babies grow poorly in utero (growth restriction) and are born small for gestational age (SGA). These babies are more susceptible to diseases such as hypertension later in life.4–6 Smoking is the most common preventable cause of poor infant outcome; 20% to 50% or more of mothers, depending on age, nationality, and ethnicity, smoke during pregnancy.7,8 Babies of these women are more at risk of dying of sudden infant death syndrome and have raised systolic blood pressure in childhood, findings that suggest, among other things, an adverse effect of tobacco products on the immature cardiovascular system.9–12 All 3 entities also may go hand in hand; smoking increases the odds of a baby being born preterm and/or SGA.13 Despite the strong epidemiology linking early events to later dysfunction, we do not know when pathophysiological changes first appear, how they evolve over time, or what mechanisms and pathways are involved. The answers to these questions may provide new opportunities for the early diagnosis, treatment, and prevention of cardiovascular disease.

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To address the first of these questions, we compared babies from uneventful and “adverse” pregnancies at the same age after conception for signs of incipient dysfunction in heart rate and blood pressure control in the latter. We analyzed reactivity to mild stress because stress may unmask physiological anomalies long before clinical symptoms such as elevated blood pressure at rest appear.14,15 Blood pressure and heart rate were recorded continuously while infants either breathed a low concentration of CO2 for several minutes or were passively repositioned (“tilted”) several times from horizontal to upright then back to lying. Both methods are routinely used to test autonomic responses, including cardio-

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vascular and control, especially during sleep. Each engages different mechanisms and elicits slightly different response profiles. The first boosts sympathetic drive to the heart, blood vessels, and adrenal, resulting in a slow escalation-deescalation in heart rate and blood pressure. The second tests short latency, including baroreflex mechanisms that resist sudden rises or falls in blood pressure. Analyzing tests short latency, including baroreflex mechanisms that resist half were tilted (head-up to 60° for 1 minute half were tilted (head-up to 60° for 1 minute and the other half were tilted (head-up to 60° for 1 minute). The total number of tests is indicated in column 7. Data are mean±SD.

*Significant difference from control and tobacco-exposed infants; †significant difference from preterm-AGA infants (ANOVA).

### Methods

#### Subjects

Our study cohort (the Table) consisted of 4 groups: (1) control infants born at term to nonsmokers (n=29), (2) infants born at term to women who smoked (n=18), (3) infants born preterm (27 to 34 weeks) and appropriately grown for gestational age (AGA) to nonsmokers (n=15), and (4) infants born preterm and SGA (or growth restricted) to nonsmokers (n=16). Term infants were born at 38 to 41 weeks, all were AGA, and all were breastfed from birth. Preterm infants were fed either expressed breast milk or a combination of expressed breast milk and formula. Smokers estimated the number of cigarettes smoked per day during each trimester of pregnancy (via questionnaire). Preterm-born infants suffered no major neonatal complications (no respiratory abnormalities except for transient tachypnea, no persistent ductus arteriosus, and no evidence of intraventricular hemorrhage or sepsis during the neonatal period). Preterm-born infants classified as SGA were below the 10th birth weight percentile for gestational age. All preterm infants were studied after discharge at a corrected age of 1 to 2 weeks (ie, at term-equivalent age). Half the term-born infants were studied just before discharge (postnatal day 2 to 3); the remainder were studied 1 to 2 weeks later.

The study complied with the Declaration of Helsinki. The relevant Institutional Ethics Review committees approved all procedures, and written informed consent was obtained from the parents of all infants who participated. The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

### Table. Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Study Group (n)</th>
<th>GA, wk</th>
<th>PNA, d</th>
<th>PMA, wk</th>
<th>Birth Wt, g</th>
<th>Study Wt, g</th>
<th>Tests, n</th>
<th>Pretest Baseline</th>
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</thead>
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<tr>
<td>Term-control (29)</td>
<td>40±1</td>
<td>7±6</td>
<td>40±1</td>
<td>3547±412</td>
<td>3584±418</td>
<td>H, 16</td>
<td>124±15</td>
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<td>Term-tobacco (18)</td>
<td>39±2</td>
<td>12±10</td>
<td>41±2</td>
<td>3320±590</td>
<td>3364±501</td>
<td>H, 12</td>
<td>123±20</td>
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<td>Preterm-AGA (15)</td>
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<td>67±19*</td>
<td>40±1</td>
<td>1755±531*</td>
<td>3179±311</td>
<td>H, 11</td>
<td>143±15*</td>
</tr>
<tr>
<td>Preterm-SGA (16)</td>
<td>32±2*</td>
<td>53±10†</td>
<td>40±1</td>
<td>1313±578†</td>
<td>2361±522†</td>
<td>H, 16</td>
<td>145±13*</td>
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<td>&lt;0.0001</td>
<td>0.8</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>H</td>
<td>0.001</td>
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<td>T</td>
<td>0.001</td>
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</tbody>
</table>

GA indicates gestation age at birth; PNA, postnatal age; PMA, postmenstrual age (GA+PNA); Wt, weight; HR, heart rate at rest (bpm); MBP, mean blood pressure at rest; SBP, systolic blood pressure at rest; and DBP, diastolic blood pressure at rest. Pretest baseline was the 1 minute immediately preceding each test (mean of all periods for each protocol is shown). All infants were studied during a routine daytime nap. Half in each group breathed 4% CO2 for 4 minutes (H), and the other half were tilted (head-up to 60° for 1 minute [T]).

**Procedures**

Infants were studied during a routine daytime nap lying supine, lightly dressed, in a warm (20°C to 21°C) room during periods of behaviorally determined quiet sleep. We tested the response to either mild hypercapnia or passive head-up tilt.

**Physiological Measurements**

Blood pressure was recorded from a wrist cuff (Finometer, FMS, Amsterdam, the Netherlands) as described previously. During the hypercapnic but not the tilt protocol, we also recorded chest and abdominal breathing movements (Respirtrac, Ambulatory Monitoring Inc, Ardsley, NY), ECG, and transcutaneous PCO2 (Radiometer TC35, Copenhagen, Denmark). A detailed description of the hypercapnic protocol can be found elsewhere. Briefly, a 10-L polycarbonate head box was continuously flushed with gas (air or 4% CO2 in air). Tests were made up of a 2-minute baseline (the box was flushed with air), followed by 4% CO2 in air for 4 minutes and then back to air for 4 minutes (total, 10 minutes); tests were repeated twice. For the tilt tests, the infant slept supine on a custom-built tilt table. Babes were comfortably padded and secured in a harness to maintain the baby’s head in the midline and to prevent limb or body movements during tilt. To eliminate hydrostatic gradients when upright, the cuffed wrist was always kept at the level of the heart. Once heart rate and blood pressure had been steady for 30 seconds, the baby was tilted head up to 60° within 5 seconds, held in this position for 1 minute, and then returned to horizontal. Tilts were repeated after a 5- to 6-minute interval.

**Analysis**

Tests interrupted by a change in state, sigh, or arousal (movement of the head or limbs for ≥2 seconds accompanied by transient loss of the blood pressure waveform) were discarded. The arterial waveform was digitized and stored at 200 Hz. We used peak-and-valley detector software (Beatscope version 1.1, FMS) to calculate instantaneous systolic, diastolic, and mean blood pressures and heart rate (peak-to-peak interval). Data were expressed as relative changes from pretest baseline (30 seconds preceding each test), averaged each 5 seconds, and plotted against elapsed time to generate hemodynamic profiles. For CO2 tests, respiratory rate (RR), breath amplitude (tidal volume, VT), and ventilation (V=RR×VT) were plotted against changes in transcutaneous PCO2 to estimate the strength (slope) of the hypercapnic ventilatory response.

**Statistical Analysis**

Data from successful tests were pooled, and an average tilt/CO2 response was calculated for each infant. One-way ANOVA was used
Results

Characteristics of the study population are shown in the Table. At term-equivalent age, preterm SGA infants weighed 13% less than preterm AGA infants and 24% less than their AGA counterparts born at term. All preterm infants regardless of birth weight studied had increased heart rates and lower blood pressures at rest than babies born at term (Table). Smokers consumed 10 to 15 cigarettes a day (self-reported average throughout pregnancy and during breastfeeding). Babies of these mothers were slightly smaller than control infants at birth (not significant), but resting blood pressure and heart rate were within normal limits.

Maternal Smoking Exaggerates the Newborn’s Circulatory Response to CO₂

Breathing 4% CO₂ raised blood CO₂ levels of all 4 infant groups by $\approx 1$ kPa (Figure 1A). Control infants had the most and tobacco-exposed and SGA infants had the least vigorous ventilatory response to this classic breathing stimulus (Figure 1B), which normally (in control babies) also increased heart rate by $\approx 10\%$. Babies of mothers who smoked had exaggerated and prolonged rises in blood pressure and heart rate. Peak responses were 50% to 100% above age-matched controls, and both parameters remained elevated long after CO₂ levels returned to baseline (Figure 1C through 1F). Premature infants regardless of size at birth had a normal pressor but little or no heart rate response to CO₂.

Complications of Pregnancy Exaggerate Sympathetic Responses to Tilt

Normally (control infants), head-up tilt while asleep caused systolic pressure to fall slightly and heart rate to rise transiently, with the peak in heart rate occurring 8 to 10 heartbeats, or about 5 seconds, after the start (ie, on reaching and stopping at 60° vertical). Subsequently, systolic, diastolic, and mean blood pressures gradually increased and settled slightly above baseline while upright and then fell slowly back to baseline on returning to horizontal (Figure 2A through 2C). For babies born to mothers who smoked or those born preterm-SGA, vertical repositioning elicited the expected rise in heart rate (Figure 2B and 2C) but an unusually exaggerated (several times greater than in control infants) and long-lasting rise in systolic, diastolic, and mean blood pressures (Figure 2A, 2D, and 2E). The pressor response of preterm-AGA infants was “intermediate” between preterm-AGA and tobacco-exposed and SGA infants had the least vigorous ventilatory response to this classic breathing stimulus (Figure 1B), which normally (in control babies) also increased heart rate by $\approx 10\%$. Babies of mothers who smoked had exaggerated and prolonged rises in blood pressure and heart rate. Peak responses were 50% to 100% above age-matched controls, and both parameters remained elevated long after CO₂ levels returned to baseline (Figure 1C through 1F). Premature infants regardless of size at birth had a normal pressor but little or no heart rate response to CO₂.
SGA and control babies. Brief arousals occurred at the onset of 20% of tilts; this proportion was the same for all groups, as was arousal duration estimated from movement artifact.

No significant associations were found between reactivity (heart rate and diastolic, systolic, and mean blood pressures) and postnatal age for babies of smokers ($P = 0.25$ to $0.6$), suggesting that stress hyperreactivity did not change during the first postnatal weeks.

**Discussion**

Here, we provide physiological evidence confirming what epidemiological reports of modest elevations in resting blood pressure suggest: that being born too soon, too small, or to a mother who smokes alters cardiovascular control from an early age.$^3$ Our findings are entirely consistent with the concept of developmental “programming”$^{20}$ and moreover suggest that functional consequences of programming may be present much sooner than previously thought (ie, within days or weeks of birth).

Evidence for the adverse impact of passive smoking on the adult cardiovascular system began to emerge nearly 20 years ago.$^2$ Our data suggest that the immature cardiovascular system also is exquisitely vulnerable to secondhand smoke exposure, despite the considerable first-pass “filtering” of toxic combustion products that must occur in the mother’s placenta and breast. Some of the physiological changes we observed in babies of smokers—cardiac, vascular, and blood pressure hyperreactivity—resemble those seen in smokers themselves. These are all classic actions of nicotine in the autonomic system.$^{22}$ Although only one of many components of cigarette smoke, nicotine is a powerful developmental toxin. Animal studies indicate that chronic exposure to nicotine before birth weakens adrenergic excitation of the heart, blunts adrenal catecholamine release, and depresses cardiovascular stress reactivity after birth.$^{24}$ We cannot explain why reactivity is paradoxically heightened in babies exposed to nicotine via maternal smoking. One important difference, however, may be that babies are not continuously exposed to nicotine as are experimental animals but probably experience adult smoker-like patterns of nicotine exposure: intermittent daytime surges followed by overnight “abstinence/withdrawal.” These different patterns of nicotine exposure may have quite different cellular and behavioral side effects.$^{27}$

The effects of prenatal smoke and nicotine tend to be strongest soon after birth and then fade.$^{24}$ yet we found no differences between babies of smokers tested before or after hospital discharge, an interval of $\approx 2$ weeks at most. Two weeks may be far too soon to expect changes in such a small cohort of babies, bearing in mind that all received ongoing nicotine exposure via milk (all smokers breastfed) and perhaps environmental smoke at home. Both pathways could prolong and exacerbate changes that begin in utero. This raises the issue of whether incipient dysfunction may improve if a nursing mother who smokes abstains from breastfeeding.

Preterm birth is known to accentuate sympathetic drive (high resting heart rate and powerful peripheral vasoconstric-

![Figure 2. The head-up tilt test. Infants were tilted to 60° while comfortably immobi-

lized. Babies born preterm and to smokers responded with an exaggerated and prolonged rise in mean blood pressure (BP; A [segments marked are expanded in B, D, and E, respectively]). The heart rate (HR) of all infants increased transiently within 8 to 10 heartbeats of tilting up (B; peak HR response is shown in C). Systolic (SBP) and diastolic (DBP) BPs always increased proportionately (D and E; means during final 30 seconds upright). Note that AGA infants had an intermediate pressor response (D and E). For clarity, an overall profile for preterm infants (AGA and SGA combined) is shown in A; only HR changes for controls and tobacco-exposed babies are compared in B. Omnibus ANOVA $P$ values for group comparisons are shown in C through E. Significant differences from all other groups ($) and from tobacco-exposed or SGA infants (¶) are indicated (multiple-range tests). Data are mean±SD.
magnified. Mechanical forces such as friction resulting from changes in sleep position or breathing pattern) also may be caused by other circumstances (medication, arousal, or positioning) that allow blood pressure to surge too high also let it to fall too low, allowing a momentary loss of blood pressure to spiral out of control.\textsuperscript{11}

**Study Limitations**

Active smoking is strongly associated with preterm delivery and intrauterine growth restriction, with an incremental increase in the prevalence of preterm birth and reduction in birth weight as the number of cigarettes smoked rises.\textsuperscript{12,38,39} A malfunction in blood pressure and/or heart rate control during sleep has long been suspected but never identified. Our data provide intriguing evidence of incipient, sleep-related cardiovascular dysfunction in both of these high-risk groups. Could dysfunction that allows blood pressure to surge too high also let it to fall too low, allowing a momentary loss of blood pressure to spiral out of control?\textsuperscript{11}

**Conclusions**

Smoking during pregnancy and preterm birth both measurably alter cardiovascular reflex function in the weeks after birth. We suggest that these changes are a direct consequence of physiological programming by adverse circumstances and that they could plausibly be early markers of underlying processes that lead to or are involved in other changes or complications later on (eg, raised blood pressure and sudden infant death syndrome).\textsuperscript{41}

**References**

8. Poets CF, Schlaad M, Kleemann WJ, Rudolph A, Diekmann U, Sens B. Sudden infant death and maternal cigarette smoking: results from the
Large-scale epidemiological studies convincingly indicate that the seeds of cardiovascular and other diseases may be planted very early in life, before or around the time of birth. Events or insults that occur during this formative developmental period are thought to alter (“program”) the body’s structure and physiology, which in turn increases vulnerability to stress and accelerates disease onset. The developmental programming concept has broad public health implications because it raises the prospect of improving the early diagnosis, treatment, and perhaps prevention of disease. This article provides some of the first direct evidence of perinatal programming of human cardiovascular physiology by revealing distinct vascular reactivity patterns in infants born preterm, small for dates, and to mothers who smoked during pregnancy. These are the 3 most common complications of pregnancy and causes of poor infant outcome in the world developing nations. Early experiences may be plausibly be an early marker of later susceptibility to complications such as raised blood pressure. Large-scale epidemiological studies convincingly indicate that the seeds of cardiovascular and other diseases may be planted very early in life, before or around the time of birth. Events or insults that occur during this formative developmental period are thought to alter (“program”) the body’s structure and physiology, which in turn increases vulnerability to stress and accelerates disease onset. The developmental programming concept has broad public health implications because it raises the prospect of improving the early diagnosis, treatment, and perhaps prevention of disease. This article provides some of the first direct evidence of perinatal programming of human cardiovascular physiology by revealing distinct vascular reactivity patterns in infants born preterm, small for dates, and to mothers who smoked during pregnancy. These are the 3 most common complications of pregnancy and causes of poor infant outcome in the world developing nations. Early experiences may be plausibly be an early marker of later susceptibility to complications such as raised blood pressure.