Hypothalamic-Pituitary-Adrenal Axis Function and the Cellular Immune Response in Former Preterm Children


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Context: Animal data suggest that adverse early experiences may affect endocrine and immune functioning in later life.

Objective: Our objective was to assess the impact of preterm delivery on hypothalamus-pituitary-adrenal axis functioning, heart rate responses, and immune function.

Participants: Former preterm children [aged 8–14 yr (n = 18)], sex and age-matched full-term born control children (n = 18), data on birth weight, gestational age, birth weight for gestational age (in SD units), actual body weight, height, and body mass index were assessed.

Design and Outcome Measures: Subjects were exposed to a standardized laboratory stressor (“Trier Social Stress Test for Children”). Cortisol in saliva was determined in 10-min intervals before and after the stress test; heart rates were obtained continuously during the stress test. Additional assessment of saliva cortisol was performed: 1) on 3 consecutive days after awakening and at +10, +20, and +30 min (morning cortisol); and 2) at 0800, 1400, 1600, and 1900 h (short diurnal profile). Measurement of the delayed type hypersensitivity reaction to seven recall antigens [Multitest cellular mediated immunity (Multitest-Immignost, Biosyn, Fellbach, Germany)].

Results: Exposure to the Trier Social Stress Test for Children yielded significantly increased cortisol levels [F (8, 232) = 19.86; P < 0.001] and heart rates [F (38, 988) = 10.46; P < 0.001], however, no difference between former preterms and full-terms could be observed. No between-group differences were found in the short diurnal cortisol profile. Former preterms showed significantly higher cortisol levels after awakening [F (3, 102) = 3.14; P < 0.05]. In addition, a significantly suppressed delayed type hypersensitivity response [reduced number of positive antigens (t = −2.64, P < 0.05); induration (t = −2.4, P < 0.05)] was found in former preterms.

Conclusion: The data suggest that preterm delivery may be associated with altered endocrine and immune functions well into later childhood.

Epidemiological data indicate that the frequency of preterm delivery (birth before 37 wk of pregnancy) in the United States has significantly increased and rose to 12% in 2002, from 9.4% in 1981. Preterm birth rates in European countries are lower and vary, for example, from 8.4% in Austria, to 6.2% in Germany, 5.2% in Sweden, and 4.2% in France (1, 2). The increased prevalence of preterm delivery is a major health concern because preterm birth is associated with both adverse neonatal outcomes and increased infant morbidity. Furthermore, preterm birth significantly increases the risk for neonatal morbidity, including infections, chronic respiratory problems, or disorders of the central nervous system, such as cerebral palsy or neurodevelopmental deficiencies (3). Besides these direct effects of preterm delivery on neonatal and infant health outcome, it has been reported that preterm birth can have long-lasting effects, including developmental impairments or motor disabilities (4). Moreover, preterm delivery appears to predict an increased risk for later metabolic and cardiovascular dysfunctions, such as hyperglycemia or hypertension (5, 6). Some authors (7, 8) have emphasized that adult metabolic and cardiovascular risk factors may be more strongly linked to intrauterine growth retardation than to premature delivery, demonstrating that low birth weight adults born full term are at higher risk for metabolic and cardiovascular disease. However, it could be demonstrated that preterm birth, whether or not associated with intrauterine growth retardation, predisposes to increased metabolic or cardiovascular risk factors in later life (5, 6).

Multiple factors such as malnutrition, drug intake, smoking, inflammation (systemic or decidual chorioamnioniotic), cervical or uterine anomalies, or preeclampsia are known to be major factors leading to preterm delivery. Moreover, accumulating evidence points to a significant role of prenatal stress in preterm birth. It is suggested that a stress-induced activation of the maternal or fetal hypothalamus-pituitary-adrenal (HPA) axis with an accelerated rate of increase in placental CRH may initiate the release of prostaglandins and oxytocin from the placenta, initiating preterm labor (9). A growing body of literature suggests that an adverse prenatal environment may have profound long-term influences and may alter physiological and behavioral functions in later life. For example, gestational stress in animals has been related to endocrine dysfunctions, i.e., an altered (re)activity of the HPA axis or the sympathetic nervous system (10). Stress in utero was further linked to significantly altered immunocompetence in the resultant offspring (11).

The observation of a close link among gestational stress,
preterm delivery, low birth weight, and the susceptibility to disease in adulthood, a concept known as “fetal programming,” has gained considerable interest. In this model, exposure to adverse intrauterine (and early extrauterine) influences may imprint on the fetus a pattern of physiological activity resulting in “programs” in the developing brain, leading to significantly altered physiological functioning and vulnerability to various diseases. These “programs” alter the set points for the release of neurochemical messengers that may lead to a long-term alteration of the behavioral and physiological repertoire of the individual throughout his or her life (12). Extensive research over the last years has shown that the HPA axis is highly susceptible for prenatal and postnatal programming. In animals, gestational stress has been linked to altered HPA axis functions in adulthood, such as a shift in the circadian rhythm of corticosterone secretion, increased basal levels of cortisol and ACTH, or a hyperresponsiveness of the HPA axis to stress (13). Accordingly, stressful neonatal events such as social isolation or maternal deprivation were associated with long-lasting alterations of HPA axis function, such as elevated basal cortisol levels (14) or an increased and prolonged HPA axis response to stress (15). In humans, preterm birth (with appropriate weight for gestational age) has been linked with lower urinary cortisol metabolite excretion in adulthood, however, this effect was only found in women, but not in men (16). In contrast, others demonstrated increased plasma cortisol levels in former preterm adults, but only in those with intrauterine growth retardation (17). Although a considerable amount of data relates a stressful prenatal and postnatal environment to physiological dysfunctions in later life, most of these findings are based on animal studies. However, several important species differences, including the neuroendocrine development, preclude extrapolation of animal data to humans. Although there is an increasing research effort in the field of early programming of behavior and physiology in humans (for review, see Ref. 12), studies on the long-term effects of preterm delivery on endocrine and immune functions have been scarce, and often limited to the assessment of developmental and cognitive deficits (4).

The specific goal of the present study was to investigate the impact of preterm delivery on HPA axis functions in adolescence. In addition, immune functioning in former preterms, i.e., the cellular immune response [delayed type hypersensitivity (DTH) response], to different recall antigens should be evaluated. It is broadly accepted that the HPA axis plays a pivotal immunoregulatory role and that a dysfunctional HPA axis may account for aberrant immune responses (18). Based on these findings, it may be speculated that if preterm birth adversely affects the development of the HPA axis leading to altered HPA axis functioning, these changes may affect the immune system. Some animal data would support this idea showing that an adverse prenatal or postnatal environment may have long-term effects on the immune system, and may impair both the humoral and cellular immune response (19). In addition, clinical observations suggest attenuated antibody titers to vaccine antigens in school children born preterm (20). These data support the idea that stress during the prenatal and postnatal period may be linked to immunosuppression in later life.

### Subjects and Methods

#### Subjects

Former preterm children \((n = 18; 9\) males, \(9\) females), aged 8–12 yr (mean 10.53 ± 1.29), were recruited by a systematic search of birth records of the pediatric department of a local hospital in Trier, Germany. As indicated by the medical birth records, all former preterms were born between 26 and 36 wk gestation (mean 31 ± 2.9), with a mean birth weight of 1560 ± 356.7 g. All children were treated in a pediatric intensive care unit for the first hours/days depending of their medical condition. Based on a parent interview assessing signs of puberty (pubic, axillary hair growth, menstruation), three girls of the experimental groups had started puberty. In the last decade, betamethasone has been used to treat women who are at risk for delivering prematurely. Synthetic glucocorticoids promote maturation of the fetal lungs, allowing the newborn to breathe after birth (21). However, human and animal data indicate that prenatal treatment with glucocorticoids may have long-lasting effects on HPA axis function (22). To control for the effect of maternal treatment with glucocorticoids on later HPA axis function in our subjects, children whose mothers had been treated with exogenous glucocorticoids (betamethasone) were excluded from the study.

To recruit an appropriate control group, former preterms were asked to bring their best same-sex friend. The control group included 18 children (8 females, 10 males; aged 10.86 ± 1.32 yr). As indicated by their birth records, all control children were born full term between 39 and 41 wk gestation (mean 39.4 ± 1.04), with a mean birth weight of 3254 ± 579.7 g. In the control group, three girls already had started puberty (parent interview). Children with any acute or chronic medical problems were excluded from the study. By definition, the two experimental groups differed significantly in gestational age \((t = −11.7; P < 0.001)\) and birth weight \((t = −10.48; P < 0.001)\). No significant between-group differences could be determined in body mass index (BMI), body weight, or body size \((all P > 0.05)\) (Table 1).

All children participated voluntarily, and written parental consent for their involvement in the study was obtained. The experimental protocol was approved by the local ethics committee. After finishing the experiment, each child received a voucher for a free movie.

#### Experimental protocol

**Psychological stress test (“Trier Social Stress Test for Children” (TSST-C)).** Experimental sessions were run between 1400 and 1700 h, with one preterm and one full-term child participating each day. The investigators were blind for the prematurity status of the participants. After 45 min, the children were guided to the experimental room and confronted with the TSST-C, which has been described elsewhere (23). Briefly, the children received the beginning of a story and were asked to finish the story in a manner as exciting as possible in front of the audience. After a preparing period, the children were exposed 4 min to the speaking task. After finishing the TSST-C, the children were given the beginning of a story and were asked to finish the story in a manner as exciting as possible in front of the audience. The experimental protocol was approved by the local ethics committee. After finishing the experiment, each child received a voucher for a free movie.

#### Table 1. Characteristics of participants

<table>
<thead>
<tr>
<th></th>
<th>Former preterms ((n = 18))</th>
<th>Controls ((n = 18))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean birth weight (g)</td>
<td>1560 ± 356.7</td>
<td>3254 ± 579.7</td>
</tr>
<tr>
<td>Mean gestational age (wk)</td>
<td>31 ± 2.9</td>
<td>39 ± 1.04</td>
</tr>
<tr>
<td>Mean age when studied (yr)</td>
<td>10.53 ± 1.29</td>
<td>10.85 ± 1.32</td>
</tr>
<tr>
<td>Mean BMI (kg/m²)</td>
<td>16.16 ± 2.16</td>
<td>17.07 ± 2.71</td>
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<tr>
<td>Mean height when studied (m)</td>
<td>1.39 ± 0.09</td>
<td>1.45 ± 0.09</td>
</tr>
<tr>
<td>Mean weight when studied (kg)</td>
<td>31.55 ± 6.89</td>
<td>36.4 ± 9.27</td>
</tr>
<tr>
<td>No. of SGA children*</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>No. of LGA children*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. of NGA children*</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Birth weight for gestational age [reference data (40)]</td>
<td>0.30 ± 1.3</td>
<td>0.47 ± 1.2</td>
</tr>
</tbody>
</table>

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*Unless noted otherwise, values are mean ± SD.  
SGA, Small for gestational age (mean birth weight [reference data (40)] − 2 SD).  
LGA, Large for gestational age (mean birth weight [reference data (40)] + 2 SD).  
NGA, Normal for gestational age (birth weight within normal range [reference data (40)]).
task. The committee then asked the children to subtract numbers serially as fast and accurately as possible.

**Cortisol analysis**

**Psychosocial stress test (TSST-C).** To assess cortisol levels in response to the stress test, saliva was sampled 35 (t₁), 25 (t₂), 15 (t₃), and 1 (t₄) min before, and 1 (t₅), 10 (t₆), 20 (t₇), 30 (t₈), and 40 (t₉) min after the TSST-C using the Salivette sampling device (Sarstedt, Rommelsdorf, Germany).

**Cortisol awakening response.** Saliva cortisol was determined in the morning after awakening (t₀), and 10 (t₁), 20 (t₂), and 30 (t₃) min after waking up, respectively. To obtain a more representative measure of morning cortisol levels in our subjects, saliva was sampled on 3 consecutive days [1 d before the TSST-C (d₀)], on the experimental day (d₁), and the day after the TSST-C (d₂)], and the mean morning cortisol level for every sample was determined.

**Short diurnal cortisol profile.** Moreover, to determine a short diurnal profile of cortisol levels, saliva was sampled the day before the TSST-C at 0800, 1400, 1600, and 1900 h. All samples were stored at −20 °C before analysis.

For cortisol determination, 100 μl saliva was removed for duplicate analysis of cortisol levels using a time-resolved fluorescence immunoassay (DELFIA), which has been described elsewhere (24).

**Heart rates**

Heart rates were monitored continuously at 1-min intervals with precision using a wireless signal transmission device (Sport Profi, Polar, Büttelborn, Germany)

**Delayed-type hypersensitivity (DTH) reaction**

To determine (DTH) reaction, the Multitest Merieux test system (Multitest Merieux, Institut Merieux, Lyon, France) was used. This system is a commercially available, skin-puncture device that simultaneously injects seven different glycerinated recall antigens and one glycerin control to test for stress-induced changes (time effect), overall between-group differences (group effect), or different response profiles between the two groups (group × time effects). To test for group differences in the area under the cortisol response curve (AUC), the cortisol increase (cortisol-diff), DTH response, as well as the BMI, body weight and body height of the children when studied, t tests for independent samples were computed. AUCs were computed as described elsewhere (25). The increase of cortisol in response to the TSST was calculated as cortisoldiff = t₉ − t₁. To obtain the mean cortisol response in the morning, the mean cortisol levels over the 3 sampling days (d₀ − d₂) of every sample (t₁ − t₉) were computed (e.g. mean morning cortisol = d₀t₁ + d₀t₉ + d₂t₉/3). Spearman rank correlations were computed for assessment of associations between birth factors (birth weight, gestational age), and the cortisol and DTH response.

**Results**

**Psychosocial stress test (TSST)**

One specific goal of the present study was to evaluate a potentially aberrant HPA axis responsiveness to psychosocial stress in former preterm children. ANOVA of the cortisol data on the experimental day yielded a significant time [F (8, 232) = 19.86; *P < 0.001], but no significant group [F (1, 29) = 3.09; *P = 0.09] or group × time [F (8, 232) = 1.72; *P = 0.26] effect. As illustrated in Fig. 1, the TSST resulted in significantly increased cortisol levels, however, no significant difference could be observed between former preterm and full-term born children. As also reflected by the AUC, there is a tendency of attenuated cortisol responses to the stressor in former preterms, however, read 48 h later by an investigator blinded to the personal history of the children, and the number of positive reactions and the average diameter of induration were recorded.

**Statistical analysis**

Because all endocrine and immune measures showed normal distributions as tested by Kolmogorov-Smirnov (all d’s <0.2), parametric statistics without prior transformation of the raw data were performed. For all physiological parameters with repeated measures (cortisol, morning cortisol, heart rates), ANOVAs were computed on the absolute levels to test for stress-induced changes (time effect), overall between-group differences (group effect), or different response profiles between the two groups (group × time effects). To test for group differences in the area under the cortisol response curve (AUC), the cortisol increase (cortisol-diff), DTH response, as well as the BMI, body weight and body height of the children when studied, t tests for independent samples were computed. AUCs were computed as described elsewhere (25). The increase of cortisol in response to the TSST was calculated as cortisoldiff = t₉ − t₁. To obtain the mean cortisol response in the morning, the mean cortisol levels over the 3 sampling days (d₀ − d₂) of every sample (t₁ − t₉) were computed (e.g. mean morning cortisol = d₀t₁ + d₀t₉ + d₂t₉/3). Spearman rank correlations were computed for assessment of associations between birth factors (birth weight, gestational age), and the cortisol and DTH response.

**Fig. 1.** Changes of cortisol levels in response to the TSST-C in former preterm and full-term born children (means ± SEM).
the difference did not reach statistical significance (preterms: 13.3, confidence interval: 4.8–21.8; controls: 21.7, confidence interval: 13.7–29.6; t = 1.28; df = 29; P = 0.21; Fig. 1). Additional analyses of the cortisol responses to the TSST in boys and girls yielded no significant sex difference in the two experimental groups (all P > 0.05). Critical birth factors, i.e. gestational age and birth weight, were not correlated to the cortisol response to the stressor (AUC; cortisoldiff; all P > 0.05). A significant correlation between gestational age and the cortisol levels t6 (r = 0.47; P < 0.05), t8 (r = 0.43; P < 0.05), and t7 (r = 0.43; P < 0.05) was found. Exposure to the TSST resulted in significantly elevated heart rates [F (38, 988) = 10.46; P < 0.001; Fig. 2], with no significant difference between preterm and full-term born children [group effect: F (1, 26) = 0.088; P = 0.77; group × time effect: F (38, 988) = 1.02; P = 0.42].

Short diurnal cortisol profile

No difference between the two experimental groups was found in the short diurnal profile of cortisol concentrations, as indicated by a nonsignificant group, and a nonsignificant group × time effect (all P > 0.05; data not shown).

Cortisol awakening response

However, analysis of the mean morning cortisol levels yielded a significant time effect [F (3, 102) = 13.33; P < 0.001] and group × time effect [F (3, 102) = 3.14; P < 0.05]. As shown in Fig. 3, former preterms showed significantly elevated morning cortisol levels after awakening when compared with the full-term controls. Cortisol levels after awakening were further found to be significantly correlated with gestational age (r = −0.47; P < 0.05).

DTH reaction [cellular mediated immunity (CMI)]

In addition to HPA axis (re)activity, cellular immune function as indicated by the DTH response to recall antigens was tested. As summarized in Fig. 4, the total number of positive reactions (t = −2.64; P < 0.05) and the total induration (t = −2.4; P < 0.05) were significantly reduced in former preterms, suggesting attenuation of cellular immune response in this specific group.

Discussion

The present study aimed to investigate whether preterm delivery may affect HPA axis and immune functioning in later life. Preterm birth is a highly stressful early experience characterized by maternal deprivation and painful medical treatment (26). Furthermore, preterm birth can be linked to adverse birth factors such as increased gestational stress or low birth weight (19, 10). However, accumulating data suggest that stressful prenatal and neonatal factors may shape the development of the HPA axis and the immune system, and may lead to significant functional alterations of these systems in later life (11, 13).

In the present paper, significantly elevated cortisol levels after awakening were observed in former preterm children. Elevated morning cortisol levels were inversely correlated with gestational age and birth weight. Our findings are in line with a number of studies indicating that low birth weight (that also has been documented in our subjects) is strongly associated with increased morning cortisol levels in young and older adults (27, 28). In these studies, higher morning cortisol levels were further found to be linked to higher systolic blood pressure, higher plasma glucose concentration or insulin resistance, suggesting a potential role of an altered programming of the HPA axis in the recently postulated relationship between low birth weight and increased risk for cardiovascular and metabolic disease (7, 8). However, whether increased basal morning cortisol is a consequence or an etiologically relevant risk factor for metabolic or cardiovascular morbidity found in these subjects is still unclear. Therefore, our data suggesting increased basal morning cor-

Fig. 2. Changes of heart rates in response to the TSST-C in former preterm and full-term born children (means ± SEM). b, Beats.
tisol levels in low birth weight children without current metabolic or cardiovascular pathology may be of specific interest.

Increased morning cortisol levels in low birth weight children has also been reported by Jones et al. (29). In contrast to our data, they found this effect only in girls, but not in boys. The same group further reported increased cortisol responses to psychosocial stress (TSST-C) in low birth weight children, which again, was gender-specific in that this effect could only be demonstrated in boys. Elevated cortisol levels in response to psychosocial stress or a low-dose ACTH stimulation test in lower birth weight male adults have also been shown by others (28, 30), supporting the idea of an increased reactivity of the HPA axis in low birth weight males. In contrast to these findings, we failed to show a hyperresponsive HPA axis to stress in our subjects. Although not statistically significant, our data more likely argue for a decreased cortisol response to stress in former low birth weight preterms. However, it should be noted that in the study by Jones et al. (29), low birth weight and HPA axis reactivity to the TSST-C were only associated when using measures of cortisol at home as a baseline for comparison with measures during the stress test. Using prestress cortisol measures as a baseline (as we did in our study), no clear relationship between birth weight and cortisol responsiveness to the TSST-C could be revealed. Another important factor that makes the study results difficult to compare is the different sample size of the two studies with a larger amount of subjects investigated in the paper by Jones et al. (29). Finally, the diversity of the subjects investigated in the different studies has to be considered. Although in the present paper the impact of lower birth weight in preterm children on HPA axis function has been evaluated, other studies tested the impact of low birth weight on the HPA axis in full terms (31), or in twins...
born full term or preterm (30). However, the effects of low birth weight on HPA axis function may vary depending on other critical birth factors. For example, an inverse correlation between basal serum cortisol and birth weight was observed only in adults born before 39 wk gestation, whereas subjects with a gestational age of 40 wk and older showed a positive correlation (31). Moreover, preterm birth was related to lower urinary cortisol metabolite excretion in adults with normal birth weight, whereas low birth weight subjects born preterm showed slightly elevated cortisol metabolite excretion (16). These data support the idea that one adverse birth factor may have different, even opposing, effects on long-term HPA axis functioning depending on other critical birth variables. Thus, when interpreting the present data of reduced cortisol levels after awakening in former preterms, it is important to keep in mind that preterm birth is characterized by a set of key factors of HPA axis programming, i.e., increased prenatal stress, low birth weight, low gestational age, increased neonatal stress exposure by stressful medical treatment, maternal separation, or altered maternal behavior (overprotection). All of these factors have been described to have long-lasting effects on HPA axis function (10, 32). Thus, future studies are warranted to clarify further which birth factors linked to preterm birth are critical for the programming of the HPA axis and, most importantly, how they interfere with each other. The latter aspect may be of specific importance because a growing number of studies show that postnatal factors such as neonatal handling or enrichment of the neonatal environment may compensate for the prenatal negative effects or even reverse them (33, 34).

Besides altered HPA axis function, significantly suppressed DTH responsiveness to recall antigens has been observed in former preterm children. The DTH reaction represents an antigen-specific, cell-mediated immune response that involves a cascade of immunological processes. It is broadly accepted that a decrease in the ability to mount a DTH response to a test antigen is a valuable indicator of a dysfunctional CMI (35). Our observation of a potentially disturbed CMI in former preterms may be of clinical relevance and raises the question whether these children display a higher risk for various diseases due to a potentially compromised host resistance. This idea would be supported by reports suggesting that a reduction or loss of CMI as indicated by a reduced DTH responsivity may predict disease progression in cancer patients or HIV infected individuals (36, 37). However, it is noteworthy that the significance of a diminished DTH responsiveness may be different in an already immunocompromised host, and attenuated CMI in a healthy organism is not necessarily linked to increased disease vulnerability. There is some evidence, however, suggesting a reduced vaccine response to hepatitis B or polio in former preterm infants (38, 39). However, this effect could only be demonstrated in children born extremely preterm (26–27 wk). Because adequate follow-up studies are missing, it is further not clear whether the defective vaccine response may be transient or whether it reflects a permanent impairment of the immune system. However, knowledge about the impact of preterm birth on various aspects of immunity and, moreover, the impact of these alterations for health and disease are scanty. Additional studies are needed to highlight further critical programming factors linked to preterm birth, and to uncover their role in the development of the human HPA axis and the immune system. Valid longitudinal studies may help to clarify further the significance of altered “programs” of HPA axis and immune functions for health and disease.

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