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Chernobyl exposure as stressor during pregnancy and hormone levels in adolescent offspring

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Abstract

Background—Animal research suggests a programming effect of prenatal stress in the fetal period, resulting in disruptions in behavioral and neuromotor development. Physiological changes that mediate these effects include alterations in the hypothalamic-pituitary-adrenal axis and in testosterone levels. This human study focuses on changes related to these physiological systems after prenatal stress exposure.

Methods— We examined the potential effect of prenatal stress associated with the Chernobyl disaster in an ongoing genetic epidemiological study in Finland. One birth cohort of twins (n= 121 twin pairs) was exposed in utero to maternal stress, and their saliva cortisol and testosterone levels at age 14 were compared with twins (n = 157 twin pairs) born one year later.

Results—Cortisol levels in both sexes and testosterone levels among females were significantly elevated after prenatal exposure to maternal stress from the second trimester onwards, compared to reference groups of non-exposed adolescents. Exposure explains 3 percent of variance ($p<0.05$) in cortisol levels and 18 percent of variance in testosterone levels ($p< 0.001$). No significant differences were found for exposure from either first or third trimester onwards.

Conclusion—Our results suggest that prenatal exposure to maternal stress in the second trimester of pregnancy may have resulted in prenatal programming of physiological systems relating to cortisol and testosterone levels.

Keywords

Adolescent; Chernobyl Nuclear Accident; Glucocorticoids; Prenatal Exposure Delayed Effects; Testosterone

Introduction

Several lines of animal research suggest a programming effect of prenatal stress in the fetal period, resulting in disruptions in behavioral and neuromotor development maintained into adulthood [1]. An overactive and impaired negative feedback regulation of the hypothalamic-

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pituitary-adrenal axis [e.g. 2] may mediate the link between prenatal stress exposure and changes in offspring behavioral outcomes. Thus far, only one study has examined the long-term effects of exposure to prenatal anxiety on hypothalamic-pituitary-adrenal axis activity beyond childhood, expressed in cortisol levels in human offspring [3]. In a sample of 74 10-year-olds, prenatal exposure to maternal stress, assessed with an anxiety questionnaire, was associated with a higher cortisol levels 30 minutes after awakening compared to those not exposed.

Other possible physiological changes after prenatal stress exposure include alterations in testosterone levels [4]. However, thus far no human studies have been carried out to examine testosterone levels in offspring after prenatal stress exposure, while several animal studies have shown an increase in testosterone levels after such prenatal exposure (e.g. [5][6][7]). It has been well established that testosterone in adults males are associated with aggressive and antisocial behavior (e.g. [8][9]). In a previous study, we showed that testosterone levels among adolescent twins were also related to more adverse alcohol drinking behavior, such as frequent intoxication [10]. Thus, changes in both cortisol and testosterone levels after exposure to prenatal maternal stress may mediate changes in adolescent behavioral outcomes.

In the present study, we had the unique possibility to test, in an ongoing study among Finnish twins, the association of prenatal stress exposure and saliva cortisol and testosterone levels in adolescent offspring of women who were pregnant during the Chernobyl disaster of 1986 with offspring of women who were pregnant a year after the disaster took place. Shortly after the Chernobyl accident, there was very little information available in Finland. A fear spread across the country with rumours, such as speculations in newspapers about e.g. whether or not milk was safe to drink, or rain water could be used for making food. In general, stress that is generated by exposure to a disaster may be linked to the uncertainty about the ultimate health consequences to the affected population as a result of radiation exposure and distrust in government information about contamination levels [11]. The suddenness of the Chernobyl disaster and the very broad media coverage, in addition to the rumours and uncertainty caused by slow risk communication will most probably have caused stress in the Finnish population, including women who were pregnant at that time.

Accordingly, the aim of the present study was to gain insight into the potential effect of the exposure in utero to maternal stress presumable associated with fear that radiation caused by the Chernobyl disaster may have detrimental effects on her unborn child on offspring measures of cortisol and testosterone.

METHODS

Sample

The full FinnTwin12 sample consists of 2724 twin pairs and their parents, representing nearly 90 percent of all twins born in the five birth cohorts, alive, resident in Finland, living with one or both biological parents and eligible for study. Nested within this population-based sample, 1035 families of twins were selected for intensive study (for details see [10][12]). Data collection was approved by the Institutional Review Board of Indiana University, Bloomington, Indiana, and the ethical committee of the Hospital District of Helsinki and Uusimaa, Helsinki, Finland. All participants signed informed consent.

For the present study, we took advantage of the opportunity afforded by the *FinnTwin12* study and selected twins that were prenatally exposed to maternal stress associated with the Chernobyl disaster from the last two birth cohorts of *FinnTwin12*. Specifically, the study compares individuals who were in utero when the actual disaster occurred (i.e., in utero on April 26, 1986) with those individuals not in utero at the time. Eight twin pairs were excluded

due to a very low birth weight (< 1500 gram) of one or both of the twins, and/or a gestational age shorter than 31 weeks. Prenatal exposure to Chernobyl was determined by subtraction of gestational age from birth date, yielding a group of twins (n=242) who were born between April 27, 1986 and February 9, 1987. All twins (n= 314) born a year after this period (n= 314) were selected to form the non-exposed reference group. The exposed group was further subdivided into three periods of pregnancy in which the Chernobyl disaster occurred; (1) first trimester (weeks 1–13 of gestation), (2) second trimester (weeks 14–27 of gestation), and (3) third trimester (>28 weeks gestation). Although the actual starting point of the exposure is known (April 26th, 1986), the endpoint of the exposure period of maternal stress associated with the disaster is unknown, and likely varied across individuals. We therefore use the terms 'exposure from first, or second trimester onwards', to refer to exposure that started in the first or second trimester, respectively. Since the third trimester reflects the final part of pregnancy, exposure in this period is referred to as 'exposure in the third trimester'. We assumed that the peak of stress was probably closely related to the actual timing of the event. The reference group was also further divided into three groups, with similar months of birth as compared to the three exposed groups, in order to control for potential confounding due to season of birth.

Procedures

Saliva samples—Two saliva samples were obtained under standard conditions immediately pre/post structured interviews when twins were aged 14 by trained interviewers, to maximize individual representation of the endogenous steroids. Saliva samples were obtained with paraffin stimulation. Twins were instructed to chew paraffin which stimulates saliva secretion and to deposit saliva into a tube. Saliva tubes were immediately refrigerated on ice and subsequently transferred to −20°C storage on the interviewer's return to the research center. At regular intervals, collected samples were transferred in dry ice to the National Public Health Institute in Helsinki, where they were stored at −70°C until laboratory determination of cortisol and testosterone. The time interval between the two saliva samples varied with the time required to complete the structured interview: the median inter-sample interval was 75 minutes and 99 percent of samples were obtained within 2 hours of one another. Most saliva samples were collected at the twins' schools, where interviews were scheduled before noon (1st sample at 11:45 a.m. on average). There was no significant difference in the timing of saliva sampling between the exposed (sampling on average at 11:48 am) and non-exposed (sampling on average at 11:46 am) groups. *Assay procedures*. On the day of the assay, frozen saliva samples were thawed, pipetted into microcentrifuge tubes, and centrifuged 13,000 g for 2 minutes. Saliva cortisol measurements reflect the biologically active free form. Salivary free cortisol is approximately 70 percent of that of serum free cortisol because of conversion of cortisol to cortisone in the salivary glands. Nevertheless, salivary cortisol levels correlate very strongly with plasma free cortisol [13][14]. We also assessed levels of free testosterone in two salivary samples. Free testosterone represents the proportion of overall testosterone that most closely reflects the bioactive fraction exerting a biological effect by interacting with intracellular androgen receptors. A previous study showed that the two assay samples of testosterone were very highly correlated (r = 0.91) across all boys [10] and therefore, the two values for each twin were averaged. Cortisol and testosterone concentrations were measured from the saliva supernatants, using commercial available radioimmunoassay kits from Orion Diagnostica, Finland. Cortisol within-assay variability (CV%) was 10.7% and between-assay was 12.9 percent at the levels of 5–7 nmol/l (n=19). Testosterone within-assay variability (CV%) was 6.2 percent, and between-assay variability was 9.7 percent at the level of 0.13 nmol/l (n = 25).

Questionnaires

Birth outcome—Information was obtained from parents on gestational age at birth, and birth weight of both twins when they were age 12. This information has been shown to be accurate when compared to birth records [15]. This is probably due to the fact that in Finland 99% of

pregnant women attend prenatal consultation clinics, where they receive a chart that keeps track of their pregnancy, and birth outcome measures are recorded on a baby chart by professionals.

Pubertal development—For each interviewed twin, the score on the 5-item Pubertal Development Scale (PDS;[16]) obtained at age 14, was available. The PDS exhibits good psychometric properties in studies of adolescents in the US [17], and in this adolescent Finnish twin sample [18].

At age 14, boys' testosterone levels are associated with their pubertal development (e.g.[16], which was previously shown in the *FinnTwin12* dataset [10]. Measures of pubertal development at age 14 were obtained, and data were used to adjust associations between prenatal exposure to maternal stress with testosterone for individual differences in pubertal maturation.

Parental socio-economic status (SES) was assessed by questionnaire and determined by the highest level of parental education, resulting in low (only primary education), moderate (secondary education), or high (college or university education) SES.

Maternal smoking and drinking during pregnancy were assessed by maternal self-report, and were included in the model as dichotomous variables (no/yes).

Analyses

Background information on birth outcome was compared by means of t-tests for the exposed and the non-exposed groups. Next, we studied the relationship between prenatal exposure to maternal stress and (1) cortisol and (2) testosterone levels for all twins as individuals by means of linear regression analyses testing for sex-specific effects. Cortisol measures were first adjusted for timing of the sample, because cortisol levels are influenced by circadian rhythms. Although we collected information on food intake and use of coffee and other beverages preceding saliva sampling, these factors did not significantly contribute to variation in cortisol levels. Therefore, we only adjusted for this timing of saliva sampling in the present paper. Analyses of testosterone were adjusted for pubertal development. In a previous study, testosterone showed significant seasonal and diurnal effects [10], and therefore, we used testosterone adjusted for these effects in our analyses. All associations were corrected for clustering of data due to twins' relatedness, by using a random effects model, and were adjusted for birth weight. First, an overall test for each outcome variable was performed in which exposed groups were compared with non-exposed reference groups. Second, the relation of exposure in each part of pregnancy with outcome measures was examined. All analyses were carried out in SPSS version 12.0, and p values less than 0.05 (two-sided) were used to determine statistical significance.

RESULTS

Descriptive analysis

T-tests show that twins at 14 years of age born after in utero exposure had slightly increased gestational ages and higher birth weights than those not exposed (Table 1). The age of the twin at interview did not differ between exposed and non-exposed twins. Maternal age at interview did not differ between exposed and non-exposed groups. The proportion of opposite sex dizygotic twins in both groups was compared by means of the Chi-square test; it did not differ. Moreover, this selected sample of twins did not differ on general background characteristics, such as maternal age at inclusion, parental education, urbanicity, or ethnicity from the full twin

cohort. Means and standard deviations of cortisol and testosterone levels of the exposed and non-exposed groups are shown in Table 2.

Linear regression analyses

1) Cortisol—To correct for skewness, the cortisol values were log transformed. After transformation cortisol values had a skewness and kurtosis between −1.0 and + 1.0. There was no interaction effect for exposure*sex, and therefore, both sexes were combined in subsequent analyses. For cortisol, we used the first saliva sample because the first and second cortisol measures were highly correlated (Spearman's Rho= 0.62, $p < .001$), and the first cortisol measure probably reflects anticipation stress, because it showed the highest level in the vast majority of participants. Linear regression analyses were performed separately for each trimester of pregnancy, after an overall test in which exposure during any trimester of pregnancy was performed.

The results in Table 3 show that cortisol levels are significantly elevated after prenatal exposure to maternal stress from the second trimester onwards, compared to reference groups of non-exposed adolescent twins. No significant differences were found for exposure from first trimester onwards, or in the third trimester.

2) Testosterone—To correct for skewness, the testosterone values were log transformed and adjusted for seasonal and diurnal effects. After correction, testosterone values had a skewness and kurtosis between −1.0 and + 1.0. There was a significant interaction effect for exposure*sex and therefore, the next analyses were performed stratified for sex. All analyses were corrected for variation in pubertal development.

The results in Table 4 show that, compared to reference groups of non-exposed adolescents, testosterone levels of females who were prenatally exposure to maternal stress from the second trimester onwards were significantly elevated with exposure explaining 17.7 percent ($p<0.001$) of variance. For males, no significant difference was found between exposed versus non-exposed groups.

In our models for cortisol and testosterone, we did not additionally control for gestational age, since birth weight is highly correlated with gestational age in general, and this was also found in our study ($r = 0.70$, $p < .001$). Moreover, we tested whether additional confounders, such as parental SES and exposure in utero to maternal drinking or smoking changed the relationship between prenatal exposure to maternal stress and testosterone or cortisol levels. These confounders did not change these associations, and therefore, we did not include them in our models. Finally, because maternal testosterone levels decrease with increasing maternal age [19], potentially effecting a relative decrease in prenatal testosterone exposure with increasing maternal age and a lower testosterone level in offspring, we tested for differences in maternal age between the exposed and non-exposed groups. None were found.

Discussion

The present study is the first to suggest that maternal prenatal exposure to stress in the second trimester of pregnancy has long-term effects on offspring hormonal levels, in particular testosterone, in adolescence. At age 14, cortisol levels of both sexes, and testosterone levels of girls, were significantly higher after prenatal exposure to maternal stress from the second trimester onwards, compared to reference groups of non-exposed adolescents. The most striking effect found was for female testosterone levels after prenatal exposure from the second trimester of pregnancy onwards; that effect explained almost 18 percent of the variation in testosterone levels. We ruled out some other between-group differences that might contribute to differences in testosterone levels, including the age and pubertal development of the twins

at the time of hormonal assays. We cautiously interpret our findings to suggest that prenatal exposure to stress, specifically in the second trimester of pregnancy, may have resulted in prenatal programming of physiological systems relating to cortisol and testosterone levels. That suggestion is consistent with animal studies reporting similar effects after prenatal exposure to stress (e.g. [2][4][5][6][7]).

If this is a correct inference, our results likely reflect effects of threat of exposure to radiation while pregnant and concerns created by overall effects of the Chernobyl disaster in a nearby environment, rather than prenatal exposure to radiation. Auvinen et al. [20] examined the relationship between the Chernobyl fallout and birth outcome, such as rate of live births and stillbirths, pregnancy loss, and induced abortions in Finland and found no association. In the present study, no adverse effects on birth outcome of in utero exposure to maternal stress, presumably associated with the Chernobyl disaster, was found. Rather, twins born after in utero exposure had slightly increased gestational ages and higher birth weights than those not exposed. Thus, if present, exposure to radiation in utero has not resulted in adverse birth outcomes. Moreover, the mothers of the twins in the present study lived in all parts of Finland, so that the exposure to actual and perceived radiation levels likely show high variability within our sample.

When we consider the possibility that the threat of being exposed to radiation while pregnant has increased levels of prenatal maternal stress and anxiety, the prenatal programming theory may apply to our findings. Welberg and Seckl [21] speculated in their thorough review on prenatal stress, glucocorticoids and the programming of the brain that prenatal programming allows environmental factors, such as exposure to stress, to alter the set-point of physiological systems to prepare the fetus optimally for the environmental conditions after birth. If the postnatal environmental circumstances are not as anticipated, such prenatal programming will produce maladaptive physiological systems. Fetuses may be exposed to excess glucocorticoid when the mother is stressed (e.g.[22][23][24][25]).

Recently, a human study showed that prenatal exposure to maternal anxiety was related to small increases in awakening cortisol in 10-year-olds, thus providing some evidence for the relationship between maternal stress in pregnancy and human offspring alterations in hypothalamic-pituitary-adrenal axis activity [3].

In addition, the effect of stress can depend on the timing of exposure. Theoretically, it seems reasonable to assume that programming effects depend on the state of differentiation of the tissue involved. In humans, similar effects as were found in hippocampus of the rat may therefore be expected for stress exposure from the 19^{th} until the 32^{nd} week of gestation, because during that period, certain hippocampal areas develop [25]. This may explain the finding of the present study that exposure from the second trimester onwards was related to increased levels of cortisol in adolescent offspring, which may reflect a somewhat increased anticipation stress response. Yet, if some women were unaware of their pregnancy at the time of the disaster, this may have resulted in a smaller group of “exposed” women from first trimester onwards, and a smaller power to find effects in their offspring. Additionally, we assumed that the peak of stress was probably closely related to the actual timing of the event. If indeed the second trimester of pregnancy is a sensitive period of the fetal brain development, our assumption may explain that we found an effect for those exposed “from second trimester onwards” and not for those exposed “from first trimester onwards”. However, our study, like many previous other studies, cannot establish during which period of pregnancy exposure to stress matters most in affecting offspring outcomes.

For the prenatal exposure effect on testosterone levels, our results suggest that only testosterone levels of females may be affected by prenatal exposure to stress, which is in line with some animal studies [26][27][28].

Several limitations have to be taken into account when interpreting our findings. First, although we assume that the Chernobyl disaster caused stress for pregnant mothers, we cannot completely rule out possible radiation effects on the measured cortisol and testosterone levels. However, because the highest momentary dose rate measured in Finland was only 5 sSv/h it is highly unlikely that exposure to radiation in utero has directly influenced the development of the twins in this study. Additionally, we did not have individual-level assessments of perceived stress in the mothers of the present study. This was due to the fact that we took advantage of the ongoing data-collection of the FinnTwin12 study, which was not specifically designed to test the hypotheses presented in this paper. There is no study that can take a disaster a priori into account, and the present study is no exception to this rule. Future studies may focus on different aspects of hypothalamic-pituitary-adrenal axis functioning, such as reactivity to stressors. Finally, other unequally distributed factors among exposed and non-exposed groups may explain our findings. This is highly unlikely, since we previously showed that all cohorts were very similar [12] with regard to urbanicity, maternal age, and ethnicity.

In sum, the present study shows that prenatal exposure to stress or anxiety may relate to some changes in hormone levels of adolescents. Whether these changes are maladaptive and relevant for future behavioral and health outcomes is unclear and may warrant future, longitudinal research.

What this paper adds

Animal studies have shown effects of prenatal stress on cortisol and testosterone levels in offspring, which may mediate the link between prenatal stress exposure and changes in offspring behavioral outcomes. The present study is the first to suggest that maternal prenatal exposure to stress has long-term effects on offspring cortisol levels of sexes, and on testosterone levels of girls, in adolescence.

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Table 1

Comparisons of background characteristics between groups of adolescents that were prenatally exposed to maternal stress or non-exposed

	Exposed (N= 242) Mean (SD)	Non-exposed (N=314) Mean (SD)	P value
Gestational age at birth in weeks	37.30 (2.00)	36.90 (2.00)	< 0.05
Birthweight twin A in grams	2842 (486)	2741 (486)	< 0.05
Birthweight twin B in grams	2784 (476)	2700 (470)	< 0.05
Age of twin at interview in years	14.17 (0.10)	14.19 (0.10)	0.08
Maternal age at interview in years	41.51 (4.60)	41.28 (4.80)	0.54
Proportion of opposite sex dizygotic twins	33.90%	28.20%	0.12

SD = standard deviation; Twin A is the oldest twin, Twin B the youngest.

Table 2

Means and standard deviation of Cortisol and Testosterone measures for adolescents who were exposed or non-exposed to prenatal maternal stress

	Exposed Mean (SD)	Exposed N	Nonexposed Mean (SD)	Nonexposed N	P value
Total group					
Cortisol (nmol/l)	6.94 (12.26)	242	6.51 (12.76)	314	0.07
Testosterone (nmol/l)	.09 (0.06)	242	.09 (0.07)	314	0.13
Testosterone boys	.12 (0.07)	133	.12 (0.08)	169	0.76
Testosterone girls	.06 (0.03)	109	.06 (0.03)	145	< 0.05
Exposure from 1st trimester onwards					
Cortisol (nmol/l)	6.06 (7.12)	68	6.28 (4.66)	112	0.99
Testosterone (nmol/l)	.09 (0.05)	68	.08 (0.05)	112	< 0.001
Testosterone boys	.10 (0.05)	48	.09 (0.05)	56	0.11
Testosterone girls	.07 (0.03)	20	.06 (0.05)	56	0.09
Exposure from 2nd trimester onwards					
Cortisol (nmol/l)	8.49 (19.55)	82	4.94 (3.97)	134	< 0.05
Testosterone (nmol/l)	.09 (0.07)	82	.08 (0.05)	134	0.09
Testosterone boys	.13 (0.09)	40	.11 (0.06)	73	0.76
Testosterone girls	.06 (0.02)	42	. 05 (0.02)	61	< 0.001
Exposure during 3rd trimester					
Cortisol (nmol/l)	6.31 (4.92)	92	4.94 (2.42)	68	0.16
Testosterone (nmol/l)	.09 (0.07)	92	. 10 (0.09)	68	0.22
Testosterone boys	.12 (0.08)	45	.12 (0.11)	40	0.84
Testosterone girls	.05 (0.02)	47	.06 (0.02)	28	< 0.05

SD = standard deviation; N = number of participants

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Table 3

Linear regression analysis of cortisol levels at age 14. Regression coefficient (and 95% CI) for exposure (yes/no), R^2 change due to the exposure to prenatal maternal stress, and significance (F-test, p-value) for models adjusted for timing of the sample, birth weight and clustered data.

Exposure	B	(95% CI)	R^2 change	F	P value
Any time of pregnancy	0.05	(−0.01 – 0.11)	0.01	2.52	0.11
Exposure: 1st trimester onwards	−0.00	(−0.10 – 0.09)	0.00	0.00	0.99
2nd trimester onwards	0.13	(0.03 – 0.22)	0.03	4.48	< 0.05
3rd trimester	0.06	(−0.02 – 0.15)	0.01	1.65	0.20

B = regression coefficient; 95 % CI: 95% confidence interval

Table 4

Linear regression analysis of testosterone levels at age 14. Regression coefficient (and 95% CI) for exposure (yes/no), R^2 change due to the exposure to prenatal maternal stress, and significance (F-test, p-value) for models adjusted for timing of the sample, pubertal development, birth weight and clustered data.

Exposure	B	(95 % CI)	R^2 change	F	P value
Any time of pregnancy					
Males	0.01	(−0.04 – 0.06)	0.00	0.04	0.84
Females	0.06	(0.01 – 0.10)	0.03	5.05	< 0.05
From 1st trimester onwards					
Males	0.10	(0.00 – 0.19)	0.05	2.29	0.14
Females	0.10	(−0.02 – 0.21)	0.04	3.05	0.09
From 2nd trimester onwards					
Males	0.00	(−0.11 – 0.11)	0.00	0.00	0.96
Females	0.16	(0.08 – 0.24)	0.18	14.15	< 0.001
During 3rd trimester					
Males	−0.02	(−0.13 – 0.09)	0.00	0.01	0.92
Females	−0.06	(−0.13 – 0.01)	0.05	3.23	0.08

B = regression coefficient; 95 % CI: 95% confidence interval