

CLINICAL STUDY

Hypothalamic–pituitary–adrenal axis activity in adults who were prenatally exposed to the Dutch famine

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Abstract

Objective: The hypothalamic–pituitary–adrenal (HPA) axis has been proposed to be susceptible to fetal programming, the process by which an adverse fetal environment elicits permanent physiological and metabolic alterations predisposing to disease in later life. It is hypothesized that fetal exposure to poor circumstances alters the set point of the HPA axis, leading to increased HPA axis activity and subsequent increased cortisol concentrations. In this study, we tested the hypothesis that prenatal exposure to famine during different periods of gestation is associated with increased activity of the HPA axis.

Design and methods: We assessed plasma cortisol concentrations after a dexamethasone suppression and an ACTH_{1–24}-stimulation test in a group of 98 men and women randomly sampled from the Dutch famine birth cohort. Cohort members were born as term singletons around the 1944–1945 Dutch famine.

Results: Cortisol profiles after dexamethasone suppression and ACTH_{1–24} stimulation were similar for participants exposed to famine during late, mid- or early gestation ($P=0.78$). Cortisol concentrations after dexamethasone suppression test did not differ between those exposed and those unexposed to famine *in utero* (mean difference -2% (95% confidence interval (CI) -27 to 23)). Neither peak cortisol concentration (20 nmol/l (95% CI -27 to 66)), cortisol increment (-5 nmol/l (95% CI -56 to 47)) or cortisol area under the curve post-ACTH_{1–24} injection (4% (95% CI -4 to 12)) differed between exposed and unexposed participants.

Conclusions: Prenatal famine exposure does not seem to affect HPA axis activity at adult age, at least not at the adrenal level. This does not exclude altered HPA axis activity at the levels of the hippocampus and hypothalamus.

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Introduction

The fetal origins hypothesis states that an adverse fetal environment may elicit permanent physiological and metabolic alterations predisposing to disease in later life, a process called fetal programming (1). The hypothalamic–pituitary–adrenal (HPA) axis has been proposed to be susceptible to fetal programming. Prenatal undernutrition may program the HPA axis by permanently altering the set point of the HPA axis *in utero*, leading to increased activity and subsequent increased concentrations of cortisol that predispose to cardiovascular disease and type 2 diabetes (2–4). *In utero* resetting of the HPA axis may thus be an important link between early life events and these chronic adult diseases.

Animal studies have established that prenatal nutrition can affect the functioning of the HPA axis in adult life. The newborn progeny of rats exposed to a low protein diet during late gestation, show increased HPA

axis activity as indicated by elevated levels of basal plasma corticotropin (ACTH) and corticosterone (5). Modest as well as severe prenatal undernutrition during early and late gestation in sheep produces increased post-natal activity of the HPA axis after a corticotropin-releasing hormone plus arginine vasopressin challenge (6, 7). Studies in guinea pigs that were undernourished during a short period in late gestation have shown clear sex-specific alterations in HPA axis activity at adult age (8). Basal ACTH and cortisol responses are reduced in male guinea pigs, while in females basal ACTH responses remain unchanged and cortisol responses are elevated compared with normally fed animals.

Fetal programming of the HPA axis has been identified in humans who were small at birth. Small size at birth is considered to reflect a poor fetal environment. Low birth weight was found to be associated with elevated fasting plasma cortisol concentrations in a variety of populations of men and women (9, 10). People who were small at birth also show

increased responsiveness to dexamethasone suppression and increased responsiveness to corticotropin releasing hormone and ACTH stimulation, although some studies failed to find evidence for these associations (11–14).

In previous studies, we have shown that prenatal exposure to undernutrition during different periods of gestation in the Dutch famine was associated with a range of risk factors for, as well as an increased prevalence of, coronary heart disease (CHD) and type 2 diabetes at adult age (15–21). The Dutch famine was a period of 5 months of severe undernutrition at the end of World War II. Although it was a historical disaster, it provides us with the opportunity to study the long-term health effects of undernutrition during different periods in pregnancy. Programming of the HPA axis may explain the association between famine exposure *in utero* and the increased risk of CHD and type 2 diabetes in later life. In a new study, we tested the hypothesis that prenatal exposure to famine during late, mid and/or early gestation is associated with increased activity of the HPA axis.

Materials and methods

The Dutch famine birth cohort

The Dutch famine birth cohort members were born as term singletons between 1 November 1943 and 28 February 1947 at the Wilhelmina Gasthuis in Amsterdam, The Netherlands. The selection procedure is described in detail elsewhere (15). At age 58, 1423 of the 2414 original cohort members (58%) were still alive, living in The Netherlands and their address was known to the investigators. These people were eligible for our study.

Exposure to famine

We defined the famine period according to the official daily food-rations for the general population older than 21 years. The official rations accurately reflect the variation over time in the total amount of food available in the west of the Netherlands (22). We considered fetuses to have been exposed to famine *in utero* if the average daily rations for those older than 21 years during any 13-week period of gestation were less than 1000 calories. According to this definition, people born between 7 January 1945 and 8 December 1945 were considered to be exposed to famine *in utero*. We defined periods of 16 weeks each to differentiate between those who were exposed to famine in late gestation (born between 7 January 1945 and 28 April 1945), in mid gestation (born between 29 April and 18 August 1945) and in early gestation (born between 19 August and 8 December 1945). People born before 7 January 1945 and conceived and born after 8 December 1945 were considered to be unexposed to famine *in utero*.

Participants

All 1423 eligible Dutch famine birth cohort members were invited to attend the clinic for a data-collection protocol, which included a 75 g oral glucose tolerance test (23). We were able to complete this test for 699 of the 810 study participants. Based on the results of the oral glucose tolerance test, we selected 100 normoglycaemic men and women for participation in a dexamethasone suppression and an ACTH_{1–24}-stimulation test using the same protocol as described by Reynolds *et al.* (13). For this purpose, 10 men and 10 women were randomly selected from each of the following study groups: people born before the famine, people exposed to famine in late gestation, people exposed in mid gestation, people exposed in early gestation and people conceived after the famine. We defined normoglycemia as 120-min plasma glucose concentrations below 7.8 mmol/l in accordance with the definition in our previous study and the 1999 World Health Organisation (WHO) recommendations (15, 24). Participants taking oral glucocorticoids were excluded from selection. The local Medical Ethics Committee approved the study, which was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Dexamethasone suppression and ACTH_{1–24}-stimulation test

The participants were asked to take 0.25 mg dexamethasone at 2200 h and to fast overnight. The following morning they attended the clinic where a cannula was placed in the antecubital vein. After a 30-min period of resting, a baseline blood sample was taken and 1 µg ACTH_{1–24} (Tetracosactid, Synacthen, Novartis Pharma, Arnhem, The Netherlands) was injected intravenously as a bolus. Venous blood samples were taken 20, 30, 40 and 60 min after injection of ACTH_{1–24}. Plasma cortisol concentrations were measured by enzyme immunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA) with a detection limit of 50 nmol/l. Cortisol concentrations in the baseline sample were used to indicate dexamethasone suppression. We used low doses of dexamethasone (0.25 mg) and ACTH_{1–24} (1 µg) instead of conventional doses (e.g. 1 mg dexamethasone and 250 µg ACTH_{1–24}) to be able to detect the subtle alterations in cortisol responses after suppression and stimulation to be expected in healthy participants. Conventional doses would induce maximal effects in all participants and minimise the possibility of detecting differences between participants exposed and unexposed to famine *in utero*.

Interview and anthropometric measurements

We measured height with a portable stadiometer and weight with a portable Tefal scale. We asked participants

about their use of medication. All other data used in this study were collected on the day the oral glucose tolerance test was performed (23). Waist circumference was measured with a flexible tape measure. Information on socioeconomic status, medical history and lifestyle was retrieved from a standardized interview. Current socioeconomic status was coded according to ISEI-92, a numeric scale based on the person's, or their partner's occupation, whichever status is highest (25).

Clinical measurements

Plasma glucose concentrations obtained during the earlier performed oral glucose tolerance test (25) were measured by a standardised enzymatic photometric assay on a Modular P analyser (Roche). Plasma insulin concentrations were measured by an immuno-luminometric assay on an Immulite 2000 analyser (Diagnostic Product Corporation). Blood was drawn for analysis of low- and high-density lipoprotein cholesterol. Blood pressure was measured in duplicate on two occasions (morning and afternoon) using an automated device (Omron 705CP/IT, Omron Healthcare UK, West Sussex, UK). Mean systolic and diastolic blood pressure was calculated using all available measurements. Standard 12-lead electrocardiograms (ECG) were taken from the participants. The presence of CHD was defined as the presence of one or more of the following: angina pectoris according to the Rose/WHO questionnaire; Q-waves on the ECG (Minnesota codes 1-1 or 1-2) or a history of coronary revascularisation (angioplasty or bypass surgery). We defined the metabolic syndrome according to the recommendations of the National Cholesterol Education Program (NCEP), modified in 2004 in accordance with the American Diabetes Association (ADA) (26, 27).

Statistical methods

We took the highest concentration of cortisol in the line of 20, 30, 40 and 60-min cortisol concentrations of each individual as the peak cortisol level. We subtracted the baseline cortisol concentration from the peak cortisol concentration to obtain cortisol increment. We calculated the area under the curve (AUC) for cortisol from 0 to 60 min post- $ACTH_{1-24}$ following the trapezoidal rule (base \times average height under cortisol curve). To obtain normality in case of a skewed distribution, we applied logarithmic transformations to the variables: body mass index (BMI), socio-economic status, 2-h insulin, systolic blood pressure, diastolic blood pressure, high density lipoprotein (HDL) cholesterol, post-dexamethasone cortisol and AUC cortisol. Spearman's rank correlations were calculated between maternal, birth, adult and cortisol outcomes. Linear and logistic regression analyses were used to detect possible differences in cortisol outcomes between the groups of people exposed and unexposed to famine

in utero. Effect sizes and confidence intervals (CI) are given as percentage difference between groups for logarithmically transformed outcome variables.

We used a general linear mixed model analysis to investigate differences between the exposed and unexposed groups in the course of cortisol concentrations over time after injection of $ACTH_{1-24}$. In all our regression analyses we adjusted for gender and BMI. Additional adjustment was performed for a range of variables including maternal and birth characteristics, smoking, socioeconomic status, treatment with estrogen, treatment with anti-depressive medication and use of inhaled corticosteroid medication. We considered differences to be statistically significant if *P*-values were smaller than 0.05.

Results

Three people who were exposed to famine during early gestation were unable to participate. Inadvertently, in the group of participants born before the famine, one person too many was included. The group of 98 participants with dexamethasone suppression/ $ACTH_{1-24}$ -stimulation tests consisted of 47 men and 51 women with a mean age of 58 years (s.d. 1 year), of which 57 (58%) had been exposed to famine during gestation.

Table 1 shows that mothers of participants exposed to famine in early gestation were younger and gained more weight during the third trimester of pregnancy compared with mothers of unexposed participants. Mothers exposed in mid gestation also gained more weight, but weighed less at the end of pregnancy. Mothers exposed in late gestation gained no weight and weighed less at the end of pregnancy. Babies exposed to famine in mid gestation were smaller at birth compared with unexposed babies.

Table 2 shows that at age 58, those exposed to famine in early gestation had a lower socioeconomic status compared with those unexposed. They had higher 2-h glucose and 2-h insulin concentrations compared with unexposed participants. The 2-h insulin concentrations were also higher for those exposed to famine during mid gestation.

Cortisol concentrations and maternal and birth characteristics

Table 3 shows correlations between maternal, birth and adult characteristics and cortisol outcomes. Post-dexamethasone and peak cortisol concentrations post- $ACTH_{1-24}$ injection were higher if the mother was older at delivery. With every year increase in maternal age, post-dexamethasone concentrations in the offspring increased by 2% (95% CI 0-4) and post- $ACTH_{1-24}$ peak cortisol concentrations increased by 4 nmol/l (95% CI 0-7 nmol/l). Plasma cortisol concentrations after dexamethasone suppression were lower in those with low

Table 1 General, maternal and birth characteristics according to timing of prenatal exposure to the Dutch famine. Results are means and s.d., except when indicated differently.

	Exposure to famine					All (s.d.)	n
	Born before	In late gestation	In mid gestation	In early gestation	Conceived after		
n	21	20	20	17	20		98
General characteristics							
Proportion of men	0.48	0.50	0.50	0.41	0.50	0.48	98
Age	58.9	58.4	58.0	57.8	57.3	58.1 (0.7)	98
Maternal characteristics							
Age at delivery (years)	30	32	29	25*	29	26 (6)	98
Weight gain 3rd trimester (kg)	1.6	0.0*	4.8*	5.7*	3.2	2.8 (3.2)	66
Weight at last antenatal visit (kg)	67.7	62.9*	63.8*	68.9	70.2	66.7 (8.2)	88
Birth outcomes							
Gestational age (days)	285	283	285	290	288	286 (11)	84
Birth weight (g)	3318	3323	3086*	3464	3727	3380 (471)	98

*Statistically significant difference ($P < 0.05$) compared with people unexposed to famine *in utero*.

birth weight. Post-dexamethasone concentrations decreased by 23% (95% CI 7–39) per kilogram decrease in birth weight. Analysis of men and women separately showed that the association between low birth weight and low post-dexamethasone cortisol concentrations was statistically significant in women only (36% decrease per kilogram decrease in birth weight (95% CI 5–57) vs 9% decrease in men (95% CI –22–32)).

Cortisol concentrations and adult characteristics

Men had higher cortisol concentrations after dexamethasone suppression (47% (95% CI 18–82)) and a lower cortisol increment after ACTH_{1–24} injection

(–75 nmol/l (95% CI –125 to –25)) compared with women (see also Table 3). Peak cortisol concentrations and cortisol AUC post-ACTH_{1–24} injection were higher with higher 2-h glucose concentrations. Peak cortisol concentrations rose by 25 nmol/l (95% CI 3–47) and cortisol AUC by 4% (95% CI 0–8) per mmol/l increase in 2-h glucose concentrations. Cortisol increment post-ACTH_{1–24} injection was higher with higher 2-h insulin concentrations. Cortisol increment increased by 0.23 nmol/l per pmol/l (95% CI 0.08–0.38) increase in 2-h insulin concentrations.

Repeated measures analyses of the cortisol profiles showed that there were positive associations between the cortisol profile and waist:hip ratio ($P < 0.01$), 2-h glucose ($P = 0.03$) and 2-h insulin concentrations, ($P = 0.04$)

Table 2 Adult characteristics according to timing of prenatal exposure to the Dutch famine. Results are means and s.d., except when indicated differently.

	Exposure to famine					All (s.d.)
	Born before	In late gestation	In mid gestation	In early gestation	Conceived after	
n	21	20	20	17	20	98
Proportion of current smokers	0.10	0.15	0.15	0.24	0.15	0.15
Current socio-economic status#	51	52	46	40*	49	47 (1.3)
Body mass index (BMI) (kg/m ²)#	26.0	27.3	27.0	27.9	27.7	27.2 (1.1)
Waist:hip ratio	91.1	91.9	91.5	93.1	90.0	91.5 (7.8)
Systolic blood pressure (mmHg)#	135	132	139	132	132	134 (1.2)
Diastolic blood pressure (mmHg)	80	78	84	82	82	81 (11)
Total cholesterol (mmol/l)	5.8	6.1	6.1	5.8	5.9	5.9 (1.0)
HDL cholesterol (mmol/l)#	1.54	1.43	1.41	1.50	1.37	1.45 (1.3)
2-h glucose (mmol/l)#	5.3	5.2	5.6	6.1**	5.1	5.4 (1.2)
2-h insulin (pmol/l)#	167	197	256**	294**	187	213 (1.2)
CHD prevalence	0.05	0.00	0.10	0.13	0.05	0.06
Metabolic syndrome prevalence†	0.24	0.15	0.45	0.18	0.25	0.26

CHD, coronary heart disease; HDL cholesterol, high density lipoprotein cholesterol; #Geometric means and s.d.; *statistically significant difference ($P < 0.05$) compared with people unexposed to famine *in utero*; **statistically significant difference ($P < 0.05$, adjusted for gender and BMI) compared with people unexposed to famine *in utero*. †According to the recommendations of the NCEP, modified in 2004 in accordance with the ADA (26, 27).

Table 3 Associations between maternal, birth and adult characteristics and plasma cortisol concentrations.

	Maternal age	Birth weight	Gender (men=1, women=2)	BMI	2-h glucose	2-h insulin
Post-dexamethasone cortisol	0.15	0.23*	-0.34*	-0.18	-0.03	-0.22*
Peak cortisol post-ACTH	0.23*	0.05	0.08	-0.12	0.23*	0.04
Cortisol increment post-ACTH	0.00	-0.07	0.36*	0.18	0.28*	0.36*
Cortisol area under curve (AUC) post-ACTH	0.18	0.17	-0.05	-0.12	0.23*	0.08

Spearman's rank correlations.

*Statistically significant correlation ($P < 0.05$).

and the prevalence of the metabolic syndrome according to the recommendations of the NCEP ($P = 0.03$). Gender, BMI, age, lifestyle variables (smoking, alcohol), socioeconomic status and other clinical outcomes (systolic and diastolic blood pressure, total and HDL cholesterol and the prevalence of CHD) were not associated with the cortisol profile.

Cortisol concentrations and prenatal famine exposure

Cortisol profiles after suppression with dexamethasone and stimulation with ACTH₁₋₂₄ for participants exposed to famine during late, mid or early gestation did not significantly differ from each other ($P = 0.78$) and were, therefore, grouped together for comparison with participants unexposed to famine *in utero*. The cortisol profiles after suppression with dexamethasone and stimulation with ACTH₁₋₂₄ are shown in Figure 1. Table 4 shows that cortisol concentrations after suppression with dexamethasone did not differ between those exposed and unexposed to famine *in utero* (mean difference -2% (95% CI -27 to 23)). Peak cortisol concentrations after injection with ACTH₁₋₂₄ did not differ between prenatally famine exposed and unexposed (20 nmol/l (95% CI -27 to 66)) subjects. There were also no differences between the exposed and unexposed groups in cortisol increment post-ACTH₁₋₂₄ (-5 nmol/l (95% CI -56 to 47) and cortisol AUC post-ACTH₁₋₂₄ (4% (95% CI -4 to 12)). Repeated measure analysis of the cortisol profiles showed that profiles of exposed and unexposed participants did not significantly differ from each other ($P = 0.62$). There was no interaction between sex and famine exposure ($P = 0.94$). Adjusting for maternal age, maternal weight gain, weight at the last antenatal visit, birth weight, smoking, socioeconomic status, treatment with estrogen, treatment with anti-depressive medication, and use of inhaled corticosteroid medication also did not alter the results.

Discussion

In the present study, we aimed to investigate whether prenatal exposure to famine is associated with increased

activity of the HPA axis at age 58. We performed a dexamethasone suppression and an ACTH₁₋₂₄-stimulation test, but found no differences in suppressed and stimulated cortisol concentrations between participants exposed and unexposed to famine *in utero*. These results suggest that the prenatal famine exposure does not program HPA axis activity, at least not at the adrenal level.

This study is a unique population-based study of men and women recruited from the original Dutch famine birth cohort still living in The Netherlands (15). The famine had a sudden beginning and end, and lasted for 5 months. This enables us to study the consequences of undernutrition during different stages of gestation. Although we found dexamethasone suppressed and ACTH₁₋₂₄-stimulated cortisol concentrations in those prenatally exposed to famine in late, mid or early gestation to be similar to cortisol concentrations of those unexposed, we think it is too early to conclude that famine exposure *in utero* does not affect functioning of the HPA axis at all. There are some methodological drawbacks and issues that have to be taken into account.

Our study sample was relatively small and the considerable fluctuations in cortisol concentrations make it difficult to detect differences between groups. Also, participants were sampled from a normoglycaemic group. HPA programming by famine exposure may be more pronounced among those with impaired glucose tolerance and prenatal famine related differences in normoglycaemic participants might be too subtle to detect. With a significance level of $\alpha = 0.05$, our study had 80% power to detect a minimal difference of 1 s.d. (about 100 nmol/l) in peak cortisol level between participants unexposed to famine and participants exposed to famine in late, mid or early gestation.

Another issue concerns the fact that the dexamethasone suppression/ACTH₁₋₂₄ activation test primarily acts at the adrenal level of the HPA axis, while higher levels of the HPA axis remain mostly unmeasured. The results of the present study suggest that prenatal famine exposure does not affect functioning of the HPA axis at the adrenal level, but it may affect functioning at a higher level. Experiments in which rats were pre- or perinatally undernourished showed physiological and functional changes at the levels of the hippocampus

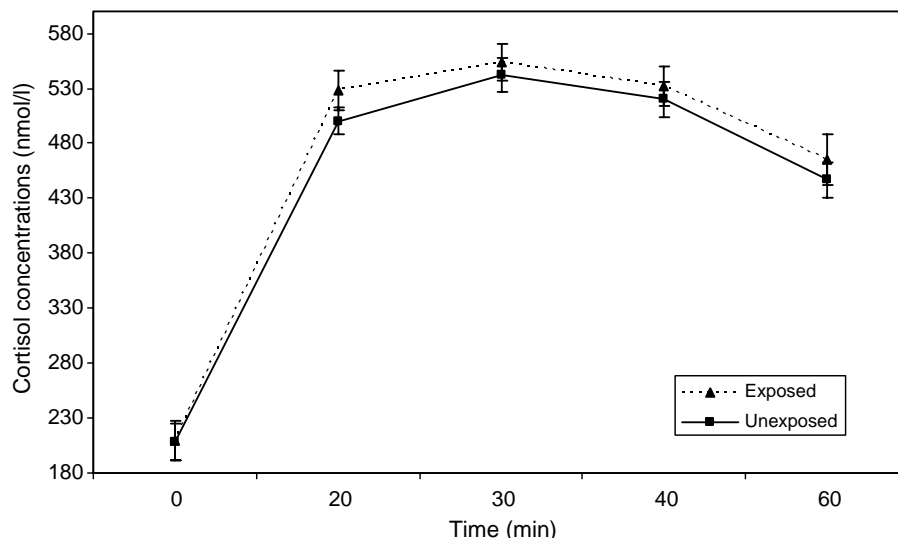


Figure 1 Cortisol profiles plotted as means (\pm S.E.M.) after dexamethasone suppression (0) and ACTH₁₋₂₄-stimulation (from 0 to 60 min) for participants exposed and unexposed to famine *in utero*. $P=0.62$ for difference between the exposed and the unexposed group based on regression analysis, adjusted for gender and BMI.

and hypothalamus (5, 28). Higher levels of the HPA axis can be tested by means of psychological stressors (29). We are currently investigating whether psychological stress leads to different HPA axis responses in humans exposed to famine *in utero*.

A final issue concerns the impact of increasing age. Various cohort studies have shown associations between low birth weight and cortisol concentrations across different age groups, implying there is no effect of age on the fetal programming of the HPA axis (10). Evidence from animal studies, however, seems to dispute this. Programming of the HPA axis by prenatal exposure to glucocorticoids was shown to be dependent on increasing age in studies of male guinea pigs (30, 31). Glucocorticoids-exposed 80-day-old guinea pigs showed a reduction in basal and activated pituitary–adrenal function compared with unexposed animals, while this effect was no longer observed at the age of 150 days. Programming effects on the activity of the HPA axis were still present, but persisted only at the level of the hippocampus. These age-dependent effects on the HPA

axis are possibly modulated by changes in mineralcorticoid receptor levels. Effects of prenatal famine exposure on HPA axis activity may also have changed with increasing age. Unfortunately, we were not able to test the possible effects of increasing age, because all participants are of about the same age.

We found elevated dexamethasone suppressed and ACTH₁₋₂₄-stimulated cortisol concentrations in participants born to mothers who were older at delivery. This result agrees with the result of a study that investigated HPA axis function in a cohort of children born in Jamaica (32). Some studies have shown associations between HPA axis responsiveness to ACTH₁₋₂₄ stimulation and low birth weight (13, 14). We found no evidence for such an association in our study, but we found that low birth weight was associated with lower cortisol concentrations following dexamethasone suppression, although in women only. While most studies report that low birth weight is linked with high morning cortisol concentrations which are suppressed normally following dexamethasone (10, 13, 14), associations

Table 4 Plasma cortisol concentrations according to timing of prenatal exposure to the Dutch famine. Results are means and s.d., except when indicated differently.

	Exposure to famine					All (s.d.)	n
	Born before	In late gestation	In mid gestation	In early gestation	Conceived after		
Post-dexamethasone cortisol (nmol/l)#	177	205	166	158	186	178 (1.8)	96
Peak cortisol post-ACTH (nmol/l)	608	600	590	584	539	584 (110)	89
Cortisol increment post-ACTH (nmol/l)	402	351	389	399	358	380 (123)	87
Cortisol AUC post-ACTH (nmol/l·min)#	27623	27496	27101	27581	26008	27369 (1.2)	84

#Geometric means and s.d.

with greater suppressibility following dexamethasone have been reported in some populations of men and women (11, 12).

To our knowledge, gender differences in the association between low birth weight and responsiveness of the HPA axis have not been reported before. Studies by Reynolds *et al.* showed gender differences in the cortisol responses to HPA manipulation, as did our results, but found no interaction effect of gender and low birth weight on HPA responsiveness (13, 14). Animal experiments have shown gender-specific HPA reactions as a consequence of prenatal undernutrition, which points to an increase in adrenal sensitivity in females (8). However, the link between small size at birth and greater suppressibility of the HPA axis in women in our study does not seem to be caused by undernutrition *in utero*. Size at birth is a summary measure of fetal growth, which only indirectly relates to maternal nutrition. It is a result of a wide range of maternal, placental and fetal factors. It has been shown that size at birth can produce permanent effects on adult health independently from prenatal undernutrition; it has also been found that prenatal undernutrition can have profound effects in the later life without affecting size at birth (18, 33, 34).

In summary, we found no evidence in support of the hypothesis that prenatal famine exposure programs response to dexamethasone suppression and ACTH₁₋₂₄ stimulation at the adrenal level of the HPA axis. At the levels of the hippocampus and hypothalamus there may be programming by famine exposure *in utero*. We are currently testing this assumption by performing psychological stress tests in our cohort.

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