

## BRIEF REPORT

# Stress Responsiveness in Adult Life: Influence of Mother's Diet in Late Pregnancy

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**Context:** Men and women whose mothers ate an unbalanced high-protein, low-carbohydrate diet in late pregnancy have raised blood pressure. We recently showed that they also have raised fasting plasma cortisol concentrations. Because raised fasting cortisol concentrations probably reflect a greater response to the stress of fasting and venesection, we suspected that this diet may have led to increased stress responsiveness in the adult offspring.

**Objective:** The aim was to determine whether an unbalanced high-protein diet during pregnancy is associated with increased cortisol secretion in response to psychological stress in the offspring.

**Design and Participants:** Salivary cortisol concentrations were measured during a modified Trier Social Stress Test in 70 men and women aged 36.3 yr whose mothers had taken part in a dietary intervention in

which they were advised to eat 1 pound (0.45 kg) of red meat daily during pregnancy and to avoid carbohydrate-rich foods.

**Results:** The offspring of women who reported greater consumption of meat and fish in the second half of pregnancy had higher cortisol concentrations during the Trier Test. Compared with the offspring of mothers who had reported eating no more than 13 meat/fish portions per week, the average cortisol concentrations were raised by 22% (95% confidence interval, 13 to 71%) and 46% (5 to 103%) in the offspring of those eating 14–16 and at least 17 portions per week, respectively.

**Conclusions:** These findings provide the first human evidence that an unbalanced high protein maternal diet during late pregnancy leads to increased cortisol secretion in response to psychological stress in the offspring. (*J Clin Endocrinol Metab* 92: 2208–2210, 2007)

THERE IS INCREASING evidence that an adverse prenatal environment can change the set-point of the hypothalamic-pituitary-adrenal (HPA) axis and the adrenocortical response to stress. In animal studies, experimental manipulations during pregnancy induce lifelong alterations in the HPA axis (1, 2). In humans, too, low birth weight, a marker of an unfavorable intrauterine environment, is associated with raised plasma cortisol concentrations, altered regulation of the HPA axis, and enhanced cortisol responses to psychological stress (3–6). Because glucocorticoids have potent biological effects on both vascular tone and glucose-insulin metabolism, these HPA axis changes may contribute to the increased susceptibility to coronary heart disease, hypertension, and type 2 diabetes, documented in people who experienced an adverse prenatal environment.

In humans, little is known as to the nature of the specific maternal factors that could affect offspring HPA function. We have recently been interested in whether maternal consumption of a high-meat, low-carbohydrate diet in pregnancy has long-term consequences for the offspring. This diet is known to reduce fetal growth (7) and has been associated with raised adult blood pressure in the offspring (8) of mothers attending a maternity hospital in Motherwell, Scotland, between 1952 and

1976. During pregnancy, the mothers in Motherwell were advised to eat a high-meat, low-carbohydrate diet in an attempt to prevent preeclampsia. The specific advice was to eat 1 pound (0.45 kg) of red meat daily during pregnancy and to avoid carbohydrate-rich foods such as potatoes, bread, or cakes. Moderate consumption of fish, eggs, and cheese was encouraged together with green vegetables twice daily. The women's actual intakes were recorded, and in subsequent studies greater maternal consumption of meat and fish in late pregnancy was linked with higher systolic blood pressure in the adult offspring at 29 yr of age (8). Recently, we found that greater maternal meat and fish intake was also associated with elevated offspring fasting plasma cortisol concentrations (9). We hypothesized that the elevated cortisol concentrations reflected increased HPA axis responsiveness to the stress of fasting, venesection, and the novel clinic setting in which the samples were obtained. We have now tested this hypothesis in a subset of the men and women born in Motherwell.

### Subjects and Methods

With ethics approval and written informed consent, we approached men and women who had been part of the previous study (9) and recruited 31 men and 39 women born in Motherwell during 1967–68. Information abstracted from the obstetric records has been described in detail previously (8) and included maternal height, age, parity, social class (derived from the father's occupation), smoking history, pregnancy weight gain, highest blood pressure during pregnancy, and dietary intakes. Trained clerical assistants had used a standard format to record in the obstetric notes the number of times and amounts that the women had eaten 10 different food commodities during the previous week:

First Published Online March 6, 2007

Abbreviation: HPA, Hypothalamic-pituitary-adrenal.

JCEM is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

meat, fish, eggs, milk, cheese, potatoes, green vegetables, bread, cakes/scones/biscuits, and sweets. Dietary data abstracted from these notes were compiled as intakes in early ( $\leq 20$  completed wk gestation) and late ( $> 20$  completed wk gestation) pregnancy. Previous analyses of these dietary data show a doubling of protein intake between early and late pregnancy in the Motherwell women (8). Information on duration of gestation, determined from menstrual data, and infant size at birth was abstracted from the obstetric records.

The psychological stressor was a modified Trier Social Stress Test (10). A public speaking task (5 min) was followed by a mental arithmetic task (3 min), and salivary cortisol was measured at eight time-points before, during, and after the stress challenge. Subjects were asked to avoid caffeine, alcohol, and smoking on the day of the test, and women were studied in the luteal phase of the menstrual cycle. Subjects taking glucocorticoids by any route within the last 3 months were excluded. Current medication including use of the oral contraceptive pill ( $n = 9$  women) was recorded. All studies were carried out in the afternoon after 1400 h. Salivary cortisol was measured by a time-resolved immunofluorescent assay (dissociation-enhanced lanthanide fluoroimmunoassay) (11). The assay had a lower limit of detection of 0.4 nmol/liter and an interassay coefficient of variation of 5–10% between 2 and 15 nmol/liter. Because the salivary cortisol concentrations were skewed, we log-transformed them and report geometric means and SD values. The salivary cortisol profile was analyzed with a repeated measures regression, using an autoregressive correlation matrix and separate variances for concentrations at different time-points (SPSS version 12; SPSS Inc., Chicago, IL).

## Results

Table 1 shows the characteristics of the study participants and their mothers. Between early and late pregnancy, the mothers' meat/fish consumption almost doubled, from 8.5 to 14.7 portions per week, whereas consumption of carbohydrate-rich foods fell from 6.7 to 2.5 portions per day. In response to the stress challenge, offspring salivary cortisol concentrations rose from 4.9 (SD 1.7; interquartile range, 3.4 to 6.3) nmol/liter to 9.6 (SD 2.1; range, 5.6 to 17.2) nmol/liter in men and from 3.9 (SD 1.9; range, 2.6 to 5.6) nmol/liter to 4.7 (SD 2.1; range, 2.7 to 6.1) nmol/liter in women. Average cortisol concentrations across all time points increased by 2.3% (95% confidence interval, 0.1 to 4.5%;  $P < 0.04$ ) per portion of meat and fish consumed by their mothers per week in late pregnancy, an effect that was somewhat, although not significantly greater in men (2.9%) than in women (2.0%). Compared with offspring of mothers who had

reported eating no more than 13 meat/fish portions per week, the average cortisol concentrations were raised by 22% (95% confidence interval, 13 to 71%) and 46% (5 to 103%) in offspring of those reporting 14–16 and at least 17 portions per week, respectively (Fig. 1). Cortisol responses were not related to maternal height, weight, pregnancy weight gain, smoking, social class, or carbohydrate-rich food intake. Nor were they related to the subject's current social class, BMI, or the use of the oral contraceptive pill. Allowing for these variables in the repeated measures regression did not diminish the association between the maternal meat/fish intake and the cortisol response to stress.

## Discussion

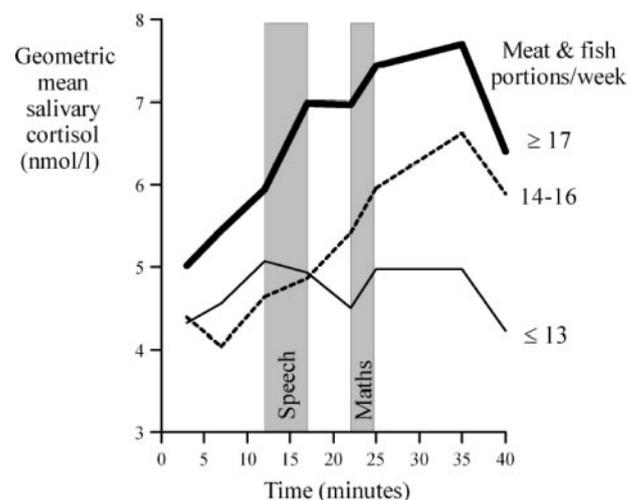
In this study we have shown that women who consumed an unbalanced diet with high meat/fish intakes during late pregnancy have offspring with greater cortisol secretion in response to psychological stress. The effect was statistically significant and graded, with the greatest offspring cortisol responses being observed in the women who reported the highest meat and fish intakes during pregnancy. It was also independent of likely confounding influences both in mother and offspring.

We previously reported that the offspring of mothers with high meat/fish consumption in late pregnancy had elevated fasting cortisol concentrations. We suspected that cortisol concentrations measured in fasting venesection samples might have reflected a stress response due to the combination of fasting, venesection, and the novel clinic setting in which the samples were obtained. The findings in the present study support this suggestion. Cortisol is a biologically potent glucocorticoid that affects both vascular responses and the metabolism of glucose and lipids. Higher fasting cortisol concentrations in fasting samples have been linked with features of the metabolic syndrome, including raised blood pressure, insulin resistance, glucose intolerance, and dyslipidemia (3) and are also predictive of the occurrence of ischemic heart disease (12). Furthermore, because these HPA axis changes are likely to have been lifelong, exposure to higher cortisol concentrations during fetal life, infancy, and childhood may

**TABLE 1.** Clinical characteristics of the subjects

	Men (n = 31)	Women (n = 39)
Age (yr)	36.3 (0.53)	36.4 (0.49)
Height (cm)	177.7 (5.9)	161.5 (5.8)
Weight (kg)	81.3 (10.1)	62.5 (11.7)
BMI (kg/m <sup>2</sup> )	27.1 (3.4)	25.0 (4.0)
Manual social class (%)	45.2	46.2
Smokers (%)	16.1	30.8
High alcohol consumption (men > 21, women > 14 U/wk) (%)	29	15
Birth weight (kg)	3.08 (0.36)	3.01 (0.34)
Gestational age (wk)	38.6 (1.34)	38.7 (1.24)
Mother's age at delivery (yr)	28.1 (5.7)	27.1 (6.6)
Weight gain in pregnancy (kg/wk)	0.25 (0.10)	0.27 (0.13)
Smoked in pregnancy (%)	42.9	43.6
Manual social class in pregnancy (%)	45.2	23.1
Mother's protein intake in early pregnancy (portions/wk)	9.0 (3.5)	8.0 (3.0)
Mother's protein intake in late pregnancy (portions/wk)	15.4 (5.5)	14.3 (6.5)
Mother's carbohydrate intake in early pregnancy (portions/d)	6.0 (3.0)	7.2 (2.5)
Mother's carbohydrate intake in late pregnancy (portions/d)	2.2 (2.9)	2.8 (3.3)

Data represent mean (SD) or percentage.



**FIG. 1.** Salivary cortisol concentrations in response to the Trier Social Stress Test in men and women according to the mother's meat and fish consumption (portions per week) in late pregnancy.

also direct the course of development toward the generation of a phenotype adapted for adult adversity, with changes in tissue and organ structure and function that predispose to disease (13). It is probable therefore that the raised cortisol concentrations observed in this study have adverse health effects and may, at least in part, account for the link between an unbalanced high-protein diet during pregnancy and raised blood pressure in the offspring.

Although we studied a small number of subjects, this is the first human study to find associations between the mother's diet in pregnancy and alterations in the HPA axis response to stress in the offspring. The findings are supported by previous human studies showing that low birth weight, a nonspecific marker of prenatal adversity, is associated with enhanced adrenocortical stress responsiveness (6, 14). They are also strongly supported by a variety of animal studies in various species showing that prenatal interventions can permanently alter the adrenocortical response to stress (1, 2). The effects on the cortisol response to stress were more evident in men than women, which accords with increasing evidence of marked gender differences in these associations (15). Because the effect is to enhance the biological response to stress, it is likely that the actual health outcomes depend on interactions between the early environmental experience and the exposure to stressful circumstances in adult life; the most marked effects would be expected in people whose mothers had the most adverse prenatal diets and who themselves were experiencing the most stressful lifestyles.

The underlying processes linking an unbalanced maternal diet with offspring cortisol responses to stress are not known. A high-protein diet is known to stimulate the HPA axis, possibly by means of the direct neuroendocrine effects of ingested neurotransmitter substrates including L-tyrosine and L-tryptophan (16). Because cortisol may cross the incomplete placental glucocorticoid barrier (17), the fetus may be exposed to excess cortisol, thereby reprogramming the developing fetal HPA axis by altering the expression of glucocorticoid receptor populations in limbic structures within the brain (2). Alternatively, as we have suggested, the type of diet consumed by pregnant women in Motherwell may have imposed a direct metabolic stress on both mother and fetus as a result of the reduced availability of nonessential amino acids (9). Meat and fish are rich in essential amino acids that must be either used for protein synthesis or oxidized. Oxidation consumes nonessential amino acids whose synthesis requires cofactors including folate and vitamin B6 that are likely to have been low in the diet consumed by the pregnant women in Motherwell. Finally, the mother's diet may have altered HPA axis regulation by means of the epigenetic modification of glucocorticoid receptor expression (18). These mechanisms will not be easily resolved in human studies and require elucidation in animal models.

The mean daily intakes of protein in Motherwell during late pregnancy have been estimated to be 88 g/d (19). This is a figure considerably higher than current dietary recommendations for protein intake during pregnancy [estimated mean of 52.8 g/d based on the UK Food Standards Agency ([www.food.gov.uk](http://www.food.gov.uk)) recommendations of 0.75 g/kg protein·d and an extra 6 g pro-

tein/d during pregnancy]. The advice given to the Motherwell mothers, which includes a very low carbohydrate intake, is unusual and precludes direct application to other populations. Nevertheless, high-protein diets, such as the Atkins diet, although not recommended during pregnancy, are popular in young women today, and this study raises the possibility that they may have long-term health effects on the offspring.

### Acknowledgments

We thank Dr. H. Simonsen and Mrs. S. Pearson for help with conducting the study, Professor D. Barker for his involvement in the design of the study, and the men and women in Motherwell who generously gave up their time to take part.

Received January 11, 2007. Accepted February 28, 2007.

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This work was supported by The Medical Research Council and The Chief Scientist's Office of Scotland.

Disclosure Statement: The authors have nothing to disclose.

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