

Maternal Consumption of a High-Meat, Low-Carbohydrate Diet in Late Pregnancy: Relation to Adult Cortisol Concentrations in the Offspring

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Recent studies have linked maternal consumption of an unbalanced high-protein, low-carbohydrate diet in late pregnancy with raised adult blood pressure in the offspring. Because high-protein diets stimulate the hypothalamic-pituitary-adrenal axis, we hypothesized that an unbalanced maternal diet might increase maternal cortisol levels, exposing the fetus to excess cortisol and programming lifelong hypersecretion of cortisol. We therefore measured fasting plasma cortisol concentrations in 251 subjects, 28–30 yr old, whose mothers had been advised to eat 0.45 kg of red meat daily during pregnancy and avoid carbohydrate-rich foods. Cortisol concentrations were higher in subjects with lower body mass index ($P < 0.0001$) and in those who reported recent

vigorous activity ($P = 0.03$) and greater alcohol consumption ($P = 0.004$). Allowing for gender, current body mass index, activity, and alcohol consumption, cortisol concentrations increased 5.4% per portion of maternal meat/fish consumption per day ($P = 0.03$), decreased 3.3% per portion of maternal green vegetable consumption per week ($P = 0.14$), and were 12.2% higher in those born into manual social class families ($P = 0.03$). The specific advice given to mothers in this study precludes direct application to other populations, but the findings provide the first human evidence that an unbalanced maternal diet during late pregnancy may program lifelong hypercortisolemia in the offspring. (*J Clin Endocrinol Metab* 88: 3554–3560, 2003)

THERE IS INCREASING evidence that raised blood pressure and related conditions, including glucose intolerance and the metabolic syndrome, may originate from adverse influences during intrauterine life that lead to permanent alterations in fetal structure and physiology (1). This process is known as fetal programming. Although the hypothesis originated in studies showing associations between low birthweight and these conditions in adult life, more recent studies suggest that fetal programming may occur in the absence of changes in fetal size (2, 3). Recent research therefore has sought to identify the nature of the adverse maternal influences that raise blood pressure and alter levels of cardiovascular risk factors in the offspring.

Substantial evidence now suggests that maternal diets of high protein density have adverse effects on the fetus and are associated with small, but significant, reductions in birthweight. In a trial of prenatal nutritional supplementation in New York City, mothers who had received a high-protein supplement experienced a mean 73 g decrease in the birthweight of their infants (4). Similar deficits associated with high-protein diets have been reported in a number of other studies of nutritional supplementation (5). High protein maternal diets have also been associated with raised blood pressure in the offspring during adult life. In a study of 253 men and women whose mothers took part in a survey of diet in pregnancy at around 30 wk of gestation, between 1948 and 1954, diets of high protein density were associated with

raised systolic and diastolic pressure in the offspring at age 40 yr (6). A more recent follow-up of men and women in Motherwell whose mothers had been advised to eat a high-animal-protein, low-carbohydrate diet in pregnancy showed that a higher maternal intake of meat/fish and a lower intake of green vegetables during pregnancy was associated with higher adult blood pressure in the offspring (7).

One explanation proposed for the adverse effects of high-meat/fish consumption is that this may increase maternal cortisol concentrations, which, in turn, affect the developing fetus, resetting its developing hypothalamic-pituitary-adrenal (HPA) axis (7). A number of studies have described robust increases in the cortisol response to a protein-rich meal. Salivary cortisol in both men and women increases as much as 2-fold above premeal levels 1 h after a protein-rich meal (8–10). Animal experiments demonstrate that raising maternal cortisol concentrations during gestation results in inappropriate fetal exposure to cortisol and can retard fetal growth. Moreover, inappropriate fetal exposure to cortisol can also lead to elevated basal or stress-induced glucocorticoid secretion and high blood pressure or other features of the metabolic syndrome in later adult life (11–12). It is likely that the same processes operate in human populations, given that we and others have reported recently that lower birthweight is associated with higher fasting morning plasma cortisol concentrations and increased adrenal responsiveness in later childhood and adult life (13–16). These studies have led us to suggest that adverse events in early life permanently alter or program cortisol secretion and that, particularly when combined with adult obesity, this leads to a high prev-

Abbreviations: BMI, Body mass index; CI, confidence interval; GTT, glucose tolerance test; HPA, hypothalamic-pituitary-adrenal.

absence of raised blood pressure and the metabolic syndrome in adult life (15).

In this study, we report analyses of fasting plasma cortisol concentrations in the Motherwell subjects in whom we found higher blood pressure in the offspring of mothers who reported higher meat/fish and lower green vegetable consumption in late pregnancy (7). Our aim was to test the *a priori* hypothesis that, in the setting of advice to follow a pregnancy diet high in protein and low in carbohydrate, an unbalanced pattern of higher meat/fish and lower green vegetable consumption in late pregnancy leads to elevated cortisol concentrations in the offspring.

Methods and Materials

As previously described (7), the 1967–1968 records from Motherwell Maternity Hospital included 1432 live, singleton births with complete names and birth records and at least 1 diet record. Information abstracted from the obstetric records has been described in detail previously (7). In summary, this 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: meat (portions), fish (portions), eggs (number), milk (pints), cheese (ounces), potatoes (number), green vegetables (portions), bread (slices), cakes/scones/biscuits (number), and sweets [quarter pounds (1/4 pounds)]. Diet data abstracted from these notes was compiled as intakes in early (≤ 20 completed weeks of gestation) and late (> 20 completed weeks of gestation) pregnancy. Information on the neonates was also taken from the obstetric records and included birth weight, placental weight, crown-heel length, and head circumference at birth. Duration of gestation at birth was determined from menstrual data.

We used the National Health Service Central Register (Scotland) and records from Lanarkshire Health Board to trace the offspring 30 yr later.

Letters inviting them to participate in our initial survey were sent out to 957 subjects alive and still living in Lanarkshire; 684 (71%) agreed to participate (7) (Fig. 1). From 1996 to 1997, subjects completed a questionnaire assessing their smoking habits, alcohol consumption, and health history and had their blood pressure measured during home visits. Alcohol intake was classified as very low, low, moderate, and high (< 1 , 1–10, 11–21, > 21 U of alcohol per week for men and < 1 , 1–7, 8–14, > 14 U of alcohol per week for women, respectively). Current social class was assessed through questions recording their occupation or, for married women who did not work, their partner's occupation and was coded into manual and nonmanual for all except 18 subjects. During the home visit, subjects were asked whether they would be willing to have an oral glucose tolerance test (GTT).

Of those who answered "yes" to taking part in a GTT, 323 attended a clinic at Motherwell Maternity Hospital one morning after an overnight fast and had a standard 75-g oral GTT including a fasting sample for plasma cortisol measurement. Subjects were questioned about maintaining the fast and asked to stay in the clinic for the duration of the test. They then gave informed written consent. We excluded from our analyses 24 subjects who had no dietary records in late pregnancy, together with 48 women taking oral contraception, because this substantially alters measured amounts of total plasma cortisol (see Refs. 19 and 20). Our final study size was therefore 251 subjects (Fig. 1), representing 37% of the cohort assembled for the blood pressure study. Ninety-three percent of the fasting blood samples were taken between 0835 and 1000 h; the remainder were taken between 1000 and 1050 h. Plasma cortisol concentrations were similar in samples taken before and after 1000 h (P for difference = 0.97) and were not related to time at sampling analyzed as a continuous variable ($P = 0.14$). The mean (SD) interval between the home interview for the blood pressure study and the clinic visit for the GTT and fasting plasma cortisol sample was 17 (5) months.

The subjects' frequency and level of physical activity was assessed using questions adapted from the 1991 Health Survey of England (17). Four types of activities were assessed: occupational, daily living, exercise for fitness and play, and home activities (such as housework and gardening). Subjects were asked to count the number of times they had participated in these activities within the past 4 wk. They also rated the intensity level of their activity as inactive, light, moderate, or vigorous.

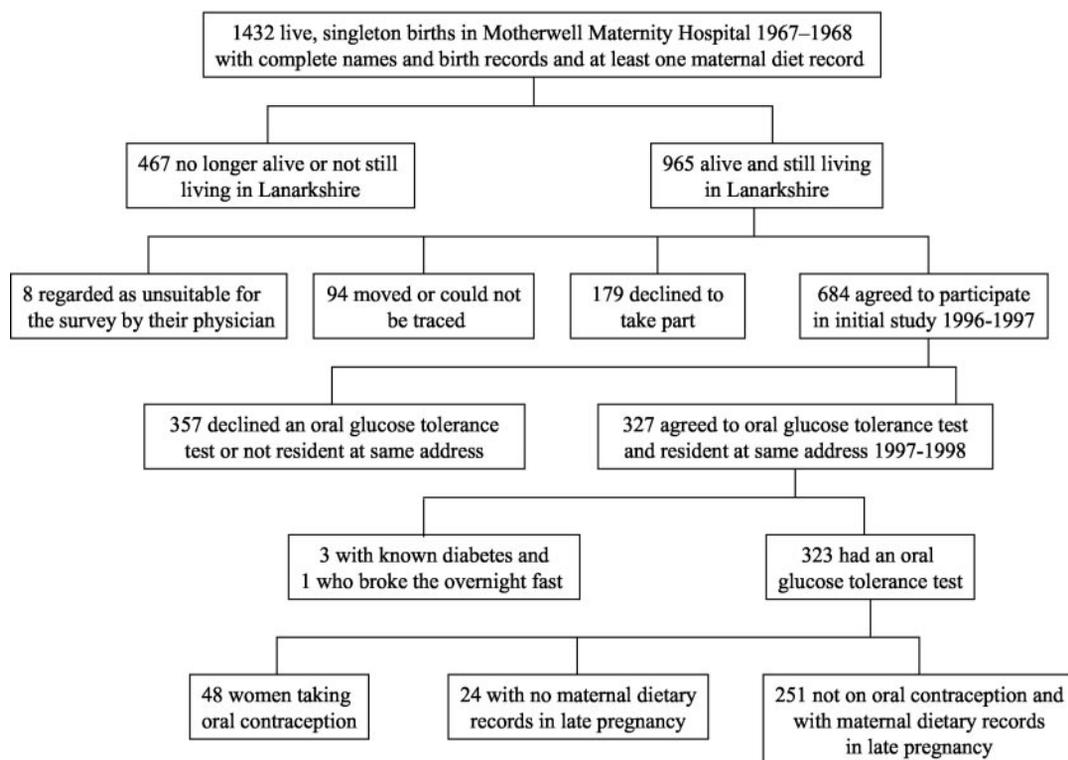


FIG. 1. Schematic representation of how subjects for the study were selected.

Participants were also asked to update their health history, and their current medications were ascertained. No subject had any current or past illness that may have indicated previous problems with the HPA axis, and no subject was taking oral steroids. To assess the subjects' body composition, height was measured with a Harpenden pocket stadiometer and weight with Seca digital scales (CHS, London, UK). The Research Ethics Committee of Lanarkshire Health Board approved all phases of the study.

Laboratory methods

Plasma cortisol measurements were performed on aliquots of the fasting blood sample that had been separated by centrifuging at 2500–3000 rpm for 20 min and then stored at -80°C . The Regional Department of Chemical Pathology, Southampton General Hospital, performed an in-house RIA (18) to determine total plasma cortisol. The interassay coefficient of variation was 8%.

Statistical analysis

Plasma cortisol concentrations and body mass index (BMI) values were positively skewed, and both were log transformed to satisfy assumptions of statistical normality; geometric means and interquartile ranges are presented for these variables. Pearson's correlation coefficient was used to test for associations between the *a priori* variables, meat/fish and green vegetable intake in late pregnancy and the maternal, neonatal, and adult characteristics. Simple linear regression was used to build a model of adult lifestyle variables and percent change in cortisol per SD change in the independent variable. The regression model controlling for gender and adult lifestyle was then used in multiple regression analysis to test associations between cortisol and maternal and neonatal attributes. All statistical analysis was performed using STATA, release 7 (Stata Corp., College Station, TX). Levels of significance refer to analysis of continuous variables by regression unless stated. Variables are grouped in the tables for clarity of presentation.

Results

Table 1 shows the characteristics of the subjects followed up at a mean age of 30 (range, 28–32) yr. Geometric mean (interquartile range) plasma cortisol concentrations in the 119 men and 132 women were 406.5 (range, 323–518) and 375.2 (range, 294–490) nM, respectively. The overall mean (SD) birth weight of the 251 subjects was 3052 (± 400) g, with a mean gestational age of 38.9 wk because of the clinical policy of inducing labor around 38 wk gestation. Table 1 also shows the characteristics and food consumption in late pregnancy of the 251 mothers.

Table 2 shows mean fasting plasma cortisol concentrations according to the offspring's adult body composition, lifestyle, and gender. Fasting plasma cortisol concentrations were higher in subjects with a lower BMI ($P < 0.0001$). Cortisol concentrations were also higher in men and women who had taken vigorous activity in the previous 4 wk and those who reported greater alcohol consumption ($P = 0.03$ and $P = 0.004$, respectively). Cortisol concentrations were not related to current height, smoking, and social class. A weak trend for plasma cortisol to be higher in men than women ($P = 0.09$) was weakened after taking account of BMI, activity, and alcohol consumption (adjusted $P = 0.36$).

Table 3 shows mean fasting plasma cortisol concentrations according to neonatal characteristics and maternal influences. Birthweight and duration of gestation at birth were not related to adult plasma cortisol concentrations. Placental weight and neonatal length and ponderal index were also unrelated to plasma cortisol in adulthood (data not shown). Although the offspring of older mothers and of mothers with

TABLE 1. Characteristics of 251 adults, neonates, and mothers

	Value \pm SD	
Mothers' characteristics^a		
Age at delivery, yr	27.2 \pm 6.0	
Height, cm	159.1 \pm 6.2	
Gestation week at first examination	17.5 \pm 6.4	
First antenatal weight, kg	59.7 \pm 9.6	
First antenatal BMI, kg/m ²	23.6 \pm 3.6	
Weight gain in pregnancy, kg/wk	0.24 \pm 0.15	
Maximum systolic pressure in pregnancy, mm Hg	129 \pm 10	
Smoked in pregnancy, %	49.8	
Primiparous, %	41.8	
Manual social class, %	75.6	
Mothers' food consumption in late pregnancy		
Meat, portions/wk	13.9 \pm 5.7	
Fish, portions/wk	1.3 \pm 0.7	
Eggs, no./wk	6.2 \pm 3.1	
Cheese, ounces/wk	4.5 \pm 2.9	
Milk, pints/d	0.6 \pm 0.4	
Green vegetables, portions/wk	5.7 \pm 2.9	
Potatoes, no./d	0.8 \pm 1.2	
Bread, slices/d	0.8 \pm 1.1	
Cakes/scones/biscuits, no./d	0.5 \pm 0.9	
Sweets, ¼ pound/wk	1.1 \pm 0.8	
	Males (n = 119)	Females (n = 132)
Neonates^b		
Birthweight, g	3102 \pm 413	3006 \pm 384
Length, cm	48.3 \pm 3.3	48.1 \pm 3.2
Ponderal index, kg/m ³	28.0 \pm 5.9	27.4 \pm 5.2
Placental weight, g	614 \pm 97	611 \pm 95
Gestation, wk	38.8 \pm 1.4	39.0 \pm 1.2
Adults		
Age, yr	30.2 \pm 0.6	30.0 \pm 0.6
Height, cm	177.4 \pm 6.0	162.1 \pm 6.3
Weight, kg	84.5 \pm 14.7	67.6 \pm 15.6
BMI, ^c kg/m ²	26.5 (24.1, 28.8)	25.2 (22.2, 28.1)
Manual social class, %	47.9	47.7

^a Pregnancy weight gain, maternal social class, and current social class missing for 5, 1, and 18 subjects, respectively.

^b Neonatal length missing for three subjects.

^c Geometric mean (inter-quartile range).

lower weight gain in pregnancy had higher cortisol concentrations, adjustment for current BMI, activity, and alcohol consumption weakened these associations (Table 3). Maternal BMI and smoking in pregnancy were not associated with the offspring's cortisol concentrations, but men and women born into manual social class families tended to have higher cortisol concentrations than their nonmanual counterparts (unadjusted $P = 0.08$, adjusted $P = 0.04$). Maternal height, parity, and blood pressure in pregnancy were not related to the offspring's plasma cortisol concentrations (data not shown).

Based on the *a priori* hypothesis, we related maternal consumption of meat/fish and of green vegetables in late pregnancy to the offspring's fasting plasma cortisol. Concentrations were higher in those whose mothers had reported greater meat/fish consumption and lower green vegetable consumption in late pregnancy, but these associations were not statistically significant in univariate analyses ($P = 0.11$ and $P = 0.15$, respectively). Simultaneous analysis of adult cortisol in relation to the combination of maternal meat/fish and green vegetable consumption tended to strengthen the effect of meat/fish intake; Fig. 2 uses the same groupings as in our previous analyses of the offspring's blood pressure (7)

TABLE 2. Univariate associations of geometric mean fasting plasma cortisol with adult body composition, lifestyle, and gender

	Plasma cortisol (nM)	P-value	No. of subjects
BMI (kg/m ²) ^a			
<20	427		18
–25	425		96
–30	374		98
>30	334	<0.001	39
Height (cm) ^a			
<162	362		65
–169	412		60
–176	393		54
≥176	395	0.2	72
Taken vigorous activity in last 4 wk ^b			
No	371		127
Yes	410	0.03	124
Alcohol consumption (units/wk) ^a			
Very low (men and women, <1)	349		47
Low (men, 1–10; women, 1–7)	378		83
Moderate (men, 11–21; women, 8–14)	392		56
High (men > 21, women > 14)	437	0.004	65
Smoker ^b			
No	386		175
Yes	399	0.5	76
Current social class ^b			
Nonmanual	398		113
Manual	386	0.51	120
Gender ^b			
Male	407		119
Female	375	0.09	132

^a Regression of a continuous variable.^b *t* Test for significance.

and shows that cortisol tended to rise with increasing maternal meat/fish intake and to be higher in those whose mothers reported lower green vegetable consumption. The highest concentrations were in people whose mothers reported consuming more than 21 portions/wk of meat/fish and less than 7 portions/wk of green vegetables in late pregnancy. Multiple regression analysis showed that simultaneously taking account of gender, current BMI, activity, and alcohol consumption had little effect on the associations between adult cortisol concentrations and social class at birth (adjusted $P = 0.03$) and maternal consumption in late pregnancy of meat/fish [adjusted $\beta = 5.4\%$ change per portion per week; 95% confidence interval (CI), 0.6–10.3, $P = 0.03$] and green vegetables (adjusted $\beta = -3.3\%$ change per portion per week; 95% CI, –7.6 to 1.1, $P = 0.14$) (Table 4).

Exploratory analyses revealed no associations between the offspring's plasma cortisol and maternal consumption of the other foods shown in Table 1, with the possible exception of milk consumption. Higher maternal milk consumption in late pregnancy tended to be associated with lower plasma cortisol in the adult offspring ($P = 0.06$). Inclusion of milk consumption in the Table 4 regression analyses had little effect on the other associations shown in the table and only marginally weakened the association between milk consumption and the offspring's cortisol concentration (adjusted $P = 0.09$). Lower maternal consumption of the carbohydrate-rich foods (potatoes, bread, and cakes/scones/biscuits) was not related to the offspring's cortisol concentration ($P = 0.78$). Additionally, including pregnancy weight gain in the Table 4 regression analyses showed a persisting weak inverse as-

TABLE 3. Associations of geometric mean fasting plasma cortisol with neonatal characteristics and maternal influences

	Plasma cortisol (nM)	P-value	Adjusted P value ^a	No. of subjects
Birthweight (pounds)				
<5.5	387			17
–6.5	384			86
–7.5	402			107
≥7.5	373	0.9	0.8	41
Duration of gestation at birth (wk)				
<37	405			15
–39	412			98
–40	363			103
≥40	404	0.4	0.3	35
Mother's age (yr)				
<25	360			112
25–30	410			66
30–35	426			38
≥35	415	0.03	0.2	35
First antenatal BMI (kg/m ²)				
≤21.0	400			56
–22.5	391			61
–25.0	391			65
≥25.0	381	0.5	0.7	69
Mother's weight gain during pregnancy (kg/wk)				
≤0.15	416			61
–0.25	400			59
–0.35	372			68
≥0.35	371	0.03	0.07	58
Smoking during pregnancy				
No	397			126
Yes	383	0.4	0.5	125
Social class at birth				
Nonmanual	363			61
Manual	399	0.08	0.04	189

^a P value adjusted for current BMI, activity, and alcohol consumption.

sociation with the offspring's plasma cortisol ($P = 0.07$) but did not alter the associations with social class at birth and maternal meat/fish and green vegetable consumption.

Discussion

Previous studies have related size at birth to adult plasma cortisol levels, but this is the first to examine the association between the mother's diet during pregnancy and plasma cortisol concentrations in the adult offspring. Following on from our study of maternal diet and adult blood pressure in the offspring (7), we examined the *a priori* hypothesis that, in the setting of advice to follow a pregnancy diet high in protein and low in carbohydrate, an unbalanced pattern of higher meat/fish and lower green vegetable consumption in late pregnancy leads to elevated cortisol concentrations in the offspring. In support of this hypothesis, we found higher fasting plasma cortisol concentrations in both the sons and daughters of women who had reported higher meat/fish and lower green vegetable consumption in the second half of pregnancy. Adult cortisol concentrations were also higher in those born into manual social class families.

The study was confined to subjects born in Motherwell Maternity Hospital and who still live in the area. We ex-

FIG. 2. Fasting plasma cortisol concentration in 251 men and women, 30 yr old, according to maternal meat/fish and green vegetable consumption in late pregnancy. Values are means and SE. $P = 0.047$ and $P = 0.059$ for associations with maternal meat/fish and green vegetable intake in late pregnancy, respectively.

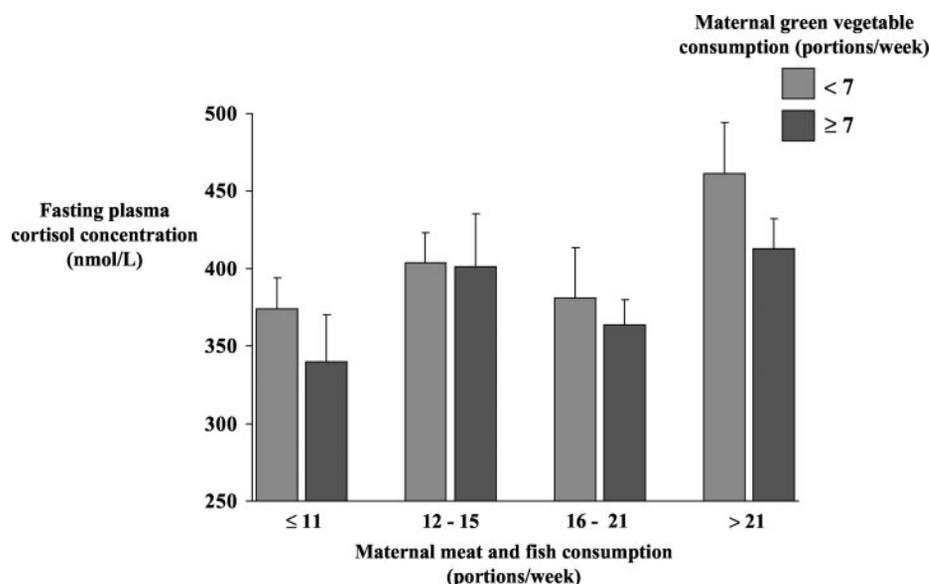


TABLE 4. Multiple linear regression analysis of the offspring's plasma cortisol concentration on the mother's consumption of meat/fish and green vegetables in late pregnancy, adjusting for offspring's gender, social class at birth, BMI, alcohol consumption, and activity level

Independent variable	Regression coefficient, % change in cortisol/1 unit change in independent variable		
	β	95% CI for β	P
Constant	295	236 to 368	<0.001
Gender			
Male	0.0	Baseline	
Female	-4.0	-12.6 to 5.6	0.40
BMI [SD score of \log^e (kg/m^2)]	-4.8	-6.8 to -2.6	<0.001
Taking vigorous activity in last 4 wk			
No	0.0	Baseline	
Yes	10.1	0.7 to 20.5	0.04
Alcohol consumption (units/wk)			
Very low (men and women, <1)	0.0	Baseline	
Low (men, 1–10; women, 1–7)	6.4	-6.3 to 20.9	0.34
Moderate (men, 11–21; women 8–14)	9.6	-4.8 to 26.2	0.20
High (men > 21, women > 14)	24	7.9 to 42.0	0.002
Social class at birth			
Nonmanual	0.0	Baseline	
Manual	12.2	1.3 to 24.4	0.03
Mother's consumption of meat/fish (portions/wk)	5.4	0.6 to 10.3	0.03
Mother's consumption of green vegetables (portions/wk)	-3.3	-7.6 to 1.1	0.14

cluded women taking oral contraception, because this substantially alters measured amounts of total plasma cortisol (19, 20). Although the men and women reported in this study represent only 17% of the original cohort of pregnancies, it is unlikely that this biased the study, because the birthweights and maternal size of the group studied was similar to that of people who had left the area or did not take part (7). Moreover, 70% of all births in the town occurred in this maternity hospital, and our analysis was based on comparisons within the sample. Bias would only have been introduced if the relationship between maternal diet and cortisol concentrations were different among people who had left the area or did not take part. The dietary data on meat and fish intakes are crude but are likely to reflect true intakes because, in a study of a small subset of women, the estimated protein intakes in late pregnancy correlated with urinary nitrogen excretion (7). Furthermore, the reduction in birthweight in

Motherwell women consuming the high-protein, low-carbohydrate diet (21) is consistent with that reported in 16 published controlled trials (5). The imprecision of the estimates of dietary intake and the use of a single fasting cortisol measurement as a measure of cortisol secretion make it likely that we have underestimated the strength of the association between prenatal exposures and the activity of the HPA axis in adult life. Future studies should use multiple cortisol measurements before and after a standardized stress challenge to provide more accurate assessments of HPA axis programming (14).

In keeping with previous studies (14, 22), we found higher fasting plasma cortisol concentrations in men and women with a lower BMI. This association is thought to reflect greater peripheral metabolism of cortisol by 5α -reductases in overweight people with a high BMI (22). We also found higher plasma cortisol concentrations in those who had taken

vigorous activity in the previous 4 wk and who reported greater alcohol consumption. The associations between maternal diet and fasting plasma cortisol concentrations in the offspring were, however, independent of current BMI, physical activity, alcohol consumption, and the other possible confounding factors that we measured. Our findings could reflect an effect of the mother's diet on the dietary preferences of her offspring; the dietary advice given to the mothers was not, however, continued into the postnatal period, and such a confounding effect seems unlikely. In our previous study, we documented that, while greater maternal meat and fish consumption was associated with higher maternal blood pressure in pregnancy, taking account of maternal blood pressure had little effect on the association between maternal diet and offspring blood pressure (7). In this study, we found that the mother's blood pressure in pregnancy was not related to the offspring's plasma cortisol concentration, and taking account of maternal blood pressure had little effect on the associations between maternal diet and offspring cortisol concentrations.

Birthweight has been shown to correlate with plasma cortisol concentrations in a number of studies (13–16). This correlation is not, however, invariable (23); and even though the high protein intakes of the women in Motherwell have previously been associated with low birthweight (21), we found no association between birthweight or other indices of size at birth and the offspring's cortisol concentrations. This accords with increasing evidence from animal studies indicating that the programming of adult physiology can occur as a result of maternal factors that either do not alter fetal growth or result in alterations in organ growth and body composition but have little or no effect on size at birth (2). Studies in human populations produce similar conclusions (3). For example, in the studies in Motherwell and Aberdeen, the effect of maternal diet on the offspring's blood pressure was independent of size at birth (6, 7).

Our findings raise the question as to the underlying processes that could link maternal consumption of a diet high in meat/fish and low in green vegetables with increased cortisol concentrations in the offspring. One of several possibilities is that meat and fish are rich in essential amino acids, which must either be used for protein synthesis or oxidized (24). Oxidation consumes nonessential amino acids, whose synthesis requires cofactors, including folate and vitamin B6. The natural sources of these cofactors include bread and potatoes, which the pregnant women in Motherwell were specifically advised to avoid; we found that low intake of green vegetables, a source of folate, accentuated the effect of high meat/fish consumption on the offspring's cortisol concentrations. In mothers with a limited capacity to synthesize nonessential amino acids, maternal amino acid oxidation could impair fetal growth as a result of reduced availability of nonessential amino acids. Consistent with this hypothesis, increased maternal amino acid oxidation during pregnancy has recently been associated with impaired fetal growth (25). Thus, in the context of advice to reduce intakes of bread, potatoes, and other carbohydrate-rich foods, consumption of a high-meat/fish, low-green-vegetable diet would impose a direct metabolic stress on the mother and fetus. This could either stimulate the fetal HPA axis directly or raise fetal

cortisol concentrations indirectly by raising maternal cortisol concentrations. This hypothesis is consistent with the observation that administration of a high-protein meal to human volunteers results in up to a 2-fold increase in salivary cortisol concentrations (8, 9). In rat studies, maternal stress or glucocorticoid administration in pregnancy have led to long-term changes in the limbic structures that control the HPA axis of the fetus and resulted in lifelong hypercortisolemia in the offspring (11, 12). The dietary data available in this study were, however, crude; and secure identification of the particular nutrients or combination of nutrients responsible for the associations we found will require studies with more detailed maternal dietary data. For example, examination of whether meat and fish have differing effects resulting from their different content of saturated and unsaturated fat would require more detailed information on the specific meat and fish products consumed by the mothers.

Our analyses found that, while adult cortisol concentrations were higher in men and women born into manual social class families, they were, however, not related to maternal height, weight, smoking, or parity or to the subject's current social class. The associations between maternal diet and the offspring's cortisol were not altered by taking account of social class at birth, suggesting that this is not a confounding influence. Though social class at birth may be a proxy for other environment effects that program HPA responses in the offspring, the association requires replication because it was not an *a priori* hypothesis in our study. Nonetheless, the association is consistent with the observation that children in low socioeconomic status families have higher salivary cortisol levels when compared with their higher socioeconomic status counterparts (26). Our finding raises the possibility of an important early-life exposure; but, in the absence of more detailed data on the mother and her infant, interpretation of the association is speculative (many early-life experiences differ between manual and nonmanual social class infants). Possible exposures worthy of examination in future studies include aspects of parenting and dietary influences not measured in this study, such as the source and quality of the meat and fish consumed by the mother.

The mothers of the men and women in this study had been given particular dietary advice: to eat 1 pound (0.45 kg) of red meat daily and avoid carbohydrate-rich foods as a means to avoid preeclampsia. The mother's intakes of carbohydrate-rich foods were remarkably low and were associated with low weight gain in pregnancy (7). This precludes direct application of our findings to other populations. We estimate that the mothers' meat and fish intakes would have given them mean daily animal protein intakes of around 58 g, which is at the upper end of those recorded in our recent prospective studies of pregnant women in Southampton, UK (27). However, the associations between meat/fish intakes and the offspring's cortisol concentrations occurred across the range of intakes and were not confined to the group with unusually high intakes (Fig. 2).

We conclude that the offspring of mothers who reported high meat/fish and low green vegetable intakes in late pregnancy tend to have increased adult fasting plasma cortisol concentrations. Because the mothers in this study were given particular advice to follow a high-protein, low-carbohydrate

diet in pregnancy, our findings cannot be applied directly to other populations. Nonetheless, our findings provide the first human evidence that an unbalanced high-meat/fish, low-green-vegetable diet during pregnancy may present a metabolic stress to the mother and program the HPA axis of the offspring, leading to lifelong hypercortisolemia. Further studies are needed to replicate the findings and to identify the nutrient or combination of nutrients responsible for the effect.

Acknowledgments

We thank the men and women in Motherwell who took part in the study. We also thank the research staff, Thora Walker, Elaine Armstrong, Karen Kennedy, Jamesina Hoey, and Ria Skelton. We thank Lin Lapper, Anne Lithgow, and Lois Kastner, who abstracted the data. Vanessa Cox processed the data.

Received February 20, 2003. Accepted April 14, 2003.

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This work was supported by grants from the Dunhill Medical Trust and the NIH (1-R01-HD41107-01).

References

1. **Barker DJP, ed.** 2000 Fetal origins of cardiovascular, lung disease. New York: National Institutes of Health
2. **O'Brien PMS, Wheeler T, Barker DJP, eds.** 1999 Fetal programming. London: Royal College of Obstetricians and Gynaecologists
3. **Godfrey KM** 2001 The gold standard for optimal fetal growth and development. *J Pediatr Endocrinol Metab* 14:1507–1513
4. **Rush D, Stein Z, Susser M** 1980 A randomised controlled trial of prenatal nutritional supplementation in New York City. *Pediatrics* 65:683–697
5. **Rush D** 1989 Effects of changes in maternal energy and protein intake during pregnancy, with special reference to fetal growth. In: Sharp F, Fraser R, Milner D, eds. Fetal growth. London: Royal College of Obstetricians and Gynaecologists; 203–229
6. **Campbell DM, Hall MH, Barker DJ, Cross J, Shiell AW, Godfrey KM** 1996 Diet in pregnancy and the offspring's blood pressure 40 years later. *Br J Obstet Gynaecol* 103:273–280
7. **Shiell AW, Campbell-Brown M, Haselden S, Robinson S, Godfrey KM, Barker DJ** 2001 High-meat, low-carbohydrate diet in pregnancy: relation to adult blood pressure in the offspring. *Hypertension* 38:1282–1288
8. **Gibson EL, Checkley S, Papadopoulos A, Poon L, Daley S, Wardle J** 1999 Increased salivary cortisol reliably induced by a protein-rich midday meal. *Psychosom Med* 61:214–224
9. **Slag MF, Ahmad M, Gannon MC, Nuttall FQ** 1981 Meal stimulation of cortisol secretion: a protein induced effect. *Metabolism* 30:1104–1108
10. **Anderson KE, Rosner W, Khan MS, New MI, Pang SY, Wissel PS, Kappas A** 1987 Diet-hormone interactions: protein/carbohydrate ratio alters reciprocally the plasma levels of testosterone and cortisol and their respective binding globulins in man. *Life Sci* 40:1761–1768
11. **Barbazanges A, Piazza PV, Le Moal M, Maccari S** 1996 Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. *J Neurosci* 16:3943–3949
12. **Levitt NS, Lindsay RS, Holmes MC, Seckl JR** 1996 Dexamethasone in the last week of pregnancy attenuates hippocampal glucocorticoid receptor gene expression and elevates blood pressure in the adult offspring in the rat. *Neuroendocrinology* 64:412–418
13. **Phillips DI, Barker DJ, Fall CH, Seckl JR, Whorwood CB, Wood PJ, Walker BR** 1998 Elevated plasma cortisol concentrations: a link between low birth weight and the insulin resistance syndrome? *J Clin Endocrinol Metab* 83:757–760
14. **Reynolds RM, Walker BR, Syddall HE, Andrew R, Wood PJ, Whorwood CB, Phillips DI** 2001 Altered control of cortisol secretion in adult men with low birth weight and cardiovascular risk factors. *J Clin Endocrinol Metab* 86:245–250
15. **Phillips DI, Walker BR, Reynolds RM, Flanagan DE, Wood PJ, Osmond C, Barker DJ, Whorwood CB** 2000 Low birth weight predicts elevated plasma cortisol concentrations in adults from 3 populations. *Hypertension* 35:1301–1306
16. **Cianfarani S, Geremia C, Scott CD, Germani D** 2002 Growth, IGF system, and cortisol in children with intrauterine growth retardation: is catch-up growth affected by reprogramming of the hypothalamic-pituitary-adrenal axis? *Pediatr Res* 51:94–99
17. **White A, Nicolaas G, Foster K, Browne F, Carey S** 1993 Health Survey for England 1991; London: The Stationary Office Books
18. **Moore A, Aitken R, Burke C, Gaskell S, Groom G, Holder G, Selby C, Wood P** 1985 Cortisol assays: guidelines for the provision of a clinical biochemistry service. *Ann Clin Biochem* 22:435–454
19. **Kirschbaum C, Kudielka BM, Gaab J, Schommer NC, Hellhammer DH** 1999 Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosom Med* 61:154–162
20. **Kirschbaum C, Pirke K, Hellhammer D** 1995 Preliminary evidence for reduced cortisol responsivity to psychological stress in women using oral contraceptive medication. *Psychoneuroendocrinology* 20:509–514
21. **Campbell-Brown M, Johnstone FD, Kerr Grieve JF** 1986 The effect on birth-weight of a high-protein, low carbohydrate diet during pregnancy. *Proc Nutr Soc* 45:90A
22. **Andrew R, Phillips DI, Walker BR** 1998 Obesity and gender influence cortisol secretion and metabolism in man. *J Clin Endocrinol Metab* 83:1806–1809
23. **Kajantie E, Phillips DI, Andersson S, Barker DJ, Dunkel L, Forsen T, Osmond C, Tuominen J, Wood PJ, Eriksson J** 2002 Size at birth, gestational age and cortisol secretion in adult life: foetal programming of both hyper- and hypocortisolism? *Clin Endocrinol (Oxf)* 57:635–641
24. **Jackson AA** 1999 Maternal and fetal demands for nutrients: significance of protein metabolism. In: O'Brien PMS, Wheeler T, Barker DJP, eds. Fetal programming. London: Royal College of Obstetricians and Gynaecologists; 246–270
25. **Duggleby SL, Jackson AA** 2002 Higher weight at birth is related to decreased maternal amino acid oxidation during pregnancy. *Am J Clin Nutr* 76:852–857
26. **Lupien SJ, King S, Meaney MJ, McEwen BS** 2000 Child's stress hormone levels correlate with mother's socioeconomic status and depressive state. *Biol Psychiatry* 48:976–980
27. **Robinson S, Godfrey K, Osmond C, Cox V, Barker DJP** 1996 Evaluation of a food frequency questionnaire used to assess nutrient intakes in pregnant women. *Eur J Clin Nutr* 50:302–308