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Does maternal nutrition in pregnancy and birth weight influence levels of CHD risk factors in adult life?

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The fetal-origins hypothesis suggests that maternal and fetal nutrition can have a profound and sustained impact on the health of the offspring in adult life. Although there is abundant literature reporting on the associations between birth weight and disease risk factors, only a handful of studies have been able to examine the relationship between maternal nutrition in pregnancy with the health of offspring in adult life directly. Between 1942 and 1944, nearly 400 pregnant women were recruited into a dietary study to determine whether the wartime dietary rations were sufficient to prevent nutritional deficiencies. Detailed biochemical and clinical assessments were conducted for each of the women, who were followed-up until after delivery. More than 50 years later, approximately one-quarter of the adult offspring were recruited into a study to explore the possible impact of maternal nutrition in pregnancy on CHD risk factors, including glucose tolerance, blood pressure and components of the lipid profile. Results from the present study provide no evidence to support the hypothesis that birth weight or maternal nutrition in pregnancy are associated with CHD risk factors in adult life.

Birth weight: Coronary heart disease: Pregnancy: Fetal nutrition

Over the last 20 years, evidence from both human epidemiological and animal studies has accumulated in support of the fetal-origins hypothesis, which proposes that suboptimal maternal and fetal nutrition can have a profound and sustained impact on the health of the individual in adult life (Barker, 1993). Much of the data in support of this hypothesis have been derived from retrospective cohort studies reporting on the associations between low birth weight, used as a surrogate marker of poor maternal and fetal nutrition, with increased levels of CHD and its associated risk factors in adult offspring (Barker, 1993).

Whereas there is a large body of literature relating the impact of maternal nutritional status (i.e. maternal pre-pregnancy BMI, weight gain in pregnancy, diet in pregnancy) on subsequent birth outcome, such as gestational age (Rondo *et al.* 1997), birth weight (Neggers *et al.* 1997) and some other measures of fetal growth (Thame *et al.* 1997), much less is known about the possible impact of maternal diet in pregnancy on the health of offspring in adult life. Possible insights have chiefly come from a small number of ecological and observational studies supported by evidence from animal dietary manipulation studies in pregnancy. For example, individuals who were conceived or born during the Dutch Winter Famine of 1944 have been suggested to be at higher risk of developing several cardiovascular risk factors, including hypertension, impaired glucose tolerance and obesity (Ravelli *et al.* 1976, 1998). However, data from the Leningrad Siege study provide little support for the hypothesis that exposure to severe maternal undernutrition during pregnancy increases an individual's likelihood of being at increased cardiovascular risk in adulthood (Stanner *et al.* 1997). Aside from these ecological studies, in which maternal dietary intakes could not be measured directly, only a few studies have been able to examine the effects of different components of the maternal diet in pregnancy on the subsequent health of adult offspring (Campbell *et al.* 1996; Belizan *et al.* 1997; Shiell *et al.* 2000; Adair *et al.* 2001). The results from these studies, however, are disparate, with both low and high maternal intakes of protein being associated with an adverse health outcome in adult offspring.

The Oxford Nutrition Survey was a nutrition study conducted by Hugh Sinclair in Oxford, UK, during World War 2, undertaken at the behest of the British Government (Huxley *et al.* 2000). At the time, the Ministry of Health was concerned about the adequacy of the wartime rationing system to meet the necessary dietary requirements of various subgroups of the population that were considered most vulnerable to dietary deficiencies, such as pregnant women and children. Between 1942 and 1944, nearly 400 pregnant women were recruited into the Oxford Nutritional Survey: they underwent detailed biochemical evaluation and were followed up until after they had delivered.

We have since traced the adult offspring of the Oxford Nutritional Survey and invited them to participate in the present study, the main purpose of which was to investigate

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whether specific components of the maternal diet influence the levels of physiological markers of risk of CHD in adult life. This retrospective cohort is, to our knowledge, one of the few studies that has been able to explore the association between levels of biochemical markers of maternal nutrition in pregnancy with CHD risk factors in adult offspring directly.

Study methods

Maternal characteristics

Two groups of pregnant women were recruited in Oxfordshire, UK, between April and August 1942 and March and August 1944 and followed up until after delivery. Full details of their recruitment are given elsewhere (Huxley *et al.* 2000). The first group consisted of 120 working-class pregnant women who were in their third trimester of pregnancy and the second group comprised 253 predominantly working-class women in various stages of pregnancy (14 % first trimester, 44 % second trimester and 42 % third trimester). Information on birth weight, parental social class and indicators of early-life environment (e.g. method of infant feeding, number of dependent persons in household) were recorded on the original case-report forms. In addition, plasma concentrations of a number of biochemical markers of maternal nutrition, including Hb, erthyrocyte count, protein, vitamins A and C, phosphatase and thiamin were measured. Details about the wartime recruitment and the biochemical information collected on the pregnant women are described more fully elsewhere (Huxley *et al.* 2000).

Laboratory methods

Full details about the biochemical assays that were performed on the maternal blood samples are published elsewhere (Huxley *et al.* 2000). In brief, vitamin C was measured colorimetrically after the plasma was oxidized and treated with hydrazine (Roe & Kuether, 1943). This assay has good precision and a sensitivity of 0·5 mg/l. Vitamin A was measured using the Carr–Price method (Kimble, 1939, cited in HM Sinclair, unpublished results), and carotene was estimated colorimetrically (Kimble, 1941, cited in HM Sinclair, unpublished results). These methods are imprecise and are non-specific, with a low sensitivity and are influenced by moisture. Protein was estimated by the biuret method (Kingsley, 1942, cited in HM Sinclair, unpublished results), which has a sensitivity of 1–15 mg/kg protein. Plasma phosphatase was measured colorimetrically (King *et al.* 1942, cited in HM Sinclair, unpublished results), a method which has a poor sensitivity and specificity due to reagent instability. Urinary riboflavin was estimated fluorimetrically (Huff & Perlzweig, 1943). This assay lacks precision and sensitivity as the fluorimetric endproduct is very unstable. Packed cell volume was assessed using the densitometric method (Wintrobe, 1933, cited in HM Sinclair, unpublished results). It has high specificity, sensitivity and precision. The Oxford Nutrition Survey used 'arbitrary' values for classifying subjects based on these biochemical assays as 'normal', 'low' and 'very low'. These values were assigned by Sinclair, but there is good agreement between the biochemical ranges that were categorized as 'normal' by the Oxford Nutrition Survey standards with more recent reference ranges quoted in the published literature (Thomas, 1994).

Participants in the follow-up study

Of 373 deliveries, there were 361 live deliveries and twelve stillbirths. Ethics committee approval was obtained before the surviving adult offspring were traced and identified using birth certificates obtained from the Office of National Statistics and National Health Service numbers. A total of 248 offspring were traced (69 %): of these fourteen had died and twenty-three had emigrated, leaving a total of 211 individuals who were living in the UK. The general practitioners for these individuals were approached and asked to provide the contact details for their patient (*n* 196), who then received a letter of invitation outlining the nature of the study and asking if they would like to participate. In total, 137 offspring (37 % of entire cohort) were recruited into the study (Fig. 1). Postal addresses of all traced individuals were supplied to QAS Systems, Manchester, UK, which used census data to classify individuals according to the Townsend deprivation index for the place of residence. This allowed a comparison to be made between those individuals who participated in the study and those individuals who declined to participate.

Methods of assessment

Individuals were visited at home where a modified version of a previously validated lifestyle questionnaire (Barker *et al.* 1990), containing sections on education level attained, health (both parental and of the individual), smoking, drinking and exercise habits, was administered. The subject's height was measured without shoes using a stadiometer and body weight measured while wearing light indoor clothing using electronic scales (Seca model 200; Seca Ltd, Birmingham, UK). The individual's blood pressure was measured after the subject had remained seated for 5 min, using an electronic sphygmomanometer (UA-731; A&D Company, Tokyo, Japan). The process was repeated three times with at least 5 min between readings, and the mean value and standard deviation of the three readings were calculated. All of these home-procedures were performed by the same researcher (R. H.).

Subjects were invited to attend a clinic for a clinical evaluation and were advised that it would involve a standard oral glucose tolerance test, collection of a 15 ml fasting venous blood sample and three consecutive early morning urine samples. During the clinical assessment the subject was invited to undergo 24 h ambulatory blood pressure monitoring. After consent was obtained, the monitor was fitted on the individual's non-dominant arm and automatically inflated at 30 min intervals. Subjects were requested to be seated during the readings and to refrain from participating in any physical exercise whilst wearing the monitor. A 24 h recording chart was provided to record the hours of sleep as well as any event that may have

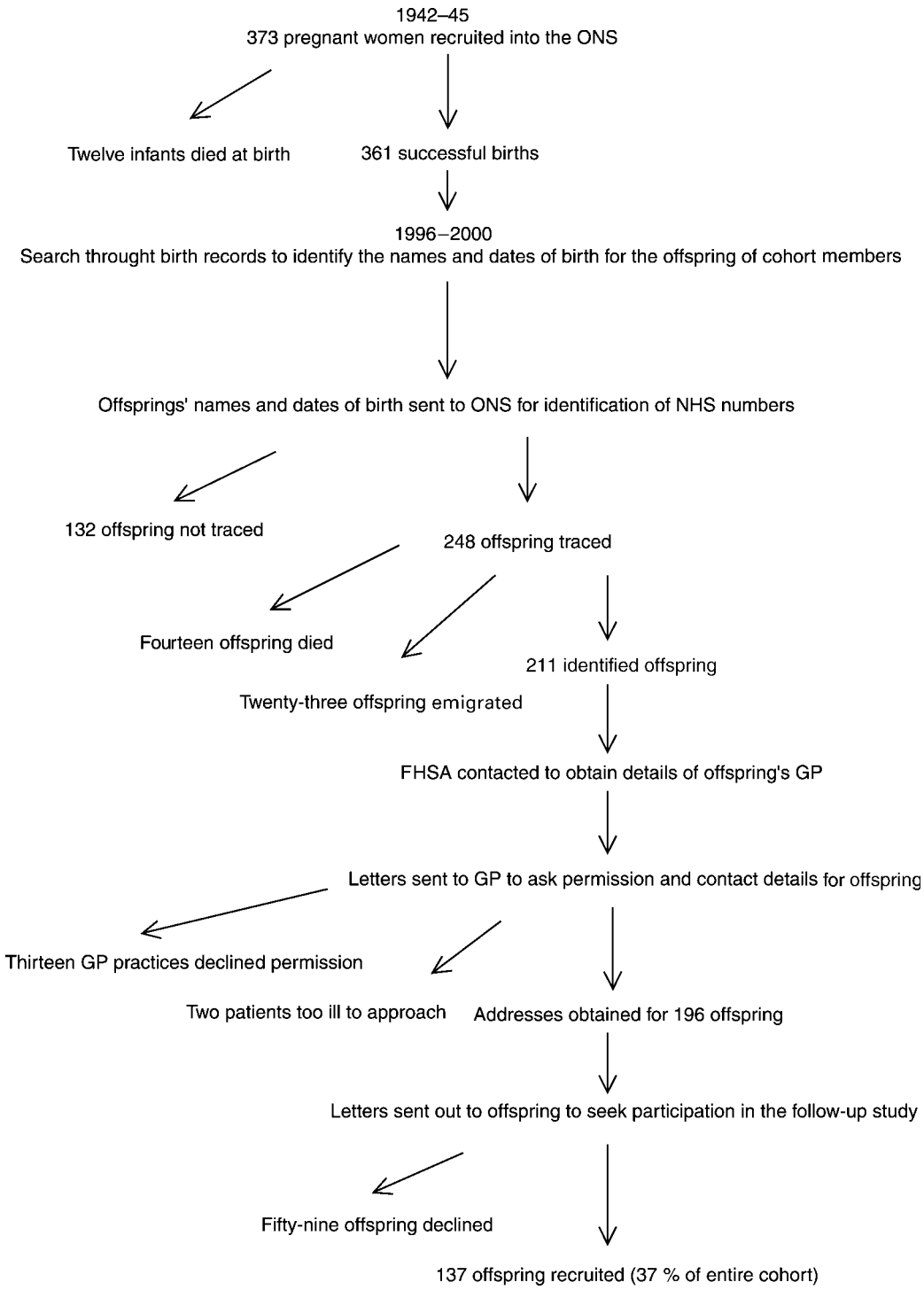

Fig. 1. Flow chart showing the identification, tracing and recruitment of the adult offspring of members of the Oxford Nutrition Survey (ONS; for details, see p. 460). NHS, National Health Service; FHSA, Family Health Services Authority; GP, general practitioner.

unduly affected blood pressure readings during the 24 h period.

Laboratory analyses

The 15 ml blood sample was centrifuged at 4000 rpm for 10 min. Portions of the serum were then placed into 2 ml Apex tubes (CamLab Ltd, Cambridge, UK) containing one of the following preservatives: fluoride for glucose, heparin for insulin, proinsulin and leptin, EDTA for LDL- and HDL-cholesterol and fibrinogen. Samples of urine (15 ml) were pipetted into 50 ml containers (CamLab Ltd) containing the preservative thiomersal. The tubes were transferred to a −70°C freezer for storage, before being

transferred to the laboratories for analysis. The biochemical assays, except cortisol, were performed by the Diabetes Research Laboratories at the Radcliffe Infirmary, Oxford, UK. Cortisol was analysed by the Department of Clinical Biochemistry at Addenbrooke's Hospital, Cambridge, UK.

Glucose was measured by an enzymatic, colorimetric endpoint method using a Beckman Synchron CX4 Delta analyser (Beckman Instruments UK Ltd, High Wycombe, Bucks., UK). Glucose concentration is directly proportional to change in absorbance due to production of NADH by hexokinase and glucose-6-phosphate dehydrogenase. The change in absorbance is detected by the analyser. The reference range for adult fasting glucose is 3·9–5·8 mmol/l. Impaired glucose tolerance was defined as fasting glucose $<$7·8 mmol/l with a 120 min glucose level between 7·8–11·0 mmol/l. The interassay CV was 0·8 %. Insulin and proinsulin were measured using a double antibody radio-immunoassay supplied by Pharmacia Ltd (Amersham, Bucks., UK) and Diagenics Ltd (Nottingham, UK) respectively. The adult fasting reference range of insulin is 21·5–115·0 pmol/l; there is currently no reference range for proinsulin. The CV for these assays were 8·9 for insulin and 7·0 % for proinsulin.

Leptin was assayed using a double antibody radio-immunoassay technique (Linco Human Leptin RIA Kit; Linco Research Inc., St Charles, MO, USA). There is no reference range for leptin levels in adults. The interassay CV was 6·2 %. Total cholesterol was measured using an enzymatic colorimetric endpoint method (Beckman synchron CXR systems CHOL 2 × 300 cholesterol reagent; Beckman Instruments UK Ltd). LDL-cholesterol was assayed using an enzymatic method (Genzyme N-geneous LDL-c kit; Beckman Instruments UK Ltd). HDL-cholesterol was assayed using a homogeneous method with a two-reagent format (N-geneous HDL cholesterol 'Biostat Direct HDL'; Beckman Instruments UK Ltd). Triacylglycerol was assayed using an enzymatic endpoint method (Beckman Synchron systems triglycerides GPO reagent; Beckman Instruments UK Ltd). The reference ranges for cholesterol, HDL-cholesterol, LDL-cholesterol and triacylglycerol are $<$6·5, 0·91–1·69, 1·5–5·3 and 0·4–1·8 mmol/l respectively and the interassay CV were 1·3, 2·1, 1·4 and 2·0 %, respectively. Rabbit anti-human fibrinogen antisera (DAKO Ltd, Ely, Cambs., UK) were added to precipitate fibrinogen to produce a turbid solution, the absorbance of which was calculated. There is no reference range for fasting levels of fibrinogen. The interassay CV was 3·3 %. Cortisol was measured using the DELFIA solid-phase time-resolved fluoroimmunoassay. As the secretion of cortisol fluctuates with a circadian rhythm, normally peaking in the morning after waking and reaching its lowest point during sleeping, there are two reference ranges for cortisol depending upon the time when the sample was taken. From 09.00 hours, the reference range is 280–650 nmol/l and from 00.00 hours the reference range is $\leq$200 nmol/l. The CV for this assay is 2·4 %. To determine the prevalence of microalbuminuria, levels of urinary albumin and creatinine were measured in three consecutive early morning urine samples. Each sample was collected and stored in 50 ml urine bottles. The adult fasting reference range for albumin is $<$64 mg/l. The interassay CV for urinary albumin was 4·1 % and for urinary creatinine 3·6 %.

Statistical analyses

Univariate relationships between birth weight, maternal markers of nutrition, offspring's blood pressure (both home and ambulatory) and physiological markers of CHD, were assessed using Pearson's correlation coefficients for normally distributed continuous variables and Spearman's correlation coefficients for ordinal variables. Where necessary, variables with skewed distributions were logarithmically transformed to approximate normality, which was assessed using Probit plots. Due to the large number of correlations performed, thereby increasing the risk of generating spurious statistical associations, Bonferonni adjustments were used to minimize the risk of obtaining false positive results. Stepwise multiple regression models were constructed with systolic blood pressure (home and ambulatory), fasting cholesterol (total and HDL), fasting triacylglycerol, fasting glucose, glucose at 120 min, fasting insulin, insulin at 120 min, fasting fibrinogen, fasting leptin, fasting cortisol and mean albumin:creatinine ratio as dependent variables; birth weight, together with the offspring's age, gender, BMI, childhood social class and current social class were independent predictor variables. Independent variables were considered predictors of the dependent variable if they attained significance at $P<0·05$ in the regression models.

A retrospective power calculation was performed on the number of individuals recruited into the study (n 137). Based on this sample size, the study had 80 % power at a 5 % level of statistical significance to detect a 6 mmHg difference in systolic blood pressure between the offspring of those mothers who were found to have had both a low protein and Hb concentration (40 % of subjects) (Huxley *et al.* 2000) and those who had a normal protein and Hb concentration (60 % of subjects) assuming a standard deviation of 12 mmHg about the difference in blood pressure between the two groups.

Results

Maternal biochemical profiles of women whose offspring were recruited into the study

A full description of the results from the wartime survey is reported elsewhere (Huxley *et al.* 2000); for the purposes of this present study only the biochemical findings from those women whose offspring were recruited into the follow-up study will be discussed. A total of 137 offspring whose mothers were participants in the wartime dietary survey were traced and recruited into the present follow-up study. Analysis of the biochemical information collated during the original wartime survey indicated that a large proportion of these women had erythrocyte counts and packed cell volume scores below the normal levels expected for pregnant women (Fig. 2), suggesting a possible deficiency in dietary intake of Fe. Similarly, more than half of the women had phosphatase concentrations

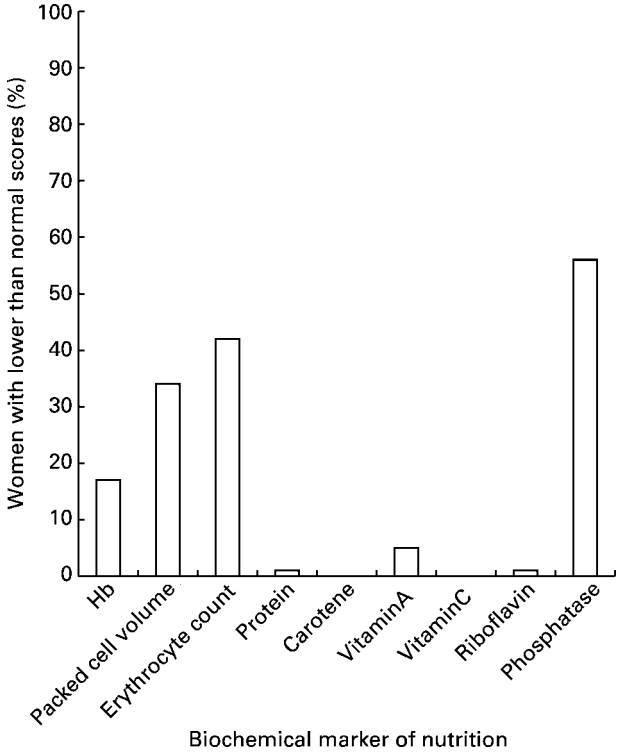

Fig. 2. Proportion of pregnant women who participated in the wartime dietary survey (1942–45) who had biochemical values outside the normal reference ranges for biochemical markers of nutrition.

outside the normal reference range, suggestive of possible deficiencies in Ca and vitamin D, but the data are difficult to interpret as fetal bone formation can also elevate concentrations of plasma phosphatase. However, nearly all of the women had normal concentrations of protein, carotene and vitamins A, C and riboflavin.

Characteristics of adult participants in follow-up study

A total of 137 individuals (sixty men and seventy-seven women) were recruited into the current study of which ninety-four (69 %) individuals were available for clinical assessment. There was no statistical evidence to suggest any difference in social class between the 137 study participants and the remaining 111 non-participants (results not shown). The mean age of the study participants was 55·4 (SD 1·2) years and approximately one-third of the men and one-fifth of the women were current smokers. As shown in Table 1, the majority of the study participants were in the higher socio-economic groups, whereas < 15 % of study participants were in groups 4 and 5, suggesting an under-representation of lower socio-economic groups. The distributions of the physiological and biochemical variables for these individuals are shown in Tables 1, 2 and 3.

Relationship of birth weight with biochemical indices of maternal nutrition and early-life environment

Information on birth weight was available for 131 (96 %) offspring. The mean birth weight was 3293 (SD 401) g, with boys being significantly heavier than girls (mean difference 217 (95 % CI 50, 385) g. In univariate analyses, birth weight was positively correlated with maternal levels of riboflavin (r 0·53, $P<0·001$), mother's parity (r 0·36, $P=0·01$), number of household dependants (r 0·23, $P=0·06$) and with the child:adult ratio in the wartime household (r 0·19, $P=0·06$), and negatively correlated with maternal Hb concentration (r −0·15, $P=0·90$). However, none of these variables remained significant predictors of birth weight in a multiple regression model.

Table 1. Characteristics of the study participants
(Mean values and standard deviations)

	Men			Women		
Characteristic	*n*	Mean	SD	*n*	Mean	SD
Age (years)	60	55·7	1·2	77	55·2	1·2
BMI (kg/m^2)	59	28·2	4·4	75	26·7	5·0
Current smokers (%)	18	30·0		16	20·8	
Social class (%)						
1	12	20·0		0	0	
2	19	31·6		21	29·2	
3	21	35·0		35	48·6	
4	6	10·0		8	11·1	
5	1	1·7		5	6·9	
Unclassified	1	1·7		3	4·2	
Blood pressure (mmHg)*						
Home SBP	47	140·3	20·1	63	127·3	17·6
Home DBP	47	84·6	10·4	63	79·9	10·8
Ambulatory awake SBP	23	126·1	13·4	28	119·8	10·2
Ambulatory awake DBP	23	80·7	6·4	28	76·9	7·7

SBP, systolic blood pressure; DBP, diastolic blood pressure.
* Excluding individuals on antihypertensive medication.

Relationship of adult blood pressure with birth weight, biochemical markers of maternal nutrition in pregnancy and adult lifestyle factors

Measurements of home blood pressure were available for 131 (96 %) of participants in the present study. No data were available for six individuals who were unable to be visited at home but who either completed the questionnaire

Table 2. Normally distributed physiological variables in study participants*
(Mean values and standard deviations)

	Men (*n* 42)		Women (*n* 50)	
Variable	Mean	SD	Mean	SD
Glucose (mmol/l)†				
Fasting	5·70	1·45	5·11	0·45
30 min	8·92	2·08	8·24	1·50
120 min	6·65	3·17	6·01	1·53
Cholesterol (mmol/l)				
Total	5·27	0·95	5·35	0·94
HDL-cholesterol	1·20	0·34	1·47	0·34
LDL-cholesterol	3·33	0·78	3·26	0·77
Cortisol (nmol/l)	365·7	90·1	355·8	113·5
Fibrinogen (g/l)	3·03	0·51	3·23	0·61

* For details of subjects, see Table 1.
† Oral glucose tolerance test.

Table 3. Non-normally distributed physiological variables in study participants*

(Median values and interquartile ranges)

	Men (*n* 42)		Women (*n* 50)	
Variable	Median	IQR	Median	IQR
Proinsulin (pmol/l)	8·10	6·05, 16·5	5·35	4·08, 7·30
Insulin (pmol/l)†				
Fasting	68·9	57·8, 116·1	58·5	47·3, 77·4
30 min	319·2	231·3, 491·2	342·2	250·6, 511·4
120 min	285·3	163·9, 505·0	263·7	169·8, 349·6
Triacylglycerol (mmol/l)	1·22	0·80, 1·63	0·95	0·69, 1·24
Leptin (mmol/l)	413·0	290·8, 683·6	1061·7	821·1, 1635·9
Urine albumin: creatinine ratio	0·34‡	0·24, 0·56	0·33‡	0·24, 0·68

IQR, interquartile range.
* For details of subjects, see Table 1.
† Oral glucose tolerance test.
‡ Mean value.

Table 4. Association of birth weight with physiological CHD risk factors in study participants*

		Unadjusted correlation coefficient†		Adjusted correlation coefficient‡	
Variable	*n*	*r*	*P*	*r*	*P*
Blood pressure					
Home SBP	110	0·06	0·51	−0·01	0·98
Home DBP	110	0·06	0·57	0·00	1·00
Ambulatory SBP	51	0·10	0·18	0·14	0·41
Ambulatory DBP	51	0·21	0·14	0·02	0·92
Glucose§					
Fasting	92	0·03	0·75	−0·01	0·96
30 min	92	−0·06	0·56	−0·00	0·99
120 min	92	0·02	0·88	−0·11	0·43
Proinsulin‖	91	0·08	0·50	0·06	0·68
Insulin§					
Fasting	91	−0·03	0·75	−0·09	0·52
30 min	91	−0·02	0·84	0·16	0·27
120 min	91	−0·22	0·05	−0·18	0·22
Cortisol‖	92	−0·08	0·48	0·01	0·96
Total cholesterol	92	0·11	0·31	0·07	0·64
LDL-cholesterol‖	92	0·14	0·21	0·04	0·79
HDL-cholesterol‖	92	−0·09	0·41	−0·12	0·41
Triacylglycerol‖	92	0·01	0·94	−0·02	0·90
Fibrinogen‖	92	−0·01	0·96	0·15	0·30
Leptin‖	73	−0·19	0·11	−0·20	0·17
Urine albumin: creatinine ratio‖	105	−0·14	0·17	0·20	0·17

SBP, systolic blood pressure; DBP, diastolic blood pressure.
* For details of subjects, see Table 1.
† Excluding individuals on antihypertensive medication.
‡ Adjusted for individual's age, gender, BMI, current social class and smoking status.
§ Oral glucose tolerance test.
‖ Fasting.

(by post) or visited the clinic for assessment. A total of 21 (15 %) individuals were taking prescribed antihypertensive medication. Categorical analysis showed there was no difference in birth weight between those individuals taking prescribed antihypertensive medication and those not on blood-pressure-lowering treatment ($P=0{\cdot}48$). The following analyses excluded individuals on antihypertensive medication. The results of the univariate analyses indicated that birth weight was not associated with home measures of blood pressure in this cohort, either before or after adjustment for potential confounders (Table 4). Similarly, there was no evidence that blood pressure was correlated with any measure of maternal nutrient status (results not shown). In multiple regression models there was some evidence that individuals whose parents were in a higher social group had a tendency towards higher blood pressures in later life (Table 5), but the only significant predictors of systolic blood pressure were gender and current body weight (Table 5).

Twenty-four h ambulatory blood pressure monitoring data was available for sixty-four individuals (thirty men and thirty-four women). Overall compliance with the monitoring was high, with most individuals (*n* 61) having at least forty valid 30 min recordings over the 24 h period. Thirteen individuals on prescribed antihypertensive medication were excluded from the analyses. In multiple regression models, there was no evidence that birth weight was associated with ambulatory blood pressure in this cohort (Table 4). There was also no apparent association between any biochemical indices of maternal nutrition with ambulatory blood pressure (results not shown).

Association of birth weight, adult lifestyle factors and maternal markers of nutrition with offspring's CHD biochemical risk profile

Glucose tolerance. A total of ninety-four individuals underwent an oral glucose tolerance test and two diabetic subjects were excluded from the analysis. In univariate analyses, there was no evidence to suggest that birth weight was associated with plasma glucose levels at 0, 30 or 120 min after a glucose load. In multiple regression models, only current body weight was a significant predictor of fasting glucose and glucose 120 min after a glucose load. Fasting insulin and insulin at 30 min after an oral glucose load were unrelated to birth weight in univariate analyses. However, birth weight was weakly and inversely associated with insulin at 120 min in univariate analyses, but the association was weakened after adjustment for current body size, age and gender (Table 6). In a multiple regression model, birth weight was weakly inversely associated with insulin at 120 min after an oral glucose load, such that a 1 kg higher birth weight was associated with a 92·5 (95 % CI −12·6, 197·6) pmol/l lower insulin concentration at 120 min. No biochemical marker of maternal nutrition was associated with either glucose or insulin levels at any time point during the oral glucose tolerance test in this cohort (results not shown).

Lipids. Measurements for components of the fasting lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol and triacylglycerol) were available for ninety-two individuals. Birth weight was unrelated to any component of the lipid profile in univariate analyses (Table 4). In multiple regression models, parental social class, gender and current body size were significant predictors of HDL-cholesterol, such that individuals whose

Table 5. Multiple regression analysis of CHD risk factors with birth weight, current weight, age, gender and measures of parental and current social class*

Dependent variable	β	95 % CI	Statistical significance of effect (*P* value)
Home systolic blood pressure (mmHg)			
Birth weight (kg)	−0·8	−5·3, 3·7	0·7
Age (years)	1·9	−9·8, 4·8	0·2
Gender	−9·9	−17·2, −2·6	0·009
BMI (kg/m^2)	1·5	0·7, 2·3	0·00
Parental social class	−4·0	−8·4, 0·5	0·08
Current social class	−0·1	−0·3, 0·2	0·6
Ambulatory systolic blood pressure (mmHg)			
Birth weight (kg)	2·3	−4·4, 8·9	0·5
Age (years)	−1·0	−3·7, 1·8	0·5
Gender	−4·6	−11·6, 2·3	0·2
BMI (kg/m^2)	0·2	−0·4, 0·8	0·5
Parental social class	2·9	−1·5, 7·3	0·2
Current social class	−0·04	−0·2, 0·1	0·6
Fasting cholesterol (mmol/l)			
Birth weight (kg)	0·2	−0·2, 0·5	0·3
Age (years)	0·004	−0·2, 0·2	1·0
Gender	0·1	−0·3, 0·6	0·5
BMI (kg/m^2)	0·007	−0·04, 0·05	0·8
Parental social class	−0·1	−0·4, 0·2	0·4
Current social class	−0·001	−0·2, 0·01	0·9
Fasting HDL-cholesterol (mmol/l)			
Birth weight (kg)	−0·04	−0·1, 0·06	0·4
Age (years)	−0·003	−0·06, 0·06	0·9
Gender	0·2	0·04, 0·3	0·01
BMI (kg/m^2)	−0·03	−0·04, −0·01	0·00
Parental social class	−0·1	−0·2, −0·03	0·01
Current social class	0·0001	−0·004, 0·004	1·0
Fasting triacylglycerol (mmol/l)			
Birth weight (kg)	−0·04	−0·3, 0·2	0·8
Age (years)	−0·007	−0·1, 0·1	0·9
Gender	−0·2	−0·5, 0·2	0·3
BMI (kg/m^2)	0·04	0·01, 0·08	0·01
Parental social class	0·3	0·05, 0·5	0·02
Current social class	0·0009	−0·01, 0·01	0·9

* For details of subjects, see Table 1.

Table 6. Multiple regression analysis of CHD risk factors with birth weight, current weight, age, gender and measures of parental and current social class*

Dependent variable	β	95 % CI	Statistical significance of effect (*P* value)
Fasting insulin (pmol/l)			
Birth weight (kg)	−13·3	−41·3, 14·7	0·3
Age (years)	−1·4	−18·2, 15·4	0·9
Gender	−44·5	−85·6, −3·4	0·03
BMI (kg/m^2)	11·2	7·4, 15·0	0·00
Parental social class	−3·3	−30·3, 23·8	0·8
Current social class	0·3	−1·0, 1·5	0·6
Fasting proinsulin (pmol/l)			
Birth weight (kg)	−1·2	−13·3, 10·9	0·8
Age (years)	0·3	−6·2, 6·9	0·9
Gender	−14·0	−29·9, 1·9	0·08
BMI (kg/m^2)	3·8	2·3, 5·2	0·00
Parental social class	−6·2	−16·6, 4·1	0·2
Current social class	0·05	−0·4, 0·5	0·8
Insulin 120 min (pmol/l)			
Birth weight (kg)	−92·5	−197·6, 12·6	0·08
Age (years)	19·8	−41·6, 18·2	0·5
Gender	−105·0	−254·1, 44·2	0·2
BMI (kg/m^2)	29·8	16·7, 43·0	0·00
Parental social class	33·4	−67·9, 134·7	0·5
Current social class	−0·8	−5·2, 3·7	0·7
Fasting glucose (mmol/l)			
Birth weight (kg)	−0·01	−0·4, 0·3	1·0
Age (years)	0·2	−0·01, 0·4	0·06
Gender	−0·5	−0·1, 0·04	0·07
BMI (kg/m^2)	0·06	0·02, 0·1	0·01
Parental social class	0·09	−0·2, 0·4	0·6
Current social class	−0·005	−0·2, 0·01	0·5
Glucose 120 min (mmol/l)			
Birth weight (kg)	−0·02	−0·8, 0·8	0·9
Age (years)	0·3	−0·2, 0·8	0·2
Gender	−0·3	−1·5, 0·9	0·8
BMI (kg/m^2)	0·13	0·02, 0·24	0·02
Parental social class	0·3	−0·5, 1·1	0·4
Current social class	−0·02	−0·06, 0·02	0·4

* For details of subjects, see Table 1.

parents were in a higher social group had the highest HDL-cholesterol levels (Table 5). Similarly, a higher parental social class was associated with lower levels of triacylglycerol in adult offspring (Table 5).

Other physiological measures. In univariate analyses, birth weight was not associated with fasting concentrations of cortisol, leptin, fibrinogen or the mean albumin:creatinine ratio among these individuals (Table 4). In addition, there was no evidence that any marker of maternal nutrition was related to any of the outcome measures mentioned earlier (results not shown). In a multiple regression model, birth weight, together with gender and current body size, was significantly associated with fasting leptin such that a 1 kg higher birth weight was associated with 181·9 (95 % CI 12·1, 351·8) mmol/l lower leptin level (Table 7).

Discussion

The present study provided an unusual opportunity to investigate the possible impact of biochemical markers of maternal diet in pregnancy on the CHD risk profile of adult offspring. The overall findings from this cohort study of middle-aged men and women born during World War 2 in Oxford, UK, provide limited support for the hypothesis that birth weight and maternal nutrition in pregnancy are associated with levels of physiological CHD risk factors in adult life. In addition, there was little evidence to suggest that individual components of the maternal diet, together with the *in utero* environment, may affect birth weight. This is in agreement with evidence from systematic reviews of randomized trials of dietary interventions in pregnancy, which on the whole have suggested that only protein–energy supplementation has a significant, but modest, benefit to birth weight (De Onis *et al.* 1998).

In the present follow-up study, there was no evidence of an inverse association of birth weight with subsequent blood pressure, an association that has been claimed to provide the strongest support for the fetal-origins hypothesis (Robinson, 2001). Moreover, there was some evidence that adult offspring of parents in the higher social class during wartime had higher rather than lower blood pressure, a finding which conflicts with previous reports that a poor post-war early-life environment is associated with adverse health outcomes in later life (Kuh *et al.* 2002).

Table 7. Multiple regression analysis of CHD risk factors with birth weight, current weight, age, gender and measures of parental and current social class*

Dependent variable	β	95% CI	Statistical significance of effect (*P* value)
Fasting cortisol (nmol/l)			
Birth weight (kg)	−11·7	−44·6, 21·1	0·5
Age (years)	9·5	−9·9, 28·9	0·3
Gender	−14·3	−62·8, 34·2	0·6
BMI (kg/m^2)	−0·2	−5·1, 4·8	0·9
Parental social class	−9·6	−40·8, 21·5	0·5
Current social class	−1·1	−2·6, 0·3	0·1
Fasting fibrinogen (g/l)			
Birth weight (kg)	0·1	−0·06, 0·3	0·2
Age (years)	0·01	−0·1, 0·1	0·8
Gender	0·3	0·03, 0·6	0·03
BMI (kg/m^2)	0·01	−0·01, 0·04	0·3
Parental social class	0·1	−0·06, 0·28	0·2
Current social class	0·001	−0·007, 0·01	0·8
Fasting leptin (mmol/l)			
Birth weight (kg)	−181·9	−351·8, −12·1	0·04
Age (years)	8·2	−55·5, 71·9	0·8
Gender	629·9	468·4, 791·4	0·00
BMI (kg/m^2)	86·0	71·9, 100·1	0·00
Parental social class	−82·9	−190·7, 24·8	0·1
Current social class	−0·6	−6·7, 5·5	0·9
Urine albumin:creatinine ratio			
Birth weight (kg)	−0·03	−0·2, 0·1	0·8
Age (years)	0·03	−0·08, 0·1	0·6
Gender	0·1	−0·2, 0·4	0·4
BMI (kg/m^2)	0·03	0·003, 0·05	0·03
Parental social class	0·03	−0·1, 0·2	0·7
Current social class	0·0006	−0·007, 0·008	0·9

* For details of subjects, see Table 1.

These current findings of a null association between birth weight and later blood pressure are, however, in agreement with a recent meta-analysis that concluded that birth weight is of little relevance to subsequent blood pressure; they suggest that previous reports of an inverse association were chiefly driven by publication bias, confounding, and inappropriate adjustment for current weight (Huxley *et al.* 2002).

Similarly, birth weight was not associated with fasting levels of glucose, proinsulin or insulin in the present follow-up study, which conflicts with some (Hales *et al.* 1991; Robinson *et al.* 1992; Fall *et al.* 1995) earlier reports of inverse associations between size at birth and later measures of glucose tolerance. There was, however, some evidence of a weak non-significant inverse association between birth weight and insulin at 120 min after an oral glucose load, similar to previous findings (Fall *et al.* 1995), but it is unclear whether this is a true causal association or simply a chance finding due to the large number of regressions that were performed. There was also no association between birth weight and any component of the lipid profile, a finding that again agrees with some (Cowin & Emmett, 2000), but not all (Barker *et al.* 1993; Mi *et al.* 2000), of the published literature. There was, however, some evidence that a better early-life environment, indicated by higher parental social class, was associated with lower levels of triacylglycerol and higher levels of HDL-cholesterol in adult life. Similarly, in contrast with previous assertions, we found no evidence of an association between birth weight and fasting levels of cortisol (Phillips *et al.* 2000), fibrinogen (Martyn *et al.* 1995) or the albumin: creatinine ratio (Hoy *et al.* 1999), which have all previously been suggested to be inversely associated with birth weight, and to potentially be on the causal pathway mediating the link between birth weight and CHD.

In the present cohort, birth weight was a significant predictor of fasting leptin levels such that those individuals born lighter had the highest leptin levels. Only two previous studies have reported on the association between size at birth and adult leptin concentrations: both reported an inverse association between birth weight and leptin levels (Lissner *et al.* 1999; Phillips *et al.* 1999), but the possible relevance of such an association to later disease risk is currently unknown. There have been speculations in the literature that high leptin levels are a risk factor for myocardial infarction and first-ever stroke, but it remains to be seen whether modification of adult leptin levels modifies CVD risk (Soderberg *et al.* 1999).

A large proportion of the pregnant women in the wartime survey whose offspring participated in this follow-up study were borderline anaemic and possibly deficient in Ca, but overall, their intakes of protein and various vitamins were adequate. There was no evidence from the present follow-up study of the adult offspring that any of the biochemical markers of maternal nutrition were associated with subsequent physiological risk factors, findings that conflict with those from an earlier study which suggested that maternal anaemia is associated with raised blood pressure in offspring (Godfrey *et al.* 1991).

The principal limitation of this current study is its small sample size, resulting in low statistical power and possible selection and recruitment bias, due in part, to the difficulties in identifying and tracing individuals after more that 50 years. There may also have been a greater likelihood for healthier individuals to have agreed to participate in this current study, the so-called 'healthy volunteer effect', as suggested by an over-representation of participants from the higher social classes. These limitations are not unique to this current study and are likely to be shared by similar historical cohort studies that have attempted to examine the early life origins of adult disease. Efforts were made, however, to compensate for the small sample size by minimizing the level of random error, for example, by the use of automated blood pressure machines and 24 h ambulatory blood pressure monitoring. Furthermore, home interviews and anthropometrical assessments were conducted by the same researcher, as were all clinical assessments, thereby minimizing inter-observer variation. The use of the Bonferroni correction in univariate analyses may be criticized as being too conservative and thereby increasing the chance of 'false-negative' results. However, unless such an adjustment is performed, it is difficult to

know whether any statistically significant result observed is real or spurious, due to the play of chance when conducting a large number of correlations on a small dataset. In addition, since only a third of the offspring were recruited into the present study, selection bias may have contributed towards the overall null findings, but it is unlikely to explain all of it since other published historical cohort studies, despite having similar rates of attrition, have suggested associations between size at birth and later chronic disease risk factors (Joseph & Kramer, 1996).

In summary, we found little evidence to support the hypothesis that maternal nutrition in pregnancy and birth weight are determinants of CHD risk factors in adult offspring. Although these findings conflict with the majority of published studies that have reported associations between birth weight and later disease, the role of publication bias (whereby small studies with extreme results are more likely to be published) in driving these associations has recently been examined and shown to be a major contributor to estimates of an inverse relationship between birth weight and subsequent blood pressure (Huxley *et al.* 2002). It is therefore possible that the negative findings reported in the present study are not solely due to its limited statistical power and selection bias, but may instead reflect the lack of any real association between maternal nutrition and birth weight with cardiovascular health in later life.

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