Does malnutrition in utero determine diabetes and coronary heart disease in adulthood? Results from the Leningrad siege study, a cross sectional study

S A Stanner, K Bulmer, C Andrès, O E Lantseva, V Borodina, V V Poteen, J S Yudkin

Department of Medicine, University College London Medical School, Whittington Hospital, London N19 3UA
S A Stanner *project coordinator*
K Bulmer, *research technician*
C Andrès, *research technician*
J S Yudkin, *professor of medicine*
Ott Institute of Obstetrics and Gynaecology, Russian Academy of Medical Science, St Petersburg, Russia
O E Lantseva, *endocrinologist*
V Borodina, *biologist*
V V Poteen, *professor of medicine*
Correspondence to: Ms Stanner sstanner@med.ucl.ac.uk

BMJ 1997;315:1342–9

Abstract

Objective: To investigate the relation between decreased maternal food intake and risk factors for coronary heart disease in adult life.
Design: Cross sectional study.
Subjects: 169 subjects exposed to malnutrition in utero (intrauterine group) during the siege of Leningrad (now St Petersburg) in 1941-4; 192 subjects born in Leningrad just before rationing began, before the siege (infant group); and 188 subjects born concurrently with the first two groups but outside the area of the siege (unexposed group).
Setting: Ott Institute of Obstetrics and Gynaecology, St Petersburg.
Main outcome measures: Development of risk factors for coronary heart disease and diabetes mellitus—obesity, blood pressure, glucose tolerance, insulin concentrations, lipids, albumin excretion rate, and clotting factors.
Results: There was no difference between the subjects exposed to starvation in utero and those starved during infant life in: (*a*) glucose tolerance (mean fasting glucose: intrauterine group 5.2 (95% confidence interval 5.1 to 5.3), infant group 5.3 (5.1 to 5.5), $P = 0.94$; mean 2 hour glucose: intrauterine group 6.1 (5.8 to 6.4), infant group 6.0 (5.7 to 6.3), $P = 0.99$); (*b*) insulin concentration; (*c*) blood pressure; (*d*) lipid concentration; or (*e*) coagulation factors. Concentrations of von Willebrand factor were raised in the intrauterine group (156.5 (79.1 to 309.5)) compared with the infant group (127.6 (63.9 to 254.8); $P < 0.001$), and female subjects in the intrauterine group had a stronger interaction between obesity and both systolic ($P = 0.01$) and diastolic ($P = 0.04$) blood pressure than in the infant group. Short adult stature was associated with raised concentrations of glucose and insulin 2 hours after a glucose load—independently of siege exposure. Subjects in the unexposed group had non-systematic differences in subscapular to triceps skinfold ratio, diastolic blood pressure, and clotting factors compared with the exposed groups.
Conclusions: Intrauterine malnutrition was not associated with glucose intolerance, dyslipidaemia, hypertension, or cardiovascular disease in adulthood. Subjects exposed to malnutrition showed evidence of endothelial dysfunction and a stronger influence of obesity on blood pressure.

Introduction

Several reports have shown that low birth weight is associated with diabetes and hypertension in adult life.[1–10] Other studies have reported that thin babies develop insulin resistance in adulthood.[11 12] These observations have led to the hypothesis that growth retardation affecting the development and vascularisation of particular organs at different stages of fetal development will predispose the individual to impaired organ function, with consequent disease in later life.[13]

Many of these reports have assumed that early growth retardation is synonymous with fetal malnutrition and that this in turn is related to maternal supply of nutrients during pregnancy. A "thrifty phenotype" hypothesis has been proposed to suggest that many of the diseases of Western civilisation, which occur in epidemic proportions when populations move from malnutrition to a Western lifestyle, may be the result of programming of the metabolism and function of a tissue or organ as a result of diminished supply of certain nutrients during critical stages of development.[14 15] Animal studies have shown that intrauterine protein deficiency is associated with impaired pancreatic β cell function[16–18] and increased blood pressure[19] in later life. Nevertheless the relation between maternal nutrient intake and either the birth weight of offspring[20] or subsequent disease[21] in humans remains poorly documented.

The siege of Leningrad (the German blockade of the city now known as St Petersburg) between 8 September 1941 and 27 January 1944 prevented supplies from reaching the city for 872 days. Of a population of 2.4 million, between 750 000 and one million people died, mostly from starvation. Most of these deaths occurred during the "hunger winter" of November 1941 to February 1942, when the siege was in full force and the bread ration of 250 g for workers and 125 g for others was all that was available.[22] The average daily ration for most of the citizens of Leningrad during this time therefore provided around 300 calories and contained virtually no protein. Although the situation improved when Lake Ladoga froze sufficiently to allow supplies to be transported across, it was April/May 1942 before food supplies increased substantially. Average male and female birth weights fell by 18% and 16% respectively.[23]

We investigated the relation between decreased maternal food intake and risk factors for coronary heart disease in adult life, with specific reference to malnutrition in utero during the siege of Leningrad.

Subjects and methods

Subjects and study design

We investigated two groups of subjects exposed to the siege of Leningrad. These two groups were identified from the register of the Society of Children of the Siege, which maintains a complete and updated record of all people living in, or born in, the city of Leningrad during the siege. Subjects exposed to the siege in utero (intrauterine group) comprised adults born in the city

of Leningrad between 1 November 1941 and 30 June 1942, the first date being 54 days after the start of the siege. Subjects exposed to the siege as infants (infant group) comprised adults also born in the city of Leningrad but between 1 January and 30 June 1941, and consequently they were at least 10 weeks old at the beginning of the siege. Historical records document the plentiful supply of food in Leningrad until rationing was imposed on 18 July 1941 in preparation for the impending siege.

We identified 1229 subjects (548 male) born between 1 January 1941 and 30 June 1942. Four of these subjects had died and 209 were not contactable or had changed address, leaving 1016 (464 male) available for invitation to the study. Of these, 443 (44%) subjects attended for screening (125 male (27% of those contacted), 318 female (58%)), of whom 10 were excluded as known diabetics and 72 were excluded either because their birth date fell between 1 July 1941 and 31 October 1941 (n=51) or because, owing to inaccurate entry on the register, the date fell outside the limits of the study group (n=21). This left a population of 169 (37 male) in the intrauterine group and 192 (62 male) in the infant group. Of the subjects in these two groups, 115/167 (69%) and 129/192 (67%) respectively remained in Leningrad until the siege ended, with most of the evacuations occurring after July 1942. Thus all the subjects in the intrauterine group would have been additionally exposed to siege in infancy.

In addition to the two groups exposed to the siege, we studied a third group. This comprised 188 adults (50 male) who were born in the province of Leningrad but outside the city (and thus the siege limits) during the same period as subjects in the other two groups (1 January 1941 to 30 June 1942). The subjects in this group (unexposed group) were invited from two sources—the radial kerotomy clinic of the local hospital, where patients had been referred for surgery for refractive eye problems, and six local workplaces. Subjects with known diabetes, glaucoma, or hypertensive or diabetic retinopathy were excluded (n=7); 102 subjects (24 male) from the kerotomy clinic attended, and 86 subjects (26 male) from the local workplaces attended. The two subgroups were not found to be significantly different in any of the criteria studied (data not shown) and were therefore combined.

Methods

Anthropometric methods

Subjects were invited to attend the department of endocrinology at the Ott Institute, St Petersburg, the morning after an overnight fast. Subjects were weighed in light clothing and without shoes on a beam balance (Seca, Birmingham) and height recorded on a stadiometer (Pribordetal Plant, Zuevo, Russia). The waist to hip ratio and the subscapular to triceps skinfold ratio were calculated.[24] Blood pressure was measured in triplicate with a random zero sphygmomanometer (Hawksley Gelman, Lancing, Sussex) that had been calibrated against a similar machine in the department of medicine at the University College London Medical School, Whittington Hospital, London. A resting 12 lead electrocardiogram was recorded. Personal and family medical histories were taken to ascertain previous health and experience of the siege, and a standardised questionnaire was administered for ischaemic heart disease.[25] Smoking and alcohol histories were also obtained.

Blood sampling and biochemical analyses

A venous blood sample was taken after an overnight fast for several measures (see below), and each patient was asked to void his or her bladder. Each patient was then given 75 g of anhydrous glucose (Fortical, Cow and Gate, Trowbridge, Wiltshire) dissolved in 300 ml water to drink over 5 minutes; 30 minutes and 120 minutes later, blood samples were taken for measurement of glucose and insulin concentrations. The patient passed urine again after the glucose tolerance test, and the time was recorded. Each subject was then given a urine collection bottle to make a timed overnight urine collection. Plasma glucose concentration was measured in samples taken after fasting and after 30 and 120 minutes with a Beckman analyser (Beckman Instruments UK, High Wycombe, Buckinghamshire). Fifty four per cent of the samples were also assayed in the department of medicine at the University College London Medical School, with good agreement between the two assays (mean difference 0.12 (SE 0.03) mmol/l, P=0.001).

Total and high density lipoprotein cholesterol were measured using the enzymatic colorimetric method (Sigma Diagnostics, Poole, Dorset), the latter after precipitation of triglyceride-rich lipoproteins with heparin and manganese. Triglyceride concentrations were also assayed with a Sigma kit (Sigma Diagnostics), and low density lipoprotein cholesterol concentration was calculated with the Friedewald formula.[26] Plasma insulin concentrations were measured with a commercial enzyme linked immunosorbent assay (ELISA) (Dako Diagnostics, Ely, Cambridgeshire), which is specific for insulin without cross reaction with either intact or des 31,32 proinsulin.[27] Intact and des 31,32 proinsulin, fibrinogen, factor VII and urinary albumin were assayed as previously described.[28] Fasting C peptide concentration was measured with a radioimmunoassay kit for human C peptide (Biodata, Rome, Italy); plasminogen activator inhibitor was measured both as activity and as antigen (Biopool, Bio-Stat, Stockport, Cheshire); and plasma concentration of von Willebrand factor was measured with an in house enzyme linked immunosorbent assay by using antibodies from Dako (Copenhagen, Denmark) and validated by using controls from the National Institute for Biological Standards and Controls. All assays were performed in a single run, except for plasminogen activator inhibitor, factor VII, and fibrinogen, for which all statistical analyses were adjusted for analytical batch.

Classification of subjects

Because of the absence of satisfactory Russian criteria for classifying social class, subjects were classified according to manual or non-manual occupation and the Office of Population Censuses and Surveys' classification of occupations.[29] Glucose intolerance was classified as impaired glucose tolerance or diabetes mellitus, according to the World Health Organisation's criteria.[30] The electrocardiographic results were coded in Britain according to Minnesota code criteria,[25] and subjects were classified in three groups. Group 1

comprised subjects with confirmed myocardial infarction according to codes 1.1,1.2, or 7.1 for electrocardiographic changes. Group 2 comprised subjects with definite or possible ischaemia (all subjects with the changes that defined group 1, and subjects fitting one or more of the codes 1.3, 4.1-4.4, 5.1-5.3). Group 3 comprised all subjects with the abnormalities that defined groups 1 and 2 or with a history of angina (on a World Health Organisation questionnaire). Microalbuminuria was defined as an albumin excretion rate of 20-200 μg/min on either a two hour sample or an overnight sample. Hypertension was defined as a systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, or treatment for hypertension. The percentage of proinsulin-like molecules was calculated as: $100\times(\text{proinsulin}+\text{des }31,32\text{ proinsulin})/(\text{insulin}+\text{proinsulin}+\text{des }31,32\text{ proinsulin})$.

Statistical analyses

We compared the distribution of variables in the intrauterine group and the infant group, as the source of the subjects in these groups was identical and most of the subjects had remained in Leningrad throughout the siege. We used analysis of variance, with logarithmic transformation for skewed variables and adjustment for sex. Categoric variables were compared by using the χ^2 test in a similar fashion. We used correlation and linear regression analysis to study associations between continuous variables, again with logarithmic transformation of skewed data. Analysis of covariance was used to test the homogeneity of regression slope of blood pressure and body mass index (weight(kg)/height(m)^2)) for these two groups. All analyses were repeated across all three study groups, with the same adjustment for sex. A P value of <0.05 was taken as significant, although, where multiple comparisons have been performed, it would be appropriate to use more rigorous criteria for significance.

From the variance of these measures in an age matched population in London[31] we calculated that 200 subjects in each group would permit the detection of a 0.22 mmol/l difference in fasting plasma glucose and 6.1 mm Hg difference in systolic blood pressure between groups at the 1% level with a power of 90%.

Results

Intrauterine and infant groups

The subjects in the intrauterine group and the infant group were similar for parents' nationality, percentage of subjects in employment, social class, and years of education. In the intrauterine group 21% (26/122) of subjects recalled the loss of a first degree relative during the siege, compared with 24% (36/151) of those exposed during infancy ($P=0.52$); the percent-

Table 1 Anthropometric variables, blood pressure, and prevalence of coronary heart disease, according to exposure to siege of Leningrad. Values are means (95% confidence intervals) unless stated otherwise

	Exposed groups			
	Intrauterine (n=169)	Infant (n=192)	Significance (P value)†	Unexposed group (n=188)
Sex:				
Male	37	62	0.03	50
Female	132	130		138
Age (years)	52.3	53.1		52.8***
Height (m):				
Male	1.72 (1.70 to 1.74)	1.74 (1.72 to 1.76)	0.43	1.73 (1.71 to 1.75)
Female	1.58 (1.56 to 1.60)	1.59 (1.57 to 1.61)	0.50	1.60 (1.56 to 1.64)
Body mass index (kg/m^2):				
Male	24.6 (23.6 to 25.6)	25.4 (24.2 to 26.6)	0.39	25.2 (24.1 to 26.3)
Female	26.9 (26.1 to 27.7)	27.0 (26.2 to 27.8)	0.89	26.7 (25.9 to 27.5)
Waist:hip ratio:				
Male	0.86 (0.84 to 0.88)	0.88 (0.84 to 0.92)	0.66	0.87 (0.85 to 0.89)
Female	0.79 (0.77 to 0.81)	0.78 (0.76 to 0.80)	0.49	0.79 (0.75 to 0.83)
Subscapular:triceps skinfold ratio:				
Male	1.26 (1.11 to 1.41)	1.32 (1.20 to 1.44)	0.50	1.41 (1.31 to 1.51)
Female	1.01 (0.93 to 1.09)	0.93 (0.87 to 0.99)	0.17	0.88 (0.82 to 0.94)*
Systolic blood pressure (mm Hg):				
All subjects	134.7 (131.0 to 138.4)	134.4 (131.3 to 137.5)	0.74‡	130.9 (127.7 to 134.1)
Excluding subjects taking antihypertensives	131.6 (127.9 to 135.3) (n=146)	133.1 (129.9 to 136.3) (n=173)	0.73‡	128.5 (125.3 to 131.7) (n=164)
Diastolic blood pressure (mm Hg):				
All subjects	82.2 (80.1 to 84.3)	82.9 (80.9 to 84.9)	0.93‡	79.0 (77.1 to 80.9)*
Excluding subjects taking antihypertensives	80.9 (78.7 to 83.1) (n=146)	82.2 (80.0 to 84.3) (n=173)	0.67‡	77.3 (75.4 to 79.2) (n=164)*
Electrocardiographic and history evidence (male:female (%)):				
Group 1§	2.7:1.5	3.2:2.3	0.88:0.64	0:2.2
Group 2§	18.9:20.0	16.1:12.3	0.72:0.10	14.3:16.7**
Angina (on questionnaire)§	13.9:27.5	29.5:40.6	0.08:0.03	14.0:21.9
Group 3§	25.0:35.1	37.7:41.4	0.20:0.30	22.0:31.9

Denominators are given in parentheses if different from whole group.
*P<0.05, **P<0.005, ***P<0.001 across all three groups.
†Analysis of variance for continuous variables and χ^2 test for categoric variables.
‡Adjusted for sex.
§See methods section for definitions.

Table 2 Glucose and insulin concentrations, according to exposure to siege. Values are means or geometric means (95% confidence intervals) unless stated otherwise

	Exposed groups			
	Intrauterine (n=169)	Infant (n=192)	Significance (P value)*	Unexposed group (n=188)
Plasma glucose (mmol/l):				
Fasting	5.2 (5.1 to 5.3)	5.3 (5.1 to 5.5)	0.94	5.3 (5.1 to 5.5)
30 min	8.0 (7.7 to 8.3)	8.4 (8.1 to 8.7)	0.29	7.9 (7.8 to 8.0)
120 min	6.1 (5.8 to 6.4)	6.0 (5.7 to 6.3)	0.99	5.7 (5.4 to 6.0)
Percentage with impaired glucose tolerance and diabetes mellitus:			0.23	
Known diabetes†	2.3	2.5		3.6
Newly diagnosed diabetes	1.8	0.5		2.7
Impaired glucose tolerance	9.6	14.1		8.6
Plasma insulin (pmol/l)‡:				
Fasting	35.0 (17.4 to 70.6) (n=168)	35.0 (17.4 to 70.6) (n=190)	0.76	32.9 (16.3 to 66.3) (n=174)
30 min	225.2 (111.7 to 454.0) (n=164)	229.4 (113.8 to 462.5) (n=184)	0.83	230.0 (115.2 to 459.3) (n=180)
120 min	141.9 (69.1 to 291.5) (n=165)	149.8 (73.6 to 304.9) (n=187)	0.28	130.0 (63.9 to 264.6) (n=182)
Fasting plasma proinsulin (pmol/l)‡	2.7 (1.3 to 5.5) (n=145)	2.9 (1.4 to 5.9) (n=164)	0.28	2.4 (1.2 to 4.8) (n=167)
Fasting plasma des 31,32 proinsulin (pmol/l)‡	1.4 (0.7 to 3.0) (n=143)	1.2 (0.6 to 2.5) (n=163)	0.29	1.5 (0.7 to 3.2) (n=162)
Proinsulin-like molecules (%)‡	11.5 (5.7 to 23.2) (n=142)	10.8 (5.4 to 21.6) (n=162)	0.53	11.3 (5.7 to 22.6) (n=152)
C peptide (ng/ml)‡	0.38 (0.19 to 0.77)	0.40 (0.20 to 0.81)	0.73	0.39 (0.19 to 0.79)

Denominators are given in parentheses if different from whole group.
All variables are adjusted for sex.
*Analysis of variance for continuous variables, with logarithmic transformation of skewed data, and χ^2 test for categoric variables.
†Excluded from any analysis.
‡Geometric mean.

ages of subjects recalling mothers and siblings lost through starvation or illness were 4% (5/122) and 5% (7/151) respectively (P = 0.83). Table 1 shows the characteristics of the subjects studied. The intrauterine and infant groups did not differ in height or in degree of central obesity (determined by waist to hip ratio), regardless of whether men and women were analysed together or separately. Blood pressure and the prevalence of coronary heart disease were similar for the two groups, although a significantly higher proportion of the infant group reported symptoms of angina. Smoking rates and alcohol intake were similar for all groups (data not shown).

There was no difference in concentrations of fasting and two hour plasma glucose during an oral glucose tolerance test (table 2) or any excess of known diabetes or glucose intolerance associated with the intrauterine or infant group. These conclusions were unaffected by using assay results from London rather than from St Petersburg. Levels of insulin-like molecules, lipid concentrations, and daytime albumin excretion rate did not differ, but the intrauterine group had a significantly lower overnight albumin excretion rate (table 3).

Concentrations of fibrinogen, factor VII, and plasminogen activator inhibitor (activity and antigen) were similar for the intrauterine and infant groups (table 4), but subjects in the intrauterine group had a significantly higher concentration of von Willebrand factor. This remained significant after further adjustment for obesity, smoking, and current coronary heart disease (P = 0.009).

Unexposed group

The subjects in the unexposed group were similar for all lifestyle and demographic factors studied. They had marginally lower diastolic blood pressure than the exposed subjects (P < 0.05). They also had significantly higher concentrations of factor VII but lower concentrations of plasminogen activator inhibitor antigen and activity.

Leon et al have suggested that adult blood pressure is more markedly affected by obesity in individuals with intrauterine growth retardation than in those without growth retardation.[9] We therefore explored the relation between exposure to the siege and adult obesity. In all subjects combined, blood pressure was positively related to obesity (systolic blood pressure

Table 3 Lipid concentrations and albumin excretion rate, according to exposure to siege. Values are means or geometric means (95% confidence intervals) unless stated otherwise

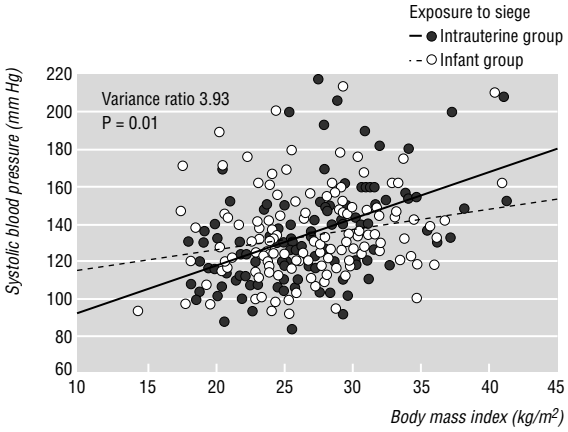
	Exposed groups			
	Intrauterine (n=169)	Infant (n=192)	Significance (P value)*	Unexposed group (n=188)
Total cholesterol (mmol/l)	5.5 (5.3 to 5.7)	5.5 (5.3 to 5.7)	0.63	5.5 (5.4 to 5.6)
Triglyceride (mmol/l)†	1.1 (0.6 to 2.2)	1.1 (0.6 to 2.2)	0.51	1.0 (0.5 to 2.0)
High density lipoprotein (mmol/l)	1.3 (1.2 to 1.4)	1.4 (1.3 to 1.5)	0.27	1. 4 (1.3 to 1.5)
Low density lipoprotein (mmol/l)	3.6 (1.5 to 5.7)	3.5 (3.3 to 3.7)	0.31	3.6 (3.5 to 3.7)
2 hour albumin excretion rate (μg/min)†	4.5 (2.2 to 9.3) (144)	4.7 (2.3 to 9.7) (175)	0.59	4.8 (2.3 to 9.9) (178)
Overnight albumin excretion rate (μg/min)†	2.2 (1.1 to 4.6) (144)	3.0 (1.5 to 6.2) (175)	0.01	3.1 (1.5 to 6.3) (178)‡

All variables are adjusted for sex.
*Analysis of variance for continuous variables, with logarithmic transformation of skewed data.
†Geometric mean.
‡P<0.005 across all three groups.

Table 4 Haemostatic and fibrinolytic variables, according to exposure to siege. Values are geometric means (95% confidence intervals) unless stated otherwise

	Exposed groups			
	Intrauterine (n=169)	Infant (n=192)	Significance (P value)*	Unexposed group (n=188)
Von Willebrand factor (%)	156.5 (79.1 to 309.5)	127.6 (63.9 to 254.8)	<0.001†	131.0 (66.2 to 259.1)§
Fibrinogen (mg/l)	313.2 (159.9 to 613.4)	315.3 (161.0 to 617.5)	0.60†	309.1 (157.8 to 605.3)
Factor VII (%)	84.2 (42.6 to 166.5)	81.5 (41.2 to 161.2)	0.81‡	95.8 (48.4 to 189.5)§
Plasminogen activator inhibitor activity (au/ml)	10.3 (4.9 to 21.6)	8.5 (4.1 to 17.8)	0.11‡	6.3 (3.0 to 13.4)§
Plasminogen activator inhibitor antigen (ng/ml)	24.0 (12.0 to 47.9)	22.9 (11.5 to 45.7)	0.12‡	21.2 (10.6 to 42.3)¶

*Analysis of variance for continuous variables, with logarithmic transformation of skewed data.
†Adjusted for sex.
‡Adjusted for sex and analytical batch.
§P<0.001 across all three groups.
¶P<0.005 across all three groups.



Relation between body mass index and systolic blood pressure in females in the intrauterine and infant groups

partial $r=0.29$, $P<0.001$, with sex, age, and exposure controlled for). However, the relation in women between body mass index and (*a*) systolic blood pressure (figure) and (*b*) diastolic blood pressure was significantly stronger in the intrauterine group than in the infant group ($P=0.01$ and $P=0.04$ respectively). This suggests that siege exposure and adult obesity may act synergistically to increase susceptibility to hypertension.

We explored the relation between adult height and glucose intolerance, hypertension, and microalbuminuria. Glucose intolerant subjects were shorter than those with normal glucose tolerance, with a mean difference of 21 mm ($P=0.02$) after adjustment for age, sex, and exposure to siege. This difference did not persist after further adjustment for adult social class and education (adjusted mean difference 13 mm, analysis of variance $P=0.935$, with age, sex, and exposure to siege controlled for). The partial correlation coefficients of height were $r=-0.10$, $P=0.019$ with two hour plasma glucose, and $r=-0.13$, $P=0.002$ with insulin two hours after glucose challenge, after adjustment for the same covariates. The height of subjects with hypertension or microalbuminuria did not differ significantly from the height of subjects without.

Discussion

We studied 549 subjects born in and around Leningrad at the time of the siege, one third of whom are likely to have been exposed to severe intrauterine starvation. This population has allowed us to explore the relation between maternal malnutrition and cardiovascular risk factors, which otherwise have been predominantly studied in vitro or in animal models. We did not find evidence for the hypothesis suggesting that intrauterine exposure to maternal malnutrition was linked with glucose intolerance, dyslipidaemia, hypertension, or cardiovascular disease in adulthood. Although these observations suggest that increased levels of cardiovascular risk factors are not consequent on maternal nutritional intake, endothelial dysfunction and a stronger relation between obesity and blood pressure were more common in the subjects who were exposed to the siege in utero.

Effect of losses to follow up

We studied 44% of eligible subjects (those in the exposed groups who were invited for screening). The nature of studies relating to fetal origin inevitably means that follow up is less complete than for the usual sort of epidemiological study. Low ascertainment rates (as low as 5.8% in some studies[32]) have been criticised,[33 34] but Martyn et al have argued that as the analyses are based on internal comparisons, selection bias would arise only if the associations of early life events and coronary heart disease were different between those studied and those lost to follow up.[35] In our study the only characteristic that distinguished the intrauterine group from the infant group was a date of birth before July or after November 1941. This thus determined whether they were exposed to the siege conditions only as an infant or additionally as a fetus, the two siege groups being selected from the same register with similar attendance rates. If programming of adult metabolism is consequent on exposure to malnutrition during certain stages of organ development in utero, substantial differences would be expected between these two groups.

Lack of reliable data on birth weight

We could not obtain reliable data on birth weight. Twenty two per cent (120) of the subjects provided a recalled birth weight, the intrauterine group having a significantly lower mean birth weight (2.7 (SD 0.9) kg *v* infancy group 3.4 (SD 1.1) kg *v* unexposed group, 3.6 (SD 1.0) kg; analysis of variance, with sex controlled for, $P<0.001$). This is consistent with the birth weights recorded during this period of the siege.[23] This study

used exposure to siege, rather than birth weight, as a surrogate marker of maternal nutrient intake. Historical records show that maternal diet in pregnancy was likely to have been good for the infant group, who were aged at least 10 weeks when the siege began, with food supply being plentiful until rationing began. We found no evidence that subjects born during the siege had parents in occupations with preferential access to food.

Alternative explanations

Other possible explanations for our negative findings include the possibility that, as observed by Godfrey et al in human studies,[20] and as recognised in sheep,[36] the effect of protein deprivation on fetal development is more marked in offspring of women with a high carbohydrate intake in pregnancy, a situation likely to prevail in malnourished populations of developing countries. Another interpretation is that malnutrition may be necessary for more prolonged periods or for more than one generation before the relations between growth retardation and adult disease are seen.[35] Studies have shown powerful intergenerational effects on birth weight—largely transmitted through the maternal line[37]—which seem to apply even when women migrate from communities with poor nutrition to an environment with an improved food supply.

Effect of malnutrition on adult height

Short stature has been related both to incidence of coronary heart disease[38] and to glucose intolerance.[39] We confirmed the relation between short stature and glucose intolerance, this association being independent of study group. Adult height is determined both by birth weight and by childhood environment. We found no difference in height between the study groups, and the relation between height and glucose intolerance seems to be explained partly by occupational grade and education, which we have used as indicators of later social deprivation. Studies of men born during or after the hunger winter in the Netherlands show that height was unaffected by exposure to famine conditions in utero, indicating the potential for "catch up" growth if later food supply is adequate.[40] We did not find in this population the described associations between microalbuminuria[31] or blood pressure[41] and adult height.

We also studied subjects born outside siege conditions, who differed in several variables from the two siege groups. However, these differences may relate to different selection criteria and not to different maternal nutritional intakes.

We are grateful to Professor D J P Barker for help in seeking financial support for this project and discussions about the design of the study, to Drs John and Simon Griffin for helping with assays in St Petersburg, and Stephanie Goubet and Dr David Leon for advice on analysis of our data. We are indebted to the staff of the Ott Institute who helped with subject recruitment and screening and are particularly grateful to Elena Yablochkina for all her help in translating questionnaires, organising trips, and coordinating this project in St Petersburg.

Funding: This study was supported by grants from the British Diabetic Association and the International Association for the Promotion of Cooperation with Scientists from the Former Soviet Union.

Conflict of interest: None.

Key messages

- Relations between intrauterine growth and adult disease such as diabetes and cardiovascular disease have been linked to poor nutrition during pregnancy
- In this study, however, intrauterine exposure to malnutrition was not associated with glucose intolerance
- Intrauterine malnutrition did not affect insulin concentration, blood pressure, or concentration of lipids or coagulation factors
- Concentration of von Willebrand factor, a marker of endothelial damage, was raised in the subjects exposed to intrauterine malnutrition
- Obesity and blood pressure were more strongly related in subjects exposed to intrauterine malnutrition than in subjects either unexposed to malnutrition or exposed to malnutrition only as infants

1 Wadsworth ME, Cripps HA, Midwinter RE, Colley JR. Blood pressure in a national birth cohort at the age of 36 related to social and familial factors, smoking, and body mass. *BMJ* 1985;291:1534-8.
2 Barker DJP, Bull AR, Osmond C, Simmonds SJ. Fetal and placental size and risk of hypertension in adult life. *BMJ* 1990;301:259-62.
3 Hales CN, Barker DJP, Clark PMS, Cox LJ, Fall C, Osmond C, et al. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ* 1991;303:1019-22.
4 Robinson S, Walton RJ, Clark PM, Barker DJP, Hales CN, Osmond C. The relation of fetal growth to plasma glucose in young men. *Diabetologia* 1992;35:444-6.
5 Phipps K, Barker DJP, Hales CN, Fall CHD, Osmond C, Clark PMS. Fetal growth and impaired glucose tolerance in men and women. *Diabetologia* 1993;36:225-8.
6 Law CM, de Swiet M, Fayers PM, Barker DJP, Cruddas AM, Fall CHD. Initiation of hypertension in utero and its amplification throughout life. *BMJ* 1993;306:24-7.
7 Martyn CN, Barker DJP, Jespersen S, Greenwald S, Osmond C, Berry C. Growth in utero, adult blood pressure, and arterial compliance. *Br Heart J* 1995;73:116-21.
8 Gennser G, Rymark P, Isberg PE. Low birth weight and risk of high blood pressure in adulthood. *BMJ* 1988;296:1498-500.
9 Leon DA, Koupilova I, Lithell HO, Berglund L, Mohsen R, Vågerö D, et al. Failure to realise growth potential in utero and adult obesity in relation to blood pressure in 50 year old Swedish men. *BMJ* 1996;312:401-6.
10 McCance DR, Pettitt DJ, Hanson RL, Jacobsson LT, Knowler WC, Bennett PH. Birth weight and non-insulin dependent diabetes: thrifty genotype, thrifty phenotype, or surviving small baby genogype? *BMJ* 1994;308:942-5.
11 Phillips DIW, Barker DJP, Hales CN, Hirst S, Osmond C. Thinness at birth and insulin resistance in adult life. *Diabetologia* 1994;37:150-4.
12 Barker DJP, Godfrey KM, Osmond C, Bull A. The relation of fetal length, ponderal index and head circumference to blood pressure and the risk of hypertension in adult life. *Paediatr Perinat Epidemiol* 1992;6:35-44.
13 Barker DJP, ed. *Fetal and infant origins of adult disease*. London: BMJ, 1992.
14 Barker DJP, Hales CN, Fall CHD, Osmond C, Phipps K, Clark PMS. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* 1993;36:62-7.
15 Barker DJP. The intrauterine origins of cardiovascular disease and obstructive lung disease in adult life. *J R Coll Physicians Lond* 1991;25:129-33.
16 Weinkove C, Weinkove EA, Pimstone BL. Insulin release and pancreatic islet volume in malnourished rats. *S A Med J* 1974;48:1888.
17 Swenne I, Crace CJ, Milner RDG. Persistent impairment of insulin secretory response to glucose in adult rats after limited period of protein-calorie malnutrition early in life. *Diabetes* 1987;36:454-8.
18 Snoeck A, Remacle C, Reusens B, Hoet J. Effect of a low protein diet during pregnancy on the fetal rat endocrine pancreas. *Biol Neonate* 1990;5:107-18.
19 Langley SC, Jackson AA. Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diets. *Clin Sci* 1994;86:217-22.
20 Godfrey K, Robinson S, Barker DJP, Osmond C, Cox V. Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. *BMJ* 1996;312:410-4.
21 Campbell DM, Hall MH, Barker DJP, Cross J, Shiell AW, Godfrey KM. Diet in pregnancy and the offspring's blood pressure 40 years later. *Br J Obstet Gynaecol* 1996;103:273-80.
22 Pavlov D. *Leningrad 1941: the blockade*. Chicago: University of Chicago Press, 1965.
23 Antonov AN. Children born during the seige of Leningrad in 1942. *J Paediatr* 1947;30:250-9.
24 Bennett KA, Osbourne RH. Interobserver measurement reliability in anthropometry. *Human Biology* 1986;58:751-9.

25 Rose GA, Blackburn H. Cardiovascular survey methods. In: *WHO Monograph Series No 56*. Geneva: World Health Organisation, 1968.
26 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499-502.
27 Robbins DC, Andersen L, Bowsher R, Chance R, Dinesen B, Frank B, et al. Report of the American Diabetes Association's task force on standardization of the insulin assay. *Diabetes* 1996;45:242-56.
28 Mohamed-Ali V, Gould MM, Gillies S, Goubet S, Yudkin JS, Haines AP. Association of proinsulin-like molecules with lipids and fibrinogen in non-diabetic subjects—evidence against a modulating role for insulin. *Diabetologia* 1995;38:1110-6.
29 Office of Population Censuses and Surveys. *Classification of occupations*. London: HMSO, 1980.
30 World Health Organisation Study Group. Report on diabetes mellitus. *WHO Tech Rep Ser* 1985;727:9-12
31 Gould MM, Mohamed-Ali V, Goubet SA, Yudkin JS, Haines AP. Microalbuminuria: associations with height and sex in non-diabetic subjects. *BMJ* 1993;306:240-2.
32 Stein CE, Fall CHD, Kumaran K, Osmond C, Cox V, Barker DJP. Fetal growth and coronary heart disease in South India. *Lancet* 1996; 348:1269-73.
33 Kramer MS, Joseph KS. Enigma of fetal/infant-origins hypothesis. *Lancet* 1996;348:1254-5.
34 Paneth N, Susser M. Early origin of coronary heart disease (the "Barker hypothesis"). *BMJ* 1995;310:423-7.
35 Martyn CN, Barker DJP, Osmond C. Mothers' pelvic size, fetal growth, and death from stroke and coronary heart disease in men in the UK. *Lancet* 1996;348:1264-8.
36 Barker DJP, Gluckman PD, Robinson JS. Conference report: fetal origins of adult disease—report of the first international study group, Sydney, 29-30 October 1994. *Placenta* 1995;16:317-20.
37 Emanuel JI, Filakti H, Alberman E, Evans SJW. Intergenerational studies of human birthweight from the 1958 birth cohort. 1. Evidence for a multigenerational effect. *Br J Obstet Gynaecol* 1992;99:67-74.
38 Marmot MG, Shipley MJ, Rose G. Inequalities in death-specific explanations of a general pattern? *Lancet* 1984;i:103-6.
39 Brown DC, Byrne CD, Clark PM, Cox BD, Day NE, Hales CN, et al. Height and glucose tolerance in adult subjects. *Diabetologia* 1991;34:531-3.
40 Stein Z, Susser M, Saenger G, Marolla F. Famine and human development. The Dutch hunger winter of 1944-1945. New York: Oxford University Press, 1975.
41 Walker M, Shaper AG, Phillips AN, Cook DG. Short stature, lung function and risk of a heart attack. *Int J Epidemiol* 1989;18:602-6.

(Accepted 26 August 1997)

Commentary: A hypothesis challenged

Janet W Rich-Edwards, Matthew W Gillman

Department of Ambulatory Care and Prevention, Harvard Medical School and Harvard Pilgrim Care, 126 Brookline Avenue, Suite 200, Boston, MA 02215, USA

Janet W Rich-Edwards, *instructor*

Matthew W Gillman, *associate professor*

Correspondence to: Dr Rich-Edwards

Critics have charged David Barker, champion of the hypothesis that the environment in the uterus programmes the risk of adult disease,[1] with overenthusiastic inductive reasoning as well as inattention to issues of selection bias and confounding.[2] A growing number of independent epidemiologists, however, have confirmed associations between birth weight and hypertension,[3 4] diabetes mellitus,[3 5–7] cardiovascular disease,[8 9] and breast cancer.[10–12] These associations have been observed in various settings and have proved robust to adjustment for socioeconomic and lifestyle factors. Thus increasing attention is being paid to what underlies these observations; Barker himself has invoked "undernutrition" of the fetus to explain the cardiovascular outcomes.[1] Now Stanner and colleagues put this explanation to the test by measuring cardiovascular risk factors among adults who were exposed to severe famine in the siege of Leningrad while in utero or in their first year of life. Their results are resoundingly null. Yet, before proclaiming this study a lethal blow to the hypothesis of in utero programming, consider whether this was a fair test of a clearly delineated hypothesis.

Stanner and colleagues' study is ecological as it lacked data on the exposure status of individuals. Thus the authors were forced to assume that every mother pregnant during the siege was malnourished and that every mother who delivered before the siege was not. The greater the deviation from this assumption, the more a bias to the null would result. At the appalling siege ration of 300 carbohydrate calories/day, however, little cause exists to second guess the extraordinary deprivation experienced by those enduring the siege. Indeed, the mean birth weight of those exposed to the siege in utero was 700 g less than those born before the siege. During the siege 27% of pregnancies delivered in Leningrad's hospitals were stillborn or perished in their first month.[13] The selective survival implied by such a grim mortality might also raise our suspicions of bias. Yet, according to the Barker hypothesis, programming should be most evident among those fetuses who successfully downregulated their growth and survived. Among those who survived infancy during the siege, 64% could not be traced or declined to participate. However, the authors found no evidence for the rather implausible scenario necessary to produce biased results from loss to follow up: that they had inadvertently "over-enrolled" healthy adults who had been exposed to the siege in utero or had "under-enrolled" ill adults who were not exposed in utero.

With some assurance of a fair test, then, what was the hypothesis? As both exposure groups experienced famine during infancy, the study measures the additional impact of having been starved in utero. One interpretation of the null result is that starvation at any point between conception and an infant's first birthday is sufficient to exert programming effects. At the other extreme, perhaps only starvation during a certain period of gestation is relevant, and the exposed group was too broadly defined to detect an effect. Or specific dietary imbalances, rather than sheer starvation, may programme the fetus. Another alternative is that intrauterine programming results from factors other than maternal diet. Finally, these null results could be evidence that intrauterine programming is a chimera of confounding by social class, as rich and poor seem to have been equally starved in the Leningrad siege.

Such broad latitude in interpretation invites new, more specific hypotheses to explain the observed associations between measures of fetal growth and the risk of adult disease. Several recent theories implicate maternal and fetal hormones[14 15] as well as trimester-specific effects of maternal diet.[1] Historical data may prove too blunt to test these increasingly specific hypotheses. Other study designs, including animal experiments and prospective studies of mothers and their offspring, are likely to yield more definitive results. These studies are important to do, because ensuring optimal wellbeing for young women may prove a powerful way to promote the lifelong health of their children.