Life course epidemiology

Effects of prenatal micronutrient and early food supplementation on metabolic status of the offspring at 4.5 years of age. The MINIMat randomized trial in rural Bangladesh

Eva-Charlotte Ekström,[1]* Emma Lindström,[1] Rubhana Raqib,[2] Shams El Arifeen,[2] Samar Basu,[3] Kerstin Brismar,[4] Katarina Selling[1] and Lars-Åke Persson[1]

[1]International Maternal and Child Health, Women's and Children's Health, Uppsala University, Uppsala, Sweden, [2]International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh, [3]Oxidative Stress and Inflammation, Department of Public Health and Caring Sciences, Uppsala University, Uppsala, Sweden, and Chaire d'Excellence Program, Department of Biochemistry, Molecular Biology and Nutrition, Universite d'Auvergne, Clermont-Ferrand, France and [4]Department of Molecular Medicine and Surgery, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

*Corresponding author. International Maternal and Child Health, Department of Women's and Children's Health, Akademiska sjukhuset, Uppsala University, 751 85 Uppsala, Sweden. E-mail: lotta.ekstrom@kbh.uu.se

Abstract

Background: Fetal nutritional insults may alter the later metabolic phenotype. We hypothesized that early timing of prenatal food supplementation and multiple micronutrient supplementation (MMS) would favourably influence childhood metabolic phenotype.

Methods: Pregnant women recruited 1 January to 31 December 2002 in Matlab, Bangladesh, were randomized into supplementation with capsules of either 30 mg of iron and 400 μg of folic acid, 60 mg of iron and 400 μg of folic acid, or MMS containing a daily allowance of 15 micronutrients, and randomized to food supplementation (608 kcal) either with early invitation (9 weeks' gestation) or usual invitation (at 20 weeks). Their children ($n = 1667$) were followed up at 4.5 years with assessment of biomarkers of lipid and glucose metabolism, inflammation and oxidative stress.

Results: Children in the group with early timing of food supplementation had lower cholesterol (difference -0.079 mmol/l, 95% confidence interval (CI) -0.156; -0.003), low-density lipoprotein (LDL) (difference -0.068 mmol/l, 95% CI -0.126; -0.011) and ApoB levels (difference -0.017 g/l, 95% CL -0.033; -0.001). MMS supplementation resulted in lower high-density lipoprotein (HDL) (difference -0.028 mmol/l, 95% CL -0.053; -0.002), lower glucose (difference -0.099 mmol/l, 95% CL -0.179; -0.019) and lower insulin-like growth factor 1 (IGF-1) (difference on log scale -0.141 μg/l, 95% CL -0.254; -0.028) than 60 mg iron and 400 μg folic acid. There were no effects on markers of inflammation or oxidative stress.

Conclusions: Findings suggest that in a population where malnutrition is prevalent, nutrition interventions during pregnancy may modify the metabolic phenotype in the young child that could have consequences for later chronic disease risks.

Key words: Prenatal, Micronutrient, Food, Supplementation, early childhood, metabolic

---

Key Messages

- Invitation to food supplementation in early pregnancy (as compared with later) was associated with lower cholesterol, LDL and ApoB levels of the child at 4.5 years of age
- Supplementation with multiple micronutrients (as compared with standard iron-folate) was associated with lower HDL, lower glucose and lower IGF-1 levels.
- Nutrition interventions during pregnancy, when plasticity is great, may modify the metabolic phenotype in the young child.

Introduction

Nutritional imbalance in fetal life and a small size at birth are associated with serious short-term consequences, such as increased neonatal and infant mortality,[1] and impaired child growth,[2] as well as with long-term health consequences highlighted in the Developmental Origins of Health and Disease (DOHaD) hypothesis.[3] Limitations in nutrition during fetal life and early infancy, when plasticity is great, result in developmental adaptations inducing permanent changes in the metabolic phenotype. This increases the risk for insulin resistance,[4] hyperlipidaemia,[5] hypertension[6] and non-communicable diseases in adulthood such as type 2 diabetes[7] and coronary heart disease.[8] Different mechanisms underlying these effects have been proposed, such as structural changes in critical organs, epigenetic programming[9] and oxidative stress,[10] to name but a few. It is implicit that improved nutrition in early life may have the potential to reduce or prevent both short- and long-term health consequences of fetal and early life undernutrition.

Systematic reviews of the relative limited number of conducted trials providing balanced energy protein supplements in pregnancy show reduction in the frequency of stillbirth, increased birthweight and reduced occurrence of small-for-gestational-age.[11] Only a few of these studies have assessed metabolic markers in the offspring in childhood, adolescence or beyond. In Guatemala, an analysis was performed of adults born in villages where the population (including mothers and children) received a protein-energy supplement across different time periods from conception to 72 months of age. The adults with mothers who received the supplement from conception through 24 months of age had higher high-density lipoprotein (HDL) cholesterol and lower triglycerides than adults from the control group.[12] In India, adolescents born in villages where pregnant women and children up to 6 years of age received a balanced protein-energy supplement, had a lower occurrence of insulin resistance in comparison with those in control villages.[13] In the Gambia, 11–17-year-old children born to supplemented mothers had marginally lower fasting glucose levels.[14]

Anaemia in pregnancy is common and prenatal supplementation of iron and folic acid has been recommended by the World Health Organization (WHO) for many years to address this problem. As women in low-income settings often suffer from multiple micronutrient deficiencies,[15] supplements containing multiple micronutrients including iron, folic acid and vitamin B_{12} have been developed and increasingly been used.[16] A large number of trials have evaluated their short-term effects and systematic reviews have found significant effects mainly on maternal anaemia and offspring size at birth.[17,18] Little is known about long-term metabolic consequences of such prenatal supplements. A trial in Nepal did not find any effect of prenatal multiple micronutrients on blood lipids, glucose, insulin or homeostasis model assessment (HOMA) in children aged 6–8 years compared with controls. [19]

In malnourished populations, pregnant women may need both energy and protein supplements as well as micronutrient supplementation. Very few trials have been designed to evaluate whether combining supplementation of micro- and macronutrients confers health benefits. Further, the outcomes for the offspring of nutritional modification appear to depend on the stage of fetal

development and the vulnerability of the fetus.[20] Data from the Dutch Hunger Winter show that the influence on the fetus depends on the timing of nutrient restriction in pregnancy.[21] Size at birth is at least partly associated with fetal size already in the first trimester.[22] The Dutch Famine studies also indicate that nutritional insults in early pregnancy may affect metabolic regulatory mechanisms with negative consequences in adulthood, without effects on birthweight.[23]

The MINIMat (Maternal and Infant Nutrition Interventions in Matlab) trial in Bangladesh evaluated treatment with prenatal multiple micronutrients, including iron and folic acid, combined with early invitation to food supplementation (around week 9 of gestation), vs a standard programme that included treatment with iron and folic acid and usual timing of invitation to food supplementation (around week 20). Primary outcomes were maternal haemoglobin level at 30 weeks' gestation, birthweight and infant mortality. The early invitation to prenatal food supplementation in combination with multiple micronutrients resulted in markedly lower infant and under-five mortality,[24] but no differential effects on the other primary outcomes. The cohort of children born in the trial has since then continuously been followed up. The early invitation to food supplements reduced stunting up to 4.5 years, whereas allocation of multiple micronutrients resulted in a higher prevalence of stunting, all observed in boys.[25] The supplementation has also had small effects on blood pressure.[26]

The aim of this study is to evaluate whether an early timing of prenatal food supplementation and multiple micronutrient supplementation (MMS) favourably influence childhood metabolic phenotype (biomarkers of lipid and glucose metabolism, inflammation and oxidative stress) and if a combination of these interventions further enhances these outcomes.

Methods

Study location and population

The MINIMat trial was conducted in Matlab sub-district, located in poor communities in rural Bangladesh. The International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) runs a Health and Demographic Surveillance System (HDSS) in a population of 220 000 in this area. As part of the HDSS activities, community health workers perform monthly household visits and collect demographic and health data. This surveillance was used to identify and enrol pregnant women over a 2-year period. Eligibility criteria for participation were a viable fetus, gestational age less than 14 weeks by ultrasound examination,

no severe illness and written consent. A previous report has described the MINIMat trial in detail.[24]

This follow-up of the MINIMat trial included recruitments that took place during one of the two full calendar years of recruitment to the trial. Children born to the 2119 pregnant women, who were enrolled in the MINIMat trial from 1 January 2002 to 31 December 2002, were selected for biomarker analyses at 4.5 years of age. Only children who had anthropometric measurements at birth were eligible for this follow-up ($n = 1667$), whereof 1354 children participated in blood sampling at 4.5 years. We randomly selected smaller subsets for analyses of growth factors and oxidative stress markers (Figure 1).

Study design and interventions

Women had been individually and randomly allocated (2 by 3 factorial design) to two food groups and three micronutrient groups resulting in six groups in total (Figure 1). The food supplement consisted of a powder made of roasted rice, roasted pulse, molasses and soybean oil, to be mixed with water. This was provided at community nutrition centres 6 days per week, giving 608 kcal of energy per day. The two food supplement groups were invited to start supplementation either (i) immediately after detection of pregnancy, usually around pregnancy week 9 (early timing of invitation), or (ii) at the time of their choice, usually around pregnancy week 20 (usual timing of invitation, i.e. standard care). The three types of micronutrient supplements were capsules containing either: (i) 30 mg of iron (fumarate) and 400 μg folic acid (Fe30F); or (ii) 60 mg iron and 400 μg folic acid (Fe60F); or (iii) multiple micronutrients (MMS)[16] containing 30 mg of iron, 400 μg of folic acid, 800 μg of RE vitamin A (retinyl acetate), 200 IU of vitamin D (D_3), 10 mg of vitamin E (α-tocopherol acetate), 70 mg of vitamin C, 1.4 mg of vitamin B_1 (thiamine mononitrate), 1.4 mg of vitamin B_2 (riboflavin), 18 mg of niacin, 1.9 mg of vitamin B_6 (pyridoxine hydrochloride), 2.6 μg of vitamin B_{12} (cyanocobalamin), 15 mg of zinc (sulphate), 2 mg of copper (sulphate), 65 μg of selenium (sodium selenite) and 150 μg of iodine (potassium iodide). The women received the micronutrients in a bottle at health clinics in gestational week 14. There was a monthly refill of bottles.

Outcomes

The primary outcomes were maternal haemoglobin at 30 weeks of gestation, birthweight and infant mortality.[24] Secondary outcomes reported in this paper are metabolic markers (lipid and glucose metabolism, inflammation, oxidative stress) at 4.5 years of age. Other secondary
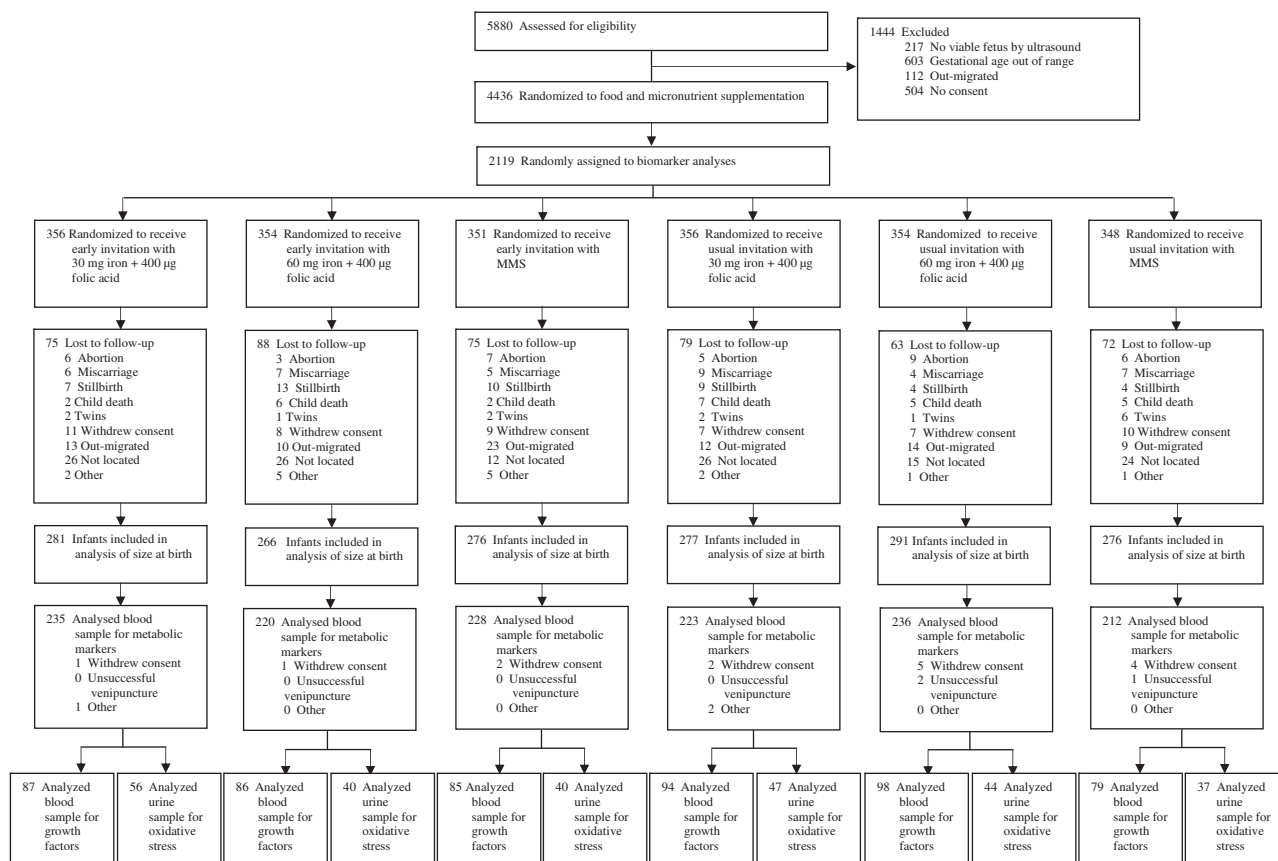
Figure 1. Flow-chart of participating women and children.

outcomes include psychomotor development in infancy,[27] effect on micronutrient status in infancy,[28] immune function,[29,30] childhood growth[31] and body composition at 4.5 years of age.[32]

Data collection

Pregnancy and delivery

At approximately gestational week 8, information was collected through household visits on household assets and was compiled into an asset score, in addition to women's health, pregnancy history and attained level of education. Trained personnel took anthropometric measurements at week 8 at the first health clinic visit. Body Mass Index (BMI) was calculated by dividing the weight (kg) by the height squared (m^2). Through a birth notification system, anthropometric data of the newborns were collected within 72 h. Maternal use of food supplements was assessed by repeated 4-week recall collected at household visits. Adherence to micronutrient supplementation was monitored by an electronic device, eDEM®, recording each opening of the capsule bottle and used as an indicator of supplement adherence.

Follow-up of children at 4.5 years

Trained nurses took anthropometric measurements of the children at health clinics. Paramedics collected overnight fasting blood samples of 5 ml venous blood using Li-heparin-treated trace-element-free tubes (Sarstedt Monevette®, Uppsala, Sweden). The plasma was centrifuged, separated within 4 h and stored at -70°C freezers at the laboratory in Matlab hospital. Urinary samples were collected in cups, transferred to plastic vials and transported cold to the laboratory where they were frozen and stored in -70°C. Plasma and urine samples were shipped on dry ice to Uppsala University, Sweden, for biomarker analysis.

Glucose was analysed in whole blood at the field site health clinics by HemoCue® (HemoCue, Ängelholm, Sweden). Plasma apolipoprotein A-1 (ApoA1), apolipoprotein B (ApoB), cholesterol, HDL, LDL, triglycerides and C-reactive protein (CRP) were measured by immunoturbidimetry on the Architect ci8200® Analyzer (Abbott Diagnostics). Plasma insulin was analysed by an immunological sandwich technique using Modular® Analytics E 170 Module (Roche Diagnostics). Insulin resistance was estimated by the homeostasis model assessment (HOMA-IR) [HOMA-IR = fasting plasma insulin level (mU/l ×

fasting plasma glucose level (mmol/l)/22.5].[33] The coefficient of variation (CV) was measured at two levels and for the lipid biomarkers CV was $\leq$ 3% in all cases. For CRP, the CV was $\leq$ 5% and for insulin $\leq$4%. IGF-1 in plasma was determined by radioimmunoassay after separation of IGF-1s from insulin growth factor BP 1 (IGFBP-1) by acid–ethanol extraction and cryoprecipitation.[34] IGFBP-1 was analysed using radioimmunoassay as described by Póvoa *et al.*[35]). 8-iso-PGF$_{2\alpha}$, a major F2-isoprostane and a reliable marker of oxidative stress *in vivo,* was analysed in urine samples in Uppsala, Sweden, by a validated radioimmuno-assay developed by Basu.[36]

Concentrations of CRP were below the detection limit of the assay (0.20 mg/l) in 24% of the plasma samples. All these samples were assigned half the value of the detection limit, i.e. 0.10 mg/l, to use as estimates in statistical analyses. Similarly, 7% of insulin samples were below the assay's detection limit (0.20 mU/l) and were thus assigned half the value of the detection limit, 0.10 mU/l. The distribution of children with concentrations below the detection limit was tested across food and supplementation groups. We found no difference in the frequency of being below the detection limit for either CRP or insulin concentrations depending on supplementation group. Further, for comparisons all key analyses were also performed in a subset of children with CRP above detection limit but, since there was no major difference in results if these were included or not, all are included in the presented results.

Randomization

Enrolled women were individually randomized into one of the six intervention groups by a computer-generated register of study identification numbers in permuted blocks of 12. Research staff and participating women were blinded for type of micronutrient supplementation and micronutrient capsules and bottles were identical. Invitation to food supplementation was randomly allocated but not blinded by design. Randomization codes were kept safe and confidential at icddr,b.

Sample size

The original sample size calculations were based on birthweight as one of the primary outcomes. A difference of 70 g was considered to be the minimum important group difference. With 90% power and a type I error of 0.05, the total sample size required was 5300 women with adjustments for 5% refusal, 11% loss during pregnancy and 9% out-migration. This paper reports the secondary outcome metabolic status assessed by biomarker concentrations in a subset of children at 4.5 years of age. For analyses of lipids,

CRP, glucose and insulin, all children to mothers enrolled during 1 calendar year were chosen to be able to cover possible seasonal differences. We used the anticipated number of children expected for biomarker analyses ($n = 1414$) to calculate the effects we would be able to detect according to the design of the trial. The effect sizes were expressed as a difference in standard deviation (SD) applying 95% confidence and 80% power. We would be able to detect a 0.16 z-score difference in any biomarker across two nutritional supplementation groups (i.e. early invitation to food supplementation vs usual timing) or a 0.48 z-score difference across the six food and micronutrient supplementation groups. For growth factors and markers of oxidative stress, smaller randomly selected subsets from the same calendar year were used. For those analyses, a sample of 600 children would enable a detection of 0.23 z-score difference between two nutritional supplementation groups or a 0.69 z-score difference across the six supplementation groups.

Statistical analysis

Baseline descriptive characteristics were compared between individuals with complete (included in main analyses) and incomplete data, and across intervention groups. The primary outcome was biomarker concentration in children at 4.5 years of age and analyses were by intention-to-treat. Biomarkers were presented in blocks divided into lipid metabolism (apoA1, apoB, apoB/apoA1, cholesterol, HDL, LDL, LDL/HDL, triglycerides), glucose metabolism (glucose, insulin, HOMA-IR), growth factors (IGF-1, IGFBP-1, IGF-1/IGFBP-1), inflammation (CRP) and oxidative stress (8-iso-PGF$_{2\alpha}$).

Normally distributed biomarkers are presented as means with 95 % confidence intervals (all assessed lipid markers and glucose). Non-normally distributed biomarkers are presented as medians with interquartile ranges (insulin, HOMA-IR, 8-iso-PGF$_{2\alpha}$, CRP and IGF-1, IGFBP-1, IGF-1/IGFBP-1). Non-normally distributed biomarkers were transformed to the natural logarithm before analyses. Due to the proportion of cases having a CRP below detection level (0.20 mg/l), we performed an additional analysis only including children with detectable CRP using log transformation. There was no association between the interventions and CRP levels using this exclusion (data not shown).

Differences between categorical variables were assessed by Pearson's chi-square test. Independent two-sample t-tests and analysis of variance (ANOVA) were used in the case of continuous variables to compare group differences (presented as differences of means). According to the original study protocol and study hypotheses, biomarkers of

Table 1. Descriptive characteristics of women and children by complete and incomplete data and by food and micronutrient supplementation groups[a]

| | Complete (n = 1351) | Incomplete (n = 316) | Maternal food supplementation | | Maternal micronutrient supplementation | | |
			Early invitation (n = 682)	Usual invitation (n = 669)	MMS (n = 439)	Fe60F (n = 454)	Fe30F (n = 458)
Household SES quintiles (% living in quintiles)[b]							
Lowest quintile	298 (22)	63 (20)	156 (23)	142 (21)	101 (23)	104 (23)	93 (20)
Highest quintile	243 (18)	79 (25)	119 (17)	124 (19)	78 (18)	75 (17)	90 (20)
Maternal characteristics pregnancy week 8							
Age (years)	26.7 (5.9)	26.1 (5.8)	26.7 (6.1)	26.7 (5.8)	26.7 (5.9)	26.5 (6.1)	26.8 (5.8)
Height (cm)	149.8 (5.3)	149.8 (5.3)	150.1 (5.3)	149.6 (5.4)	149.9 (5.5)	149.9 (5.1)	149.7 (5.4)
BMI (kg/m^2)	19.9 (2.6)	20.2 (2.7)	19.9 (2.6)	20.0 (2.6)	20.1 (2.7)	19.8 (2.6)	19.8 (2.6)
Parity[c]							
0	402 (30)	126 (40)	210 (31)	192 (29)	129 (29)	143 (32)	130 (28)
1	358 (27)	84 (27)	170 (25)	188 (28)	122 (28)	113 (25)	123 (27)
≥ 2	591 (44)	106 (34)	302 (44)	289 (43)	188 (43)	198 (44)	205 (45)
Schooling (years)							
0	500 (37)	90 (29)	256 (38)	244 (37)	173 (39)	160 (35)	167 (37)
1–4	139 (10)	44 (14)	73 (11)	139 (10)	47 (11)	42 (9)	50 (11)
≥ 5	712 (53)	182 (58)	353 (52)	359 (54)	219 (50)	252 (56)	241 (53)
Child characteristics							
Male sex	709 (53)	161 (51)	360 (53)	349 (52)	229 (52)	237 (52)	243 (53)
Age (months)	54.4 (1.7)	54.8 (2.3)	54.3 (1.7)	54.5 (1.8)	54.4 (1.7)	54.4 (1.7)	54.5 (1.8)
Weight (kg)	13.7 (1.6)	14.3 (2.1)	13.7 (1.5)	13.7 (1.7)	13.6 (1.5)	13.7 (1.6)	13.8 (1.7)
Height (cm)	99.5 (4.4)	100.3 (5.6)	99.6 (4.3)	99.4 (4.5)	99.3 (4.4)	99.5 (4.3)	99.8 (4.5)

[a]Data are presented as number (%) or mean (SD).
[b]Significantly different from incomplete with chi-square test, $P = 0.017$.
[c]Significantly different from incomplete with chi-square test, $P = 0.001$.

children born into the MMS supplement group were compared with the standard of micronutrient care provided in the Fe60F group. Biomarker concentrations in the Fe30 group are presented for descriptive purposes, although that intervention group mainly was motivated in relation to the primary outcome haemoglobin at 30 weeks of gestation. The early timing of food supplementation was compared with the standard of care, i.e. usual timing. As stated in the study protocol, and seeing that an important interaction between food and micronutrient supplementation groups has been observed previously,[24] we evaluated the interaction between food and micronutrient supplementation groups according to the 2 x 3 factorial design of the trial at $P < 0.05$.

Due to the possibility of a biased selection of individuals lost to follow-up, we performed additional analyses controlling for potential confounding factors, although the study design is a randomized controlled trial. We performed analyses including the variables of maternal height, maternal BMI at recruitment (around gestational week 9), socioeconomic status of the household (represented by asset score), child age and child sex. The inclusion of any

of these variables only caused minor changes to the effect estimates and made no difference to the interpretation of results. We are therefore only presenting the crude effect estimates.

Sex differences have been reported previously from this cohort[31] and also suggested in related literature.[37,38] Thus, regardless of whether any significant interactions between interventions and sex on the different outcomes were found, we present additional analyses stratified for sex of child in supplementary tables (available as Supplementary data at *IJE* online).

Analyses were done using the IBM SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, USA).

Ethical considerations

Informed consent was obtained from the participating women for the original trial and each step in the child follow-up. The study was approved by the ethical review committee at icddr,b, and the 4.5-year follow-up including analyses of metabolic biomarkers was approved by the regional ethical review board at Uppsala University, Sweden.

Results

The number of women lost to follow-up did not differ across the supplementation groups. Women with complete data had a higher percentage in the highest household asset score quintile and had more previous births as compared with women with incomplete data (Table 1).

Women were short and lean, with approximately one-third having BMI < 18.5 kg/m^2 and one-third being illiterate. There were no differences in baseline characteristics across supplementation groups. At 4.5 years of follow-up, boys had a mean weight of 14.0 kg (SD 1.6) or weight-for-age Z-score (WAZ) -1.36, mean height of 100.2 cm (SD 4.5) or height-for-age Z-score (HAZ) -1.46 and 31% were stunted (HAZ-score $<$-2.0). Girls had a mean weight of 13.4 kg (SD 1.6) or WAZ -1.38, a mean height of 98.9 cm (SD 4.3) or HAZ -1.60 and 36% were stunted.

By design, those women allocated to the early invitation to food supplementation consumed more packages of supplements than those allocated to the usual timing of invitation (mean difference 30 packages from enrolment to week 30 examination). On average the women took 77 micronutrient capsules and the MMS group took slightly fewer capsules (75) than the other groups.

Effects of timing of prenatal food supplementation

Children whose mothers had been allocated to early food supplementation had significantly lower apoB, lower cholesterol and lower LDL levels than children born to mothers allocated to food supplements with the usual timing (Table 2). In sex-stratified analyses, the overall effects on apoB, cholesterol and LDL were found in boys (Supplementary Table 1, available as Supplementary data at *IJE* online).

We found no differential effect by timing of food supplementation groups on markers of glucose metabolism (Table 2). Allocation to timing of food supplementation had no effect on the assessed child growth factors (Table 2). There were no associations between timing of food supplementation and child inflammation marker (assessed by plasma CRP) or marker of oxidative stress (assessed by urinary 8-iso-PGF$_{2\alpha}$) (Table 2). There was no interaction between the food and micronutrient groups for any of the biomarkers.

Effects of prenatal micronutrient supplementation

Children born to women allocated to MMS had significantly lower HDL than children with mothers randomized to Fe60F (Table 2). We found no other differences in lipid concentrations related to maternal micronutrient group.

Allocation to prenatal MMS supplementation resulted in lower fasting glucose concentrations in the children compared with the Fe60F group (Table 2). In sex-stratified analyses, this was only found among boys (Supplementary Table 2, available as Supplementary data at *IJE* online). Overall insulin concentrations were relatively low, but MMS supplementation had no differential effect on the child's fasting insulin concentration at 4.5 years. Children whose mothers had been allocated to the MMS group had lower IGF-1, corresponding to 14% difference in median, higher IGFBP-1 and consequently lower IGF-1/IGFBP-1 ratio compared with children from the Fe60F group (Table 2). In sex-stratified analyses, this association was only observed in boys with higher IGFBP-1 and lower IGF-1/IGFBP-1 concentrations (Supplementary Table 2). There were no associations between type of micronutrient supplement with the child CRP or urinary 8-iso-PGF$_{2\alpha}$ concentrations (Table 2).

Discussion

This trial was performed in a population where maternal and child chronic undernutrition is prevalent. An early start of food supplementation during pregnancy, when plasticity is great, influenced the metabolic phenotype at 4.5 years with a more favourable lipid profile. Children of women allocated to multiple micronutrient supplementation (as compared with standard iron-folate) had lower levels of fasting glucose and insulin-like growth factor 1 and also lower levels of HDL. The combination of the different food and micronutrient alternatives did not further enhance these effects.

We report on of the secondary outcomes of a prenatal individually randomized controlled trial with a follow-up of the offspring to the age of 4.5 years. We found small differences between women with complete vs incomplete data, where those included in the analyses had higher household asset scores (wealth) and more children. There was no association between participation and intervention groups. This implies that the results presented should not be influenced by any selection bias. Additional analyses were also performed controlling for potential confounders, without any important changes of effect estimates. All data collection followed standard operating procedures, and appropriate refresher training was conducted throughout the study period. The Matlab area, as well as other parts of the delta region in Bangladesh, has varying levels of arsenic contamination of drinking water from tube wells. There was no association between drinking water arsenic levels and the MINIMat randomized interventions,[39] which implies that arsenic exposure could not confound the reported associations between interventions and metabolic markers.

Table 2. Metabolic markers by food and micronutrient supplementation groups and mean differences between supplementation groups

	Maternal food supplementation				Maternal micronutrient supplementation			
	All children	Early invitation	Usual invitation	Mean difference early- usual (95% CI)	MMS	Fe60F	Fe30F	Mean difference MMS-Fe60
Lipid metabolism	(*n* = 1335)	(*n* = 672)	(*n* = 663)		(*n* = 435)	(*n* = 449)	(*n* = 451)	
ApoA1 (g/l)[a]	0.98 (0.97 to 0.99)	0.98 (0.97 to 0.99)	0.98 (.97 to 1.00)	−0.005 (−0.023 to 0.012)	0.97 (0.96 to 0.99)	0.99 (0.97 to 1.00)	0.98 (0.97 to 1.00)	−0.014 (−0.035 to 0.007)
ApoB (g/l)	0.72 (0.71 to 0.73)	0.71 (0.70 to 0.72)	0.73 (0.72 to 0.74)	−0.017 (−0.033 to −0.001)	0.72 (0.70 to 0.73)	0.72 (0.71 to 0.74)	0.72 (0.70 to 0.73)	−0.004 (−0.024 to 0.016)
ApoB/ApoA1	0.74 (0.74 to 0.75)	0.74 (0.72 to 0.75)	0.75 (0.74 to 0.77)	−0.015 (−0.034 to 0.004)	0.75 (0.74 to 0.77)	0.74 (0.72 to 0.76)	0.74 (0.73 to 0.76)	0.011 (−0.012 to 0.034)
Cholesterol (mmol/l)	3.78 (3.74 to 3.82)	3.74 (3.69 to 3.80)	3.82 (3.77 to 3.88)	−0.079 (−0.156 to −0.003)	3.75 (3.68 to 3.82)	3.82 (3.75 to 3.88)	3.78 (3.71 to 3.85)	−0.064 (−0.158 to 0.031)
HDL (mmol/l)	0.91 (0.90 to 0.92)	0.90 (0.89 to 0.92)	0.91 (0.90 to 0.93)	−0.007 (−0.027 to 0.014)	0.89 (0.87 to 0.91)	0.92 (0.90 to 0.94)	0.91 (0.90 to 0.93)	−0.028 (−0.053 to −0.002)
LDL (mmol/l)	2.07 (2.04 to 2.09)	2.03 (1.99 to 2.07)	2.10 (2.06 to 2.14)	−0.068 (−0.126 to −0.011)	2.04 (1.99 to 2.09)	2.10 (2.05 to 2.15)	2.06 (2.01 to 2.11)	−0.054 (−0.124 to 0.017)
LDL/HDL	2.32 (2.29 to 2.35)	2.29 (2.25 to 2.34)	2.35 (2.30 to 2.39)	−0.054 (−0.117 to 0.008)	2.33 (2.28 to 2.39)	2.32 (2.27 to 2.38)	2.30 (2.25 to 2.35)	0.010 (−0.067 to 0.087)
Triglycerides (mmol/l)	1.28 (1.26 to 1.31)	1.29 (1.25 to 1.33)	1.27 (1.24 to 1.31)	0.016 (−0.037 to 0.070)	1.28 (1.24 to 1.33)	1.27 (1.23 to 1.32)	1.29 (1.25 to 1.34)	0.011 (−0.054 to 0.077)
Glucose metabolism	(*n* = 1351)	(*n* = 682)	(*n* = 669)	Log mean difference early-usual (95% CI)	(*n* = 439)	(*n* = 454)	(*n* = 458)	Log mean difference MMS- Fe60 (95% CI)
Glucose (mmol/l)	4.75 (4.72 to 4.78)	4.73 (4.68 to 4.77)	4.78 (4.73 to 4.82)	−0.050 (−0.115 to 0.015)	4.70 (4.64 to 4.75)	4.80 (4.74 to 4.85)	4.76 (4.70 to 4.81)	−0.099 (−0.179 to -0.019)
	(n = 1290)	(n = 649)	(n = 641)		(n = 416)	(n = 432)	(n = 442)	
Insulin (pmol/l)	8.70 (4.56 to 15.00)	8.58 (4.56 to 14.40)	8.76 (4.53 to 15.60)	−0.009 (−0.123 to 0.105)	8.10 (4.38 to13.80)	8.79 (4.56 to 15.60)	9.06 (4.77 to 15.15)	−0.071 (−0.212 to 0.069)
HOMA-IR	0.30 (0.16 to 0.54)	0.30 (0.16 to 0.52)	0.30 (0.16 to 0.56)	−0.018 (−0.137 to 0.101)	0.28 (0.15 to 0.49)	0.31 (0.17 to 0.56)	0.30 (0.16 to 0.58)	−0.095 (−0.241 to 0.052)
Growth factors	*n* = 529	*n* = 258	*n* = 271		*n* = 162	*n* = 184	*n* = 181	
IGF-1 (μg/l)	83 (58 to 117)	83 (61 to 113)	85 (57 to 120)	0.001 (−0.091 to 0.093)	77 (53 to 106)	88 (61 to 122)	85 (62 to 124)	−0.141 (−0.254 to -0.028)[b]
IGFBP-1 (μg/l)	147 (126 to 171)	150 (124 to 170)	146 (126 to 172)	−0.024 (−0.074 to 0.027)	152 (129 to 174)	144 (124 to 167)	148 (127 to 168)	0.063 (0.001 to 0.126)
IGF-1/IGFBP-1	0.57 (0.37 to 0.86)	0.56 (0.39 to 0.82)	0.57 (0.35 to 0.87)	0.021 (−0.099 to 0.141)	0.51 (0.33 to 0.76)	0.62 (0.42 to 0.89)	0.57 (0.38 to 0.94)	−0.199 (−0.347 to -0.051)
Inflammation	(*n* = 1335)	(*n* = 672)	(*n* = 663)		(*n* = 435)	(*n* = 449)	(*n* = 451)	
CRP (mg/l)	0.48 (0.21 to 1.40)	0.48 (0.21 to 1.30)	0.48 (0.21 to 1.40)	−0.046 (−0.200 to 0.109)	0.54 (0.23 to 1.40)	0.44 (0.20 to 1.30)	0.48 (0.21 to 1.50)	0.100 (−0.090 to 0.290)
Oxidative stress	(n = 264)	(n = 136)	(n = 128)		(n = 77)	(n = 84)	(n = 103)	
8-iso- PGF$_{2\alpha}$ (nmol/ mmol creatinine)	0.97 (0.74 to 1.27)	0.99 (0.74 to 1.32)	0.95 (0.72 to 1.20)	0.069 (−0.047 to 0.184)	0.97 (0.75 to 1.29)	0.96 (0.73 to 1.20)	0.97 (0.74 to 1.28)	0.058 (−0.091 to 0.206)

[a]Data are presented as mean (95% CI) and mean difference (95% CI) for normally distributed variables, and median (IQR) and log mean difference (95% CI) for non-normally distributed variables.
[b]Corresponding to a 14% difference in median between MMS and Fe60F.

Lipid metabolism

The DOHaD hypothesis implicitly suggests that improving the nutritional status of malnourished mothers may have favourable effects on the metabolic phenotype of the child and influence later risks for chronic diseases. Bangladesh is undergoing a rapid nutritional and epidemiological transition, and has reportedly very high population-based prevalence of metabolic syndrome,[40] type 2 diabetes[41] and insulin resistance.[42] We consistently found that children born to women who had been invited to an early start of food supplementation in pregnancy, had a more favourable lipid profile at 4.5 years, such as lower apoB, cholesterol and LDL cholesterol. Effect sizes were relatively small (2–3%). In boys alone, the differences between supplementation groups were slightly higher (3–4%) and this is of similar size as the effects of the STRIP trial in Finland, where dietary counselling regarding low-fat food initiated in infancy resulted in 2–6% lower serum lipids (apoB, cholesterol and HDL) as compared with controls at 5 years of age.[43] In spite of the relatively small effect sizes, such differences may constitute risk indicators for future metabolic trajectories with long-term health consequences.

Our study differs from the previously mentioned protein-energy supplementation trials[12,14] in that the food supplementation was available to all women but the timing of the start of supplementation was randomized. Nutritional constraints in fetal life may have different effects depending on when they occur. The importance of timing of nutritional insults has been observed in animal models.[20] In ecological studies such as the Dutch Famine research, it has been shown that placenta development and function differ according to whether exposure to famine was in early, mid or late gestation.[44] Undernourished women starting food supplementation around gestational week 9 in the early invitation group may have benefited from the improved balanced food intake, resulting in developmental adaptations and permanent metabolic changes for the fetus in this period of early organogenesis and rapid growth.[45]

Women included in this trial had a high prevalence of several micronutrient deficiencies when assessed at baseline in early pregnancy,[46] including vitamin B_{12} and zinc deficiencies. These micronutrients were provided in the multiple micronutrient supplements. Women in the MMS group had significantly lower HDL, indicative of an unfavourable effect. No such effects on lipids were found in the Nepal trial that included multiple micronutrient supplements and allocated vitamin A only as control.[19]

Glucose metabolism

Children of mothers receiving MMS had significantly lower fasting glucose levels as compared with those allocated to the standard Fe60F. A lower fasting blood glucose may be indicative of a favourable effect of maternal multiple micronutrients. Average fasting glucose levels of these children were within the normal range, comparable with healthy children in Finland[47] and India,[48] and slightly higher than those reported from Nepalese children.[19]

The average insulin levels were low, which consequently also was reflected in low HOMA-IR. The observed insulin levels had a median value around the lower 25th percentile of children in both Nepal[19] and India.[48] Animal models of protein restriction during pregnancy have shown changes in pancreatic islets resulting in impaired insulin secretion in the offspring.[49] We did not find any major differential effects of food or micronutrient supplementations on insulin or HOMA-IR. In India, adolescents born to pregnant mothers who received food supplements, had lower insulin resistance compared with controls.[13]

Growth factors

Levels of IGF-1 vary widely in healthy children depending on age, sex and stage of puberty.[50] However, our observed IGF-1 concentrations were low, approximately 35% lower than those reported in healthy Danish children in the same age group.[51] IGF-1 levels are affected by current nutrition,[52] and protein and energy restriction has been associated with lower IGF-1 in humans. As previously reported, 31% of the children were stunted at 4.5 years,[25] and our observations of low IGF-1 concentrations may be a reflection of poor nutrition and of potential concern for these growing children.

We found that micronutrient supplementation in pregnancy was associated with the assessed growth factors. IGF-1 was lower in children of mothers receiving multiple micronutrients in pregnancy compared with the standard treatment with Fe60F which, in consideration of their generally low IGF-1 levels, may be considered unfavourable. As a crude measure of biologically active IGF-1, the IGF-1/IGFBP-1 ratio supports this interpretation. We have previously reported that children of mothers who were allocated to MMS more frequently were stunted in infancy and childhood up to 4.5 years of age.[25] This matches our finding of MMS and IGF-1 levels. In a primate model, maternal undernutrition during pregnancy was associated with reduced IGF-1 levels in the fetus,[53] and in humans, reduced IGF-1 levels were observed in children growth-restricted *in utero* compared with normal children.[54] This suggests an adaptive response of IGF-1 to nutrition in fetal life. In our study, we also observed higher IGFBP-1 levels in the children whose mothers were allocated to MMS. As IGFBP-1 is a marker of insulin sensitivity,[55] this suggests a potentially reduced future risk of CVD. In a Swedish study, adult

women born small for gestational age were shorter and had lower IGFBP-1 levels compared with full-term controls, suggesting reduced growth together with relative insulin resistance.[56] In our studied children, however, lower IGF-1 and stunted growth were accompanied by higher IGFBP-1. Our results indicate that the growth factors IGF-1 and IGFBP-1 in the children were modified by maternal micronutrient supplements, but the mechanisms are unclear.

Conclusion

This trial was performed in a population where chronic malnutrition still is common among mothers and children, and where rapid epidemiological and nutrition transitions take place. An early start of food supplementation during pregnancy influenced the metabolic phenotype at 4.5 years with a more favourable lipid profile. Children of mothers allocated to multiple micronutrient supplementation had lower levels of fasting glucose and IGF-1 but also lower levels of HDL. These findings suggest that nutrition interventions during pregnancy when plasticity is great may modify the metabolic phenotype in the young child, which could have consequences for later chronic disease risks.

Supplementary Data

Supplementary data are available at *IJE* online

References

1. Black RE, Allen LH, Bhutta ZA *et al*. Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet* 2008;**371**:243–60.
2. Arifeen SE, Black RE, Caulfield LE *et al*. Infant growth patterns in the slums of Dhaka in relation to birthweight, intrauterine growth retardation, and prematurity. *Am J Clin Nutr* 2000;**72:** 1010–07.
3. Barker DJ. The developmental origins of adult disease. *J Am Coll Nutr* 2004;**23**(Suppl 6):588–95S.
4. de Rooij SR, Painter RC, Phillips DI *et al*. Impaired insulin secretion after prenatal exposure to the Dutch famine. *Diabetes Care* 2006;**29**:1897–901.
5. Roseboom TJ, van der Meulen JH, Osmond C, Barker DJ, Ravelli AC, Bleker OP. Plasma lipid profiles in adults after prenatal exposure to the Dutch famine. *Am J Clin Nutr* 2000;**72**:1101–06.
6. Adair L, Dahly D. Developmental determinants of blood pressure in adults. *Annu Rev Nutr* 2005;**25**:407–34.
7. Whincup PH, Kaye SJ, Owen CG *et al*. Birthweight and risk of type 2 diabetes: a systematic review. *JAMA* 2008;**300**:2886–97.
8. Rich-Edwards JW, Stampfer MJ, Manson JE *et al*. Birthweight and risk of cardiovascular disease in a cohort of women followed up since 1976. *BMJ* 1997;**315**:396–400.
9. Hochberg Z, Feil R, Constancia M *et al*. Child health, developmental plasticity, and epigenetic programming. *Endocr Rev* 2011;**32**:159–224.
10. Grattagliano I, Palmieri VO, Portincasa P, Moschetta A, Palasciano G. Oxidative stress-induced risk factors associated with the metabolic syndrome: a unifying hypothesis. *J Nutr Biochem* 2008;**19**:491–504.
11. Ota E, Hori H, Mori R, Tobe-Gai R, Farrar D. Antenatal dietary education and supplementation to increase energy and protein intake. *Cochrane Database Syst Rev* 2015;**6**:CD000032.
12. Stein AD, Wang M, Ramirez-Zea M *et al*. Exposure to a nutrition supplementation intervention in early childhood and risk factors for cardiovascular disease in adulthood: evidence from Guatemala. *Am J Epidemiol* 2006;**164**:1160–70.
13. Kinra S, Rameshwar Sarma KV, Ghafoorunissa *et al*. Effect of integration of supplemental nutrition with public health programmes in pregnancy and early childhood on cardiovascular risk in rural Indian adolescents: long term follow-up of Hyderabad nutrition trial. *BMJ* 2008;**337**:a605.
14. Hawkesworth S, Walker CG, Sawo Y *et al*. Nutritional supplementation during pregnancy and offspring cardiovascular disease risk in The Gambia. *Am J Clin Nutr* 2011;**94**(Suppl 6):1853–60S.
15. Jiang T, Christian P, Khatry SK, Wu L, West KP Jr. Micronutrient deficiencies in early pregnancy are common, concurrent, and vary by season among rural Nepali pregnant women. *J Nutr* 2005;**135**:1106–12.
16. UNICEF/UNU/WHO. *Composition of a Multi-Micronutrient Supplement to Be Used in Pilot Programmes Among Pregnant Women in Developing Countries. Report of a UNICEF, WHO and UNU Workshop*. New York, NY: UNICEF, 1999.
17. Pena-Rosas JP, De-Regil LM, Dowswell T, Viteri FE. Daily oral iron supplementation during pregnancy. *Cochrane Database Syst Rev* 2012;**12**:CD004736.
18. Haider BA, Bhutta ZA. Multiple-micronutrient supplementation for women during pregnancy. *Cochrane Database Syst Rev* 2012;**11**:CD004905.
19. Stewart CP, Christian P, Schulze KJ, Leclerq SC, West KP Jr, Khatry SK. Antenatal micronutrient supplementation reduces metabolic syndrome in 6- to 8-year-old children in rural Nepal. *J Nutr* 2009;**139**:1575–81.
20. Hoet JJ, Hanson MA. Intrauterine nutrition: its importance during critical periods for cardiovascular and endocrine development. *J Physiol* 1999;**514**(Pt 3):617–27.

21. Roseboom TJ, van der Meulen JH, Ravelli AC, Osmond C, Barker DJ, Bleker OP. Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. *Mol Cell Endocrinol* 2001;**185**:93–98.

22. Bukowski R, Smith GC, Malone FD *et al*. Fetal growth in early pregnancy and risk of delivering low birthweight infant: prospective cohort study. *BMJ* 2007;**334**:836.

23. Ravelli AC, van Der Meulen JH, Osmond C, Barker DJ, Bleker OP. Obesity at the age of 50 y in men and women exposed to famine prenatally. *Am J Clin Nutr* 1999;**70**:811–16.

24. Persson LA, Arifeen S, Ekstrom EC, Rasmussen KM, Frongillo EA, Yunus M. Effects of prenatal micronutrient and early food supplementation on maternal hemoglobin, birthweight, and infant mortality among children in Bangladesh: the MINIMat randomized trial. *JAMA* 2012;**307**:2050–59.

25. Khan AI, Kabir I, Ekstrom EC *et al*. Effects of prenatal food and micronutrient supplementation on child growth from birth to 54 months of age: a randomized trial in Bangladesh. *Nutr J* 2011;**10**:134.

26. Hawkesworth S, Wagatsuma Y, Kahn AI *et al*. Combined food and micronutrient supplements during pregnancy have limited impact on child blood pressure and kidney function in rural Bangladesh. *J Nutr* 2013;**143**:728–34.

27. Tofail F, Persson LA, El Arifeen S *et al*. Effects of prenatal food and micronutrient supplementation on infant development: a randomized trial from the Maternal and Infant Nutrition Interventions, Matlab (MINIMat) study. *Am J Clin Nutr* 2008;**87**:704–11.

28. Eneroth H, El Arifeen S, Persson LA *et al*. Maternal multiple micronutrient supplementation has limited impact on micronutrient status of Bangladeshi infants compared with standard iron and folic acid supplementation. *J Nutr* 2010;**140**:618–24.

29. Moore SE, Prentice AM, Wagatsuma Y *et al*. Early-life nutritional and environmental determinants of thymic size in infants born in rural Bangladesh. *Acta Paediatr* 2009;**98**:1168–75.

30. Moore SE, Fulford AJ, Wagatsuma Y, Persson LA, Arifeen SE, Prentice AM. Thymus development and infant and child mortality in rural Bangladesh. *Int J Epidemiol* 2014;**43**:216–23.

31. Khan AI, Kabir I, Ekstrom EC *et al*. Effects of prenatal food and micronutrient supplementation on child growth from birth to 54 months of age: a randomized trial in Bangladesh. *Nutr J* 2011;**10**:134.

32. Khan AI, Kabir I, Hawkesworth S *et al*. Early invitation to food and/or multiple micronutrient supplementation in pregnancy does not affect body composition in offspring at 54 months: follow-up of the MINIMat randomized trial, Bangladesh. *Matern Child Nutr* 2015;**11**:385–97.

33. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;**28**:412–19.

34. Bang P, Eriksson U, Sara V, Wivall IL, Hall K. Comparison of acid ethanol extraction and acid gel filtration prior to IGF-I and IGF-II radioimmunoassays: improvement of determinations in acid ethanol extracts by the use of truncated IGF-I as radioligand. *Acta Endocrinol* 1991;**124**:620–29.

35. Povoa G, Roovete A, Hall K. Cross-reaction of serum somatomedin-binding protein in a radioimmunoassay developed for somatomedin-binding protein isolated from human amniotic fluid. *Acta Endocrinol* 1984;**107**:563–70.

36. Basu S. Radioimmunoassay of 8-iso-prostaglandin F2alpha: an index for oxidative injury via free radical catalysed lipid peroxidation. *Prostaglandins Leukot Essent Fatty Acids* 1998;**58**:319–25.

37. Eriksson JG, Kajantie E, Osmond C, Thornburg K, Barker DJ. Boys live dangerously in the womb. *Am J Hum Biol* 2010;**22**:330–35.

38. Barker DJ, Lampl M, Roseboom T, Winder N. Resource allocation in utero and health in later life. *Placenta* 2012;**33**(**Suppl 2**):e30–34.

39. Rahman A, Vahter M, Smith AH *et al*. Arsenic exposure during pregnancy and size at birth: a prospective cohort study in Bangladesh. *Am J Epidemiol* 2009;**169**:304–12.

40. Bhowmik B, Afsana F, Siddiquee T *et al*. Comparison of the prevalence of metabolic syndrome and its association with diabetes and cardiovascular disease in the rural population of Bangladesh using the modified National Cholesterol Education Program Expert Panel Adult Treatment Panel III and International Diabetes Federation definitions. *J Diabetes Invest* 2015;**6**:280–88.

41. Akter S, Rahman MM, Abe SK, Sultana P. Prevalence of diabetes and prediabetes and their risk factors among Bangladeshi adults: a nationwide survey. *Bull World Health Organ* 2014;**92**:204–13, 13A.

42. Alam DS, Talukder SH, Chowdhury MAH *et al*. Overweight and abdominal obesity as determinants of undiagnosed diabetes and pre-diabetes in Bangladesh. *BMC Obes* 2016;**3**:19.

43. Rask-Nissila L, Jokinen E, Ronnemaa T *et al*. Prospective, randomized, infancy-onset trial of the effects of a low-saturated-fat, low-cholesterol diet on serum lipids and lipoproteins before school age: The Special Turku Coronary Risk Factor Intervention Project (STRIP). *Circulation* 2000;**102**:1477–83.

44. Roseboom TJ, Painter RC, de Rooij SR *et al*. Effects of famine on placental size and efficiency. *Placenta* 2011;**32**:395–99.

45. Sadler TW. Susceptible periods during embryogenesis of the heart and endocrine glands. *Environ Health Perspect* 2000;**108**(**Suppl 3**):555–61.

46. Lindstrom E, Hossain MB, Lonnerdal B, Raqib R, El Arifeen S, Ekstrom EC. Prevalence of anemia and micronutrient deficiencies in early pregnancy in rural Bangladesh, the MINIMat trial. *Acta Obstet Gynecol Scand* 2011;**90**:47–56.

47. Kaitosaari T, Ronnemaa T, Viikari J *et al*. Low-saturated fat dietary counseling starting in infancy improves insulin sensitivity in 9-year-old healthy children: the Special Turku Coronary Risk Factor Intervention Project for Children (STRIP) study. *Diabetes Care* 2006;**29**:781–85.

48. Joglekar CV, Fall CH, Deshpande VU *et al*. Newborn size, infant and childhood growth, and body composition and cardiovascular disease risk factors at the age of 6 years: the Pune Maternal Nutrition Study. *Int J Obes (Lond)* 2007;**31**:1534–44.

49. de Oliveira CA, Latorraca MQ, de Mello MA, Carneiro EM. Mechanisms of insulin secretion in malnutrition: modulation by amino acids in rodent models. *Amino Acids* 2011;**40**:1027–34.

50. Juul A. Determination of insulin-like growth factor I in children: normal values and clinical use. *Horm Res* 2001;**55**(**Suppl 2**):94–99.

51. Juul A, Bang P, Hertel NT *et al*. Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. *J Clin Endocrinol Metab* 1994;**78**:744–52.

52. Clemmons DR, Underwood LE. Nutritional regulation of IGF-I and IGF binding proteins. *Annu Rev Nutr* 1991;**11**:393–412.

53. Li C, Schlabritz-Loutsevitch NE, Hubbard GB *et al*. Effects of maternal global nutrient restriction on fetal baboon hepatic insulin-like growth factor system genes and gene products. *Endocrinology* 2009;**150**:4634–42.

54. Cutfield WS, Hofman PL, Vickers M, Breier B, Blum WF, Robinson EM. IGFs and binding proteins in short children with intrauterine growth retardation. *J Clin Endocrinol Metab* 2002;**87**:235–39.

55. Heald AH, Cruickshank JK, Riste LK *et al*. Close relation of fasting insulin-like growth factor binding protein-1 (IGFBP-1) with glucose tolerance and cardiovascular risk in two populations. *Diabetologia* 2001;**44**:333–39.

56. Kistner A, Jacobson SH, Celsi G, Vanpee M, Brismar K. IGFBP-1 levels in adult women born small for gestational age suggest insulin resistance in spite of normal BMI. *J Intern Med* 2004;**255**:82–88.