Body composition and cardiometabolic risk markers in children of women who took part in a randomized controlled trial of a pre-conceptional nutritional intervention in Mumbai, India

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Abbreviations:

DXA	Dual X-ray absorptiometry
HeLTI	Healthy Life Trajectories Initiative
HOMA	Homeostasis model assessment
IHD	Ischemic heart disease
ISRCT	International Standard Randomised Controlled Trial Number
LMIC	Low- or middle income country
T2DM	Type 2 diabetes mellitus
RCT	Randomized controlled trial
RNI	Reference nutrient intake
SD	Standard deviation
SES	Socio-economic status
UNIMAPP	United Nations International Multiple Micronutrient Preparation
WHO	World Health Organization

Abstract

Background: Maternal nutrition influences fetal development and may permanently alter ('program') offspring body composition and metabolism, thereby influencing later risk of diabetes and cardiovascular (cardiometabolic) disease. The prevalence of cardiometabolic disease is rising rapidly in India.

Objective: To test the hypothesis that supplementing low-income Indian women with micronutrient-rich foods pre-conceptionally and during pregnancy has a beneficial impact on the children's body composition and cardiometabolic risk marker profiles.

Design: Follow-up of 1,255 children aged 5-10 years whose mothers took part in the Mumbai Maternal Nutrition Project (Project "SARAS"; ISRCTN62811278). Mothers were randomized to receive a daily micronutrient-rich snack or a control snack of lower micronutrient content, both made from local foods, in addition to normal diet, from before pregnancy until delivery. Children's body composition was assessed using anthropometry and dual X-ray absorptiometry (DXA). Their blood pressure, plasma glucose, insulin and lipid concentrations were measured. Outcomes were compared between allocation groups with and without adjustment for confounding factors.

Results: Overall, 15% of children were stunted, 34% were wasted and 3% were overweight. In the intention to treat analysis, there were no differences in body composition or risk markers between children in the intervention and control groups. Among children whose mothers started supplementation ≥3 months before conception (the 'per protocol' sample) the intervention increased adiposity among girls, but not boys. BMI in girls was increased relative to controls by 2% (95%CI 1, 4; p=0.01); fat mass index by 10% (95%CI 3, 18; p=0.004); and fat% by 7% (95%CI 1, 13; p=0.01) unadjusted, with similar results in adjusted models.

Conclusions: Overall, supplementing women with micronutrient-rich foods from before pregnancy until delivery did not alter the body composition or cardiometabolic risk markers in the children. Sub-group analyses showed that, if started at least 3 months before conception, supplementation may increase adiposity among female children.

Key words: maternal micronutrient supplementation, randomized controlled trial, India, children's body composition, children's glucose, children's insulin, children's lipids, DOHaD

Introduction

Ischaemic heart disease (IHD) and type 2 diabetes (T2DM) are leading causes of disability and death worldwide (1). While mortality from IHD is falling in the UK and other high-income countries, a trend attributed to both a falling incidence and improving medical treatment, it is increasing in low and middle-income countries (LMICs) (1). The prevalence of T2DM is rising in all countries, along with obesity, but the most rapid increases are in LMICs, despite relatively low obesity rates (2,3).

Thirty years ago Barker, Hales and others showed in a series of birth cohort studies that lower birth weight is associated with a higher risk of IHD and T2DM in adult life (4,5). They proposed that fetal undernutrition is an important risk factor for cardiometabolic disease in later life, due to impaired development of metabolic tissues such as the pancreas, liver, kidneys and skeletal muscle (6,7); this became known as the 'fetal programming' hypothesis. The same cohort studies showed that the highest risk of disease occurs in people who were small at birth but later became overweight (5,8). This led to the concept that sub-optimal fetal development results in reduced 'metabolic capacity' throughout life, which leads to disease at a lower threshold of 'metabolic load', for example from later life obesity (9). This could explain high rates of cardiometabolic disease, out of proportion to current obesity levels, in LMICs where maternal undernutrition and low birth weight remain common problems (9).

Animal experiments, showing that under-nourishing mothers leads to both fetal growth restriction and adult hypertension and diabetes in the offspring, support the fetal programming concept (10,11). However evidence for developmental programming in humans is still largely based on observational studies. Randomized controlled trials (RCTs) of nutritional interventions in undernourished women during pregnancy have shown that protein-energy and/or micronutrient supplements increase birth weight (12,13). Follow-up of

the children has shown reductions in blood pressure (14), fasting glucose (15), insulin resistance (16), LDL-cholesterol (15), triglyceride concentrations (17), arterial stiffness (16), metabolic syndrome (17) and adiposity (18). However, these changes have been small, inconsistent across studies, and sometimes transient (19,20) and some studies showed no beneficial effect on cardiometabolic outcomes (21,22). Most of these trials started the nutritional intervention between 12 and 20 weeks' gestation, which would have missed events in early pregnancy that are potentially important for programming, such as placental development, the period of rapid fetal organogenesis, and peri-conceptional epigenetic changes (23).

The Mumbai Maternal Nutrition Project (Project "SARAS", ISRCTN62811278) was an RCT of a food-based micronutrient supplement, starting pre-conceptionally, for women from low-income families living in slum communities in Mumbai, India (24). The intervention was a daily snack made from micronutrient-rich local foods as a supplement to women's normal diet. It reduced the incidence of gestational diabetes (25) and among women who started the supplement at least three months prior to conception, increased birth weight, with larger effects among women who had a higher pre-conception BMI (24). We have now followed up the children to measure body composition and cardiometabolic risk markers at the age of 5-10 years. We hypothesised that children of women in the intervention group would have lower cardiometabolic risk markers (blood pressure, serum lipids, plasma glucose, insulin resistance and a healthier body composition (greater height and lean mass and lower body fat percent) than children of mothers in the control group.

Subjects and Methods

The trial

Project SARAS was a non-blinded individually randomized nutritional supplementation trial

among women who were recruited before pregnancy in 2006-2011 (ISRCTN62811278) (24). The intervention was a daily snack made fresh each day in a trial kitchen from micronutrient-rich vegetarian local foods (green leafy vegetables, fruit and milk) (26). Control snacks contained foods of lower micronutrient content (e.g. potato and onion). The aim was for women to take one snack every alternate day or more, for at least three months before conception, and throughout pregnancy. On average, intervention snacks contained 10-23% of the WHO Reference Nutrient Intake (RNI) for β-carotene, riboflavin and vitamin B12, folate, calcium and iron, and 0.7 MJ of energy and 6 g of protein, compared with 0-7% RNI for the micronutrients, 0.4 MJ of energy and 2 g of protein in control snacks (26,27). Women were offered one snack daily; intake was supervised and the amount eaten was recorded (none, at least half, all). Women who became pregnant continued supplementation until delivery. All women were prescribed daily iron (100 mg) and folic acid (500 µg) supplements from the diagnosis of pregnancy, as per Indian government guidelines (28). Data were analysed according to 'intention-to-treat' (all women randomized) and in the 'per-protocol' sub-set of women who started supplementation >3 months before conception. Of 6,513 women recruited, 2,291 became pregnant, leading to 1,962 live singleton deliveries between 2007 and 2012.

Children's follow-up

The 'SARAS KIDS' follow-up study took place in 2013-2018, when the children were aged 5-10 years. Ethics approval was obtained from the Intersystem Biomedica Ethics Committee, Mumbai (ISBEC/NR-54/KM/JVJ/2013). Community health workers re-contacted families by telephone or home visit, explained the study, and invited parents and children to attend a local clinic for investigations. Informed parental consent and the children's assent were obtained. All investigations were carried out on one day except for blood samples, which were done on a separate day after an overnight fast.

Anthropometry: Weight was measured once to the nearest 10 g using digital scales (ATCO Ltd. Mumbai). Height was measured once to the nearest millimetre using a wall-mounted stadiometer (Microtoise, CMS Instruments, London). Head and mid-upper-arm circumferences were each measured three times to the nearest millimetre using anthropometric tapes, and the mean value used in the analysis. Biceps, triceps, subscapular and supra-iliac skinfolds were each measured to the nearest millimetre three times using Holtain skinfold callipers (CMS Instruments, London), and the mean value used in the analysis.

Blood pressure and pulse rate: Systolic and diastolic blood pressures and pulse rate were measured using an Omron 705IT digital monitor. Three measurements were made after the child had been seated for 5 minutes, removing and re-applying the cuff between measurements. The three values were averaged for analysis.

Plasma glucose, insulin and lipids: Parents were asked to ensure that the child had nothing to eat or drink except water for at least 8 hours before blood sampling. They were supplied with lidocaine anaesthetic cream and shown how to apply this to the venepuncture site before leaving home. Fasting venous blood samples were taken for plasma glucose and insulin and serum lipid concentrations and additional samples were taken at 30 minutes after an oral glucose load of 1.75 g/kg anhydrous glucose dissolved in 300 ml of water for glucose and insulin, and after 120 minutes for glucose. Samples were placed on ice and centrifuged within one hour. Plasma glucose concentrations were measured in a commercial laboratory (Dr Dharap's Laboratory, Dadar, Mumbai) using the glucose oxidase and peroxidase method, on the day of collection, using Accurex kits (Accurex Biomedical Ltd, Mumbai, India) and an EM200 auto-analyser (Transasia Biomedicals Ltd,, Mumbai, India). Plasma insulin and lipid samples were stored at -80^{O}C and assayed at the end of the study in the laboratory of the Diabetes Unit, King Edward Memorial Hospital, Pune. Insulin was measured using ELISA

kits (Mercodia AB, SE-754 50 Uppsala, Sweden) and Victor X4 multilabel plate reader (Perkin Elmer Life Sciences, Turku, Finland); their detection limit is 1.0 mU/L (ISO11843-Part-4). The kit is calibrated against the 1st International Reference Preparation 66/304. Inter- and intra-assay coefficients of variation (CVs) were <7%. All the lipids were measured on an automated analyser (Dialab, Wiener Neudorf, Austria) using Dialab ready-to-use kits. LDL-cholesterol was measured by a 2-step enzymatic selective protection method. HDL-cholesterol was measured using a homogeneous method without centrifugation steps; antibodies against human lipoproteins form antigen-antibody complexes with LDL, VLDL and chylomicrons in a way that only HDL-cholesterol is selectively determined by an enzymatic measurement. Triglycerides were measured using standard enzymatic kits. Inter- and intra-batch CVs for all three lipid measurements were <5%. Insulin sensitivity (HOMA-S) was estimated using the iHOMA2 online calculator (29). The insulogenic index [ln{Insulin(30-minute/fasting)/Glucose(30-minute/fasting)}] and the product of insulogenic index and insulin sensitivity, calculated as [insulogenic index + ln HOMA-S]) were calculated as measures of pancreatic beta cell function.

Body composition: Whole body and regional fat and lean mass were measured using dual X-ray absorptiometry (DXA). Scans were carried out at the Department of Radiology, Nanavati Hospital, Vile Parle, Mumbai on a Lunar Prodigy fan beam DXA scanner, using paediatric software. The machine was calibrated daily according to the manufacturer's instructions. Hand grip strength was measured as a marker of muscle function using a Jamar dynamometer. Three measurements were made with each hand, and the maximum value used in the analysis.

Socio-economic status: The family's socio-economic status (SES) was assessed using the Standard of Living Index questionnaire, developed for India's National Family Health

Survey, which creates a score based on the size and quality of housing and amenities and ownership of land and household assets; a higher score reflects higher SES (30).

Definitions: Height and BMI were converted into Z-scores based on the WHO 2007 standard (31,32). Stunting was defined as a height-for-age less than 2 standard deviations (SD) below the WHO median. BMI-for-age was categorised as wasting if less than 2 SD below the WHO median; 'normal' if between -2 and +1 SD of the WHO median; and overweight/obese if greater than 1 SD above the WHO median.

Statistical methods

Descriptive data are presented as mean (SD) for continuous normally distributed variables, median (interquartile range [IQR]) for skewed variables and n (%) for categorical variables. We tested the representativeness of the study sample by comparing maternal and newborn characteristics of a) the children studied with the remainder of births in the original trial, and b) within the study sample, between intervention and control groups. For comparisons of child outcomes between allocation groups, skewed variables were log-transformed, and all variables (except WHO Z-scores and their derivatives) were adjusted for the child's age and sex. They were compared using two-sided two sample t-tests for continuous variables and Chi-square tests for categorical variables in a) the full intention to treat sample and in b) the per protocol sub-group. We tested for interactions between allocation group and maternal pre-pregnant BMI and height as continuous variables, and the child's sex. Differences between groups are presented as mean difference and 95% confidence intervals (CI) for normal continuous variables, as a multiplicative difference for log-transformed variables, and as odds ratios for binary outcomes. Statistical significance was set at $p<0.005$ for comparisons of outcomes between intervention and control groups, using the Bonferroni correction for multiple testing and based on 10 'families' of outcomes (height, adiposity,

lean/muscle, blood pressure, three lipid variables, glucose, and indices of insulin sensitivity and secretion). We used a significance level of $p<0.05$ for interaction tests. Significant differences in outcomes between allocation groups were further examined using multiple linear regression, adjusting for potential confounders including maternal age, BMI, height, and parity, socio-economic status and the child's birth weight and gestation. Kernel density plots were used to examine and compare the distribution of selected variables between allocation groups. Analyses were carried out using Stata SE v16.1 (33) and R v3.6.0 (34).

Results

Of the 1,962 live singleton births in the trial, 51 children had died, 485 could not be re-traced, and 171 declined to take part in the follow-up, leaving 1,255 children (66% of survivors) who were studied (**Figure 1**). Their height- and BMI-for-age were low (mean WHO Z-scores -1.0 and -1.5 respectively); 18% of boys and 13% of girls were stunted and 34% of boys and girls were wasted. Only 4% of boys and 2% of girls were overweight or obese (**Table 1**). Girls were more adipose than boys, while boys had higher lean mass and grip strength. Boys had higher systolic blood pressure, fasting glucose concentration and insulin sensitivity, while girls had higher pulse rate, LDL-cholesterol and triglycerides (Table 1). Children studied were similar to those who were lost to follow-up in the proportions in each allocation group, maternal pre-pregnancy height and BMI and gestational diabetes status, birth weight and sex ratio, but their mothers were older and of higher socio-economic status (**Table 2**). Among the children studied, maternal age, height and SLI score were similar between the control and intervention groups, while maternal BMI was slightly lower in the intervention group (**Table 2**).

Effect of the intervention

There were no significant differences in any of the outcomes between children whose mothers were in the control and intervention groups, in either the intention to treat or per protocol samples (**Table 3**). The results were similar when the sample was limited to women who were fully adherent with supplementation (**Supplementary table 1**).

In the per protocol sample, there were significant sex interactions (allocation group X child's sex) for the adiposity outcomes: BMI, skinfolds, and fat mass and fat percent measured by DXA (Table 3). Among girls only, these adiposity measures were higher in the intervention group (**Table 4**); BMI was increased by 2% (95%CI 1, 4; p=0.01); fat mass index by 10% (95%CI 3, 18; p=0.004); and fat% by 7% (95%CI 1, 13; p=0.01). The prevalence of wasting was decreased, and that of normal BMI and overweight/obesity increased, though none of these effects were statistically significant (Table 4). Kernel density plots suggested an approximately symmetrical right shift in fat mass and fat mass index (**Figure 2**). Regression analysis, adjusting for confounding factors, showed that the increased adiposity among girls in the intervention group remained significant after adjusting for maternal characteristics, and may be partly influenced by the higher birth weight in the intervention group (shown for fat mass index in **Table 5** and for other adiposity measures in **Supplementary Table 2**). There were no interactions between allocation group and maternal BMI or height.

Discussion

Summary of findings

This study examined the impact of a pre-conceptional maternal nutritional intervention in a randomized controlled trial on cardiometabolic risk markers and body composition in the

children. The intervention, a micronutrient-rich food supplement from before conception and throughout pregnancy, had no effect overall on the children's cardiometabolic risk markers or body composition. In the sub-group of children whose mothers started supplementation $\geq$ 3 months before conception, girls had a higher BMI and were more adipose in the intervention group compared with controls.

Cardiometabolic risk markers

Possible reasons for a lack of effect on risk markers are that: 1) the intervention did not sufficiently improve maternal nutritional status; 2) a nutritional intervention alone is not sufficient to improve fetal development among women living with multiple environmental stresses likely to influence outcomes (poverty, over-crowding, pollution, inadequate sanitation); 3) a lack of obesity among these children meant that risk markers remained low in both groups; 4) the children were too young to see an effect or 5) maternal diet and nutrition are not important influences on children's cardiometabolic risk markers. We chose a food-based supplement based on findings from the Pune Maternal Nutrition Study, and for greater acceptability and potential greater scalability in future, but the 'dose' of micronutrients that it supplied was low (maximum 23% RNI) compared with other nutritional interventions used in trials, such as the UNIMAPP tablet (100% RNI). In a separate study among non-pregnant women in Mumbai we showed that the SARAS supplement increased circulating beta-carotene (35) and n-3 fatty acid concentrations (36), but did not significantly alter ferritin, retinol, ascorbate, folate or vitamin B12 status (35). Additionally, despite our efforts to make the snacks tasty and varied, it was challenging to sustain full adherence to supplementation over the long period of time required in a pre-conceptional trial. For these reasons, the effect of pre-conceptional nutritional supplementation requires testing in further trials, and it will be interesting to see the results of several completed or ongoing pre-conceptional trials which set out to deliver higher doses of multiple micronutrients in tablet or

other ready-made form and achieved higher compliance rates (37-41). Ultimately, sustainable ways of improving diet quality, using food, will be necessary. Improved maternal diet on its own may not be sufficient to achieve optimal fetal development; in preventing childhood stunting, another widespread problem in LMICs caused by complex multiple exposures combinations of interventions, targeting both health and nutrition outcomes, have proved most successful (42). That improved maternal nutrition alone may not be sufficient for optimal fetal development in the face of multiple environmental challenges is recognised in the ongoing HeLTI (Healthy Life Trajectory Initiative) and WINGS (Women and Infants Integrated Growth Study) randomized trials, which aim to improve maternal mental health as well as nutrition, and reduce infection and environmental pollution (43,44).

Body composition

Daughters of women in the intervention group who started supplementation well before conception (>3 months) were more adipose than daughters of women in the control group. The trial was designed to test this group separately (24), the rationale being that we would expect around 3 months' supplementation to be required to achieve its full impact on maternal nutritional status. The effect of the intervention on adiposity in girls was physiologically significant, approximately a 10% increase in fat mass index. Overall, the prevalence of wasting was 34% among the study children, while that of overweight/obesity was only 3%, and increased adiposity may therefore indicate more optimal nutrition. Greater adiposity provides opportunity for better future childhood and pubertal statural growth and (in girls) later reproductive outcomes. However, a gain in body fat without concomitant gains in height and lean mass could also have adverse cardiometabolic effects in adult life. Greater adiposity could reflect advanced maturation; it was not possible to determine this, although the differences in adiposity between allocation groups were not greater at older ages. Some of these possibilities will become clear with further follow-up. Most previous trials (all starting

in mid-pregnancy) using multiple micronutrients (14,45,46), protein-energy (16,22) or n-3 fatty acids (47-49) reported no increase in adiposity in the children. However, in three multiple micronutrient supplementation trials in Burkina Faso, Nepal and Bangladesh, children of mothers who received multiple micronutrients had higher BMI or weight-for-height Z-scores at age 30 months, 8.5 years and 9 years respectively (50,19,51). In the Nepal trial, as in Mumbai, this effect was present only in girls (19). In the Burkina Faso trial the positive effect on weight-for-height was accompanied by an increase in height (50).

Programming of adiposity

There is observational evidence in humans and interventional evidence in experimental animals that maternal *under-nutrition* during pregnancy increases later adiposity in the children/offspring. Exposure of previously well-nourished women to the Dutch Famine in early gestation was associated with greater adult adiposity in their children, of both sexes (52,53). In rats, both dietary restriction (either global restriction or a low-protein diet) and *over-feeding* of mothers during pregnancy increases adiposity in the adult offspring (11,54-56). None of these dietary experiences or experimental manipulations remotely corresponds to our intervention in Mumbai (supplementation of mothers, many of whom were chronically undernourished, with physiological doses of micronutrient-rich foods) but they show that adipose tissue is 'programmable' by maternal diet in pregnancy. including under- and over-feeding. Animal studies have shown that various mechanisms play a role in such experimental programming, including altered appetite (eg. hyperphagia), food choices (eg. junk food preference), reduced physical activity or resting energy expenditure, altered levels of or sensitivity to hormones (eg cortisol and leptin) or inflammatory markers, impaired mitochondrial function, altered mesenchymal stem cell commitment (to adipocyte as opposed to muscle/bone/cartilage lineages), and epigenetic changes (11,54-56). Perhaps the closest animal experiment to our study was the 'thrifty jerry' rat model, in which rats were globally

under-nourished for many generations, followed by recuperation onto normal feeding (57). During the under-nourished phase, newborn pups were smaller than controls but became excessively adipose as adults. After a return to normal feeding (ad libitum standard chow) birth weight was restored to control levels, but adult adiposity remained, and exceeded that in the multi-generationally under-nourished offspring. This was associated with epigenetic changes in the insulin-2 promoter region, which persisted after recuperation (57). Unlike our study, the increased adiposity among recuperated offspring was associated with elevated glucose, insulin and lipid concentrations.

Sex differences

There is an extensive literature from experimental animals reporting sex differences in phenotypic outcomes in offspring following maternal nutritional deprivation or over-feeding (58,59). For example in rats, maternal protein deprivation during pregnancy consistently leads to raised adult blood pressure in male but not female offspring. There are isolated examples of sex differences in the human developmental programming literature, but no consistent pattern has emerged linking particular exposures or outcomes to one or other sex (58,59). Apart from the Nepal trial described above (19) none of the child follow-ups from maternal supplementation trials in pregnancy have reported sex differences in cardiometabolic or body composition outcomes, but only a minority formally tested for sex differences. Mechanisms underlying sex differences in developmental programming in animals are still poorly understood (58-60). The fact that we observed a sex difference in the effect on adiposity only in the per protocol sample of children suggests that the critical period of exposure was peri-conceptional or in very early pregnancy, possibly related to sex differences in peri-conceptional gene expression or epigenetic characteristics in the embryo or placenta (59). In rodents, both maternal nutrient restriction and overfeeding lead to sex-specific changes in DNA methylation in the placenta (60).

Strengths and limitations

We studied a large sample of children, and cardiometabolic risk markers and body composition were measured using standard methods. A limitation was that we studied only 64% of the children born in the original trial. The greatest loss to follow-up was from families moving out of the study area, either through migration or re-location after local authority slum clearance. These losses were minimised by community health workers continually updating mobile phone numbers and attempting to retain contact with parents. We reimbursed families' expenses to come to the clinic from the main re-location areas ~20-30 km away. The children studied were similar to those lost to follow-up in key characteristics, but their mothers were older and of higher SES (Table 2). This could be because, in our experience, better-off families were more likely to own rather than rent their dwelling and therefore less likely to get moved out, and more likely to have a permanent mobile phone number. However, SES did not differ between allocation groups and our results were unchanged after adjusting for maternal age and SES and other potential confounding factors.

Conclusions and implications

The intervention, a pre-conception and pregnancy daily snack made from micronutrient-rich local foods, which increased birth weight and reduced the incidence of gestational diabetes did not alter the children's cardiometabolic risk markers. Girls of mothers who started the intervention more than 3 months before conception had a higher BMI, were less likely to be wasted, and were more adipose. We do not know the significance of this for future health outcomes and will continue to follow up these children.

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Data sharing: Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

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Table 1: Characteristics of the study sample[1]

Outcome	INTENTION TO TREAT SAMPLE (all children studied, Maximum N=1,255)					PER PROTOCOL SAMPLE (children of mothers who started supplementation ≥3m before conception, Maximum N=1,016)				
	BOYS		GIRLS		P [4]	BOYS		GIRLS		P [4]
	n		n			n		n		
Anthropometry										
Height Z-score	670	**-1.0** (1.1)	584	**-1.0** (0.9)	**0.44**	552	**-1.0** (1.1)	463	**-1.0** (0.9)	**0.45**
Stunted [2] (N(%))	670	**118** (17.6)	584	**76** (13.0)	**0.03**	552	**98** (17.8)	463	**61** (13.2)	**0.05**
BMI Z-score	670	**-1.5** (1.2)	584	**-1.6** (1.1)	**0.49**	552	**-1.5** (1.2)	463	**-1.6** (1.1)	**0.29**
BMI categories [2] (N (%))										
Wasting	670	**231** (34.5)	584	**196** (33.6)	**0.73**	552	**187** (33.9)	463	**163** (35.2)	**0.66**
Normal BMI	670	**414** (61.8)	584	**377** (64.6)	**0.31**	552	**344** (62.3)	463	**292** (63.1)	**0.81**
Overweight/obese	670	**25** (3.7)	584	**11** (1.9)	**0.05**	552	**21** (3.8)	463	**8** (1.7)	**0.05**
Sum of skinfolds [3] (mm)	670	**20.9** (18.2, 23.8)	584	**23.3** (20.2, 26.8)	**<0.001**	552	**20.8** (18.0, 23.8)	463	**23.0** (20.0, 26.7)	**<0.001**
Grip strength (kg)	669	**7.0** (1.6)	584	**6.2** (1.4)	**<0.001**	551	**7.0** (1.6)	463	**6.2** (1.4)	**<0.001**
Body composition (DXA)										
Fat mass [3] (kg)	660	**2.1** (1.7, 2.8)	567	**2.8** (2.1, 3.5)	**<0.001**	545	**2.1** (1.7, 2.7)	450	**2.8** (2.1, 3.4)	**<0.001**
Lean mass (kg)	660	**13.5** (1.6)	567	**12.3** (1.4)	**<0.001**	545	**13.5** (1.6)	450	**12.3** (1.4)	**<0.001**
Fat % [3]	660	**12.9** (10.6, 15.9)	567	**17.2** (14.0, 20.5)	**<0.001**	545	**13.0** (10.6, 15.9)	450	**17.0** (13.9, 20.2)	**<0.001**
Cardiometabolic risk markers										
Systolic BP (mmHg)	660	**93.7** (8.6)	583	**91.9** (8.7)	**<0.001**	543	**93.8** (8.6)	462	**92.0** (8.6)	**<0.001**
Diastolic BP (mmHg)	660	**56.4** (7.8)	583	**56.4** (7.0)	**0.95**	543	**56.4** (7.7)	462	**56.4** (7.1)	**0.94**
Pulse rate (beats/min)	667	**95.7** (11.3)	583	**98.5** (11.5)	**<0.001**	549	**95.9** (11.1)	462	**98.7** (11.8)	**<0.001**
LDL cholesterol (mmol/l)	651	**2.29** (0.61)	559	**2.42** (0.67)	**<0.001**	535	**2.29** (0.59)	444	**2.43** (0.64)	**<0.001**
HDL cholesterol (mmol/l)	651	**1.08** (0.23)	560	**1.06** (0.22)	**0.17**	535	**1.07** (0.23)	445	**1.07** (0.22)	**0.62**
Triglycerides [3] (mmol/l)	651	**0.82** (0.68, 1.02)	560	**0.88** (0.72, 1.11)	**0.01**	535	**0.82** (0.68, 1.01)	445	**0.88** (0.72, 1.11)	**0.01**
Fasting glucose (mmol/l)	660	**4.72** (0.56)	572	**4.62** (0.54)	**0.001**	544	**4.74** (0.54)	454	**4.62** (0.54)	**<0.001**
120-min glucose (mmol/l)	634	**4.59** (0.87)	549	**4.73** (1.05)	**0.01**	521	**4.60** (0.84)	434	**4.73** (0.95)	**0.03**
HOMA-S [3]	644	**238** (151, 422)	552	**207** (126, 323)	**<0.001**	528	**244** (153, 414)	437	**207** (129, 347)	**0.004**
Insulogenic index	639	**1.7** (1.1)	545	**1.5** (1.2)	**0.003**	525	**1.7** (1.1)	434	**1.5** (1.2)	**0.004**
Disposition index	632	**7.1** (1.7)	538	**6.7** (1.8)	**<0.001**	518	**7.1** (1.6)	427	**6.7** (1.8)	**0.001**

[1] Values are mean (SD) unless otherwise specified. All body composition and cardiometabolic outcomes were adjusted for the child's age except for Z-scores and BMI categories.
[2] Categorical variables are expressed as number (N) and percent.
[3] Skewed variables are expressed as median and inter-quartile range.
[4] P values denote the significance of differences between boys and girls.
DXA: Dual X-ray absorptiometry; HOMA-S: insulin sensitivity by Homeostasis Model Assessment.

Table 2: Representativeness of the study sample: maternal and newborn characteristics for the children included in the study sample compared with those lost to follow-up and, within the study sample, compared between maternal allocation groups [1]

Variable	Included in this study Maximum N=1,255 n		Lost to follow-up Maximum N=707 n		P [4]
Among all live singleton births in the original trial:					
Allocation group [2] (N((%)					
Control	649	51.7	356	50.4	**0.56**
Intervention	606	48.3	351	49.6	
Maternal age (years)	1255	24.5 (3.9)	707	23.5 (3.4)	**<0.001**
Maternal height (cm)	1255	151.3 (5.5)	706	151.6 (5.4)	**0.19**
Maternal pre-pregnancy BMI [3] (kg/m^2)	1254	19.8 (17.8, 22.6)	706	19.8 (17.9, 22.1)	**0.64**
Maternal SLI score	1221	25.7 (5.7)	683	23.7 (6.4)	**<0.001**
Maternal GDM status [2] (N(%))					
No GDM	660	89.8	230	91.3	**0.50**
GDM	75	10.2	22	8.7	
Child's birthweight (g)	960	2611 (381)	407	2611 (419)	**0.92**
Child's sex [2] (N (%))					
Male	671	53.5	233	53.2	**0.53**
Female	584	46.5	205	46.8	
Among children studied in this follow-up:	**Control group Maximum N=649**		**Intervention group Maximum N=606**		
Maternal age (years)	649	24.7 (3.9)	606	24.4 (3.8)	**0.17**
Maternal height (cm)	649	151.2 (5.4)	606	151.3 (5.6)	**0.91**
Maternal pre-pregnancy BMI [3] (kg/m^2)	649	19.9 (17.9, 22.6)	606	19.6 (17.7, 22.5)	**0.04**
Maternal SLI score	629	25.7 (5.6)	592	25.7 (5.8)	**0.90**
Maternal GDM status [2] (N(/%))					
No GDM	336	87.7	324	92.1	**0.05**
GDM	47	12.3	28	8.0	
Child's birth weight (g)	499	2594 (393)	461	2629 (368)	**0.11**
Child's sex [2] (N(/%))					
Male	282	56.3	248	53.6	**0.40**
Female	219	43.7	215	46.4	

[1] Values are mean (SD) unless otherwise specified.
[2] categorical variables are expressed as number (N) and percent.
[3] Skewed variable are expressed as median and inter-quartile range.
[4] P values denote the significance of differences between the groups shown.
GDM: maternal gestational diabetes mellitus.

Table 3: Outcomes at 5-10 years according to allocation group [1]

Outcome	INTENTION TO TREAT SAMPLE (all children studied, Maximum N=1,255)						PER PROTOCOL SAMPLE (children of mothers who started supplementation ≥3m before conception, Maximum N=1,016)					
	CONTROL GROUP		INTERVENTION GROUP		p[1]	p[2]	CONTROL GROUP		INTERVENTION GROUP		p[1]	p[2]
	n		n				n		n			
Anthropometry												
Height Z-score	649	**-1.0** (1.0)	605	**-1.0** (1.0)	**0.78**	**0.72**	534	**-1.0** (1.0)	481	**-1.0** (1.0)	**0.91**	**0.88**
Stunted [2] (N(%))	649	**97** (15.0)	605	**97** (16.0)	**0.60**	-	534	**85** (15.9)	481	**74** (15.4)	**0.82**	-
BMI Z-score	649	**-1.6** (1.1)	605	**-1.5** (1.2)	**0.45**	**0.16**	534	**-1.6** (1.1)	481	**-1.5** (1.2)	**0.23**	**0.04**
BMI categories [2] (N (%))												
Wasting	649	**224** (34.5)	605	**203** (33.6)	**0.72**	-	534	**190** (35.6)	481	**160** (33.3)	**0.44**	-
Normal BMI	649	**411** (63.3)	605	**380** (62.8)	**0.85**	-	534	**332** (62.2)	481	**304** (63.2)	**0.74**	-
Overweight/obese	649	**14** (2.2)	605	**22** (3.6)	**0.12**	-	534	**12** (2.3)	481	**17** (3.5)	**0.22**	-
Sum of skinfolds [3] (mm)	649	**21.9** (19.1, 24.9)	605	**22.0** (19.1, 25.5)	**0.36**	**0.18**	534	**21.7** (19.0, 24.8)	481	**21.8** (19.0, 25.4)	**0.36**	**0.02**
Grip strength (kg)	648	**6.7** (1.6)	605	**6.6** (1.5)	**0.63**	**0.88**	533	**6.7** (1.6)	481	**6.6** (1.5)	**0.87**	**0.88**
Body composition (DXA)												
Fat mass [3] (kg)	637	**2.4** (1.9, 3.1)	590	**2.5** (1.9, 3.1)	**0.40**	**0.06**	525	**2.3** (1.9, 3.0)	470	**2.5** (1.9, 3.1)	**0.31**	**0.01**
Lean mass (kg)	637	**13.0** (1.5)	590	**13.0** (1.5)	**0.97**	**0.72**	525	**13.0** (1.5)	470	**13.0** (1.5)	**0.62**	**0.53**
Fat % [3]	637	**14.8** (12.0, 18.0)	590	**15.0** (12.3, 18.2)	**0.40**	**0.08**	525	**14.7** (11.9, 17.9)	470	**14.9** (12.3, 18.0)	**0.39**	**0.02**
Cardiometabolic risk markers												
Systolic BP (mmHg)	641	**92.9** (8.4)	602	**92.8** (8.9)	**0.75**	**0.34**	526	**92.9** (8.5)	479	**93.1** (8.8)	**0.75**	**0.14**
Diastolic BP (mmHg)	641	**56.2** (7.3)	602	**56.6** (7.4)	**0.28**	**0.60**	526	**56.1** (7.3)	479	**56.8** (7.5)	**0.14**	**0.28**
Pulse rate (beats/min)	646	**97.7** (11.5)	604	**96.3** (11.3)	**0.03**	**0.46**	531	**98.1** (11.2)	480	**96.2** (11.5)	**0.01**	**0.44**
LDL cholesterol (mmol/l)	627	**2.38** (0.65)	583	**2.31** (0.61)	**0.05**	**0.18**	515	**2.39** (0.63)	464	**2.32** (0.59)	**0.07**	**0.29**
HDL cholesterol (mmol/l)	627	**1.07** (0.23)	584	**1.07** (0.22)	**0.69**	**0.14**	515	**1.07** (0.23)	465	**1.07** (0.23)	**0.71**	**0.14**
Triglycerides [3] (mmol/l)	627	**0.85** (0.68, 1.05)	584	**0.84** (0.70, 1.07)	**0.98**	**0.89**	515	**0.85** (0.69, 1.05)	465	**0.84** (0.70, 1.07)	**0.69**	**0.49**
Fasting glucose (mmol/l)	640	**4.68** (0.52)	592	**4.67** (0.58)	**0.54**	**0.85**	526	**4.69** (0.52)	472	**4.69** (0.55)	**0.91**	**0.74**
120-min glucose (mmol/l)	610	**4.66** (0.88)	573	**4.65** (1.04)	**0.89**	**0.74**	500	**4.65** (0.87)	455	**4.67** (0.92)	**0.82**	**0.65**
HOMA-S [3]	619	**223** (142, 387)	577	**223** (137, 376)	**0.56**	**0.71**	507	**225** (148, 403)	458	**228** (137, 367)	**0.45**	**0.74**
Insulogenic index	614	**1.63** (1.11)	570	**1.50** (1.16)	**0.06**	**0.66**	507	**1.66** (1.13)	452	**1.53** (1.17)	**0.08**	**0.99**
Disposition index	606	**7.0** (1.7)	564	**6.9** (1.7)	**0.17**	**0.82**	499	**7.0** (1.7)	446	**6.9** (1.7)	**0.19**	**0.96**

[1] Values are mean (SD) unless otherwise specified. All body composition and cardiometabolic outcomes were adjusted for the child's age and sex except for Z-scores.
P1: significance of difference between control and intervention groups; P2: significance of interaction between allocation group and sex.
[2] Categorical variables are expressed as number (N) and percent.
[3] Skewed variables are expressed as median and inter-quartile range.
DXA: Dual X-ray absorptiometry; HOMA-S: insulin sensitivity by Homeostasis Model Assessment.

Table 4: Adiposity measurements in the children according to the mother's allocation group, stratified by sex (per protocol sample) [1]

Outcome	BOYS				GIRLS			
	CONTROL n = 299	INTERVENTION n = 253	Difference [intervention-control] (95% CI) [4]	P [5]	CONTROL n = 235	INTERVENTION n = 228	Difference [intervention-control] (95% CI) [4]	P [5]
Anthropometry								
Body mass index [3] (kg/m^2)	13.5 (12.8, 14.1)	13.4 (12.8, 14.0)	1.00 (0.98, 1.01)	**0.62**	13.0 (12.3, 13.9)	13.2 (12.6, 14.1)	1.02 (1.01, 1.04)	**0.01**
BMI Z-score (WHO)	-1.5 (1.2)	-1.5 (1.2)	-0.0 (-0.2, 0.1)	**0.62**	-1.7 (1.1)	-1.4 (1.2)	0.3 (0.0, 0.5)	**0.02**
BMI categories [2] (N(%)								
Wasting	100 (33.4)	87 (34.4)	1.04 (0.73, 1.49)	**0.82**	90 (38.3)	73 (32.0)	0.76 (0.52, 1.11)	**0.16**
Normal BMI	189 (63.2)	155 (61.3)	0.92 (0.65, 1.30)	**0.64**	143 (60.9)	149 (65.4)	1.21 (0.83, 1.77)	**0.32**
Overweight/obese	10 (3.3)	11 (4.4)	1.31 (0.55, 3.15)	**0.54**	2 (0.9)	6 (2.6)	3.15 (0.63, 15.77)	**0.14**
Biceps skinfold [3] (mm)	4.4 (3.9, 5.3)	4.3 (3.9, 5.1)	0.98 (0.94, 1.02)	**0.28**	4.8 (4.1, 5.5)	5.0 (4.3, 6.0)	1.05 (1.01, 1.10)	**0.02**
Triceps skinfold [3] (mm)	6.9 (5.8, 7.9)	6.7 (5.8, 7.9)	0.99 (0.94, 1.03)	**0.61**	7.3 (6.1, 8.4)	7.6 (6.4, 9.1)	1.06 (1.01, 1.11)	**0.01**
Subscapular skinfold [3] (mm)	5.4 (4.8, 6.3)	5.3 (4.7, 6.3)	0.98 (0.94, 1.02)	**0.25**	5.9 (5.0, 6.7)	6.2 (5.4, 7.4)	1.07 (1.03, 1.12)	**0.002**
Suprailiac skinfold [3] (mm)	4.0 (3.3, 4.8)	3.9 (3.3, 4.6)	0.98 (0.94, 1.03)	**0.53**	4.6 (3.8, 5.5)	4.6 (4.0, 5.7)	1.02 (0.97, 1.07)	**0.55**
Sum of skinfolds [3] (mm)	20.8 (18.3, 24.2)	20.6 (17.8, 23.6)	0.98 (0.94, 1.02)	**0.37**	22.5 (19.7, 25.7)	23.3 (20.3, 28.0)	1.05 (1.01, 1.10)	**0.01**
Body composition (DXA)								
Fat mass [3] (kg)	2.13 (1.71, 2.83)	2.15 (1.63, 2.66)	0.97 (0.90, 1.04)	**0.37**	2.55 (2.01, 3.21)	2.86 (2.23, 3.62)	1.11 (1.03, 1.19)	**0.01**
Fat mass index [3] (kg/m^2)	1.8 (1.4, 2.3)	1.7 (1.4, 2.2)	0.97 (0.91, 1.03)	**0.32**	2.2 (1.7, 2.7)	2.4 (1.8, 2.9)	1.10 (1.03, 1.18)	**0.004**
Lean mass (kg)	13.5 (1.7)	13.5 (1.7)	-0.0 (-0.3, 0.3)	**0.95**	12.3 (1.4)	12.4 (1.4)	0.1 (-0.1, 0.4)	**0.36**
Lean mass index (kg/m^2)	11.0 (0.7)	11.0 (0.7)	0.0 (-0.1, 0.1)	**0.99**	10.1 (0.7)	10.2 (0.7)	0.1 (-0.1, 0.2)	**0.26**
Fat % [3]	13.0 (10.8, 16.3)	12.9 (10.4, 15.6)	0.98 (0.92, 1.03)	**0.36**	16.8 (13.6, 19.8)	17.2 (14.7, 20.6)	1.07 (1.01, 1.13)	**0.01**
Android fat [3] (kg)	0.15 (0.11, 0.20)	0.14 (0.11, 0.18)	0.98 (0.90, 1.06)	**0.61**	0.18 (0.13, 0.23)	0.19 (0.15, 0.25)	1.10 (1.01, 1.20)	**0.03**
Gynoid fat [3] (kg)	0.57 (0.48, 0.73)	0.56 (0.44, 0.67)	0.97 (0.91, 1.03)	**0.26**	0.69 (0.57, 0.81)	0.72 (0.60, 0.88)	1.06 (1.00, 1.12)	**0.04**

[1] Values are mean (SD) unless otherwise specified. All outcomes are adjusted for the child's age.
[2] Categorical variables are expressed as number (N) and percent.
[3] Skewed variables are expressed as median and inter-quartile range.
[4] Differences between allocation groups: For continuous normally distributed variables, these are expressed as raw values in the intervention group minus those in the control group, with 95% confidence intervals. For skewed variables[3], which were log-transformed for the analysis, the differences are exponentiated, and indicate the multiplicative difference between control and intervention groups; for example: a value of 1.07 means that the outcome was 7% higher in the intervention group than in the control group, while a value of 0.97 means that the outcome was 3% lower in the intervention group. For categorical variables[2], the differences between groups are expressed as odds ratios, with the control group as the reference category.
[5] P values denote the significance of differences between control and intervention groups. DXA: Dual-energy absorptiometry.

Table 5: Multiple linear regression analysis of allocation group as a predictor of log fat mass index in girls only, adjusted for maternal and newborn characteristics (per protocol sample)

Exposure	MODEL 1, N=438			MODEL 2, N=317		
	Linear regression coefficient	95% confidence intervals	p value	Linear regression coefficient	95% confidence intervals	p value
Maternal						
Allocation group (control=0, intervention=1)	0.104[1]	0.037, 0.170	0.003	0.085[1]	0.004, 0.167	0.04
Age (years)	-0.009	-0.018, -0.000	0.05	-0.016	-0.028, -0.005	0.005
BMI (kg/m^2)	0.025	0.016, 0.034	<0.001	0.024	0.013, 0.036	<0.001
Height (cm)	-0.004	-0.010, 0.002	0.20	-0.005	-0.012, 0.002	0.18
Parity - primiparous	-0.059	-0.139, 0.020	0.15	-0.054	-0.158, 0.050	0.31
Parity - multiparous	-0.112	-0.218, -0.005	0.04	-0.115	-0.243, 0.014	0.08
Socio-economic status score	-0.000	-0.007, 0.006	0.91	-0.003	-0.010, 0.005	0.49
Child						
Age (years)	0.053	0.006, 0.0101	0.03	0.032	-0.029, 0.093	0.31
Birth weight (kg)	-	-	-	0.128	0.111, 0.245	0.03
Gestational age (weeks)	-	-	-	-0.012	-0.035, 0.010	0.29

[1] For allocation group, the regression coefficient represents the difference in log fat mass between the intervention and control groups. To translate this into a more meaningful value the coefficient is anti-logged (exponentiated: values become 1.11 for Model 1 and 1.09 for Model 2) and this value indicates the multiplicative difference between control and intervention groups; for example: an exponentiated value of 1.11 means that fat mass index was 11% higher in the intervention group than in the control group.

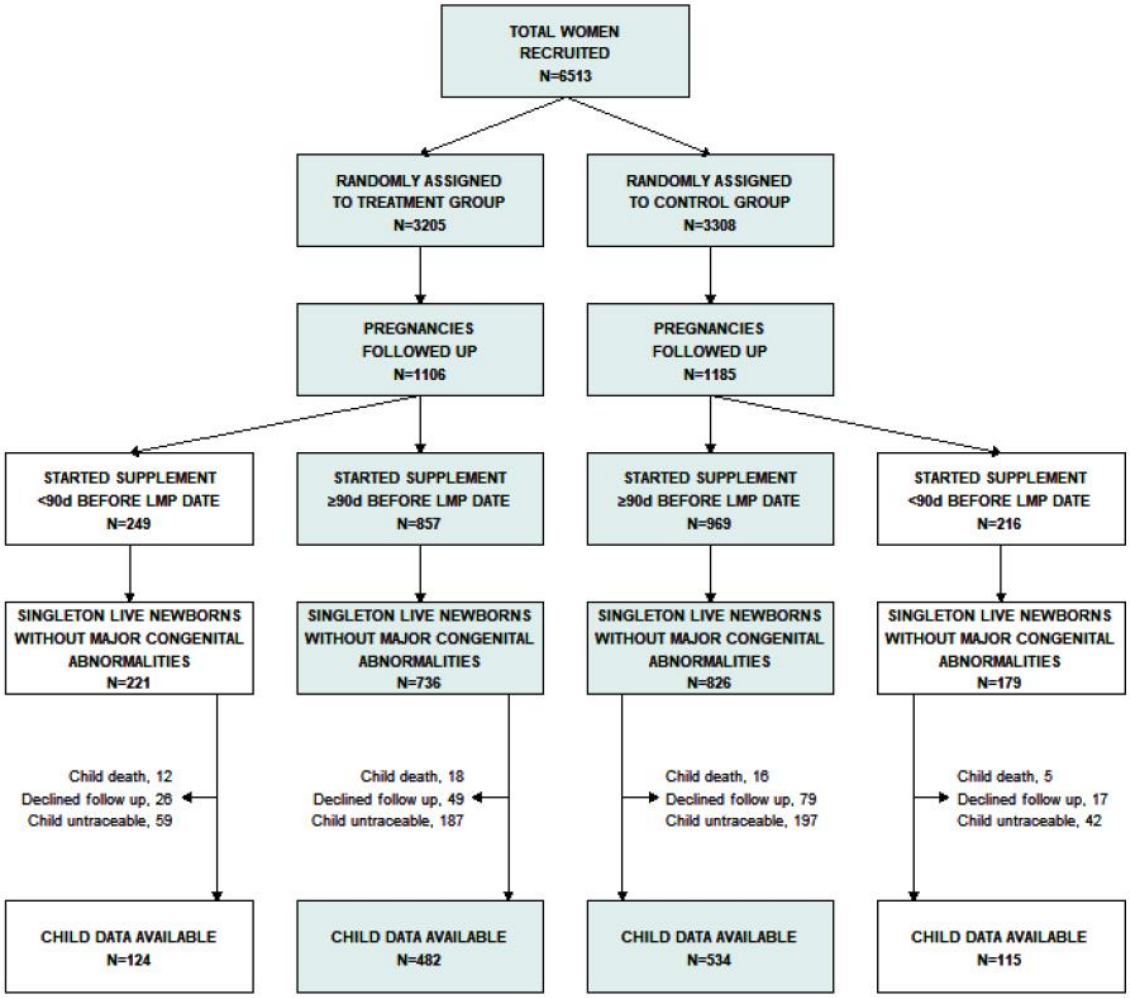

Figure 1: Participant flowchart

Figure 2

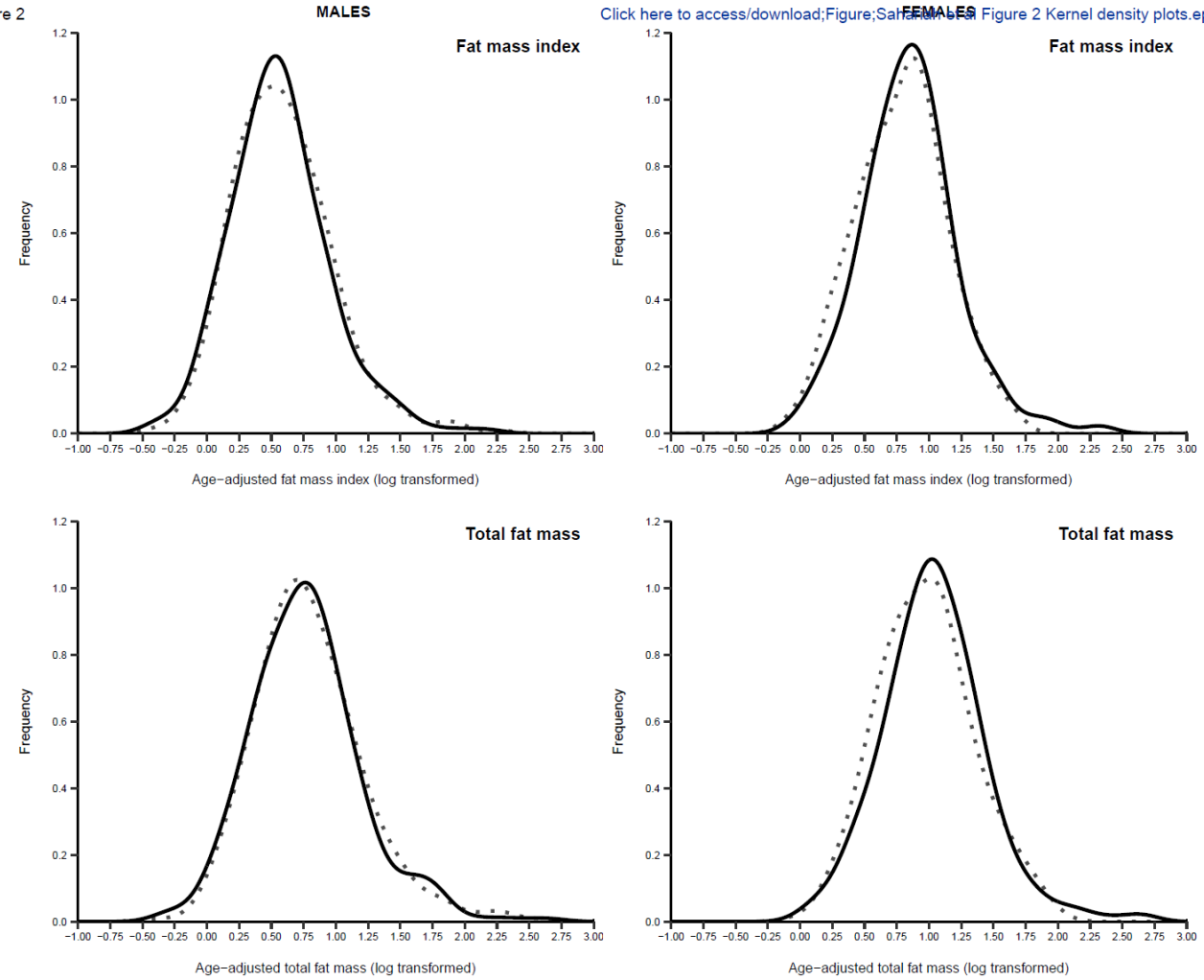

Figure 2: Kernel density plot of fat mass and fat mass index by intervention group in girls and boys