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Exploiting dietary supplementation trials to assess the impact of the prenatal environment on CVD risk

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Animal studies have demonstrated that altering the maternal diet during pregnancy affects offspring disease risk. Data from human subjects on the early-life determinants of disease have been derived primarily from birth-weight associations; studies of the impact of the maternal diet are scarce and inconsistent. Investigating CVD risk factors in the offspring of women who have participated in maternal supplementation trials provides a useful resource in this research field, by virtue of employing an experimental design (as compared with observational studies). To date, follow-up studies have been published only for a small number of trials; these trials include the impact of maternal protein–energy, multiple-micronutrient and Ca supplementation on offspring disease risk. In Nepal maternal micronutrient supplementation has been shown to be associated with lower offspring systolic blood pressure at 2 years of age. Data from Guatemala on a pre- and postnatal protein–energy community intervention have suggested long-term improvements in fasting glucose and body composition but not in blood pressure. In The Gambia no association has been found between prenatal protein–energy supplementation and markers of CVD risk including body composition, blood pressure and fasting glucose and insulin in childhood and adolescence. Little evidence of an effect of maternal Ca supplementation on offspring blood pressure has been demonstrated in four trials, although the risk of high systolic blood pressure was found to be reduced in one trial. The present paper reviews the current evidence relating maternal nutritional supplementation during pregnancy to offspring CVD risk and explores the potential explanations for the lack of association.

Developmental origins of health and disease: Maternal supplementation trials: CVD risk

The field of research currently known as the developmental origins of health and disease (DOHaD) grew from initial observations that infant mortality rates at the turn of the last century were highly correlated with standard mortality rates approximately 60 years later[1,2]. One explanation given for these findings is that a 'nutritional deficit' operating in early life results in 'a lifelong vulnerability to aspects of an affluent adult lifestyle'[1]. After 30 years the field of DOHaD has grown into a vibrant multi-disciplinary research area, although controversy remains about the nature of the early-life nutritional deficits and the mechanisms through which they might affect later health.

CVD is one of the main diseases of affluence faced by the world today. Evidence that CVD risk may be related to early-life, specifically intrauterine, exposures was initially derived from the inverse association between birth weight and CVD mortality[3–5] and/or risk factors such as blood pressure[6,7] insulin resistance[8] and the metabolic syndrome[9]. One of the most-widely-cited hypotheses, termed the 'thrifty phenotype'[10], suggests that when exposed to deprived conditions during development fetal adaptations (metabolic, endocrine and/or anatomical) occur that allow for immediate survival but may be maladapted to cope with the demands of a more-affluent lifestyle in later life

Abbreviation: DOHaD, developmental origins of health and disease.

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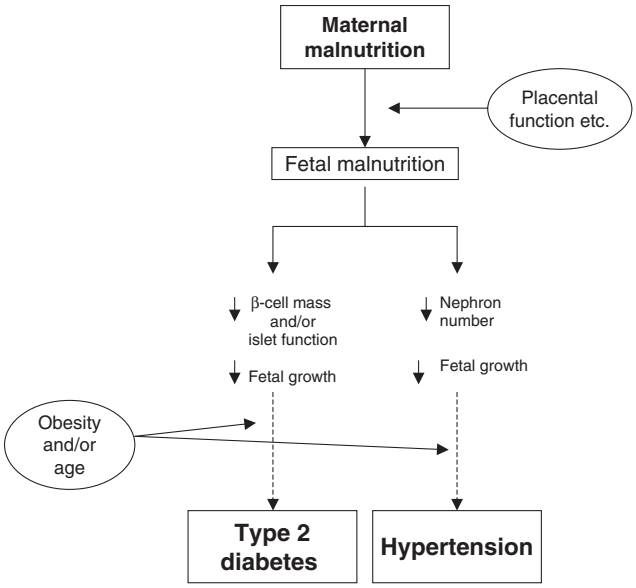

Fig. 1. Schematic diagram of the 'thrifty phenotype' hypothesis showing potential pathways to type 2 diabetes and hypertension. (Adapted from Hales & Barker[10].)

(Fig. 1). Maternal nutrition is one of the potentially-modifiable determinants of fetal nutrition and is thus a focus of research seeking to understand the association between early life and later disease.

A wealth of animal studies, extensively reviewed elsewhere[11–13], have confirmed that perturbations in the maternal diet during pregnancy can have a profound impact on offspring disease risk. Pregnant rats fed a low-protein diet give birth to offspring that develop hypertension, which persists into adult life[14]. Indeed, some or all the components of the metabolic syndrome can be produced by a variety of maternal exposures, including global nutrient restriction[15], placental insufficiency[16] and a low-Fe diet[17] as well as a low-protein diet. The animal data have allowed for increasingly sophisticated exploration of potential mechanisms and have greatly enhanced the DOHaD field. Nevertheless, the translation of these findings into human studies remains challenging.

The majority of data from human subjects still centres on the inverse association between birth weight and later disease. These observations are primarily from retrospective cohort studies and the evidence base has been criticised for inappropriate statistical methods[18], publication bias and inadequate adjustment for socio-economic status[19]. Perhaps the most important criticism is that birth weight is a poor measure of exposure, primarily as it is multifactorial in origin, prompting calls to move towards more direct assessments of exposure. Studies relating maternal diet in pregnancy and offspring CVD risk are rare, however, and have shown little or no associations to date[20,21]. Furthermore, cohort studies investigating diet during pregnancy retain many of the same study design issues as those that have utilised birth weight. The Dutch Hunger Winter Study (1944–5) has been used as a pseudo-experiment with which to study the impact of nutritional deprivation during pregnancy on offspring disease risk. Results focusing on CVD risk have been inconsistent[22–24], however, and by their nature the cohorts have widely-recognised design issues; including large loss to follow-up and a lack of data on individual dietary and/or nutrient intakes.

One emerging resource in this field is the follow up of trials of maternal supplementation during pregnancy. Although primarily conducted to evaluate the effectiveness of interventions to improve pregnancy and birth outcomes, these trials represent a useful resource for the DOHaD field by providing randomised controlled trial data. To date, the only exposures that have been studied in this manner are protein–energy, multiple-micronutrient and Ca supplementation. The remainder of the present paper will summarise these follow-up studies insofar as they relate to offspring CVD risk.

Protein–energy

In the late 1960s the Institute of Nutrition of Central America and Panama conducted a community trial of protein supplementation in rural Guatemala. Two villages received the 'Atole' supplement, a protein–energy-dense drink, whilst two control villages received the 'Fresco' supplement, a drink that contained no protein and approximately one-third of the energy[25]. The study design is summarised in Table 1; supplement drinks were provided twice daily and their consumption by pregnant women and children up to the age of 7 years was recorded. A number of follow-up studies have been conducted on the trial participants and the findings have been extensively summarised[26]. Findings most relevant to CVD risk include a marginally-greater fat-free mass in adolescent girls born in intervention villages[27], reduced fasting glucose in 25-year-old women born in intervention villages[28] but no difference in blood pressure between individuals born in intervention and control villages[29] (Table 2). A major limitation of this study in relation to the current review is that it is not possible to distinguish between pre- and postnatal enhanced nutrition in the intervention analysis. Furthermore, because of the small number of villages the cluster design of the original trial has not been accounted for in the analysis. Although often viewed as a trial of protein supplementation, the two drinks also provided different levels of certain micronutrients (Ca, P, Zn, folic acid and vitamin B_{12}), which may affect interpretation of the results[26].

The Barry Caerphilly Growth Study is a follow up of children whose mothers took part in a trial to improve their cow's milk intake during pregnancy that was conducted in South Wales, UK in the early 1970s[30] (Table 1). Women were randomised to receive milk tokens throughout pregnancy and for the first 5 years of their child's life or to receive no tokens (control). Milk tokens were equivalent to 284 ml milk/d from the milkman. At 25 years of age the offspring were enrolled into a follow-up study and it was found that those whose mothers had received the intervention had lower serum levels of insulin-like growth factor 1 compared with those born to control mothers (Table 2)[30]. Insulin resistance[31] and IHD[32] have been associated with low insulin-like growth factor 1 concentrations in observational studies. For the purposes of the present review

Table 1. Characteristics of trials and follow-up studies of maternal protein–energy supplementation

	Guatemala	Wales, UK	The Gambia
Original trial	Martorell *et al.*[25]	Ben-Shlomo *et al.*[60]	Ceesay *et al.*[33]
Design	Community-level randomised trial, 1969–77	Randomised controlled trial, 1972–4	Community-level randomised trial, 1989–94
Randomisation	Village level: two intervention villages, two 'control'	Individual level using random number tables	Village level: sixteen intervention and twelve 'control'
Participants	All village residents: intake of pregnant women and children up to age 7 years recorded	Pregnant women from two towns in South Wales attending primarycare clinics Enrolled for pregnancy and until their child was 5 years of age	All pregnant women in participating villages From 20 weeks of gestation until term
Supplement	Drink-based supplement, provided twice daily in central location Intervention villages: 'Atole'; 682 kJ energy, 11·5 g protein Control villages: 'Fresco'; 247 kJ energy, 0 g protein (consumed twice as frequently as Atole)	Milk tokens; entitled owner to free milk delivery (equivalent to 284 ml/d) Intervention arm: milk tokens Control arm: no tokens	Two biscuits provided daily (4250 kJ energy), 22 g protein, 56 g fat, 47 mg Ca, 1·8 mg Fe Intervention arm: biscuits from 20 weeks gestation until delivery Control arm: biscuits for 20 weeks post partum
Follow-up	Summary: Martorell[26] Body composition: Rivera *et al.*[27] Blood pressure: Webb *et al.*[29] Glucose: Conlisk *et al.*[28]	Ben-Schlomo *et al.*[30]	Blood pressure: Hawkesworth *et al.*[34] Body composition: Hawkesworth *et al.*[35]
Date	Rivera *et al.*[27]: 1988–9; (subjects exposed during pregnancy until 3 years of age) Webb *et al.*[29] and Conlisk *et al.*[28] 1997–8 (subjects exposed during pregnancy until 7 years of age)	1997–9	2005–6
Age of offspring	Rivera *et al.*[27]: 14–20 years Webb *et al.*[29] and Conlisk *et al.*[28]: 20–29 years	Men: 23·2–27·2 years (mean 25 years) Women: 23·1–26·8 years (mean 25 years)	11·2–17·0 years (mean 14·1 years)
Loss to follow-up	Rivera *et al.*[27]: 1574 of 2169 (73%) original trial offspring recruited for entire follow-up 460 children (exposed during pregnancy and to 3 years of age) included in analysis Webb *et al.*[29]: 450 of 585 (77%) eligible and traceable subjects; lost to follow-up associated with lower birth weight Conlisk *et al.*[28]: 478 of 585 (82%) eligible and traceable subjects	633 of 1163 (54%) of original trial offspring recruited Lost to follow-up associated with lighter birth weight and younger maternal age	1317 of 2047 (64%) of original trial offspring recruited No differences associated with lost to follow-up
Outcomes	Anthropometry Fasted blood from fingerpick assessed for plasma glucose Blood pressure (digital sphygmomanometer; UA-767; A&D Instruments Ltd, Abington, Oxon., UK)	Fasted blood samples collected. Serum levels of IGF-1, IGFBP-3 and IGF-1: IGFBP-3 were determined	Blood pressure (Omron 705IT; Morton Medical Ltd, London, UK) Body composition (Tanita BC-418MA; Chasmors Ltd, London, UK; with population-specific equations[61]) Triceps skinfold thickness Fasted blood sample analysed for glucose and insulin

IGF, insulin-like growth factor; IGFBP-3, IGF-binding protein.

Table 2. Effect of maternal protein–energy supplementation on CVD risk factors in the offspring from trials conducted in Guatemala[27–29], Wales, UK[30] and The Gambia[34,35]*

			Supplement			Control			Mean difference‖		
Outcome	Study	Gender	*n*	Mean	SD or IQR	*n*	Mean	SD or IQR	Mean	95% CI	*P*
Blood pressure											
Systolic (mmHg)	Webb *et al.*[29]	M	119	120	9·9	106	120	11·2	0·6	−2·17, 3·37	0·67
		F	109	103	9·2	116	104	11·4	−0·5	−3·23, 2·23	0·72
	Hawkesworth *et al.*[34]		608	111	8·8	659	110	9·3	0·46¶	−1·12, 2·04	0·57
Diastolic (mmHg)	Webb *et al.*[29]	M	119	73·4	7·8	106	72·4	8·1	1·0	−1·09, 3·09	0·35
		F	109	65·6	7·7	116	64·6	8·0	1·0	−1·07, 3·07	0·34
	Hawkesworth *et al.*[34]		608	64·6	7·6	659	64·7	7·7	0·09¶	−1·31, 1·13	0·89
Body composition											
Whole-body fat (%)†	Hawkesworth *et al.*[35]	M	315	12·6	2·8	337	12·6	3·0	0·01¶	−0·52, 0·55	0·96
		F	288	19·5	4·7	321	19·3	4·3	0·19¶	−0·68, 1·06	0·67
FFM (kg)‡	Rivera *et al.*[27]	M	118	42·5	3·3	127	42·3	3·4	0·2	−0·64, 1·04	0·64
		F	116	36·9	4·3	99	35·7	4·0	1·2	0·08, 2·32	0·04
Triceps skinfold thickness (mm)	Hawkesworth *et al.*[35]	M	321	6·4	1·9	338	6·3	2·0	−0·1¶	−0·04, 0·06	0·68
		F	290	11·0	5·0	321	10·8	4·5	0·003¶	−0·08, 0·08	0·92
Blood analysis											
Fasted glucose (mmol/l)	Hawkesworth *et al.**		608	49·1	30·2	653	48·2	28·4	−0·05¶	−0·10, 0·01	0·08
	Conlisk *et al.*[28]	M		NR			NR		0·02	−0·29, 0·33	0·80
		F		NR			NR		0·29	−0·23, 0·80	0·03
Fasted insulin (pmol/l)	Hawkesworth *et al.**		607	4·8	0·5	655	4·9	0·4	0·62¶	−4·19, 5·42	0·80
IGF-1 (ng/ml)	Ben-Shlomo *et al.*[30]		344	136§	110–172	306	144§	111–178	−8·5**	−15·1, −1·8	0·01
IGFBP-3 (g/ml)	Ben-Shlomo *et al.*[30]		344	6·44§	5·51–7·53	306	6·43§	5·55–7·52	−0·04**	−0·28, 0·21	0·73
IGF-1:IGFBP-3	Ben-Shlomo *et al.*[30]		344	21·8§	17·1–26·1	306	22·9§	17·6–28·5	−1·2**	−2·33, −0·04	0·04

FFM, fat-free mass; IGF, insulin-like growth factor; IGFBP-3, IGF-binding protein; M, males; F, females; NR, not reported; IQR, interquartile range.
*S Hawkesworth, AM Prentice, AJC Fulford and SE Moore (unpublished results).
†Whole-body fat assessed by bioelectrical impedance analysis utilising population-specific prediction equations.
‡Calculated from prediction equations using arm diameter for males and waist circumference for females.
§Because of skewness of data these values are the median and IQR.
‖Unadjusted regression analysis unless otherwise stated.
¶Generalised estimating equations used to take village clustering into account.
**Regression adjusted for age and gender.

milk tokens have been viewed as a protein–energy supplement, but it should be acknowledged that the increased consumption of Ca and/or growth factors may also be relevant.

In 2005 a follow-up study was conducted in The Gambia, West Africa involving offspring (aged 11–17 years) who had been born during a randomised trial of supplementation with protein–energy-dense biscuits during pregnancy or lactation. The original trial[33] provides some of the strongest evidence yet that protein–energy supplements can improve birth weight in nutritionally-deprived populations. The trial used a cluster randomised design in twenty-eight villages; pregnant women from intervention villages received protein–energy-dense biscuits from 20 weeks of gestation until delivery whilst women in control villages received the same supplement for 20 weeks post partum. The supplement given during pregnancy was shown to increase birth weight by 136 g overall, with a greater difference of 201 g during the nutritionally-poor 'hungry season' (June–October)[33]. The follow-up study has found no evidence that these early-life differences correspond to later differences in risk profile in the offspring; no effect of maternal supplementation during pregnancy on offspring blood pressure[34], body fat[35] or fasting insulin and glucose was found (S Hawkesworth, AM Prentice, AJC Fulford and SE Moore, unpublished results; Table 2). The limitation of this study is that the women in the control arm were provided with the same protein–energy-dense biscuits during lactation. However, as there is little evidence to suggest that supplementation of lactating women affects breast-milk quality or quantity[36,37] the comparison is considered to be appropriate. Although no overall effect of maternal protein–energy supplementation on offspring CVD risk was found, an interaction with body composition was found. For offspring who were relatively lean (in the lowest quartile of percentage body fat) at follow up the intervention was associated with raised systolic blood pressure (Fig. 2). It is possible that providing supplements to the mother whilst the fetus is developing provides better conditions than those that are experienced postnatally, although only for offspring who remain particularly lean. This interaction may reflect the 'mismatch' theory, which suggests that if conditions during *in utero* development are 'mismatched' to the later environment this mismatch promotes the development of disease[38]. The pattern more often seen in the literature is the interaction between low birth weight and overweight in adulthood[38]; in the less-developed setting of The Gambia it seems that the reverse of this pattern may have been observed.

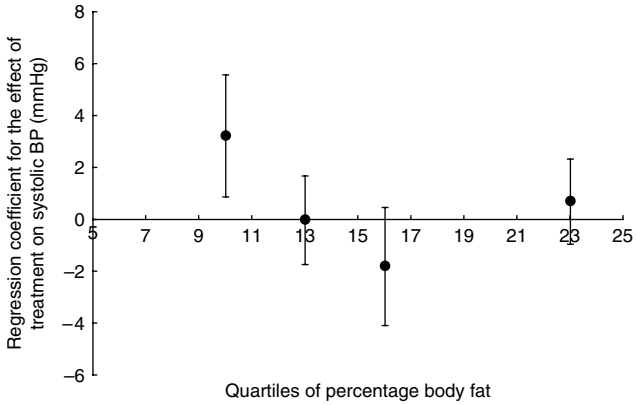

Fig. 2. Interaction between body composition (percentage body fat) and maternal protein–energy supplementation for systolic blood pressure (BP) in Gambian adolescents at follow up. Values are means with 2 SE represented by vertical bars. (Reproduced from Hawkesworth *et al.*[34])

Multiple micronutrients

In recent years there has been considerable interest in the potential of maternal micronutrient supplementation during pregnancy to provide important benefits for both the mother and her infant. Recently, the results of the many trials have been summarised, with the focus on short-term outcomes such as birth weight and infant survival[39]. Only one trial has so far published findings of follow up beyond infancy (Table 3), reporting lower systolic blood pressure and slightly greater triceps skinfold thickness for 2–3-year-old children born to Nepalese mothers who received a combination of fifteen micronutrients compared with 'control' women receiving only Fe and folic acid[40] (Table 4). The authors suggest caution in the interpretation of this finding from a single trial and recognise the importance of replication in other studies[40]. It should also be noted that there was no difference in diastolic blood pressure, and that blood pressure is notoriously difficult to measure in very young children. Similar follow-up studies in Bangladesh[41] and a different area of Nepal[42] are currently nearing completion and will soon add to the current limited knowledge.

Calcium

A number of maternal Ca supplementation trials have been conducted on pregnant women in recent years, primarily to investigate the potential for reducing the risk of pre-eclampsia[43,44]. To date three trials have published follow-up data on the offspring, focusing on blood pressure as an outcome[45–47] (Table 5). The follow-up of a trial conducted in the USA has provided evidence that maternal Ca supplementation is associated with lower systolic blood pressure in the offspring at 2 years, although only 10 % of eligible participants were recruited[46]. Data from The Gambia (S Hawkesworth, Y Sawo, AJC Fulford, GR Goldberg, LMA Jarjou, A Prentice and SE Moore, unpublished results), Australia[47] and Argentina[45] have shown no association between maternal Ca supplementation and offspring blood pressure at 5–10, 4–7 and 5–9 years respectively (Table 6). Despite no overall association with mean blood pressure, in Argentina the intervention was found to be associated with a reduced risk of having high systolic blood pressure (defined by age- and height-specific cut-offs)[45].

In the Argentinian study an interaction was also found between childhood BMI and maternal Ca supplementation on offspring blood pressure[45]. For children with a BMI

Table 3. Characteristics of a Nepalese trial and follow-up study of maternal multiple-micronutrient supplementation

		Reference
Original trial		Osrin *et al.*[62]
Design	Double-blind randomised controlled trial, 2002–4	
Randomisation	Computer-generated randomisation; allocation concealment	
Participants	Pregnant women attending antenatal clinic in Janakpur Singleton pregnancies Enrolled at ≤20 weeks of gestation	
Supplement	Tablets taken daily from enrolment until delivery Intervention arm: fifteen multiple micronutrients (800 μg vitamin A, 10 mg vitamin E, 5 μg vitamin D, 1·4 mg thiamin, 1·4 mg riboflavin, 18 mg niacin, 1·9 mg vitamin B_6, 2·6 μg vitamin B_{12}, 400 μg folic acid, 70 mg vitamin C, 30 mg Fe, 15 mg Zn, 2 mg Cu, 65 μg Se, 150 μg iodine). Control arm: 60 mg Fe and 400 μg folic acid	
Follow up		Viadya *et al.*[40]
Date	2005–6	
Age of offspring	2–3 years (mean 2·7 years)	
Lost to follow up	917 of 1107 (83%) original children enrolled Lost to follow-up associated with urban location and husbands who were salaried or owned small business Other confounders equally distributed	
Outcomes	Anthropometry and blood pressure (Omron electronic sphygmomanometer CEO197; Omron, Milton Keynes, Bucks., UK)	

Table 4. Effect of maternal micronutrient supplementation on CVD risk factors in the offspring in the follow up[40] to the original Nepalese trial[62]

	Supplement*			Control†			Difference‡		
Outcome	*n*	Mean	SD	*n*	Mean	SD	Mean	95% CI	*P*
Systolic blood pressure (mmHg)	460	99·4	13·7	454	102	17·5	−2·5	−4·54, −0·46	0·02
Diastolic blood pressure (mmHg)	460	62·1	12·8	454	63·4	14·7	−1·3	−3·09, 0·49	0·15
Triceps skinfold thickness (mm)	462	7·15	1·61	455	6·95	1·45	0·2	0·00, 0·40	0·05

*Intervention arm: fifteen multiple micronutrients (800 μg vitamin A, 10 mg vitamin E, 5 μg vitamin D, 1·4 mg thiamin, 1·4 mg riboflavin, 18 mg niacin, 1·9 mg vitamin B_6, 2·6 μg vitamin B_{12}, 400 μg folic acid, 70 mg vitamin C, 30 mg Fe, 15 mg Zn, 2 mg Cu, 65 μg Se, 150 μg iodine).
†Control arm: 60 mg Fe and 400 μg folic acid.
‡Unadjusted regression analysis.

above the mean at follow up the intervention was shown to be associated with lower blood pressure[45]. In the data from The Gambia an interaction with body composition was observed, but in the opposite direction; for individuals in the highest quartile of percentage trunk fat at follow up maternal Ca supplementation was found to be associated with raised diastolic blood pressure (S Hawkesworth, Y Sawo, AJC Fulford, GR Goldberg, LMA Jarjou, A Prentice and SE Moore, unpublished results).

Interpretation and implications

The interpretation of the data presented in the present review is that, to date, with the possible exception of multiple micronutrients, there is little evidence to suggest that supplementation of pregnant women affects their offspring's risk factors for CVD. It is therefore important to explore the reasons for this outcome. The first point that should be highlighted is that there are very little data in this area and more studies are required, including studies with nutrients for which there is currently no evidence (e.g. Fe, vitamin A, long-chain PUFA) or for which the evidence base is weak (protein–energy).

The animal data in this field of DOHaD in relation to CVD risk are extremely strong and many mechanisms have been identified that explain the impact of nutrient restriction during pregnancy on disease risk in the offspring[11,12]. It may therefore seem surprising that, to date, these findings have not been replicated in human subjects. However, the majority of animal studies involve interventions that are at the extreme end of nutrient restriction; equivalent treatments will not be given in human intervention studies because they are both unethical and not relevant to real life. In addition, animal studies have focused on restricting nutrients whereas studies of human pregnancy are designed to increase intake relative to control or placebo, therefore making the results difficult to compare. An additional explanation may be that the incremental nutrient requirements for human reproduction are very low compared with other species (because human subjects have evolved to have very slow pre- and postnatal growth rates) and the developing fetus is protected by a number of evolved adaptations[48].

One relevant issue for interpretation is that of sample size. For example, in order to detect a difference of 2 mmHg in systolic blood pressure (commonly recognised

Table 5. Characteristics of trials and follow-up studies of maternal calcium supplementation

	USA	Argentina	Australia	The Gambia
Original trial	Levine *et al.*[63]	Belizan *et al.* [64]	Crowther *et al.*[65]	Jarjou *et al.*[66]
Design	Randomised double-blind placebo-controlled trial	Randomised double-blind placebo-controlled trial	Randomised double-blind placebo-controlled trial	Randomised double-blind placebo-controlled trial
Randomisation	Computer-generated randomisation; allocation concealment	Computer generated randomisation; allocation concealment	Computer generated randomisation, stratified by centre; allocation concealment	Random number tables, in blocks of four to ensure seasonality equally distributed; allocation concealment
Participants	Nulliparous pregnant women attending five medical centres Enrolled between 13 and 20 weeks of gestation Passed a compliance test before randomisation	Nulliparous pregnant women attending three hospitals Singleton pregnancies Enrolled at 20 weeks of gestation	Nulliparous pregnant women from five centres Singleton pregnancy Enrolled <24 weeks of gestation	Nulliparous and multiparous pregnant women, from twenty-six villages in rural Gambia Singleton pregnancy Enrolled <20 weeks of gestation
Supplement	Four tablets taken daily until delivery Intervention arm: 2g $CaCO_3$ (500 mg per tablet)/d Control arm: maize-starch placebo	Four tablets taken daily until delivery Intervention arm: 2 g $CaCO_3$ (500 mg per tablet)/d Control arm: lactose placebo	Three tablets daily from 20 weeks of gestation until delivery Intervention arm: 1·8 g $CaCO_3$ (600 mg per tablet)/d Control arm: lactose placebo	Three tablets daily from 20 weeks of gestation until delivery Intervention arm: 1·5 g $CaCO_3$ (500 mg per tablet)/d Control arm; cellulose–lactose placebo
Follow up	Hatton *et al.*[46]	Belizan *et al.*[45]	Hillier *et al.*[47]	Hawkesworth *et al.**
Date	Not reported	1995–6	2000–2	2005–6
Age of offspring	Two study periods: 3 months and 2 years	5–9 years (mean 7·1 years)	4–7 years	5–10 years (mean 7·4 years)
Lost to follow-up	Study limited to one site (representing 12% of the original trial) 3 months: 260 of 559 (47%) from this site enrolled 2 years: fifty-seven of 559 (10%) from this site enrolled No data on lost-to-follow-up differences	Study limited to one hospital (representing 50% of original trial) 518 of 614 (84%) from this site enrolled Lost to follow up associated with younger maternal age and lower maternal blood pressure	179 of 456 (39%) original trial offspring Lost to follow up associated with younger mothers, with lower Ca intake and lower compliance.	389 of 519 (75%) original trial offspring No differences associated with lost to follow-up
Outcomes	Blood pressure (3 months: sphygmomanometer with ultrasonic amplification; 2 years automated sphygmomanometer; Critikon Tampa, FL, USA) Left ventricular wall thickness (echocardiography)	Blood pressure (Hg sphygmomanometer)	Blood pressure (automated: Dinamap 845XT; Critikon Tampa, FL, USA)	Blood pressure (automated: Omron 705IT; Omron, Milton Keynes, Bucks., UK) Body composition (Tanita BC-418MA; Tanita UK Ltd, West Drayton, Middlesex, UK; with population-specific equations [61]) Triceps skinfold thickness Fasted blood sample analysed for glucose and insulin

*S Hawkesworth, Y Sawo, AJC Fulford, GR Goldberg, LMA Jarjou, A Prentice and SE Moore (unpublished results).

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Table 6. Effect of maternal calcium supplementation on CVD risk factors in the offspring from trials conducted in USA[46], Argentina[45], Australia[47] and The Gambia*

Outcome	Study	Supplement			Control			Difference‡		
		n	Mean	SD	*n*	Mean	SD	Mean	95% CI	*P*
Systolic BP (mmHg)	Hatton *et al.*[46]: 3 months	130	111	14·3	130	114	12·6	−2·2	−5·5, 1·1	0·20
	Hatton *et al.*[46]: 2 years	35	95·4	7·6	18	100	7·9	−4·8	−9·2, −0·3	0·04
	Belizan *et al.*[45]	261	105	11·0	257	104	10·6	1·4	−0·5, 3·3	0·14
	Hillier *et al.*[47]	91	95·4	7·4	88	95·5	8·5	−0·1	−2·4, 2·3	0·94
	Hawkesworth *et al.**	179	98·0	8·4	171	98·1	8·6	−0·1	−1·9, 1·7	0·91
Diastolic BP (mmHg)	Belizan *et al.*[45]	261	65·8	9·3	257	65·4	9·3	0·4	−1·2, 2·0	0·63
	Hillier *et al.*[47]	91	57·1	7·2	88	56·6	7·1	0·5	−1·6, 2·6	0·65
	Hawkesworth *et al.**	179	58·0	7·7	171	57·9	7·2	0·1	−1·5, 1·7	0·90
Left ventricular mass (g/m^2)	Hatton *et al.*[46]: 2 years	35	49·7	8·7	18	50·2	5·9	−0·5	−4·5, 3·5	0·80
		n	% total		*n*	% total		Relative risk		
Risk of having high systolic BP†	Belizan *et al.*[45]	29/257	11·4		50/261	19·3		0·59	0·39, 0·90	0·01
Risk of having high diastolic BP†	Belizan *et al.*[45]	26/257	10·2		33/261	12·7		0·80	0·49, 1·30	0·41

BP, blood pressure.
*S Hawkesworth, Y Sawo, AJC Fulford, GR Goldberg, LMA Jarjou, A Prentice and SE Moore (unpublished results).
†Defined by age, gender and height centile specific cut-offs[67].
‡Unadjusted regression analysis unless otherwise stated.

as the population effect size associated with 1 kg higher birth weight[7]) with 80% power at $P<0{\cdot}05$ a study would require a sample size of 948 (474 per arm) if for blood pressure the SD is 11 mmHg, a value reported recently for Swedish military conscripts aged 18 years[49]. Only a small number of follow-up studies in the present review have larger sample sizes than 474 per arm, highlighting again the importance of further research in this area.

Another issue is that studies often suffer from large losses to follow up (for example, see Hatton *et al.*[46]), particularly if conducted in developed countries where individuals are difficult to trace. It has recently been discussed that it is necessary to allow for much larger attrition rates in DOHaD studies than are usually deemed to be acceptable for randomised controlled trials involving drugs[50]. This requirement is mainly the result of the practicalities of tracing individuals several years after their mothers were enrolled into a trial. It has been argued that provided information on attrition rates and the association of characteristics with loss to follow up are reported, then a judgement can be made on the quality of the data presented, even with large losses to follow up[50]. The majority of studies presented in the current review were conducted in developing country settings where close family ties can mean that tracing subjects is less problematic. This factor is reflected in the retention rates, most of which are >60%.

A number of the original trials presented here have their own limitations, which question the usefulness of associated follow-up data. Two of the protein–energy randomised controlled trials were randomised at the community level[25,33] and one of these trials (Institute of Nutrition of Central America and Panama, Guatemala[26]) was unable to take clustering into account for statistical analysis. Both the trial in Guatemala[25] and that in Wales[30] provided supplementation for infants and children as well as pregnant women, making it impossible to distinguish between pre- and postnatal intervention. In the multiple-micronutrient trial in Nepal[40] 'control' women received Fe and folic acid supplements that contained double the amount of Fe received by the multiple-micronutrient group. The authors raise this factor as an issue; any effect of supplementation could be the result of a lower Fe intake rather than an increase in other micronutrients[40]. Only the Ca trials are true double-blind placebo-controlled trials and even then the background Ca intakes vary greatly between the populations studied.

Another factor that may explain the lack of association seen between maternal supplementation and offspring CVD risk is the timing of the follow-up studies. In the majority of studies subjects are children or adolescents. It may be that the effect of maternal supplementation only becomes apparent when individuals reach adulthood. In addition, the context of the study is important. The lack of association seen in the studies in rural areas of The Gambia (a transition country) may reflect the fact that the prevailing nutritional exposures experienced postnatally have yet to reach the levels seen in more affluent countries; such levels may be required to reveal a disease response. Finally, pregnancy represents just one time point within the life course and may not be the optimum time to intervene

to influence offspring health. Fetal nutrition is influenced by the mother's nutrient stores and by her own exposure *in utero*[51]; the window of opportunity during pregnancy may actually be too late to affect offspring risk.

The final explanation is that it may actually be the early postnatal environment that explains the inverse association between birth weight and disease, a possibility that features strongly in the DOHaD literature. It has been proposed that this association, which is often only apparent after adjustment for current size, can be explained by the rate of growth of the individual after birth[18,52]. The evidence from randomised controlled trials of infant feeding indicates that faster postnatal growth is associated with a range of CVD risk factors including blood pressure[53] and insulin resistance[54].

The data presented here may question the importance of the fetal environment for the programming of human CVD risk in later life. However, only some aspects of the fetal environment have been reviewed, that of the maternal diet insofar as it is affected by supplementation with protein–energy, multiple micronutrients and Ca. Other exposures such as maternal smoking[55], maternal age[55], stress[56] or placental function[57] during pregnancy have not been considered here and may influence fetal systems as they develop. However, supplementation has the potential to be more easily adopted into public health strategy than altering other exposures that influence the fetal environment.

It has been suggested that the DOHaD field is of particular relevance to less-developed countries that carry the greatest burden of growth-retarded infants whilst concurrently experiencing an increasingly rapid nutrition transition[58]. Concerns have been raised that intervening in pregnancy in such settings may act to increase the risk of obesity and related disorders[59]. Perhaps one of the positive conclusions that can be drawn from the present review is that maternal interventions during pregnancy do not seem to promote adverse CVD risk in the offspring, at least within the age-groups presented here. Furthermore, supplementation during pregnancy confers short-term benefits, and may confer long-term benefits, to the offspring that are outside of the scope of the review. For example, in Bangladesh there is some evidence that food and multiple-micronutrient supplementation of undernourished women during pregnancy may improve offspring cognitive development[41].

Concluding remarks

Conducting follow-up studies of offspring born to mothers who have participated in supplementation trials during pregnancy will provide a useful evidence base for the DOHaD field. To date there is little evidence to suggest that maternal protein–energy or Ca supplementation affects offspring CVD risk factors (blood pressure, body composition, fasting glucose), but further data are required. One study of the impact of maternal multiple-micronutrient supplementation suggests an effect on offspring systolic blood pressure, but requires replication in other settings.

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