DIABETICMedicine

DOI: 10.1111/dme.12390

Review Article

The role of the one-carbon cycle in the developmental origins of Type 2 diabetes and obesity

S. Finer[1], P. Saravanan[2], G. Hitman[3] and C. Yajnik[4]

[1]University of Cambridge Metabolic Research Laboratories, Wellcome Trust-MRC Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge UK, [2]Warwick Medical School, University of Warwick, Coventry & George Eliot Hospital, Nuneaton, UK, [3]Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London and [4]Kamalnayan Bajaj Diabetology Research Centre, King Edward Memorial Hospital Research Centre, Pune, India

Accepted 12 December 2013

Abstract

Vitamin B12 deficiency is common in certain populations, such as in India, where there is also a rising prevalence of Type 2 diabetes, obesity and their complications. Human cohorts and animal models provide compelling data suggesting the role of the one-carbon cycle in modulating the risk of diabetes and adiposity via developmental programming. Early mechanistic studies in animals suggest that alterations to the cellular provision of methyl groups (via the one-carbon cycle) in early developmental life may disrupt DNA methylation and induce future adverse phenotypic changes. Furthermore, replacement of micronutrient deficits at suitable developmental stages may modulate this risk. Current human studies are limited by a range of factors, including the accuracy and availability of methods to measure nutritional components in the one-carbon cycle, and whether its disruptions exert tissue-specific effects. A greater understanding of the causal and mechanistic role of the one-carbon cycle is hoped to generate substantial insights into its role in the developmental origins of complex metabolic diseases and the potential of targeted and population-wide prevention strategies.

Diabet. Med. 31, 263–272 (2014)

Developmental origins of Type 2 diabetes and obesity

The concept of fetal programming, or the developmental origins of disease, has become increasingly described and studied over the last two decades. Fetal programming describes how adverse environmental factors *in utero* can have lasting effects on the structural and functional development of the fetus that can influence, and increase, its propensity to develop certain diseases in later life, such as Type 2 diabetes. This theory is gaining increasing recognition from a multidisciplinary perspective, combining basic scientists with clinicians, epidemiologists and social scientists. Alan Lucas first described fetal programming via nutrition [1] and Hales and Barker *et al.* [2] applied this theory through retrospective analysis of historic birth records from Hertfordshire, UK, during the 1920s/1930s .During the 1990s, Barker and Hales were able to study 463 men in their 60s (mean age 64 years) for whom birth records had been kept and, in doing so, noted that there was an inverse relationship between birthweight and risk of adult diseases, including Type 2 diabetes and cardiovascular disease [2,3].

Correspondence to: Sarah Finer. E-mail: sf494@medschl.cam.ac.uk

Several large studies based on periods of severe and widespread famine have provided evidence that a restricted diet *in utero* can predispose to diabetes in the later life of exposed offspring. The Dutch Winter Hunger was a 5-month period of extreme famine in 1944 after Allied forces retook possession of land in Northern Europe and imposed a railway strike in the Netherlands to protect further movement of occupying German forces. As retaliation, German forces banned all food transportation and, even after this embargo was lifted, harsh weather conditions prevented transport of food by canal from the east to west of the Netherlands. Food stocks ran out in the urban west and daily food rations fell to between 400 and 800 calories per day until the liberation of the Netherlands in 1945 [4]. Epidemiological studies [5] have examined retrospectively those offspring exposed to the extreme nutritional deprivation during the periconceptual period or during fetal development and compared them with their younger siblings who were not exposed to famine. Those offspring who were exposed to famine *in utero* were more likely to develop Type 2 diabetes, obesity and other disorders, such as schizophrenia, in later life, compared with their unexposed siblings. Similar data come from studies of offspring born during the severe Chinese famine in 1958–1961 [6]. In this study, the severely

What's new?

- Defects in the one-carbon cycle in pregnancy, including vitamin B12 deficiency, have been associated with increased future risk of diabetes and obesity in exposed offspring.
- Specific populations, including lactovegetarian Asians, are known to be at high risk of B12 deficiency.
- Animal studies and pilot human studies suggest that epigenetic disruptions may mediate gene-environment interactions in models of disease programming.
- Further work is needed to understand the causes and prevalence of one-carbon cycle defects in wider populations, the mechanisms by which they may increase susceptibility to diabetes and obesity through programming, and whether nutritional intervention may ameliorate this risk.

famine-exposed (n = 503) had a fourfold increased risk of fasting hyperglycaemia, compared with those in areas unaffected by famine (odds ratio 3.92, 95% CI 1.64–9.39, P = 0.002) in adulthood (age 39–42 years). Importantly, for those exposed to severe famine who subsequently followed a Western/affluent diet in later life, the increased risk of diabetes was even greater (odds ratio 7.63, 95% CI 2.41–24.1, P = 0.0005 after stratification for dietary pattern). Interestingly, mean BMI across both exposed and unexposed offspring was no different and within the normal weight range (23 kg/m^2 in both groups). In contrast to the Chinese and Dutch famine studies, a study of the long-term effects of *in utero* famine during the Siege of Leningrad did not show programming of cardiometabolic outcomes [7]. This negative finding highlights the need for caution in retrospective analysis of famine-based studies, although the authors of the Leningrad Study have suggested that different exposure windows, low follow-up rates and the use of a control group that may itself have been exposed to nutritional deprivation may have obscured programmed phenotypic differences.

These famine-based epidemiological studies provide important proof of concept, but do not reveal the contribution of different micronutrients and macronutrients to programming, limiting translation into mechanistic studies and disease prevention. Furthermore, famine-based studies are also extreme environmental exposures, limited to specific populations and times, and many researchers propose that fetal programming can occur via less extreme and more prevalent nutritional deficiencies. Evidence for the role of specific micronutrient deficiencies in the developmental origins of Type 2 diabetes and obesity is now emerging from animal models and some human cohorts, and some suggest that disruption of the one-carbon metabolic cycle, via vitamin B12 deficiency (and/or altered folate status), may be one such micronutrient with an important role [8,9]. Whether it is the deficiencies of these micronutrients that underlie famine-based programming, or their presence in combination with wider deficits, is not clear.

One-carbon cycle

One-carbon metabolism comprises a complex set of biochemical reactions in which methyl groups (CH_3) are generated or utilized (see Fig. 1) [10]. Central to this is the methylation cycle that produces S-adenosylmethionine from methionine and adenosine triphosphate (ATP). S-adenosylmethionine is converted to S-adenosylhomocysteine by methyltransferase enzymes in a transmethylation reaction that generates a methyl group that is available for DNA methylation, protein lipid and carbohydrate synthesis. The S-adenosylhomocysteine produced from transmethylation is converted to homocysteine. Methionine can be regenerated from homocysteine in the presence of a methyl donor such as 5-methyltetrahydrofolate and the enzyme methionine synthase, which itself requires vitamin B12 (cobalamin) as a co-factor. 5-Methyltetrahydrofolate is generated, in part, from dietary folate and, when not utilized in the methylation cycle, folate species can be redirected to the production of thymidine and purines for DNA/RNA synthesis and generation of high-energy molecules and cofactors including ATP, NAD and coenzyme A. Transmethylation reactions are also regulated by the availability of other substrates, including the dietary micronutrients betaine, choline and the non-essential amino acid serine and glycine.

Homocysteine concentrations are tightly controlled by the transmethylation cycle and its interaction with transulfiration. Vitamin B6 (pyridoxine) is needed to transulfirate homocysteine to cysteine (via cystathione), an amino acid required for synthesis of the antioxidant glutathione.

Aside from its role in the one-carbon cycle, vitamin B12 is a cofactor for the mitochondrial enzyme methylmalonyl-CoA mutase, which catalyses methylmalonyl CoA from odd-chain fatty acids, branched chain amino acids and cholesterol to form succinyl-CoA. Succinyl-CoA is a key substrate of the citric acid cycle that has a crucial role in cellular energy metabolism, and it is thought that it is via disruption to this pathway that can lead individuals with vitamin B12 deficiency to have neurological sequelae.

It is therefore evident that the one-carbon cycle relies on the provision of dietary micronutrients to function, including B vitamins, tetrahydrofolate and essential amino acids. Deficiencies of these micronutrients could lead to disruption of the one-carbon cycle and its downstream metabolic processes. The dietary sources of one-carbon metabolites will be discussed later. An individual's B12 and folate status is also known to vary according to the presence/absence of certain genetic polymorphisms; for example, the FUT2 A

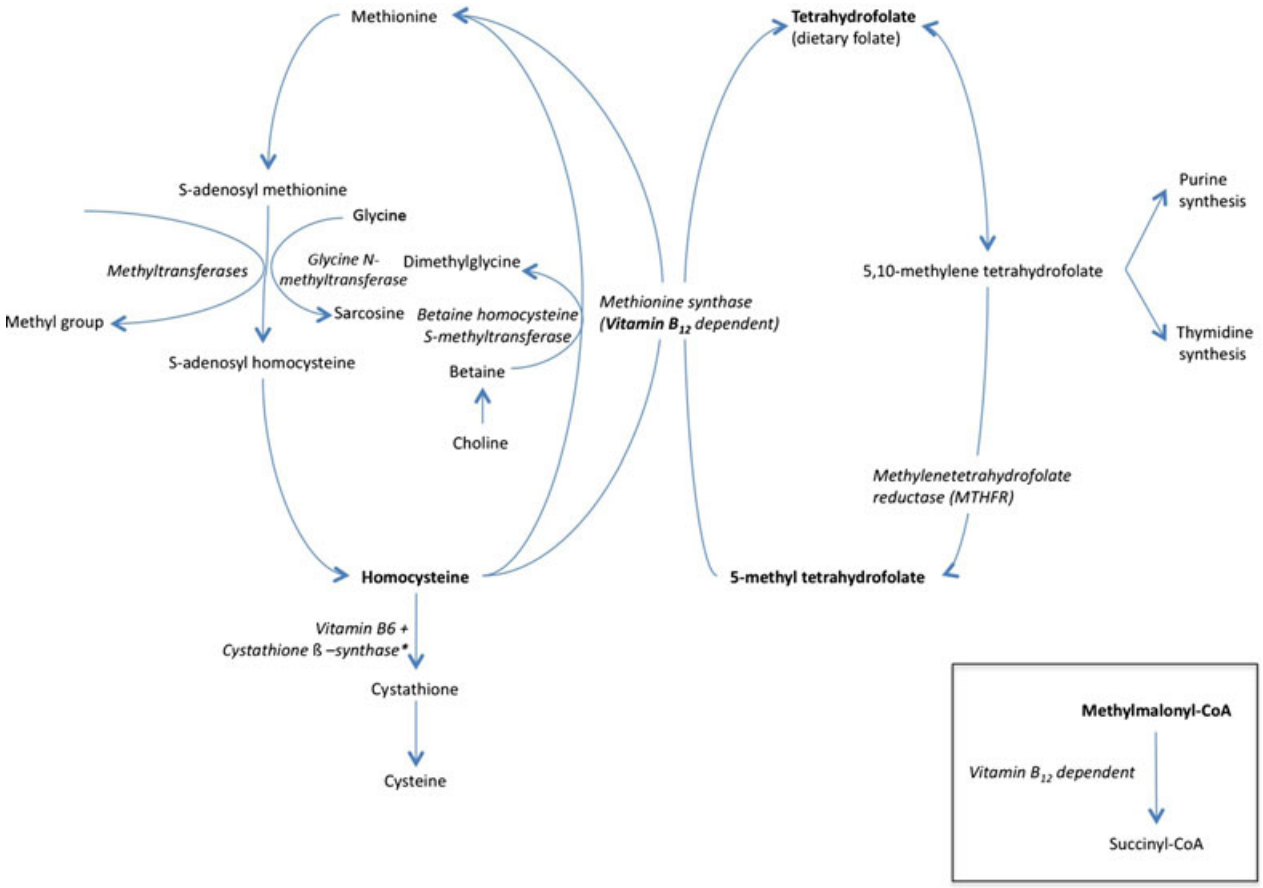

FIGURE 1 The one-carbon cycle. Metabolites readily assayed in clinical laboratories are highlighted in bold.

allele is associated with a 44.2-pg/ml higher concentration of vitamin B12 (allele frequency 0.49) [11], and the methyltetrahydrofolate (MTHFR) C677T polymorphism (allele frequency 53% in European populations) is highly associated with homocysteine concentrations [12].

Association of one-carbon disturbance with the developmental origins of diabetes and obesity

Over recent years, human and animal studies have shown the potential for maternal nutritional deficiency of one-carbon metabolites to programme a phenotype of diabetes and obesity in offspring. The role of B12 and folate in human fetal programming has been described elegantly in the Pune Maternal Nutrition Study, a prospective study of a rural population in Maharashtra, India [9]. The Pune Maternal Nutrition Study recruited non-pregnant women of reproductive age from six villages near Pune, India in the early 1990s. These women were seen regularly with monitoring of their body anthropometry and menstrual cycle to establish careful confirmation and dating of incipient pregnancy. Pregnant women were followed up at 18 and 28 weeks' gestation, undergoing further anthropometry, questionnaire-based assessment of diet and physical activity and blood sampling. Assays of circulating one-carbon micronutrients were performed, including red cell folate, vitamin B12, methylmalonic acid and homocysteine. Anthropometry of offspring born to these mothers was performed within 72 h of their birth. These children were followed up at 6 years of age with anthropometry, body composition [using whole-body dual-energy X-ray absorptiometry (DEXA)], glucose tolerance testing, the homeostatic model assessment of insulin resistance (HOMA-IR) and blood sampling for nutritional and metabolic assays. High follow-up rates were achieved with 700 of the 762 live births studied at 6 years. Longitudinal follow-up of offspring (now 18 years of age) is currently underway.

In the Pune Maternal Nutritional Study, over 60% of women had a low concentration of vitamin B12 (< 150 pmol/l) at 18 and 28 weeks' gestation, and B12 showed inverse correlation with homocysteine and methylmalonic acid, reflecting true deficiency. These findings are considered to be dietary in origin because of the lacto-vegetarian diet consumed by much of this population. In contrast, only one woman out of the 618 studied had a low red cell folate concentration. Offspring born to these

women were studied at 6 years of age and an association between low maternal vitamin B12 at 18 weeks with higher HOMA-IR in children was found ($P = 0.03$), but higher maternal erythrocyte folate concentrations at 28 weeks predicted higher offspring adiposity and higher HOMA-IR (both $P < 0.01$). Regression modelling showed that the offspring of mothers with a combination of high folate and low vitamin B12 concentrations were the most insulin resistant, even when adjusted for the child's own fat mass and plasma B12 concentration. There were no differences in birthweight in Pune offspring according to maternal B12 concentrations; however, a cohort study in Bangalore, India, has shown that women in the lowest tertile of serum vitamin B12 concentration during pregnancy were more likely to have a growth-restricted baby (< 10th birthweight centile) (odds ratios, adjusted for possible confounders, 5.98, 9.28, 2.81 for trimesters 1, 2 and 3, respectively) [13]. The unusual finding that normal/high folate concentrations were found in association with these other nutritional deficiencies was thought to be attributable to women being vegetarian and being given folic acid supplementation before and during pregnancy; this supplementation is routine throughout the world, but in India was being given at much higher doses than are considered necessary. In the UK, 400 μg folic acid is recommended to all women who are planning a pregnancy, but in contrast, a 5-mg tablet (5000 μg) is given in India. The potential harmful role of high folic acid intake (dietary and supplement intake) has also been suggested by the detection of unmetabolized folic acid in the plasma of healthy individuals, and a possible detrimental impact on natural killer cell activity, an aspect of immune function [14]. The 'methylfolate trap' has been proposed as a mechanism by which folate repletion can exacerbate coexistent B12 deficiency via the impaired conversion of homocysteine to methionine [15]. The methylfolate trap also highlights the need for further investigation of the effects of high-dose folic acid supplementation in pregnancies complicated by diabetes (women with Type 1 and Type 2 diabetes are recommended to take 5 mg of folic acid periconceptually in the UK) [16], folic acid fortification of flour to reduce the risk of neural tube defects [17], and the possible role of metformin in inducing vitamin B12 deficiency [18]. Recent data from the Metformin in Gestational Diabetes (MiG) trial suggest that metformin use in pregnancy may cause maternal vitamin B12 depletion [19], and this is an important area of further study in view of the results of the Pune Maternal Nutrition Study.

A model of periconceptual methyl group deprivation in ewes has also been described and provides further support to the role of one-carbon metabolites in programming a 'cardiometabolic' phenotype in offspring [8]. In this model, ewes were deprived of a range of methyl donors in their diet, including vitamin B12 and folate as well as methionine, pre-conceptually and at the time of conception. The ewes receiving the deprived diet showed biochemical deficiency of B12, folate and methionine with an expected rise in homocysteine. Embryo transfer was performed from donor ewes to normally fed surrogate ewes for the remainder of pregnancy and weaning, allowing a defined window of periconceptual exposure. Granulosa cell lysates were collected at the time of embryo transfer and these showed a reduced S-adenosylmethionine:S-adenosylhomocysteine ratio. All methyl-deficient exposed offspring were heavier at birth and exhibited higher growth rates into their adulthood, and male offspring exhibited higher fat mass [judged by computerized tomography (CT) scan] at 12 months of age compared with control subjects. Male offspring also showed insulin resistance, independent of differences in adiposity, with similar, but smaller, differences seen in female offspring. By 23 months of age, male offspring showed elevated body fat-adjusted blood pressure in the exposed group vs. controls.

Data from a rat model also provide evidence of the role of one-carbon metabolite deficiencies in the pathogenesis of obesity and diabetes [20]. In this model, female (pre-pregnancy) Wistar rats were exposed to either folate, B12 or combined folate and B12 deficiency and these were compared with controls (normal diet). After 3 months of dietary restriction and pectin administration (to reduce intestinal absorption of these nutrients), nutritional deficiencies were confirmed biochemically and were said to be equivalent to human dietary deficiency. Female rats studied after 3 months revealed higher body weight ($P < 0.05$) in the folate-deficient and B12-deficient groups compared with controls. Increased total body fat was observed in B12-deficient animals and increased visceral fat mass in folate-deficient rats. Exposure to these dietary deficiencies was continued in 48 rats during pregnancy, weaning and into the male F1 generation until 12 months of age. By 12 months of age, male offspring exposed to folate, B12 and combined deficiencies showed significantly increased ($P < 0.01$) body weight, body fat and visceral adiposity, compared with controls. In addition, all three exposed groups showed higher plasma cholesterol, triglycerides and some features of a pro-inflammatory state, including elevated tumour necrosis factor alpha (TNFα), interleukin (IL)-6, IL-1β and reduced adiponectin. These findings provide convincing evidence of the direct effects of variation in one-carbon metabolism on fat mass. By measuring the adipogenic proteins fatty acid synthase and acetyl-CoA-carboxylase and showing increased activity in livers of the 12-month-old male exposed offspring, the authors suggest that this altered fat mass is attributable to increased adipogenesis. Unfortunately, this rat model does not define the window of exposure that is responsible for the 'programming' effect of maternal deficiency of one-carbon metabolites and, rather than a transgenerational effect on offspring via the maternal pregnancy environment, the effect on offspring could be mediated by altered post-natal diet in exposed offspring. This study does not mirror the nutritional imbalance seen in the Pune Maternal Nutrition Study in which combined maternal B12 deficiency and folate repletion was associated with the highest risk of adiposity and insulin resistance in offspring. Other studies have shown a link between vitamin B12 deficiency and accumulation of liver fat [21].

Putative mechanisms by which one-carbon can exert its actions

The conversion of S-adenosylmethionine to S-adenosylhomocysteine reflects the transmethylation reaction in the one-carbon cycle, generating methyl groups that can be used for DNA methylation. The ratio of S-adenosylmethionine: S-adenosylhomocysteine has therefore been used as a marker of 'cellular methylation capacity' [22] and several studies have suggested that it has a direct impact on DNA methylation, presumably because of the availability of methyl groups from transmethylation. DNA methylation is an epigenetic modification that may alter gene expression without any change in DNA sequence and recent studies suggest that such phenomena may play an important role in mediating gene–environment interactions, especially in early life when the developing epigenome is susceptible to a perturbed environment [23].

The study of sheep pregnancy described above showed phenotypic changes in offspring exposed to periconceptual methyl group deficiency and variation in offspring DNA methylation [8]. The researchers used methylation-sensitive restriction enzymes to characterize DNA methylation at 1400 CpG dinucleotides, capable of methylation, mostly at promoter sequences of genes in the fetal livers of 18 control and 16 methyl-deficient animals. In their analysis, they found that 57 loci (4% of the CpG sites studied) exhibited altered methylation in two or more animals in methyl-deficient offspring and suggest that this is more than would be expected by chance. Furthermore, the authors report that hypomethylation was more common than hypermethylation, as could be expected from methyl deficiency, and that the differences were more common in males than females, consistent with the phenotypic outcome. Although this method of studying DNA methylation provides only a limited perspective of the potential complex epigenomic phenomena, and has now been superseded by genome-wide technologies, this finding has given an early, and much--needed support of the potential mechanisms underlying this fetal programming hypothesis.

Other researchers have shown small methylation differences in target genes in association with maternal and infant one-carbon status and folic acid supplementation in healthy populations in the UK and USA [24,25]. These studies used bisulphite-pyrosequencing assays to detect small methylation differences (of up to 8%) and weak correlations in association with parameters of one-carbon status. More importantly, McKay and colleagues have provided evidence in their paper that epigenetic–genetic interactions may be important in determining methylation status. In their study, analysis of common genetic variants that regulate folate metabolism, such as the MTHFR gene, identified an association between maternal and infant genotype with infant methylation. Although only preliminary findings, these highlight how one-carbon metabolism may contribute to a complex interaction between gene and environment, through epigenetic processes, that modify gene function.

A study by Ahmad *et al.* [26] has expanded this hypothesis by looking at variation in the liver proteome of rat pups exposed to normal vs. deficient maternal B12 levels during pregnancy. They identify specific gene expression and protein differences in peroxisome proliferator-activated receptors (PPAR)α and PPARγ signalling pathways that have important roles in lipid, carbohydrate and amino acid metabolism in liver. Furthermore, they describe that supplementation of B12 to rat offspring at parturition rescues the expression differences seen in earlier life associated with maternal deficiency.

Dietary sources of one-carbon metabolites

The common dietary sources of one-carbon metabolites, are shown in Table 1, and are presented as the Dietary Reference Value (DRV), a statistical estimate of the requirements for a particular group of people within a population. These values differ slightly from the Reference Nutrient Intake (RNI) or its equivalent, the Recommended Daily Allowance (RDA); these refer to the amount of a nutrient that is enough to ensure that the needs of 97.5% of a group are met. These definitions explain the discrepancy between the Dietary

Table 1 Common dietary sources of one-carbon cycle substrate, Reference Nutrient Intakes (UK) and groups at risk of deficiency [27]

	Vitamin B12	Folate	Essential amino acids	Choline
Reference Nutrient Intake	1.5 μg/day (adults). 2.0 μg/day (lactation)	200 μg/day (adults). 300 μg/day (pregnancy) 260 μg/day (lactation)	0.75 g kg^{-1} day^{-1}	425 mg/day (women), 550 mg/day (men). 450 mg/day (pregnancy), 550 mg/day (lactation)
Main food sources	Meat, fish, milk, cheese, eggs, yeast extract, fortified breakfast cereals	Green leafy vegetables (e.g. spinach), brown rice, peas, oranges, bananas, fortified breakfast cereals	Animal proteins, e.g. meat, fish, eggs, milk, cheese. Plant proteins, e.g. cereals, pulses, nuts, seeds, soya	Lethicin, fatty meat and fish, cruciferous vegetables
People at risk of deficiency	Vegans, vegetarians, pernicious anaemia	Pregnant women, megaloblastic anaemia	Vegetarians	Pregnant and breastfeeding women

Reference Value of folate for women in pregnancy (300 μg/day) and the recommended supplementation that they should take (400 μg/day) to prevent deficiency in almost all individuals.

Measuring components of the one-carbon cycle

B12

The standard assay of cobalamin measures total circulating B12 in serum, including approximately 6–20% that is bound to transcobalamin (known as holotranscobalamin) and in an 'active' form, and the 80% bound to haptocorrin and which has no known functions (see Table 2). The sensitivity of the standard B12 test in detecting true and functional B12 deficiency is thought to be poor, and it is not reliable to detect deficiency in pregnancy because of the effects of haemodilution after the first trimester of pregnancy [28]. Standard B12 assays are also thought to be prone to assay malfunction and falsely normal levels in the presence of anti-intrinsic factor antibodies, of particular concern when applied to the investigation of B12 deficiency in individuals with pernicious anaemia [29]. Methylmalonic acid is an intracellular metabolite that is converted to succinyl-CoA for use in the Krebs cycle and requires B12 as a cofactor for its conversion. Multiple studies now show that methylmalonic acid is a sensitive functional marker of cobalamin deficiency at a tissue level and this is now increasingly used to diagnose true B12 deficiency in routine clinical practice; however, it is affected by pregnancy, age and renal function [30]. Holotranscobalamin is emerging as another useful marker of B12 status, representing the fraction of 'active' cobalamin bound to the transcobalamin II molecule and ready for tissue uptake. This test is not commonly available in clinical laboratories, but is thought to offer a useful first-line marker of B12 deficiency, with an area-under-the-curve analysis of 0.90 (95% CI 0.86–0.93) suggesting higher accuracy than serum B12 (0.80; 95% CI 0.75–0.85) and methylmalonic acid (0.78; 95% CI 0.72–0.83) [31]. However, other researchers have suggested that the diagnostic power of holotranscobalamin is insufficient on its own to distinguish deficiency from non-deficiency in its middle range or 'grey zone', but that adding methylmalonic acid into an algorithm to detect deficiency can help detect an additional 18% of deficient individuals from it [32].

Murphy *et al*. have addressed the difficulty of characterizing B12 status in pregnancy attributable to haemodilution reducing the serum B12 level [33]. They identified that pre-conception methylmalonic acid was significantly associated with methylmalonic acid in pregnancy (at 8, 20 and 32 weeks) and in cord blood, and also had a significant inverse relationship with maternal B12 and holotranscobalamin during pregnancy and with cord blood holotranscobalamin and suggest that methylmalonic acid is a useful biomarker of B12 status at the materno–fetal interface. Unfortunately, this study did not include assays of folate status, or other intermediates of the one-carbon cycle such as homocysteine, both of which may be useful to determine the relevant influences on fetal programming via the one-carbon cycle.

Folate

The standard clinical assay of folate status is usually performed in serum or red cell lysates, and different measurement techniques include immunoassay (see Table 2), microbiological measurement or liquid chromatography–mass spectrometry/mass spectrometry. These different plat-

Table 2 Examples of one-carbon metabolite assays available in a specialist UK clinical laboratory (courtesy of St Thomas' Nutristasis Laboratory)

One-carbon metabolite	Common clinical assay	Normal ranges (unless stated otherwise)	Units of measurement
Serum B12	Electrochemilluminescent competitive immunoassay	191–900	ng/l
Holotranscobalamin	Immunoassay	< 25—suggests vitamin B12 deficiency 25–50—'grey zone'—measure methylmalonic acid 51–165—vitamin B12 replete > 165—recent cobalamin treatment	pmol/l
Methylmalonic acid	Gas chromatography–mass spectrometry	≤ 280 (< 65 years) ≤ 360 (> 65 years)	nmol/l
Serum folate (including multiple folate species)	Electrochemilluminescent competitive immunoassay	3.8–20.0	μg/l
Red cell folate	Competitive binding paramagnetic particle assay	160–640	μg/l
5-methyltetrahydrofolate	High-performance liquid chromatography	7.6–42.0	nmol/l
Homocysteine	High-performance liquid chromatography	< 10	μmol/l

forms have different precision for measuring the range of different folate species in blood (including 5 methyltetrahydrofolate, folic acid, tetrahydrofolate, 5-formyltetrahydrofolic acid and 5,10 methenyl-tetrahydrofolic acid) [34]. In pregnancy, the red cell folate assay is the optimal measure of folate status as it reflects folate status over 90–120 days (the lifecycle of an erythrocyte) and is therefore unlikely to show falsely normal levels attributable to the recent ingestion of folate supplementation. The specific 5-methyltetrahydrofolate assay is not commonly used but allows assessment of the principal circulating component of dietary folate that is also the 'active' one-carbon donor and will be true reflection of folate status in individuals carrying the common polymorphism in the 5MTHFR gene.

Essential amino acids

Essential amino acids are not routinely measured and the standard means to assess dietary protein intake is to use a food frequency questionnaire, such as that used in the National Health and Nutrition Examination Survey (NHANES) study [35].

Choline and betaine

Despite their important role in the one-carbon cycle, betaine and its precursor choline are rarely assayed. Higher dietary intakes of choline and betaine (measured by a food frequency questionnaire) were associated lower plasma homocysteine and were independent of folate and B vitamin intake in 1260 healthy adults in the Framingham Offspring Study [36]. However, the complexity of these relationships in varying nutritional environments is highlighted by the positive association of plasma homocysteine with plasma betaine and choline (measured by liquid chromatography-mass spectrometry) in 62 young adult women from rural Gambia where multiple macro- and micronutrient deficiencies are seen and exhibit seasonal variation [37].

Intermediate and downstream metabolites in the one-carbon cycle

Homocysteine, methioinine, S-adenosylhomocysteine and S-adenosylmethionine can be measured in plasma using techniques including high-performance liquid chromatography, although only homocysteine is measured in standard clinical settings (see Table 2). King *et al.* [38] have shown that the B12 and folate status of 581 healthy volunteers predicted, in part, homocysteine levels, but not the intermediates either side of the transmethylation reaction, S-adenosylmethionine and S-adenosylhomocysteine. However, another study showed correlations between choline and betaine with S-adenosylmethionine and S-adenosylhomocysteine [39]. These unpredictable relationships are likely to highlight the complex relationships between the many metabolites in the one-carbon cycle, the need for accurate assays if they are to be measured, but also that the functions of the cycle, for example methylation capacity, are likely to be cell- and tissue-specific. Other functions of this cycle, such as purine and thymidine synthesis, and the additional functions of B12 in mitochondrial enzyme pathways, are also difficult to quantify and it is not known what impact nutritional deficits have on these specific roles. A detailed study of dietary intake and biochemical assays of one-carbon metabolites and related cofactors was performed in non-pregnant women from Gambia with well-characterized seasonal variation in nutrient intake [37]. This study was performed to investigate the availability of nutrients with 'DNA methylation potential' and showed that food intake data was inconsistently associated with their biochemical parameters and did not predict S-adenosylmethionine:S-adenosylhomocysteine ratio.

Limitations of current research and evidence and future direction

Nature of the environmental exposure

In the Pune Maternal Nutrition Study, maternal vitamin B12 deficiency with folate repletion was associated with the adverse childhood phenotype of insulin resistance and adiposity and thought to be the programming influence. One of the strengths of this study, in contrast to the retrospective famine-based cohorts described above, was the careful characterization of the maternal diet using semi-quantitative 24-h recall and food frequency questionnaires to determine macronutrient intake (i.e. protein, fat, energy and carbohydrates) and other dietary intake, for example dairy products and green, leafy vegetables. Yajnik *et al.* [9] performed an analysis to identify whether there was an association between any of these variables and the phenotype of insulin resistance, but did not find that macronutrient was not related but intake frequency of micronutrients was related. This provides reassurance that macronutrient status, for example protein intake, did not confound the association with maternal one-carbon status in this study that could be considered possible given their similar dietary sources. A study of gestational diabetes in Mysore, India, has shown an association between B12 deficiency and gestational diabetes, raising the possibility of other causal mechanisms or confounding influences on the programming of disease risk. Notably, the work of Dominguez-Salas *et al.* (37) has identified the complex variation in components of the one-carbon cycle, whether measured by questionnaires of food intake or biochemical assay, in a population under varied nutrient availability, and an unknown relationship of circulating metabolites with 'methylation potential' (S-adenosylmethionine:S-adenosylhomocysteine). Whether normative data on circulating

one-carbon cycle metabolites can be attained is yet to be determined.

Timing of the exposure

Researchers have highlighted the importance of the variable timing of exposure on programming [40]. In the Pune Maternal Nutrition Study, biochemical and questionnaire data was collected at 18 and 28 weeks' gestation to characterize maternal nutritional status in a population for whom pre-pregnancy weight and BMI had previously been characterized [41]. Data collected from pregnant women may not fully characterize pre-pregnancy micronutrient status; the biochemical measure of red cell folate is unlikely to change during pregnancy, but serum vitamin B12 levels fall from 8 weeks of gestation as a result of haemodilution [33]. In the sheep model of methyl group deficiency previously described, the exposure was periconceptual, a time point that it can be assumed that mothers in the Pune Maternal Nutrition Study were also exposed to. One potential difficulty with interpreting the findings of this clinical study lies in the exposure lasting throughout pregnancy and possibly also during lactation and post-natally and this concern has only partly been addressed by measuring vitamin B12 levels in 6-year offspring and finding that there was no association of this with phenotypic outcome. The importance of the post-natal period in modulating risk of future disease is highlighted in the rat study described above in which rescue of gene expression differences set down *in utero* was achieved through B12 supplementation of offspring at parturition [26]. Human studies of post-natal growth provide convincing evidence that variation in post-natal growth can also impact on future disease risk, most notably the rapid 'catch-up' of weight in children born at low birthweight [42]; however, it is not known whether *in utero* factors may themselves 'programme' these post-natal growth patterns.

Causation vs. association

The studies described show association between one-carbon metabolites and programming of diabetes and obesity in offspring across various human and animal studies, but do not prove causality. Cross-sectional and observational study design of human cohorts, whilst useful at identifying patterns and associations, cannot elucidate whether these are in fact causal, and this can only be achieved through randomized intervention studies. The need for such studies is great, and their results will also facilitate the effective translation of molecular mechanisms and guide future nutritional interventions, guided by an insight into the susceptible windows of exposure. These issues are being addressed in the recently launched B12 and multi-micronutrient intervention in adolescents in the Pune Maternal Nutrition Study called the Pune Intervention Study (ISRCTN 32921044).

Molecular mechanisms by which one-carbon exerts its actions

At present, there is no definitive evidence that one-carbon abnormalities exert their effects via epigenetic mechanisms. Preliminary evidence of altered DNA methylation in different one-carbon environments does exist, but, to prove this molecular mechanism, future studies will need to combine epigenetic studies with those of gene expression to identify functionality. The tissue specificity of epigenetic phenomena may be a barrier to understanding these molecular mechanisms in humans, and complementary studies in animal models are likely to be informative.

Population-wide and global relevance of one-carbon cycle defects

Certain populations, for example lacto-vegetarian South Asians, are known to have a high prevalence of B12 deficiency; however, prevalence studies in non-vegetarian populations are limited and hampered by the limitations of the commonly used B12 assay. Recent preliminary data from our group show that B12 deficiency is not uncommon in the UK pregnant population [43,44] and two prospective studies are underway (PRiDE study, www.warwick.ac.uk/go/pride-study; GIFTS study, http://www.gifts-project.eu/drupal/), which will study the prevalence and role of one-carbon metabolites in pregnancy, their association with maternal diabetes and obesity and their relationship to offspring anthropometry and later cardiometabolic risk. Understanding programming from one-carbon disorders may also come from non-nutritional determinants, such as common genetic variants that affect its components, as well as the interaction with other nutritional variables, such as protein deficiency. Large studies with adequate characterization of these complex environmental and genetic factors will be necessary to determine the likely contribution of the one-carbon cycle to programming via altered growth and development in early life.

Conclusions

The Pune Maternal Nutrition Study and animal models described above provide compelling data suggesting the role of the one-carbon cycle in modulating the risk of diabetes and adiposity via developmental programming of susceptible metabolic pathways. The advent of epigenetic research and improvements in nutritional profiling are hoped to yield insight into the precise mechanisms by which the nutritional environment of a developing organism can regulate DNA methylation, gene function and induce phenotypic change. Further understanding of such gene–environment interac-

tions in complex disease, and their reversibility through intervention, may contribute significant advances to the development of preventative strategies to reduce the increasing global burden of disease from diabetes and obesity.

References

1 Lucas A. Does early diet program future outcome? *Acta Paediatr Scand Suppl* 1990; **365**: 58–67.
2 Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C *et al*. Fetal and infant growth and impaired glucose tolerance at age 64. *Br Med J (Clin Res Ed)* 1991; **303**: 1019–1022.
3 Osmond C, Barker DJ, Winter PD, Fall CH, Simmonds SJ. Early growth and death from cardiovascular disease in women. *Br Med J (Clin Res Ed)* 1993; **307**: 1519–1524.
4 Roseboom T, De Rooij S, Painter R. The Dutch famine and its long-term consequences for adult health. *Early Hum Dev* 2006; **82**: 485–491.
5 Ravelli AC, Van der Meulen JH, Michels RP, Osmond C, Barker DJ, Hales CN *et al*. Glucose tolerance in adults after prenatal exposure to famine. *Lancet* 1998; **351**: 173–177.
6 Li Y, He Y, Qi L, Jaddoe VW, Feskens EJM, Yang X *et al*. Exposure to the Chinese famine in early life and the risk of hyperglycemia and type 2 diabetes in adulthood. *Diabetes* 2010; **59**: 2400–2406.
7 Stanner SA, Bulmer K, Andrès C, Lantseva OE, Borodina V, Poteen VV *et al*. Does malnutrition *in utero* determine diabetes and coronary heart disease in adulthood? Results from the Leningrad siege study, a cross sectional study. *Br Med J (Clin Res Ed)* 1997; **315**: 1342–1348.
8 Sinclair KD, Allegrucci C, Singh R, Gardner DS, Sebastian S, Bispham J *et al*. DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. *Proc Natl Acad Sci U S A* 2007; **104**: 19351–19356.
9 Yajnik CS, Deshpande SS, Jackson AA, Refsum H, Rao S, Fisher DJ *et al*. Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. *Diabetologia* 2008; **51**: 29–38.
10 Rush E, Katre P, Yajnik C. Vitamin B12: one carbon metabolism, fetal growth and programming for chronic disease. *Eur J Clin Nutr* 2013; **68**: 2–7.
11 Hazra A, Kraft P, Lazarus R, Chen C, Chanock SJ, Jacques P *et al*. Genome-wide significant predictors of metabolites in the one-carbon metabolism pathway. *Hum Mol Genet* 2009; **18**: 4677–4687.
12 Tanaka T, Scheet P, Giusti B, Bandinelli S, Piras MG, Usala G *et al*. Genome-wide association study of vitamin B6, vitamin B12, folate, and homocysteine blood concentrations. *Am J Hum Genet* 2009; **84**: 477–482.
13 Muthayya S, Kurpad A V, Duggan CP, Bosch RJ, Dwarkanath P, Mhaskar A *et al*. Low maternal vitamin B12 status is associated with intrauterine growth retardation in urban South Indians. *Eur J Clin Nutr* 2006; **60**: 791–801.
14 Troen AM, Mitchell B, Sorensen B, Wener MH, Johnston A, Wood B *et al*. Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. *J Nutr* 2006; **136**: 189–194.
15 Scott JM, Weir DG. The methyl folate trap. A physiological response in man to prevent methyl group deficiency in kwashiorkor (methionine deficiency) and an explanation for folic-acid induced exacerbation of subacute combined degeneration in pernicious anaemia. *Lancet* 1981; **2**: 337–340.
16 National Institute for Health and Clinical Excellence. *Diabetes in Pregnancy: Management of Diabetes and its Complications from Pre-Conception to the Post-Natal Period*. CG63 N clinical guidelines. 2008. Available at http://publications.nice.org.uk/diabetes-in-pregnancy-cg63 Last accessed 25 November 2013.
17 Crider KS, Bailey LB, Berry RJ. Folic acid food fortification—its history, effect, concerns, and future directions. *Nutrients* 2011; **3**: 370–384.
18 Ting RZ-W, Szeto CC, Chan MH-M, Ma KK, Chow KM. Risk factors of vitamin B(12) deficiency in patients receiving metformin. *Arch Intern Med* 2006; **166**: 1975–1979.
19 Gatford KL, Houda CM, Lu ZX, Coat S, Baghurst PA, Owens JA *et al*. Vitamin B12 and homocysteine status during pregnancy in the Metformin in Gestational Diabetes trial: responses to maternal metformin compared with insulin treatment. *Diabetes Obes Metab* 2013; **15**: 660–667.
20 Kumar KA, Lalitha A, Pavithra D, Padmavathi IJN, Ganeshan M, Rao KR *et al*. Maternal dietary folate and/or vitamin B(12) restrictions alter body composition (adiposity) and lipid metabolism in Wistar rat offspring. *J Nutr Biochem* 2013; **24**: 25–31.
21 Smith RM, Osborne-White WS. Folic acid metabolism in vitamin B12-deficient sheep. Depletion of liver folates. *Biochem J* 1973; **136**: 279–293.
22 James SJ, Melnyk S, Pogribna M, Pogribny IP, Caudill MA. Elevation in S-adenosylhomocysteine and DNA hypomethylation: potential epigenetic mechanism for homocysteine-related pathology. *J Nutr* 2002; **132**: 2361S–2366S.
23 Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. *Nat Rev Genet* 2007; **8**: 253–262.
24 McKay JA, Groom A, Potter C, Coneyworth LJ, Ford D, Mathers JC *et al*. Genetic and non-genetic influences during pregnancy on infant global and site specific DNA methylation: role for folate gene variants and vitamin B12. *PloS One* 2012; **7**: e33290.
25 Hoyo C, Murtha AP, Schildkraut JM, Jirtle RL, Demark-Wahnefried W, Forman MR *et al*. Methylation variation at IGF2 differentially methylated regions and maternal folic acid use before and during pregnancy. *Epigenetics* 2011; **6**: 928–936.
26 Ahmad S, Kumar KA, Basak T, Bhardwaj G, Yadav DK, Lalitha A *et al*. PPAR signaling pathway is a key modulator of liver proteome in pups born to vitamin B12 deficient rats. *J Proteomics* 2013; **91**: 297–308.
27 Committee on Medical Aspects of Food and Nutrition Policy and Scientific Advisory Committee on Nutrition. London: British Nutrition Foundation, 2012. Available at http://www.nutrition.org.uk/nutritionscience/nutrients/nutrient-requirements Last accessed 6 January 2014.
28 Wheeler S. Assessment and interpretation of micronutrient status during pregnancy. *Proc Nutr Soc* 2008; **67**: 437–450.
29 Carmel R, Agrawal YP. Failures of cobalamin assays in pernicious anemia. *N Engl J Med* 2012; **367**: 385–386.
30 Vogiatzoglou A, Oulhaj A, Smith AD, Nurk E, Drevon CA, Ueland PM *et al*. Determinants of plasma methylmalonic acid in a large population: implications for assessment of vitamin B12 status. *Clin Chem* 2009; **55**: 2198–2206.
31 Valente E, Scott JM, Ueland P-M, Cunningham C, Casey M, Molloy AM. Diagnostic accuracy of holotranscobalamin, methylmalonic acid, serum cobalamin, and other indicators of tissue vitamin B status in the elderly. *Clin Chem 2011*; **57**: 856–863.
32 Obeid R, Jung J, Falk J, Herrmann W, Geisel J, Friesenhahn-Ochs B *et al*. Serum vitamin B12 not reflecting vitamin B12 status in patients with type 2 diabetes. *Biochimie* 2013; **95**: 1056–1061.
33 Murphy MM, Molloy AM, Ueland PM, Fernandez-Ballart JD, Schneede J, Arija V *et al*. Longitudinal study of the effect of pregnancy on maternal and fetal cobalamin status in healthy women and their offspring. *J Nutr* 2007; **137**: 1863–1867.

34 Yetley EA, Pfeiffer CM, Phinney KW, Bailey RL, Blackmore S, Bock JL *et al*. Biomarkers of vitamin B-12 status in NHANES: a roundtable summary. *Am J Clin Nutr* 2011; **94**: 313S–321S.
35 Fulgoni VL. Current protein intake in America: analysis of the National Health and Nutrition Examination Survey, 2003–2004. *Am J Clin Nutr* 2008; **87**: 1554S–1557S.
36 Cho E, Zeisel SH, Jacques P, Selhub J, Dougherty L, Colditz GA *et al*. Dietary choline and betaine assessed by food-frequency questionnaire in relation to plasma total homocysteine concentration in the Framingham Offspring Study. *Am J Clin Nutr* 2006; **83**: 905–911.
37 Dominguez-Salas P, Moore SE, Cole D, Da Costa K-A, Cox SE, Dyer RA *et al*. DNA methylation potential: dietary intake and blood concentrations of one-carbon metabolites and cofactors in rural African women. *Am J Clin Nutr* 2013; **97**: 1217–1227.
38 King WD, Ho V, Dodds L, Perkins SL, Casson RI, Massey TE. Relationships among biomarkers of one-carbon metabolism. *Mol Biol Rep* 2012; **39**: 7805–7812.
39 Imbard A, Smulders YM, Barto R, Smith DEC, Kok RM, Jakobs C *et al*. Plasma choline and betaine correlate with serum folate, plasma S-adenosyl-methionine and S-adenosyl-homocysteine in healthy volunteers. *Clin Chem Lab Med* 2013; **51**: 683–692.
40 Tobi EW, Lumey LH, Talens RP, Kremer D, Putter H, Stein AD *et al*. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Hum Mol Genet* 2009; **18**: 4046–4053.
41 Rao S, Yajnik CS, Kanade A, Fall CH, Margetts BM, Jackson AA *et al*. Intake of micronutrient-rich foods in rural Indian mothers is associated with the size of their babies at birth: Pune Maternal Nutrition Study. *J Nutr* 2001; **131**: 1217–1224.
42 Crowther NJ, Cameron N, Trusler J, Gray IP. Association between poor glucose tolerance and rapid post-natal weight gain in seven-year-old children. *Diabetologia* 1998; **41**: 1163–1167.
43 Antonysunil A, Tripathi G, Sivakumar K, Lawson A, Webster C, Wood C *et al*. Maternal B12 insufficiency independently predicts the metabolic risk factors of the offspring at birth. *Developmental Origins of Health and Disease (DOHaD) Conference*, Singapore, 17–20th November 2013. Submission number 13-1437. 2013.
44 Saravanan P, Wood C, Anderson N. B12 deficiency is more common than folate deficiency in early pregnancy: Do we need to consider B12 fortification? *Diabetologia* 2010; **53**(Suppl 1): S151–S2.