OPINION

# Folic acid — vitamin and panacea or genetic time bomb?

*Mark Lucock and Zoë Yates*

Abstract | We live in a health-conscious age — many of us supplement our diet with essential micronutrients through the discretionary use of multivitamin pills or judicious selection of foods that have a health benefit beyond that conferred by the nutrient content alone — the so-called 'functional foods'. Indeed, the citizens of some nations have little choice, with a mandatory fortification policy in place for certain vitamins. But do we ever stop to consider the consequences of an increased exposure to micronutrients? We examine this issue in relation to the B-group vitamin folic acid, and ask whether supplementation with this vitamin could introduce a strong genetic selection pressure — one that has the side effect of increasing the prevalence of some of the most significant, human life-threatening diseases. Are we affecting our genetics — is this a case of human evolution in progress by altering our diet?

Folic-acid (or folate) supplementation offers substantial and well-defined health benefits. However, mandatory fortification of grain with this vitamin in some countries, and increased discretionary intake as a preventive measure against SPINA BIFIDA (see Glossary), might increase the genetic selection of one particular variant folate metabolism gene — 677T methylenetetrahydrofolate reductase (677T *MTHFR*) — which has been associated with a range of developmental and degenerative conditions. Because such conditions are those that are actually likely to be prevented by adequate folate nutrition, we explore whether, by selecting for 677T *MTHFR*, society is in fact engineering its own acute dependency on folate. We also discuss the underlying cellular mechanisms that make folic acid such an important vitamin in life processes, and how the vitamin interacts with this key gene to influence cellular events and modulate disease risk. Finally, we look at how certain other key micronutrients interact with cellular-replication mechanisms to maintain the integrity and function of DNA.

## Folate — a nutritional panacea

Our understanding of how nutrition can influence fundamental life processes has advanced markedly in recent years (BOX 1). There are few better examples of this than for the B-group vitamin folic acid. Scientists studying the role of this vitamin in disease development have come to embrace various disciplines that range from nutrition to molecular biology[1–3]. Collectively, these sub-disciplines form the area of 'folate nutritional genetics'[4]. The importance of this focus is unsurprising given that folate nutritional status and/or genetics have in fact been linked to a plethora of conditions that range from mood disorders[5] to Alzheimer disease[6], and an array of cancers including those of the colon[7], breast[8], pancreas[9], cervix[10], bronchus[11], as well as leukaemia[12]. It is also implicated in the aetiology of vascular disease[13], birth defects[14], Down syndrome[15], complications during pregnancy[16–18], and most recently in male subfertility[19]. However, although folate and/or its related genes have a role in these conditions, it must be recognized that a complex set of gene–gene and nutrient–gene interactions is invariably involved in defining any given clinical phenotype and that the 677T *MTHFR* variant and low levels of folate do not, by themselves, cause these conditions.

The reason for this panoply of effects is based on several B-group vitamin-sensitive molecular mechanisms that subserve the genomic machinery. The cellular folate status, and/or the interaction between folate nutrition and folate-dependent enzyme polymorphisms might be vital determinants of such mechanisms, which include maintenance of genomic CpG-methylation patterns for regulated gene expression[20] and synthesis of pyrimidine nucleotides to prevent uracil misincorporation and therefore DNA instability[21].

A third mechanism that is unrelated to epigenetic phenomena places folate at the centre of athero- and thrombogenic vascular disease — folate and its dependent enzymes are crucial for lowering plasma homocysteine (Hcy), a vasculotoxic sulphur amino acid that is converted into methionine by methyl-$H_4$ folate[13]. If this form of folate is in short supply, Hcy levels increase, which leads to a risk of vascular pathology. FIGURE 1 shows the biochemical events that are associated with this process.

## Genetic selection and dietary folate

Despite the enormous proven benefit of folate nutrition[4], recent findings indicate that exposure to elevated levels of this vitamin during the periconceptional period could select embryos that carry the mutant 677T *MTHFR* allele[22]. By contrast, in the presence of low folate reserves, this allele is associated with elevated Hcy and aberrant epigenetic processes, both of which are thought to be key factors in vascular disease and cancer[22].

The effect of 677C>T *MTHFR* on fetal survival has been reviewed elsewhere[22] (see also FIG. 1). By contrast, the interaction of this

Box 1 | **Essential micronutrients and their genomic interactions**

Folate is not the only important essential nutrient; even marginal deficiency of many other micronutrients can influence the integrity and function of DNA.

**Vitamin A**

Retinoic acid (RA) interacts with nuclear receptors that are related to steroid and thyroid hormone receptors, a family of ligand-dependent proteins that function as transcription factors. The all-*trans* form of RA binds to the RA receptor (RAR), whereas 9-*cis* RA binds to the retinoid receptor (RXR). RAR/RXR heterodimers bind to specific 'RA response elements' to regulate transcription.

In addition to its role in vision, most of the physiological effects of vitamin A are a consequence of its role in cellular differentiation. In particular, the morphogenetic properties of RA might stem from activation of developmentally regulated genes by RAR complexes. Excess vitamin A is considered to be TERATOGENIC, and women who are pregnant or planning a pregnancy should limit their intake to avoid the potential risk of birth defects.

**Vitamin D**

Calcitriol interacts with the vitamin D receptor (VDR) in the nucleus. Together with RXR, this complex interacts with the vitamin D response element to alter transcription. This mechanism increases vitamin-D-related $Ca^{2+}$ transport in the small intestine, regulates $Ca^{2+}$ mobilization by the bone and $Ca^{2+}$ reabsorption by the kidneys.

Vitamin $D_3$ (calciol) is thought to protect against breast cancer. VDR polymorphisms are associated with breast cancer risk and might be associated with progression of this disease. It has been suggested that VDR polymorphisms modulate the interaction between vitamin $D_3$ and the VDR, and that they could lead to a differential responsiveness of target cells to the vitamin $D_3$ (REF. 39).

The influence of ultraviolet light on vitamin D synthesis in the skin might have provided a selection pressure for the development of skin pigmentation[40]. In 1967, W. F. Loomis[41] suggested that as hominids moved beyond the tropics (particularly at latitudes above 40°N), skin depigmentation evolved to facilitate synthesis of vitamin $D_3$ from 7-dehydrocholesterol in the skin. Today it is believed that the latitudinal gradient in skin colour might result from a balance between natural and sexual selection[42].

**Vitamin $B_2$**

Riboflavin (vitamin $B_2$) supplements might improve methylenetetrahydrofolate reductase (MTHFR) activity and therefore improve folate-dependent one-carbon flux to important pathways such as *de novo* methionine biosynthesis that is needed to maintain genomic CpG-methylation patterns.

**Vitamin $B_3$**

Poly-ADP-ribose polymerases (PARPs) catalyse the transfer of ADP-ribose pairs from $B_3$-derived NAD (nicotinamide adenine dinucleotide) to arginine, lysine or asparagine residues of proteins that are involved in DNA repair and replication. A deficiency of vitamin $B_3$ leads to DNA instability.

**Vitamin $B_{12}$, $B_6$ and folate**

These vitamins function by potentially reducing intracellular folate pools that donate one-carbon units for nucleotide formation and for methyl groups that are used in the CpG methylation. The former affects DNA stability, the latter, gene expression.

Folate's ultraviolet instability and its role in reproduction might have had a role in the evolution of skin pigmentation[40]. The finding that folate can rescue lethal homeobox gene-family mutations in mice shows how effective folate nutrition is at masking harmful mutations[43].

**Vitamins C and E, iron, zinc, magnesium and selenium**

Deficiency causes ssDNA and dsDNA breaks and oxidative lesions. Vitamin C has general antioxidant properties, including salvage of the inactive tocopheroxyl radical back to α-tocopherol (vitamin E). Zinc has a wide range of effects on several proteins that are involved in maintaining replication and the normal functioning of DNA. Selenium, in the form of selenocysteine at the catalytic site of glutathione peroxidase, is also important in neutralizing reactive oxygen species — for example, it is known to protect developing sperm from oxidative damage to DNA[44] and has a similar role in somatic cells. Selenium deficiency has also been shown to decrease genomic DNA methylation in Caco-2 colon cancer cells and in rat liver and colon[45,46]. Magnesium is an essential cation with a role in maintaining optimum fidelity in DNA transcription. Iron, by contrast, might cause DNA strand breaks.

Although antioxidants such as vitamins C, E and β-carotene have a general role in preventing oxidative damage to chromosomes at high concentrations, they might function as pro-oxidants and promote the formation of free radicals that interfere with many carefully orchestrated genomic mechanisms.

phenomenon with a mandatory public-health policy of fortifying grain at the source with folic acid[23,24], and an increased their discretionary intake of folate as a functional food, has not been considered.

Sufficient evidence now supports the view that increasing dietary folate as a population measure through government mandate or through unregulated discretionary use can increase representation of the 677T allele, and

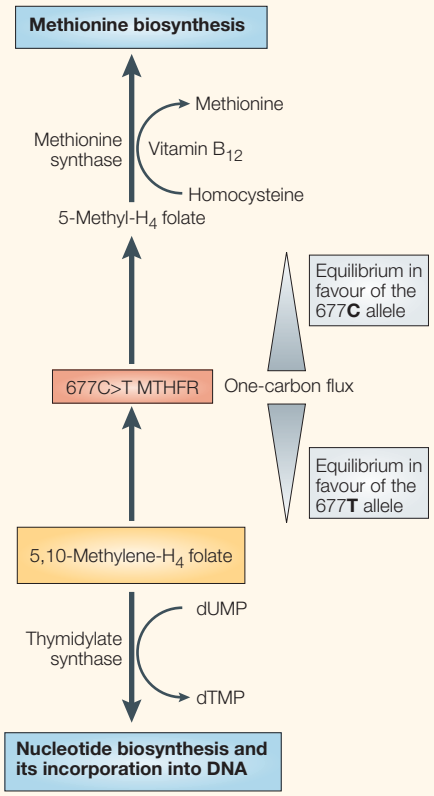


Figure 1 | **The putative cellular mechanism for genetic selection that is based on folate gene variant and dietary folate.** The balance between the use of methylene-$H_4$ folate for DNA synthesis rather than for methionine synthesis might depend on the presence of the 677T variant of methylenetetrahydrofolate reductase (MTHFR) and the nutritional folate status. Given adequate dietary folate, the 677T variant of MTHFR functions as a 'valve', preferentially routing one-carbon units to DNA at the expense of methionine. If folate nutritional status is poor, the 677T variant might promote the misincorporation of uracil into DNA, leading to chromosome breakage. This mechanism might therefore select embryos that are best suited to reproductive success for a given environmental abundance of dietary folate by augmenting dTMP (deoxythymidine monophosphate) synthesis. Therefore, given the current practice of increasing dietary folate before conception, *in utero* selection of the 677T variant might lead to a negative, long-term effect on the prevalence of cancer and vascular disease for which this mutation is a potential risk factor[21,47]. dUMP, deoxyuridine monophosphate.

potentially influence the prevalence of chronic degenerative diseases, with a possible effect on the morbidity and mortality of future generations. For example, there might be a survival advantage for fetuses that are homozygous recessive (TT) for 677C>T *MTHFR*, as there is a four-fold higher prevalence of this genotype in neonates compared with miscarried fetuses[25]. In an earlier study, the use of periconceptional folic-acid supplements as a preventive agent for neural-tube defects had already been shown to increase the prevalence of individuals with the *MTHFR* 677 TT genotype[26]. Interestingly, this is the opposite to what happens in the absence of folic-acid supplementation[27–29]. Taken together, these reports indicate a possible beneficial effect on survival of fetuses with the recessive *MTHFR* 677 TT genotype when folate supply is unrestricted[30].

Tracking the geographical distribution of this allele has produced some interesting results[22]. It has been suggested that the natural abundance of folate can explain the diverse global distribution of the 677C>T *MTHFR* variant. In Europe, a *MTHFR* 677T gradient that runs from north to south might depend on the higher folate content of the Mediterranean diet. In the Americas, a similar tendency can be detected. To further highlight the possible relationship between the abundance of foods that are rich in folate and frequency of the 677T *MTHFR* allele, it has been suggested that the atypically low prevalence of the recessive TT genotype in black Africans might also be related to a deficiency in nutritional sources of folate in a country that is subject to malnourishment and disease[30]. Interestingly, the frequency of the 677T allele seems to have increased in African-Americans: its frequency of 0.06 in sub-Saharan Africans has risen to 0.12–0.35 in Americans of African descent[30,31]. It is not unreasonable to speculate that this might have arisen as a result of improved folate nutrition among African-Americans, although population ADMIXTURE is also a likely factor.

## Mechanism of selection by folate

Collectively, the demographical and genetic information that is available on the 677T SNP would seem to indicate a significant embryonic or fetal survival advantage for TT genotypes when folate nutrition is adequate[21,22]. Such a phenomenon probably results from the fact that 677T MTHFR increases levels of methylene-$H_4$ folate, the crucial coenzyme that is required for nucleotide biosynthesis[32] (FIG. 1). When we consider that the enzymes of nucleotide biosynthesis have $K_M$ VALUES that are higher than the concentration of their cellular folate substrates, it becomes clear that these enzymes are highly sensitive to folate coenzyme concentration. Therefore, with adequate folate nutrition, an accumulation of methylene-$H_4$ folate might actually protect against uracil misincorporation and problems in the synthesis and subsequent stability of DNA. However, when levels of folate are low, the same SNP might compromise the accumulation of cellular methylene-$H_4$ folate at a level that would be required to maintain normal conversion of dUMP (deoxyuridine monophosphate) into dTMP (deoxythymidine monophosphate) (FIG. 1), and so augment uracil misincorporation[21], leading to genomic instability. This provides a putative but elegant explanation to account for the loss of the developing embryo. An accumulation of Hcy might also compound this negative *in utero* effect[33].

Recent research on 677C>T *MTHFR* supports a model in which genotype influences folate metabolism in the above manner. Colon cancer cells with endogenous 677C *MTHFR* and the same cell line transfected with 677T *MTHFR* differed with respect to growth and metabolism[32]. The 677T variant had enzyme activity that was 35% lower than the wild type (677C), with 10% lower levels of methyl-$H_4$ folate and a 12% increase in folate enzymes destined for nucleotide biosynthesis. This small change in cellular methylene-$H_4$ folate was sufficient to alter chemosensitivity to antifolate drugs and as such elicits a potentially significant biological effect[32]. BOX 2 lists many of the conditions that are associated

### Box 2a | The 677T variant and mechanims in important clinical conditions

**The 677T allele encodes a small change in protein structure (as a result of alanine to valine substitution), with potentially large biological effects. It has been associated with a number of clinical conditions: adult acute lymphocytic leukaemia[12], colon cancer[5,32,47], complications during pregnancy (PRE-ECLAMPSIA, recurrent early pregnancy loss, fetal-growth restriction)[48], Down syndrome[15], elevated homocysteine; a risk for vascular disease[49,50], oral cleft[51] and spina bifida[52].**

**Biological mechanisms**

***Direct nutrient–protein interaction.*** **Guenther and colleagues[53] showed that folate coenzymes can stabilize the polymorphic enzyme that is encoded by the 677T allele. This stabilization prevents the enzyme from losing its bound flavin cofactor. Dietary folate, in the form of unmetabolized synthetic pteroylmonoglutamic acid (PGA), might also function as a modulator of regulatory (allosteric) enzymes such as methylenetetrahydrofolate reductase (MTHFR), in which structurally similar native dihydrofolate would normally have a regulatory role[4,54]. Allosteric inhibition of MTHR by dihydrofolate provides vital regulation of nucleotide biosynthesis in that it diverts the flow of one-carbon units away from methionine to pyrimidine-nucleotide production when required (FIG. 1). PGA could therefore, theoretically, function like a drug to enhance the synthesis of DNA and prevent chromosome breakage by a mechanism other than by functioning as a precursor to 'activated' one-carbon forms of the vitamin (FIG. 1). Structurally similar dihydrobiopterin might inadvertently have a similar effect in patients with phenylketonuria (PKU)[55]. The implications, if any, for a direct interaction of PGA with the product of the 677T allele are unclear, but might be worthy of study. Any increase in methylene-$H_4$ folate owing to excess PGA (FIG. 2) might help the mutant enzyme to bind its flavin-adenine dinucleotide (FAD) cofactor, and might therefore improve its catalytic activity. Vitamin $B_2$, the precursor of FAD, might also be an independent determinant of enzyme activity, as indicated by homocysteine level[56]. A daily intake of 0.6 mg of vitamin $B_2$ results in a modest reduction of plasma homocysteine[57]. Other data also indicate that vitamin $B_2$ status specifically influences enzyme activity, and therefore the homocysteine level in 677T homozygotes[58,59].**

**De novo *methylation.* Our diet does not provide adequate methyl groups for all cellular methylation reactions. Therefore, *de novo* synthesis of *S*-adenosylmethionine through the folate one-carbon pool is important to maintain the regulated methylation of proteins, lipids, biogenic amines and DNA. Folate-dependent DNA methylation of specific CpG sites regulates gene expression and has a crucial role in the cell cycle[20]. CpG islands lie in 5′-promoter regions or within the first few exons of half the human genes[60]. As a rule of thumb, CpG islands are unmethylated[61], allowing gene expression. In the germ line, DNA is generally fully methylated; demethylation is tissue-specific[62]. Most housekeeping genes are usually maintained in an unmethylated state in both germline and somatic cells[62].**

**The polymorphic enzyme that is encoded by the 677T allele might reduce the availability of *de novo* (folate-derived) methyl groups that are destined for CpG methylation, which might be important in oncogenesis because a deficiency of methyl groups could modify proto-oncogene expression. Hypomethylation might also induce DNA strand breaks and subsequent mutations[20].**

with the 677C>T genotype and briefly summarizes some of the functional consequences of the polymorphic protein that is encoded by the 677C>T *MTHFR* gene variant.

### Effects of excess synthetic folate

In considering the biological and genetic effects of dietary folate, it is necessary to recognize that there is more to folate status than a simple measure of blood level. Food folates occur mainly as methyl-$H_4$ folate and formyl-$H_4$ folate[34]. However, folate that is in fortified foods and in supplements is in the form of pteroylmonoglutamic acid (PGA), which is a cheap and stable synthetic analogue that does not occur in nature. The body readily metabolizes PGA into methyl-$H_4$ folate, the normal transport form of the vitamin that is found in plasma. Research shows that this absorption and biotransformation process is readily saturated at doses in the region of 400 μg of PGA or less[35,36]. In other words, at or just below a 400-μg dose of PGA (the recommended dose to prevent spina bifida), this entire synthetic analogue is converted into biologically active methyl-$H_4$ folate during absorption. Above this dose, synthetic PGA is transported in an unmodified form into the blood in a manner that is directly proportional to dose. Given the current mandatory folate-fortification policy in some countries, and a general increase in discretionary intake of folate supplements, what are the likely cellular consequences of exposure to unmetabolized PGA, and are there any genetic considerations?

**Box 2b | The 677T variant and mechanims in important clinical conditions**

***Synthesis of DNA.*** **Thymidylate synthase catalyses the synthesis of dTMP (deoxythymidine monophosphate) from dUMP (deoxyuracil monophosphate). This step requires the one-carbon unit of methylene-$H_4$ folate. If cellular folate levels are low owing to a poor diet, uracil misincorporation occurs, leading to DNA strand breaks — an important antecedent of cancer. The 677T allele augments dTMP synthesis if folate nutritional status is good (a bottleneck in transfer of one-carbon units to methionine favours diversion to nucleotides as shown in FIG. 1), which is thought to confer protection against certain cancers (such as colon cancer[7,32,47] and leukaemia[12]).**

**If folate nutritional status is inadequate, the SNP might instead promote disease. The 677T allele might be linked to this phenomenon through its influence on the role of folate at chromosome fragile sites. Common fragile sites are site-specific breaks that are seen on metaphase chromosomes after partial inhibition of DNA synthesis using folate or thymidylate deprivation. Fragile sites and their associated genes are often deleted or rearranged in cancer cells, clearly showing their importance in genome instability and tumorigenesis[63]. This process is likely to be exacerbated by both reduced DNA-excision repair, an event that is linked to folate depletion[64], and impaired mismatch repair, which also helps to maintain genomic integrity. Cells that are proficient in mismatch repair are highly sensitive to folate deficiency compared with cells that are defective in mismatch repair. That is, wild-type, but not mutant cells undergo apoptosis after extensive folate depletion[65].**

**Differences in the activity of folate-related DNA-repair enzymes, particularly as a consequence of polymorphisms, might also contribute to disease incidence, especially of cancers. Such variants are likely to further interact with the SNP in the *MTHFR* gene.**

***Homocysteine and vascular disease.*** **The functional product of the 677T allele diminishes one-carbon flux from methylene-$H_4$ folate to methyl-$H_4$ folate, the donor for transformation of homocysteine into methionine. The 677T allele might therefore elevate homocysteine that has several effects — it promotes blocked blood vessels and clot formation, is a hypertensive agent and inhibits collagen–elastin cross-linking, which leads to connective-tissue abnormalities[4]. Studies such as that by Johanning *et al.*[66], and others mentioned elsewhere in this article, which show an increase in the TT genotype owing to increased periconceptional exposure to folate, are therefore probable barometers for a potential increase in future homocysteine-related pathologies, if folate intake is subsequently restricted.**

No work has been done so far to evaluate the biological and/or genetic consequences of excess long-term exposure to PGA. However, because there is an increasing tendency for clinicians to give patients with vascular problems, or with elevated Hcy, 5-mg doses of PGA (more than 10 times that needed to give maximal methyl-$H_4$ folate levels) it should be possible to gain some insight into what might happen. We recently examined B-group vitamins and polymorphic markers as a risk factor for life-threatening blood clots[37]. Plasma-folate measurements are normally in the range 3–30 ng $ml^{-1}$, but in this study some individuals had unusually high values (100–200 ng $ml^{-1}$). These values were mirrored by a correspondingly high level of red blood cell folate (FIG. 2). HIGH-PRESSURE LIQUID CHROMATOGRAPHY (HPLC) examination of these high levels of plasma folate found that only around half the plasma folate measured by routine haematological assay was methyl-$H_4$ folate. The remainder was unmodified PGA (REF. 38). This finding provided the ideal opportunity to explore what might happen to the various cellular-folate pools when the body is exposed to excess PGA.

Limited, but detailed evaluation[4] of the red blood cell folate-coenzyme composition in three subjects that were treated with long-term, high-dose PGA indicated a consistent accumulation of the folate-coenzyme substrates methylene-, methenyl-, formyl- and unsubstituted $H_4$ folate at the expense of the MTHFR-downstream folate-coenzyme product, methyl-$H_4$ folate (FIG. 2) — an equilibrium that probably promotes DNA synthesis through a putative pharmacological inhibitory effect of PGA on MTHFR (FIG. 1; BOX 2), as well as by the one-carbon transfer reactions that are normally associated with folate. Although genotype data were collected for these subjects, it is impossible to draw any conclusions on how excess PGA might specifically influence the 677T-*MTHFR* encoded protein. However, as PGA increases methylene-$H_4$ folate (FIG. 2), it is likely that the variant form of MTHFR will be stabilized by an improved ability to bind its FAD (flavin-adenine dinucleotide) cofactor (BOX 2).

**Glossary**

ADMIXTURE
Gene flow between differentiated populations.

HIGH-PRESSURE LIQUID CHROMATOGRAPHY
(HPLC). A rapid variant of column chromatography used for high-resolution separation of molecules of low-to-moderate molecular weight.

$K_M$ VALUES
The affinity of enzymes for a substrate.

PRE-ECLAMPSIA
Also known as toxaemia, it is a condition that can occur in a woman in the second half of her pregnancy that causes high blood pressure, protein in the urine, blood changes and other problems such as low birth weight.

SPINA BIFIDA
A condition that occurs at birth in which part of the spinal cord protrudes through a small indentation in the spinal column resulting in partial to total loss of voluntary movement in the lower body.

TERATOGENIC
A factor that causes malformation of an embryo.

Although this encouraging finding lends support to a policy of mandatory folate fortification, it is hard to ignore the counter argument that increasing folate status during the periconceptional period might lead to selection of 677T *MTHFR*, and potentially increase chronic degenerative diseases in society. In addition, to be used, PGA must first be metabolized by dihydrofolate reductase for which it has a lower affinity than dihydrofolate itself, which could result in a mild antifolate effect through competitive interaction[4].

### Folate, 677T and diseases of old age

Adequate folate nutrition leads to a reduction of neural-tube defects in today's society. However, adequate folate nutrition might also lead to an increase of the 677T allele frequency in the population and could potentially lead to a future society in which serious degenerative diseases that are associated with high morbidity and mortality are even more prevalent than they are today. Such diseases are in fact those that can be ameliorated by folate nutrition, indicating that society will have even greater need for maintaining adequate B-group vitamin intake at the population level. Might we then be creating our own folate habit? This might be an interesting example of a nutritional selection pressure having a rapid effect on the prevalence of key diseases in society, and if shown to be true, is likely to ignite interesting debate on the nature versus nurture issue. Gaining irrefutable proof of such an effect is hampered by the length of the human lifespan. Nonetheless, large population studies to show an association between periconceptional folate status and infant genotype are possible, and could be done prospectively or maybe even retrospectively by following up mothers (and subsequently offspring) who took part in the original large-scale folate-supplementation studies designed to show the protective effect that is afforded by this vitamin against spina bifida.

This model also raises an important and more fundamental scientific question: could this SNP exist to tailor the one-carbon metabolism to the prevailing environmental (nutrient availability) conditions — that is, to select embryos that are best suited to reproductive success (enhanced dTMP synthesis) for a given abundance of dietary folate?

It seems that few nutrients have such a vital role as folate in maintaining health and ensuring disease prevention. It is therefore not so much a question of whether we should fortify grain with this vitamin, but more at what level of enrichment fortification should occur.

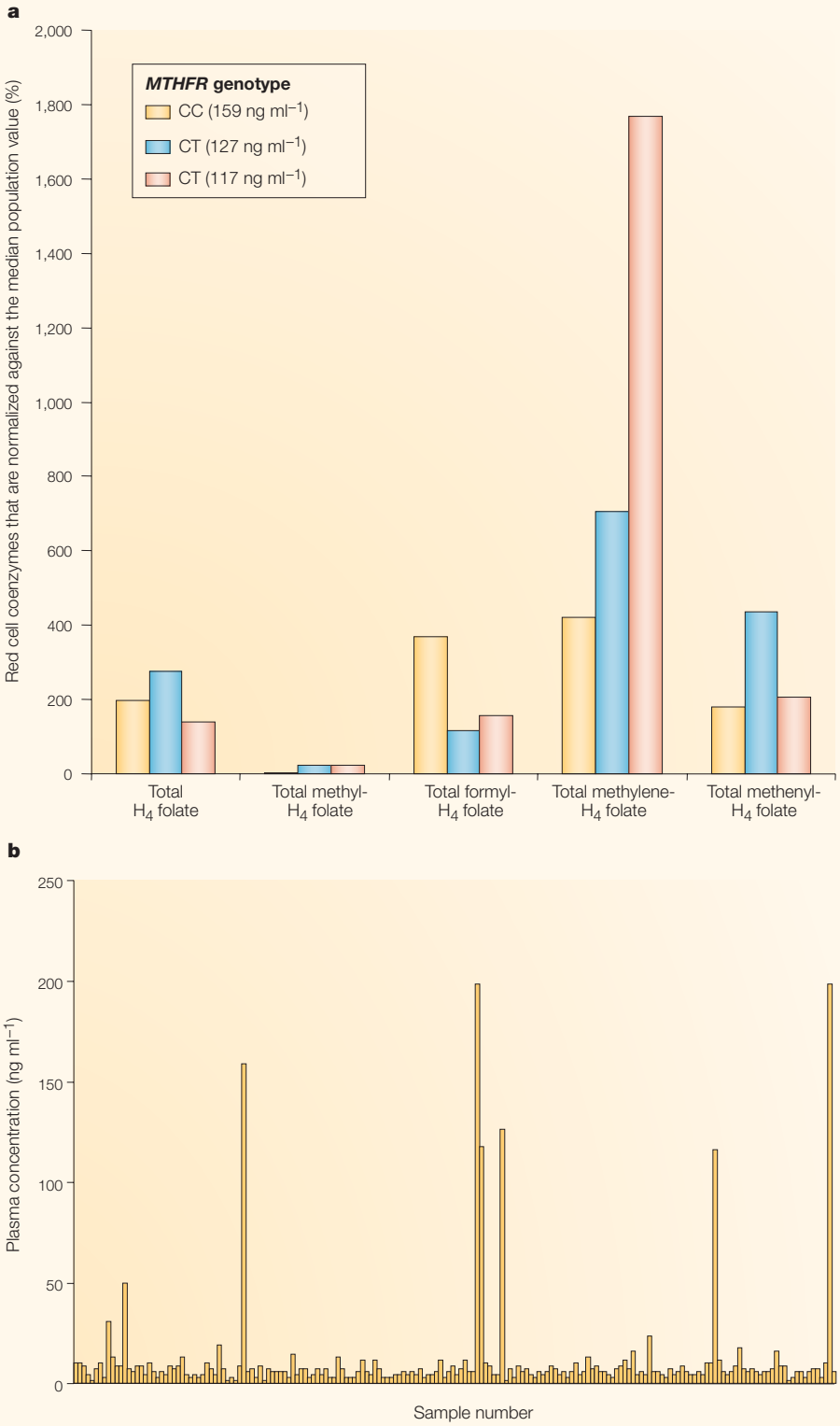


Figure 2 | **Cellular effect of excess synthetic folate.** The effect of sustained high-dose pteroylmonoglutamic acid (PGA) on cellular-folate coenzyme disposition in three individuals from a vascular patient control population. The proportion of each folate pool relative to the total folate in individuals receiving excess PGA has been expressed as a proportion (%) of the median population control value (n = 97 for patients with a plasma folate below 50 ng ml$^{-1}$). The 677C>T methylenetetrahydrofolate reductase (MTHFR) genotype is given for each individual, along with the anomalous plasma folate value (part **a**). To show the extent of excess folate status arising from pharmacological use of PGA in patients with vascular problems, part **b** shows the range of plasma folate values that were encountered in a recent study of patients with a vascular condition[37]. The most profound effect of PGA seems to be on the methylene-$H_4$ folate pool.

*Mark Lucock is at the School of Applied Sciences, University of Newcastle, Brush Rd, Ourimbah, NSW 2258, Australia.*

*Zoë Yates is at the Academic Unit of Paediatrics and Obstetrics and Gynaecology, University of Leeds, West Yorkshire LS2 9NS, UK.*

*Correspondence to M.L.*
*e-mail: Mark.Lucock@newcastle.edu.au*

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## Competing interests statement

The authors declare no competing financial interests.

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