Supplementary Material 1: Additional information on the role of nutritional factors within one-carbon metabolism

An overview of one-carbon metabolism

S-adenosyl methionine (SAM) is the methyl donor for hundreds of transmethylation reactions[1], and its importance in epigenetics stems from its ability to methylate cytosine bases and histones. The loss of a methyl group from SAM forms S-adenosyl homocysteine (SAH), a build-up of which can reduce methylation rates through allosteric inhibition[2]. The SAM:SAH ratio can be used as a proxy indicator of methylation potential[3]. SAH is further hydrolysed to homocysteine (Hcy)[4] and, since this reaction is reversible, Hcy needs to be removed from the system to maintain favourable methylation conditions. Hcy can be methylated to form methionine or be irreversibly degraded in the transsulfuration pathway. In the case of methionine formation, Hcy can either receive the methyl group from N^5-methyl tetrahydrofolate ('methyl-THF'), produced through the reduction of dietary folates and folic acid, or from betaine in the liver and kidneys, a product formed through the oxidation of choline. Methionine can then be condensed with ATP to form SAM and complete the cycle. Genetic variants in one-carbon enzymes can also affect the flow of metabolites through these pathways[5].

The role of individual nutrients

Folate: Dietary folates and folic acid are reduced to form tetrahydrofolate (THF), which is a scaffold upon which one-carbon units can be attached and activated. THF in turn is reduced to methylene-THF, then to 5-methyl-THF, which donates its methyl group to homocysteine using vitamin B12[6].

B vitamins: Several B vitamins act as cofactors in one-carbon pathways (B12, B2, B6). Vitamin B12 is a cofactor in the methylation of homocysteine by 5-methyl-THF. Vitamin B6 (in the active form of pyridoxal-5'-phosphate; PLP) is required to reduce THF to methylene-THF, and is also a cofactor in the transsulfuration pathway converting homocysteine to cysteine[7]. B2 is required as a precursor to FAD, which is a cofactor for MTHFR to reduce methylene-THF to methyl-THF[3]. The different forms of THF are interconvertible, except the methyl-THF form. In the absence of sufficient B12 to utilise the methyl group from methyl-THF in homocysteine methylation (a process which re-generates THF), methyl-THF can accumulate at the expense of other THF forms. This is termed the 'folate trap' or 'methyl trap' and means pathways dependent on other forms of THF (e.g. purine synthesis and the thymidylate pathway) can become impaired[8].

Choline can be synthesised endogenously or obtained from the diet (good sources include red meat, poultry, milk, eggs and fish[9]). A two-step oxidation reaction converts it to **betaine**[10], which can donate its methyl group to homocysteine via betaine-homocysteine methyl transferase (BHMT)[10]. Betaine can also be directly sourced from the diet (e.g. wheat bran, wheat germ, spinach, beets[9]).

Polyunsaturated fatty acids (PUFAs) influence the 1-carbon pathway through two mechanisms. Firstly, ω-3 PUFAs upregulate enzymes responsible for the methylation of homocysteine to methionine[11,12]. Secondly, the availability of PUFAs can influence methyl balance via phosphatidylcholine (PC). PC is used in the production of very low density lipoproteins (VLDLPs), bile and surfactants. It is formed via two main pathways: the cytidine diphosphocholine (CDP)–choline ('Kennedy') pathway and the PEMT pathway, which converts phosphatidylethanolamine (PE) to PC (Fig. 1). PC differs in composition according to the pathway of its formation; the former incorporates more saturated fatty acids and the latter more PUFAs, such as arachidonic acid (AA) and

docosahexaenoic acid (DHA)[13]. In the PEMT pathway the conversion of a molecule of PE to PC requires three methyl groups from SAM. In rodent studies imbalances in methyl donor supply and the coenzymes involved in 1-carbon metabolism affect PEMT activity, manifested in disturbances to tissue levels of PUFAs[12,14], since the PEMT pathway is the major way phospholipids are transported from the liver to the plasma and other tissues[15,16]. These effects have been most pronounced in pregnant dams consuming a diets designed to resemble a rural Indian intake, with excess folic acid and restricted vitamin B12 lowering offspring placental and brain DHA, as well as lowering placental global methylation[17,18]. In these experiments, supplementation with ω-3 PUFAs reversed the effect of the micronutrient imbalances[12]. The PEMT pathway represents a major demand for methyl groups[19,20]. Hence any factor that reduces the demand for methyl groups in the PEMT pathway (e.g. low PUFA intake) could in theory make more methyl groups available for DNA methylation, with potential consequences for epigenetic outcomes[21].

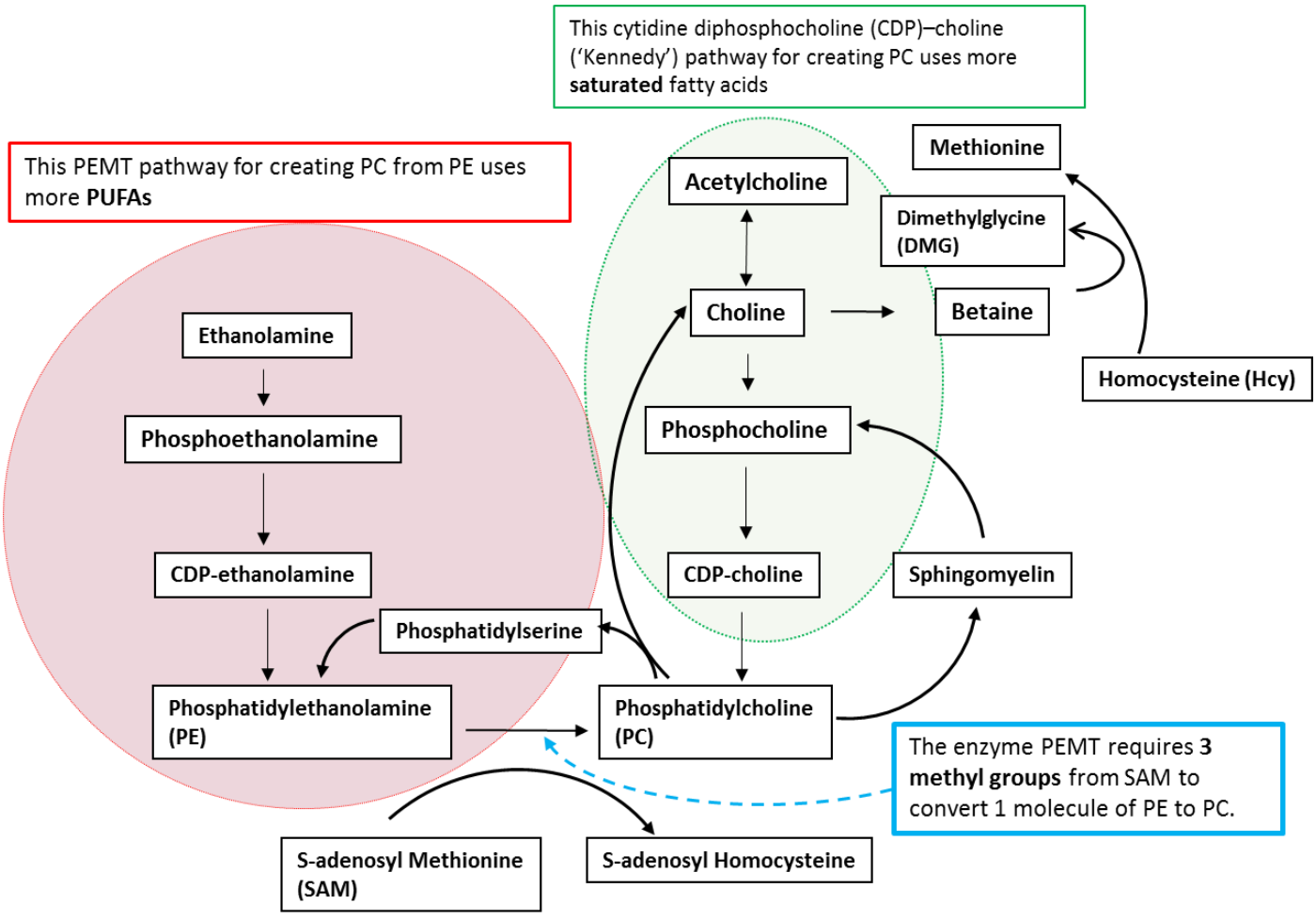

Fig 1: Formation of phosphatidylcholine by two metabolic pathways

Abbreviations: CDP, cytidine diphosphocholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine N-methyltransferase; PUFA, polyunsaturated fatty acids; SAM, S-adenosyl methionine.

Amino acids: Several amino acids are thought to contribute 1-carbon units[22], and in turn deficiencies in 1-carbon-related analytes may affect amino acid metabolism[23–25]. The most documented amino acids in this context are serine and glycine. Serine donates a 1-carbon unit at the stage of converting THF to methylene-THF through the action of Serine Hydroxymethyltransferase (SHMT) and PLP[26]. In the process serine is converted to glycine. Serine is also involved in the transsulfuration pathway at the steps of converting homocysteine to cystathionine and then to cysteine. Glycine is not simply a by-product of reactions involving serine, however. For example, it accepts methyl groups from SAM via GNMT, especially when SAM concentrations are high[27]. Its catabolism by the glycine cleavage system in the mitochondria generates methylene-THF with the involvement of THF, which is then used as a carbon donor in the 1C pathways[28]. The catabolism of amino acids tryptophan and histidine is of also of particular interest within 1-carbon metabolism as this produces formate[22,29]. Formate is produced in the mitochondria and then released into the cytosol, forming formyl-THF by condensation with THF. Formyl-THF can then either be used in purine synthesis or be interconverted into other THF oxidation states as part of folate metabolism. Taken together, formate, serine and glycine help link the mitochondrial and cytosolic folate pools[30].

Methionine: Methionine is obtained from the diet or formed by the methylation of homocysteine. Its adenylation forms SAM, making it a key metabolite in the 1-carbon pathways. Methionine also upregulates glycine N-methyltransferase (GNMT) which can use excess SAM to methylate glycine to sarcosine.

Homocysteine: Homocysteine is an intermediary metabolite that is hydrolysed from SAH. Since a build-up of SAH inhibits transmethylation reactions[2] homocysteine is often inversely associated with methylation. For example, van Mil *et al.* (2014) found an increase in maternal plasma homocysteine was associated with 0.04% lower methylation at *NR3C1* in the infant epigenome ($p=0.03$)[31]. Although it is not a nutritional exposure *per se*, nutritional inputs can influence the concentration of homocysteine. Trials that have aimed to decrease homocysteine as part of lowering blood pressure could therefore provide additional information as to exposures with the potential to influence the epigenome. For example, several studies indicate that increased consumption of ω-3 PUFAs is associated with reduced homocysteine levels[32], and that when combined with folic acid and other B vitamins the association is even stronger[33]. Betaine supplementation has also been shown to reduce homocysteine levels[27]

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