Interdependence of Folate with Choline
for Optimal Methylation

The Choline Factor in One Carbon Metabolism

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The goal of this white paper is to explore the precise role of choline in contributing to one-carbon metabolism (1CM) as it pertains to the intersect with folate in the methionine cycle and elsewhere.

Methionine – A Methylation Lynchpin

Choline and folate are metabolically entwined. Methyl groups from both are used for the re-methylation of homocysteine to form methionine, an essential amino acid that cannot be produced de-novo in the body. The body can use several substrates to convert homocysteine to methionine to ensure a sufficient supply of this crucial amino acid needed in 1CM and protein synthesis. Homocysteine can form methionine via two routes (Figure 1):[1]

1. **The Methionine Synthase (MS) pathway** – receiving methyl group from 5-Methyltetra-hydrofolate in the presence of vitamin B12 or
2. **The Betaine Homocysteine Methyltransferase (BHMT) pathway** – receiving methyl group directly from betaine.

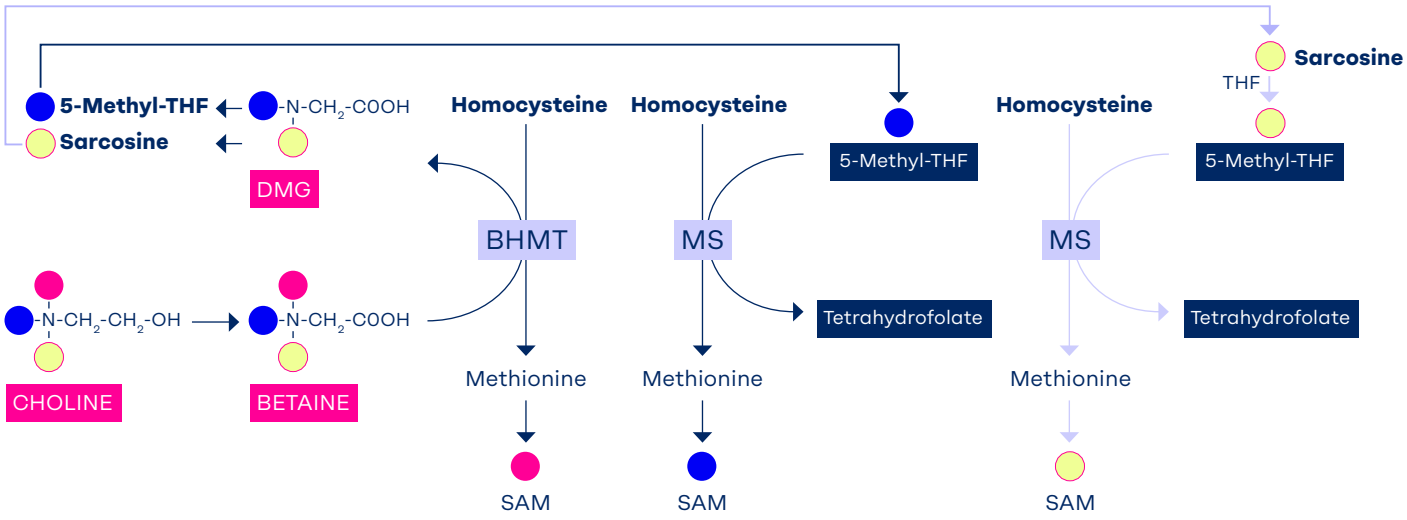


Figure 1
Source of methyl groups for methionine and SAM. Adapted from Caudill, 2018[1]
SAM: S-Adenosylmethionine; DMG: Dimethylglycine; 5-Methyl-THF: 5-Methyltetrahydrofolate; BHMT: Betaine Homocysteine Methyltransferase; MS: Methionine Synthase; THF: Tetrahydrofolate

Choline, under the control of Choline Dehydrogenase (CHDH) forms betaine in the mitochondria and then Betaine Homocysteine Methyltransferase (BHMT) adds a methyl group to homocysteine to form methionine. The interplay between the BHMT and MS interdependent metabolic pathways speaks to the importance of feeding the methionine cycle.[2]

S-Adenosylmethionine (SAM) – Universal Methylator

In this cycle, methionine is converted to S-adenosylmethionine (SAM, or AdoMet) by Methionine Adenosyltransferase in an ATP-dependent process. Methionine is thereby transformed into a high energy molecule (by reacting with the adenosyl component of ATP) that can transfer its methyl group to various substrates like DNA, RNA, proteins and lipids for example, via the PEMT (phosphatidylethanolamine methyltransferase) pathway. SAM is thereby transformed into a universal one-carbon donor for cellular methylation reactions. The byproduct of SAM's methyl donation is S-adenosylhomocysteine (SAH) which is then converted back to homocysteine via the transmethylation pathway.[3]

Liver – A Key Methylation Engine

Most SAM is generated in the liver and more than 90% of SAM molecules are utilized for methylation reactions. Emphasizing the role that choline and betaine play in maintaining methionine adequacy is the fact that more than half of betaine is metabolized to dimethylglycine which enables the conversion of homocysteine to methionine. 85% of the methylation reactions of the whole body take place in the liver.[3] Therefore, it should not be surprising that the liver is very active in taking up choline (60% of administered ^{14}C) within 2 hours[4] of administration and releases choline metabolites back into the circulation. The main choline metabolites measured in the liver are betaine and phosphocholine (within 30 minutes – demonstrating very rapid utilization)[4]. Phosphocholine is the form in which fatty acids are transported to tissues for utilization in cell membrane synthesis and betaine is the main metabolite of choline that plays a key role in 1CM.

Choline/Betaine – Methyl Groups

One methyl group of betaine goes to form SAM and the other 2 methyl groups stay on dimethylglycine. One methyl group of dimethylglycine goes to provide C1 unit that is used to convert tetrahydrofolate (THF) to 5,10-methylene-THF and sarcosine is produced; the other methyl group of dimethylglycine (now on sarcosine) goes to convert THF to 5,10-methylene-THF and glycine.[5]

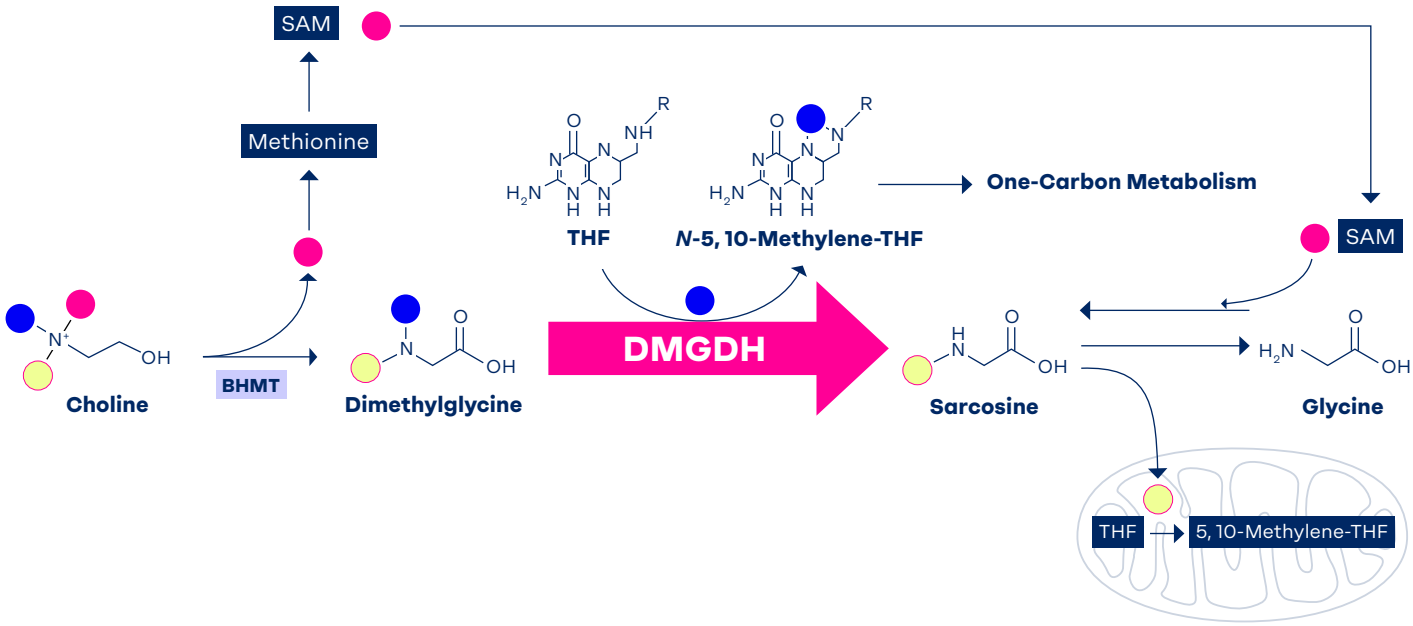


Figure 2
Choline degradation to dimethylglycine via betaine and is catalyzed by BHMT. Adapted from Fig. 1. Augustin et al. (2016)[5]
BHMT: Betaine Homocysteine Methyltransferase; DMGDH: Dimethylglycine Dehydrogenase

Thus, one of choline's methyl groups is used to produce SAM and the remaining 2 methyl groups reenter the 1CM pool as 5, 10-methylenetetrahydrofolate (5, 10-Methylene-THF) via formaldehyde. Choline enriches folate pathway with 2 carbon units that can be made available as methyl groups via 5-Methyl-THF (Figure 1).

Choline, Betaine, Folate – Interdependence

It has also been previously reported that 60% of choline is converted to betaine in the liver[6] and this was duplicated in an in-vitro study by DeLong et al (2002)[7] in which the levels of betaine were found to be three times as high as choline in normal hepatocyte culture. When hepatocytes were exposed to d_9-choline (radioisotope labeled choline) in the absence of unlabeled choline, the ratio of d_9-labeled betaine to choline was the same (red line in Figure 3). When choline is absent from the cell system, no betaine can be detected, demonstrating that oxidation of choline is the sole source for betaine in hepatocytes.[7] RH7777 Hepatoma (liver cancer) cells do not have the enzyme apparatus to convert choline to betaine and hence the 3:1 ratio of betaine to choline is extinguished – another proof that choline contributes its methyl groups to betaine in the liver.

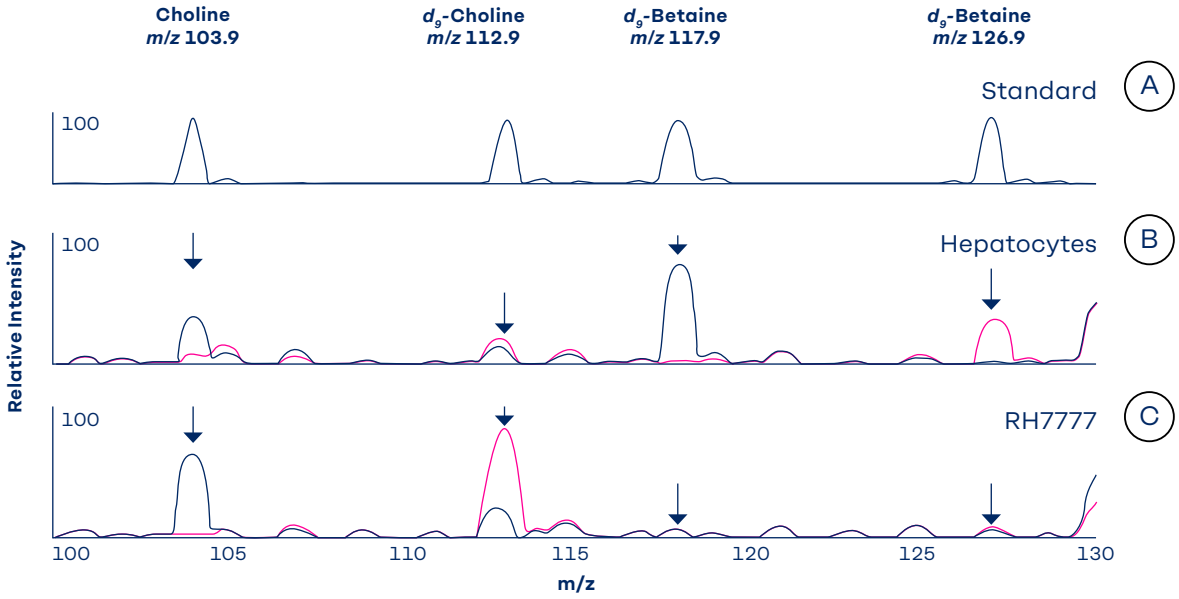


Figure 3
In-vitro measurement of choline and betaine in liver cells and liver cancer cells. Adapted from Fig. 5 DeLong et al. (2002) [7]

Because choline, methionine and folate metabolism interact at the point that homocysteine is converted to methionine, any deficiency in one substrate results in a greater dependence on the other methyl donor compounds and any excess in one compound results in sparing the other substrates. As described by Niculescu & Zeisel (2002):[2]

"Perturbing the metabolism of one of these pathways [e.g. due to nutritional deficiency or genetic variants] **results in compensatory changes in the others. For example, methionine can be formed from homocysteine using methyl groups from 5-Methyl-THF, or using methyl groups from betaine that are derived from choline."[2]** (Figure 4)

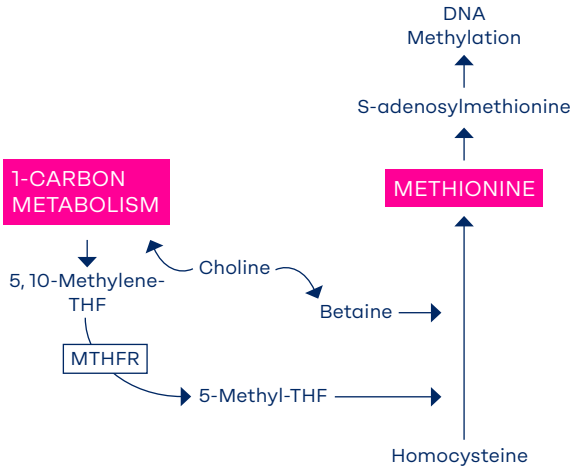


Figure 4
Methylation of homocysteine via folate and/or choline betaine pathways. Adapted from Fig. 1 Niculescu and Zeisel[2]. MTHFR: Methylenetetrahydrofolate Reductase

In the presence of a choline/betaine deficiency, there is greater dependence on 5-Methyl-THF to generate phosphatidylcholine (PtdCho) via the PEMT pathway (Figure 8). A choline deficient diet for 2 weeks in rats caused a 31 – 40% reduction in liver folate content, which was reversable when choline refeeding occurred.[8,9] Rats fed diets deficient in both methionine and choline for 5 weeks had hepatic folate concentrations that were 50% of those in controls[10]. Tetrahydrofolate deficiency, induced by methotrexate[11-15] or by dietary folate deficiency[16], resulted in decreased hepatic total choline, with the greatest decrease occurring in hepatic phosphocholine concentrations. During choline deficiency, hepatic SAM concentrations also decreased by as much as 50%[17-20]. In rats, choline deficiency doubled plasma homocysteine levels[21].

Human studies have also demonstrated an inverse relationship between choline intake and homocysteine levels. In the Framingham Offspring Study (1991-1994 prior to fortification with folic acid)[22], higher intakes of choline (expressed as the sum of choline and betaine) were related to lower homocysteine levels independent of other determinants including folate and other B group vitamins.

To reiterate, there are three possible sources for providing methyl groups to SAM:

1. 5-Methyl-THF via Methionine Synthase pathway.
2. Oxidation of choline to betaine via the BHMT pathway.
3. Exogenous (dietary) betaine and methionine as direct substrates.

MTHFR 677TT SNP – Window into Methyl Group Sourcing

The interdependence of these trans-methylation pathways and interplay with dietary sources of methyl groups is highlighted by the inverse association between choline intake and plasma homocysteine being more pronounced after a methionine load (such as in a postprandial situation) and in the presence of low folate intake.[23] Yan et al (2011)[24] states that **"The metabolic requirement for choline is likely higher in individuals with compromised folate status because betaine (oxidized from choline) shares the homocysteine remethylation step in one-carbon metabolism with 5-Methyl-THF."**[24] The availability of 5-Methyl-THF, the main active folate form in circulation, is significantly compromised by a common single nucleotide polymorphism, 677C/T, in the MTHFR gene when expressed as the homozygous variant. This interconnectedness is well demonstrated when methionine and betaine levels become depleted in MTHFR knockout mice that cannot provide sufficient 5-Methyl-THF to convert homocysteine to methionine. In this mice model, betaine is increasingly used to maintain homocysteine remethylation because 5-Methyl-THF is unavailable.[24]

In contrast to premenopausal women, whose estrogen responsive PEMT pathway can meet higher choline requirements in pregnancy, men have less ability to synthesize choline de novo. Therefore, 70% reduction in MTHFR activity due to the TT genotype would more likely result in a low folate status and consequently depend more heavily on the choline-betaine pathway for generating methionine. That's why men with MTHFR homozygous SNP are ideal to study in this context, to explore the degree upon which the body relies on this redundancy when one or more of the methyl feeder pathways for methionine and SAM are compromised.

Sixty men, mean age 27 years (MTHFR wild type 677CC, n=31 and MTHFR mutant 677TT, n=29) were randomly assigned to receive 12 weeks of 300mg, 550mg, 1100mg or 2200mg choline/d, of which a controlled diet provided 300mg choline/d, 173mg betaine/d and 319mg natural food folate/d along with 70µg supplemental folic acid to ensure 438µg folate equivalents/d.[24]

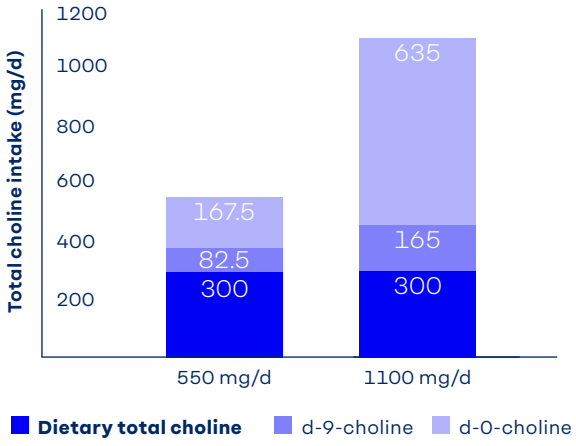


Figure 5
Mean intake of dietary total choline and choline as unlabeled (d0) or isotope labeled (d-9) choline. Adapted from Fig 2. Yan et al. (2011)[24]

During the last 3 weeks of the study (weeks 10-12), the 550mg cohort (n=11;677CC n=4 and 677TT n=7) and 1100mg cohort (n=12; 677CC n=4 and677TT n=8) received 15% of choline dose as d-9-choline chloride (Figure 5). The isotope label enrichment of choline was measured at 12 weeks in plasma and urine.

Variable	Choline intake		*P* values		
	550 mg/d	**1100 mg/d**	**Genotype**	**Choline**	**Interaction**
Serum folate (nmol/L)			0.003	0.676	0.915
677CC	12.3 ± 1.7[a]	11.6 ± 2.7			
677TT	7.3 ± 0.9	6.9 ± 0.8			
Total	9.1 ± 1.1	8.5 ± 1.2			
Plasma choline (μmol/L)			0.655	0.052	0.841
677CC	7.0 ± 0.8	9.4 ± 2.2			
677TT	7.4 ± 0.7	10.2 ± 1.1			
Plasma betaine (μmol/L)			0.418	0.014	0.755
677CC	46.0 ± 5.2	66.0 ± 14.0			
677TT	42.6 ± 3.1	58.3 ± 4.7			

Tabel 1
Biochemical variables at the end of the controlled feeding study (week 12) in men with the MTHFR 677CC or TT genotype who had choline intakes of 550 or 1100 mg/d. Adapted from Table 1 in Yan et al. (2011)[24]

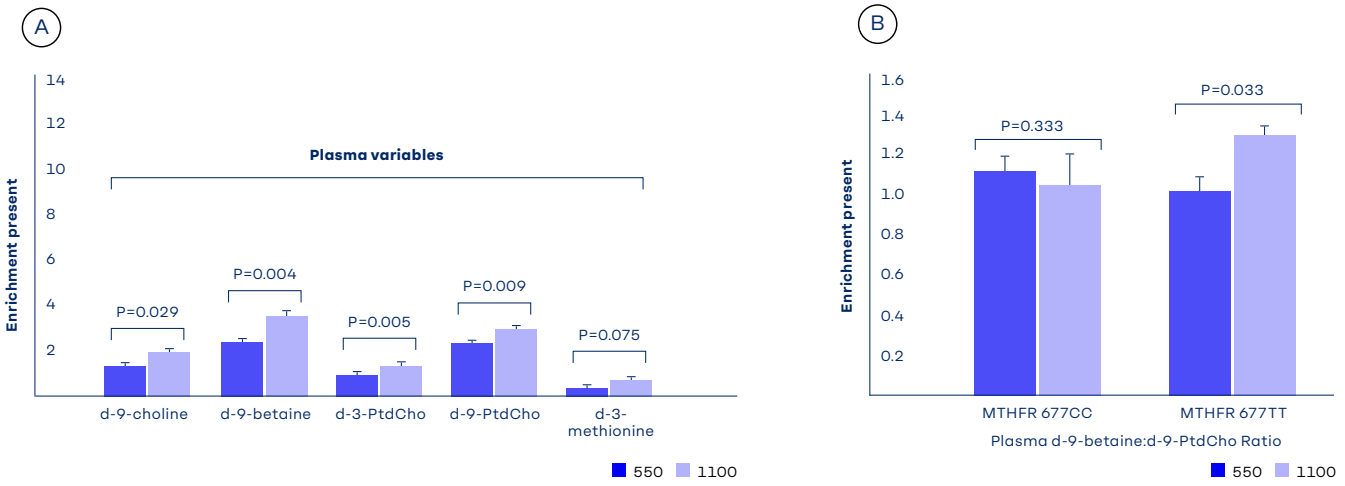


Figure 6
Isotopic enrichment of plasma choline metabolites [labeledmetabolite/(labeled + unlabeled metabolite)] grouped A; B (MTHFR) C677T or 677CC genotypes. Adapted from Fig. 3 Yan (2011)[24]

There was greater isotope enrichment of plasma choline (p=0.029), betaine (p=0.004), d-3-PtdCho (p=0.005) and d-9-PtdCho (p=0.009) in the 1100mg/d cohort compared to the 550mg/d group (Figure 6 A.). The d-3-PtdCho is representative of the phospholipid being produced via the PEMT pathway (which produces d-3-, d6 and much less likely d-9-products) vs. the CDP pathway which produces d-9-PtdCho. d-3 enrichment tended to be higher in the 1100mg group as well (p=0.075) but the higher choline dose did not impact the precursor:product ratios, indicating that the precursors were enriched equivalently. This was not the case when the genotype was taken into account. The 677TT MTHFR genotype and choline intake interacted to affect the plasma enrichment ratio of betaine to PtdCho derived from the Kennedy (CDP-choline) pathway (Figure 6 B.). The 1100mg group demonstrated a higher betaine:PtdCho ratio suggesting that higher choline intake in the 677TT group resulted in upregulation of the betaine oxidation pathway. This was in contrast to the 677CC genotype on the same high dose of choline where no shift between the oxidation pathway and the Kennedy pathway was observed.

On the other hand, there was no statistically significant difference in methionine enrichment between the two labeled choline doses, suggesting that cellular pools of SAM were similarly labeled and served as methyl donors in PtdCho synthesis via the PEMT pathway.

Also, the timing of measuring the various derivatives will significantly impact the results because the rate of metabolic conversion will vary. The rapid carboxylation of choline to betaine explains why betaine levels seem to be consistently higher than choline levels (e.g. 3-4 fold).

This study also shows that 47% of the subjects with the 677TT genotype had serum folate concentrations in the deficient range (≤ 6.8 nmol/L) and therefore, the demand for betaine as a source for methyl groups to generate methionine from homocysteine is likely higher in individuals with this genotype. The higher plasma betaine:CDP-choline ratio in this group is also consistent with the increased demand for betaine and therefore, **the imperative to channel higher proportion of choline into the choline oxidation pathway in these homozygous 677TT subjects.**

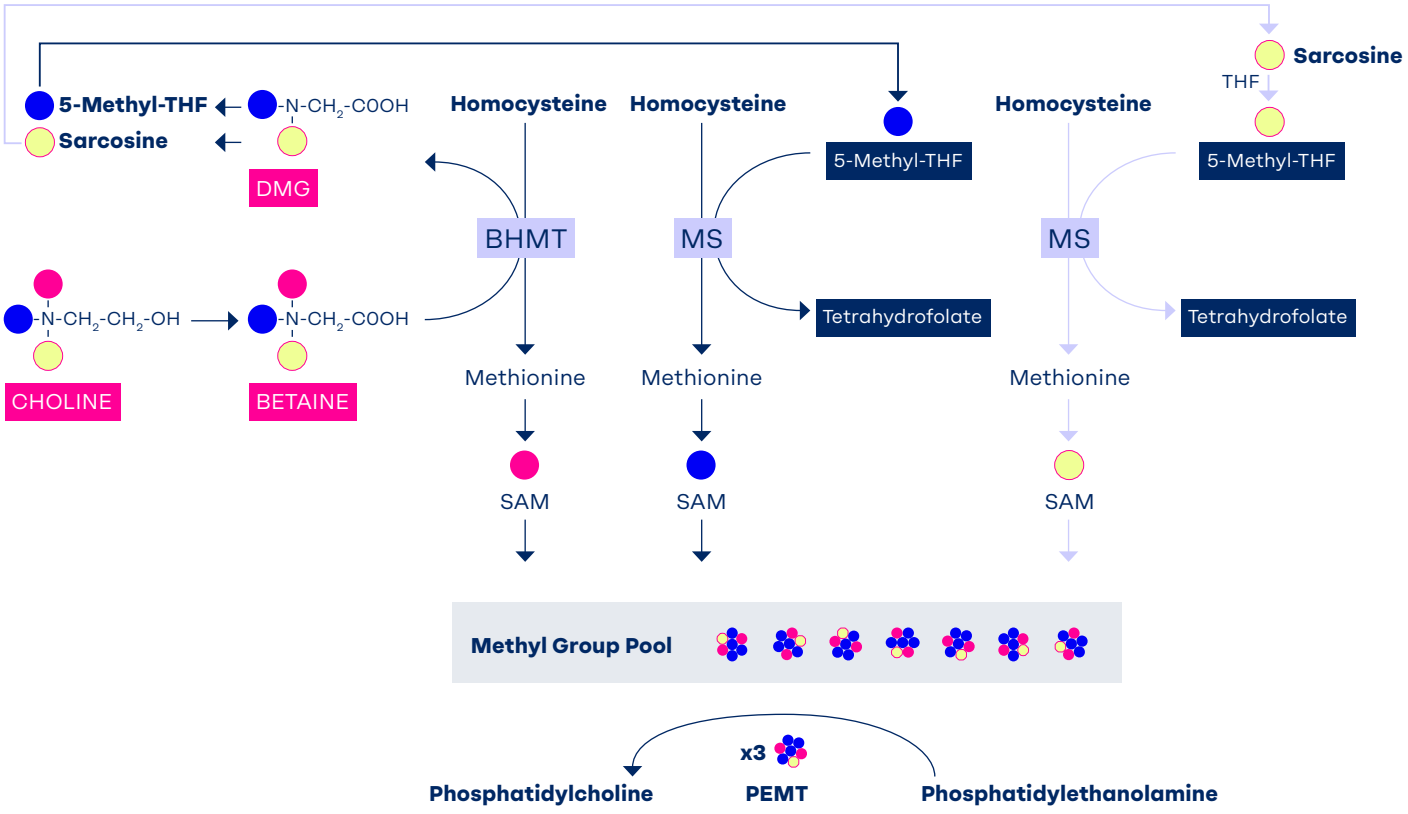
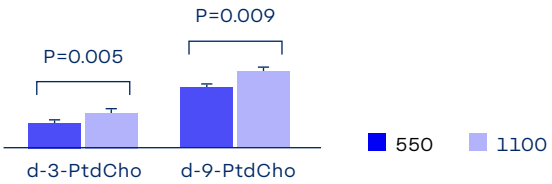


Figure 7
Choline and betaine deficiency increase the demands of folate to generate choline and phosphatidylcholine de novo via the PEMT pathway that requires 3 SAM groups to generate one phosphatidylcholine molecule. Adapted from Caudill, 2018.[1]
SAM: S-Adenysylmethionine; DMG: Dimethylglycine; BHMT: Betaine Homocysteine Methyltransferase; MS: Methionine Synthase; THF: Tetrahydrofolate; PEMT: Phosphatidylethanolamine Methyltransferase

The results of this study showed that doubling the intake of choline did not change the proportion of choline being metabolized down one pathway (CDP) as measured by d-9-PtdCho vs. the other (PEMT, d-3-PtdCho) as was anticipated by the research hypothesis, even though the absolute amount of choline directed down both pathways will increase. According to the authors; **"The d-3 labeling of PtdCho indicates for the first time in humans that choline itself is a source of methyl groups for the de novo biosynthesis of the choline moiety through the PEMT pathway – a finding consistent with a cell culture model using rat primary hepatocytes."** [24] The d-3 enrichment of methionine confirms the PEMT pathway contribution to d-3-PtdCho via SAM and all d-9-PtdCho is proof of production via the Kennedy pathway. The quantitative difference between the d-3 and d-9 distribution tracks with the 70/30 rule of choline compartmentalization.



Adapted from Figure 6 A

The authors continue: "Simple one-pool constant infusion kinetics require that enrichments decrease as the label moves from pool to pool. Our observed increase in isotope enrichment from precursor d-9-choline to several products in blood and urine indicates that **pools are in flux and that more than one precursor pool exists for derivation of subsequent products."**[24]

This highlights the need to appreciate the existence of methyl pools that can be a source for multiple 1CM protagonists and that 1CM participants can tap into multiple methyl pools. This underscores the importance of methyl donors in the diet or nutritional supplements to be constantly replenishing the methyl pool(s) to maintain substrate for methylation reactions. Good examples of these different pools are choline and betaine. Choline deficiency can draw from the PtdCho making up the bilayer cell membranes and preponderant in liver, muscle and mitochondria. Hence, early biomarkers of choline deficiency are liver enzymes alanine aminotransferase, (ALT)[25] and muscle enzymes creatine phosphokinase, (CPK) which leak out of cells due to mobilization of choline from the bi-layer membrane pool which can result in a reduction of membrane integrity. Betaine, whether from the diet or produced from choline is stored in large amounts in tissues where it functions as an osmolyte. This pool can also be pressed into service as a source of methyl groups.[26]

The upregulation of the choline-to betaine-oxidation pathway which typically metabolizes 30% of the choline (70% choline typically metabolized by the Kennedy or CDP pathway) has implications beyond the generation of methionine and SAM for methylation reactions. This is because the PtdCho generated via the PEMT pathway will form the phospholipids that transport the chain fatty acids DHA and arachidonic acid – so essential for brain development in the fetus, infant and child as well as brain health in the adult whereas phospholipids derived through the CDP pathway carry shorter chain fatty acids, like oleic acid.

When choline is labeled with deuterium, each of the three methyl groups (-CH_3) is now labeled as -CD_3 (Figure 8). When labeled choline is channeled down the Kennedy pathway, the choline diphosphate (CDP) methyl groups (now deuterated) can be detected as d-9-PtdCho. When choline is driven down the PEMT pathway via methionine, then only one deuterated methyl group can be detected as d-3-PtdCho (Figure 8). Phosphatidylcholine derived from the PEMT pathway requires three sequential methylation reactions of phosphotidylethanolamine (PE) that will generate d-3-PtdCho, d-6-PtdCho (if it has two methyl groups from the originally labeled choline or betaine) or rarely d-9 (three deutereum labeled methyl groups). Metabolites can also be identified by this method (d-6-dimethylglycine, d-3-sarcosine, d-3-methionine and d-3-SAM). Figure 8 illustrates the metabolic fate of choline's methyl groups as demonstrated by compartmentalization studies.

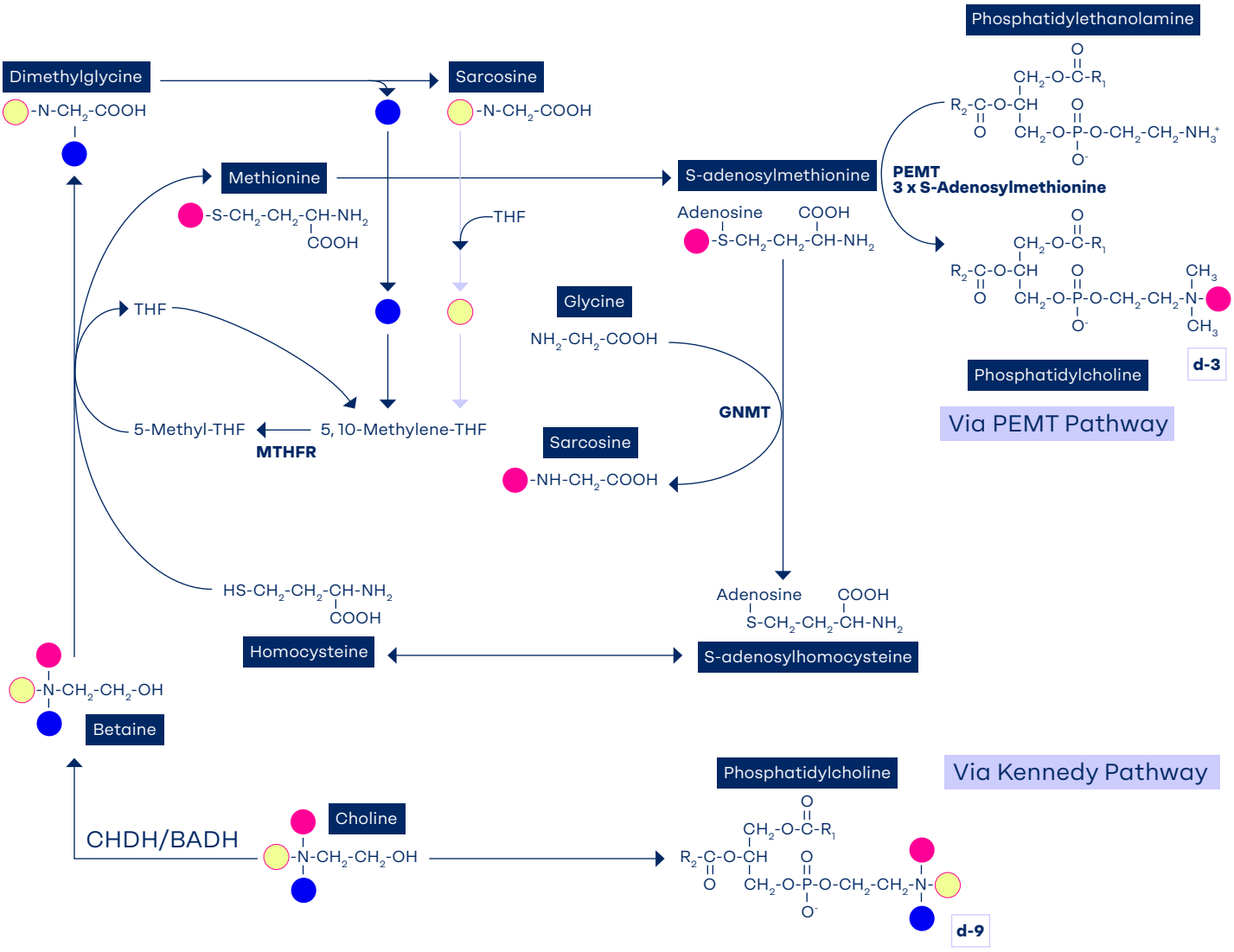


Figure 8
Metabolic fate of the orally consumed deuterium-labeled choline. The d-9-choline tracer contained deuterium-labeled methyl groups facilitating the examination of the metabolic fate of choline-derived methyl groups in addition to the intact molecule. Adapted from Yan et al. (2011)[24]
CHDH: Choline Dehydrogenase; BADH: Betaine Aldehyde Dehydrogenase; GNMT: Glycine N-Methyltransferase; MTHFR: Methylenetetrahydrofolate Reductase; PEMT: Phosphatidylethanolamine Methyltransferase

Conclusion

In conclusion, the multiple scientific studies and reviews are all quite clear on the ability of choline and betaine to provide methyl groups for methionine generation. That 5-Methyl-THF plays a dominant role in methylating methionine is indisputable, but not exclusive. Under circumstances of folate deficiency and B_{12} deficiency, choline and betaine sourced methylation is upregulated.[27] So too, under conditions of choline deficiency, there is a heavier than normal reliance on methyl groups coming from folate, which again highlights the baseline ongoing methyl donation to methionine generation by these non-folate sources. Stable isotope labeled studies have been able to track and quantify the compartmentalization of choline metabolism and demonstrate effective methylation of homocysteine by choline and betaine. Demonstration of isotope enrichment in methionine and phosphatidylcholine means (by deduction) that SAM is enriched too. Add to this a prevalence of homozygous MTHFR (677TT) of 20% and 50% heterozygous MTHFR (677CT) in Caucasians in the US and Europe, and Hispanics in Central, South and North America, it is easy to see how the redundancy described in this review will play an even more important role.

Methyl Group Mathematics

Finally, the value of a nutrient or a nutritional supplement that has more methyl groups, has (mole for mole) an advantage of contributing to the methyl pool and phosphatidylcholine over those that have less. For example, the average intake in several population studies of choline is 313mg/d (ARIC and Framingham Offspring Study)[28,29] and the average betaine intake was 85.2mg/d and 208mg/d respectively. That means that in the ARIC study, participants received 3.7X the amount of methyl groups from choline than from betaine and in the Framingham study, it was 1.5X as much from choline. Choline in these diets provides a methyl advantage over betaine. Choline of course is converted to betaine in the oxidation pathway, and it is the betaine that dominates in the measurable plasma as a result.

So too, a 5-Methyl-THF salt that has 7 methyl groups (di-Choline-5-Methyl-THF in Figure 9) has the potential for contributing more to the methyl pool compared to a 5-Methyl-THF that has 1 methyl group and compared to folic acid that has no methyl groups. Even though homocysteine methylation to form methionine favors methylation by methylcobalamin (methylated by 5-Methyl-THF), choline and betaine also play a significant role as methylators in the Methionine cycle as demonstrated by reduction in homocysteine levels independent of folate. Although the total size of the methyl pool in the body is not known, it can be confidently stated that Optifolin⁺ offers a 7-fold methyl group advantage over other 5-Methyl-THF salts in contributing to the methyl pool(s) in the body. The methylation advantage is being studied in a series of experiments to quantify any differences in DNA methylation between the 5-Methyl-THF salts.

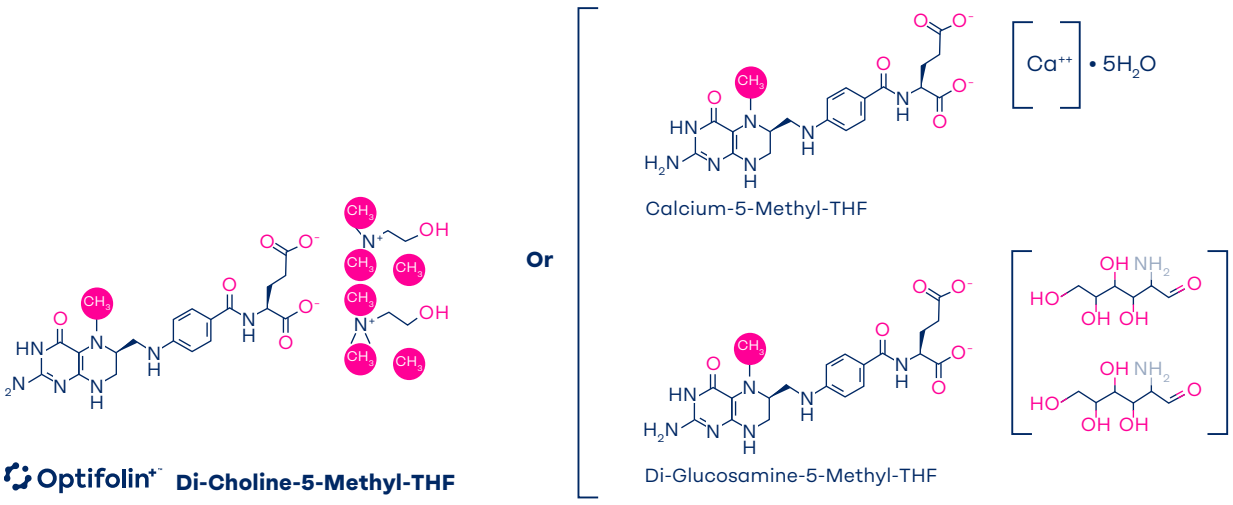


Figure 9
Chemical representations of Optifolin⁺, Calcium-5-Methyl-THF and Di-Glucosamine-5-Methyl-THF salts

Comment

This white paper (drafted by Jonathan Bortz, MD) provides fundamental explanation of the role of choline in enrichment of the body methylation reservoir. The paper summarizes the contribution of choline to cellular methylation as part of the multifaceted contributions of choline to cell functions. Each choline molecule contains three methyl groups in its chemical structure (3 x CH_3). Once choline is oxidized to betaine in the cell mitochondria, one of the three methyl groups can be used to produce S-adenosylmethionine (SAMe), the universal methyl donor in the cell. The other two methyl groups are highly preserved as di-methyl-glycine that is then used to generate folate species, thus indirectly upgrading the methylation potential of the cell. Although evidence is still needed to quantify the relative contribution of choline to the total cellular methylation capacity and the relevance to health and disease, the evidence discussed in this document strongly suggests that the body intentionally makes use of each and every single methyl group. I thoroughly read the white paper and contributed to the content, especially the scientific argumentations and the objective interpretation of available evidence. The white paper is presenting a biological concept that is supported by several clinical and experimental observations.

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Prof. Rima Obeid is a world-recognized researcher and thought leader in One Carbon Metabolism (1CM) and has published over 150 scientific papers which include many landmark original studies, population studies, meta-analyses and systematic reviews impacted by choline, folate, vitamin B12 and betaine. Her expertise and critical thinking, combined with a reputation for scrupulous scientific rigor has secured her place as a respected voice in the complex and crucial area of methylation and the important contribution of nutrition in health and well-being. She is a frequent contributor to reputable expert and consensus panels publishing nutrient guidelines, to regulatory agencies, non-profit advocacy groups and industry – all the while bringing her seasoned experience and rigorous training in scientific methodology, epidemiology, public health, meta-analysis and statistics.

Prof. Obeid studied pharmacy and public health. She earned her Doctorate in Theoretical Medicine at the Saarland University, in Germany in 2003. In 2010 she achieved the status of Habilitation (highest academic achievement in research, education and teaching in the German system) and soon after, became a tenured professor and the head of Research and Development in the Department of Clinical Chemistry and Laboratory Medicine at Saarland University Hospital, Germany. Over the years she has received multiple awards, including a Fellowship from Alexander von-Humboldt Foundation and a Marie Curie Research Fellowship. She has lectured all over the world on a variety of aspects of 1CM and in 2023, she joined Balchem's Scientific Advisory Board and has been working closely with their Nutrition Science team and many of their outside research collaborators.

Jonathan Bortz M.D.

Dr. Jonathan Bortz, a diabetes specialist with 15 years of clinical experience, founded a successful and widely recognized, multidisciplinary diabetes clinic in St. Louis in the 1990s. He pioneered one of the first web-based disease management programs for diabetes, which was licensed both in the US and internationally. His innovative clinical approach led to his recruitment by a pharmaceutical company, where he developed prescriptive nutritional supplements that became market leaders in the US.

Over his 20-year industry career, Dr. Bortz has gained extensive expertise in nutrition science, developing numerous novel, patent-protected products with Balchem ingredients. As Balchem's Head of Nutrition Science for Human Health and Nutrition, he has established a global network of leading scientists and overseen a prolific research pipeline, resulting in multiple landmark publications.

Dr. Bortz earned his medical degree from the University of the Witwatersrand in Johannesburg, South Africa, completed his Internal Medicine Residency at Mount Sinai Medical Center in Cleveland, Ohio, and his Fellowship in Diabetes, Endocrinology, and Metabolism at Washington University in St. Louis, Missouri.

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