Review

Steven H. Zeisel*

Metabolic crosstalk between choline/1-carbon metabolism and energy homeostasis

Abstract: There are multiple identified mechanisms involved in energy metabolism, insulin resistance and adiposity, but there are here-to-fore unsuspected metabolic factors that also influence these processes. Studies in animal models suggest important links between choline/1-carbon metabolism and energy homeostasis. Rodents fed choline deficient diets become hypermetabolic. Mice with deletions in one of several different genes of choline metabolism have phenotypes that include increased metabolic rate, decreased body fat/lean mass ratio, increased insulin sensitivity, decreased ATP production by mitochondria, or decreased weight gain on a high fat diet. In addition, farmers have recognized that the addition of a metabolite of choline (betaine) to cattle and swine feed reduces body fat/lean mass ratio. Choline dietary intake in humans varies over a three-fold range, and genetic variation exists that modifies individual requirements for this nutrient. Although there are some epidemiologic studies in humans suggesting a link between choline/1-carbon metabolism and energy metabolism, there have been no controlled studies in humans that were specifically designed to examine this relationship.

Keywords: betaine; choline; insulin sensitivity; obesity; phosphatidylcholine.

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Introduction

Obesity, physical inactivity and insulin resistance are public health problems that are increasing rapidly [1]. People with insulin resistance are at increased risk of developing type 2 diabetes mellitus [2] and the prevalence of diabetes is predicted to double between the years 2000 and 2030 [3]. Though there is a well-developed understanding of the mechanisms involved in energy metabolism, insulin resistance and adiposity, there are here-to-fore unsuspected metabolic factors that also influence these processes. These factors are potentially modifiable, hence opening the door for dietary or pharmacologic interventions.

There is substantial evidence suggesting that there is important crosstalk between choline/1-carbon metabolism and the pathways of insulin sensitivity, fat deposition and energy metabolism (Figure 1). Choline is an important methyl donor, a precursor for membrane formation, and it is needed for acetylcholine biosynthesis [4] (Figure 2). There is a recommended adequate intake for choline (about 550 mg/d) [4], but there is a wide variation in choline intake in the diet. In several human cohorts choline intake has been estimated to vary by as much as three-fold – the lowest quartile (or quintile) and the highest quartile of intake were approximately 150 mg and 500 mg/d choline equivalents, respectively, in the Framingham Offspring Study [5], the Atherosclerosis Risk In Communities study [6, 7] and the Nurse’s Health Study [8]. Intake of choline is likely lower in low income countries [9]. Normal dietary intake of betaine is 100 mg/d in the USA [7].

Choline and betaine metabolism are interrelated to folate metabolism, as the methylation of homocysteine can use either a methyl group derived from betaine or a methyl-group derived from 1-carbon/folate metabolism [10]. Methyl-folate can deliver methyl-groups thereby sparing choline for use in PC synthesis; betaine can deliver methyl-groups thereby sparing folate for use in DNA synthesis. For this reason dietary folate intake can moderate the dietary requirement for choline (and betaine) and vice versa [11–13].

In humans, diets very low in choline (50 mg/d) are associated with fatty liver and with liver and muscle damage in almost all men and postmenopausal women, but premenopausal women may have a reduced requirement for choline (as discussed later) [4, 14–18]. Dietary intake of 550 mg/d choline in most people is sufficient to
reverse this liver or muscle dysfunction, but in approximately 10% of people 850 mg/d choline was needed [16]. Women eating diets low in choline during pregnancy (about 200 mg/d) are more likely to give birth to a child with birth defects than are women eating 500 mg/d [19–21]. In addition, low dietary intake of choline (about 150 mg/d) was associated with decreased cognitive function in the Framingham Offspring Cohort [22]. The lower quartile of plasma choline concentrations were associated with decreased cognitive function in elderly subjects in the Hordaland study [23]. At the same time, diets high in choline (probably >500 mg/d) may be associated with an increased risk for prostate cancer progression [24], for colorectal adenomas [8] and for heart disease [25]. Thus, it appears that diets at the lower end of normal intake for choline have adverse health consequences, while diets at the higher end of normal intake also have adverse consequences. A U-shaped risk curve defines a very narrow range for optimal intake. Given this dilemma, expert panels deciding on dietary recommendations need to know more about benefits and risks of choline in the diet. Effects of choline on energy metabolism and insulin sensitivity have not been considered as part of estimating optimal dietary intake; a better understanding of

Figure 1  Hypothetical pathways for crosstalk between choline metabolism and insulin sensitivity. Available data suggest that there is metabolic crosstalk between choline/1-carbon metabolism and energy homeostasis pathways. Though not yet proven to be the source of this crosstalk, several possible signaling pathways are influenced by choline or its metabolites. FGF21, fibroblast growth factor 21; IRS-2, insulin receptor substrate 2; PC, phosphatidylcholine; PPARα, peroxisome proliferator-activated receptor α; PTEN, phosphatase and tensin homolog; ROS, reactive oxygen species; SAM, S-adenosylmethionine; SREBP1, sterol regulatory element-binding protein 1.

Figure 2  Genes that influence choline metabolism and dietary requirements. Choline is a precursor for formation of the neurotransmitter acetylcholine, it can be phosphorylated to form phosphatidylcholine, or it can be oxidized to form betaine (catalyzed by choline dehydrogenase; CHDH). Betaine is used as a methyl donor in the formation of methionine (catalyzed by betaine homocysteine methyltransferase, BHMT). 5-methyltetrahydrofolate is an alternative methyl-group donor for the formation of methionine (a critical step in the formation of 5-methyltetrahydrofolate is catalyzed by methylene tetrahydrofolate dehydrogenase; MTHFD). Methionine is a precursor of S-adenosylmethionine, which in turn can be used to form phosphatidylcholine (catalyzed by phosphatidylethanolamine methyltransferase; PEMT). Homocysteine is a precursor for cysteine synthesis. Genetic polymorphisms in CHDH, PEMT and MTHFD1 have been identified which increase the dietary requirement for choline. In mice with Chdh genes deleted, mitochondrial function is abnormal; with Bhmt deleted mice have abnormal body fat pads and energy metabolism.
the crosstalk between choline/1-carbon metabolism and energy homeostasis will allow for the proper weighting of the risk/benefit ratio for choline intake in diets.

**Genetic variation in choline metabolism**

Diet recommendations for choline intake are made more complex because many people have single nucleotide polymorphisms (SNPs) in genes of choline/1-carbon metabolism that cause metabolic inefficiencies that either decrease endogenous production of choline moiety, or increase demand for choline [17, 26] (Figure 2) (Table 1). As noted earlier, most people must eat a diet containing choline or they develop organ dysfunction [16]. Though everyone has some capacity to endogenously synthesize phosphatidylcholine (PC) via phosphatidylethanolamine-N-methyltransferase, many premenopausal women have a substantial added capacity for this biosynthesis because the gene encoding this enzyme (PEMT) is induced by estrogen [27], thereby decreasing the dietary requirement for choline. Other premenopausal women (about 40%) have a SNP in the PEMT gene (rs12325817) that abrogates estrogen induction of PEMT [28] and increases the dietary requirement for choline [26]. Other common SNPs in genes of choline and folate metabolism found in 10%–50% of the population also increase the requirement for choline [17, 26, 29]. People with these SNPs have metabolic inefficiencies that can be detected using metabolomic profiling [30]; importantly, the SNP-dependent abnormal metabolic profile can be detected in people on a normal diet and is exacerbated by a diet low in choline (<50 mg/d) [30].

**Choline metabolism and energy metabolism**

Mice fed a methionine and choline deficient diet became hypermetabolic, lost weight [31], and had better insulin sensitivity and glucose tolerance [32]. Gene deletions that decrease the flux of choline through its metabolic pathways have similar effects. Betaine homocysteine methyltransferase (BHMT) uses the choline metabolite betaine to methylate homocysteine. Bhmt−/− mice had diminished hepatic choline and increased betaine concentrations and had increased energy expenditure and increased insulin sensitivity [33]. Bhmt−/− mice gained less body weight than did their wildtype littermates and had reduced adiposity [34] (Figure 3). Choline can be derived from the PC formed by PEMT. Pemt−/− mice had decreased hepatic choline, betaine and PC concentrations and when fed a high fat diet for 10 weeks, they did not gain weight and remained insulin sensitive, while similarly fed wildtype littermates increased in body mass by 60% and became insulin resistant [36]. Compared with wildtype, the Pemt−/− mice had increased energy expenditure [36]. These differences disappeared when Pemt−/− mice were supplemented with choline [36].

**Increased betaine increases insulin sensitivity and energy metabolism**

Betaine is formed from choline. Betaine administration enhances insulin sensitivity in diet-induced-obese mice [37] and increased insulin signaling pathways in isolated adipocytes [38]. Betaine is used as an animal feed additive and is promoted as a “carcass modifier” generating leaner meat [39]. Dietary betaine supplementation resulted in reduced abdominal fat in poultry, and reduced carcass fat (by 10%–18%) in pigs [39–42]. This effect was not observed in humans in which a hypocaloric diet (~500 kCal/d) was

<table>
<thead>
<tr>
<th>Gene</th>
<th>rs number</th>
<th>Base pair and sequence change</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFD1</td>
<td>rs2236225</td>
<td>+1958 G → A</td>
</tr>
<tr>
<td>PEMT</td>
<td>rs12325817</td>
<td>−744 G → C</td>
</tr>
<tr>
<td>CHDH</td>
<td>rs9001</td>
<td>+318 A → C</td>
</tr>
<tr>
<td>CHDH</td>
<td>rs12676</td>
<td>+432 G → T</td>
</tr>
</tbody>
</table>

**Table 1** Common genetic polymorphisms (SNPs) that create metabolic inefficiencies in choline metabolism and increase dietary demand for the nutrient.

Each SNP is mapped to the genome and assigned a RefSNP accession ID (rs number). Base pair and sequence changes, also listed, are subject to revision when genes are resequenced. PEMT SNP base pair numbers are numbered from transcription start site.
supplemented with 6 g betaine/d [43]. It is possible that the effects of the 25% calorie reduction obscured the effects of betaine on energy expenditure, and the method these authors used for assessing both body fat and energy expenditure (spot indirect calorimetry) were not optimal. Other studies assessing the effects of betaine supplementation on exercise performance in humans did not assess energy expenditure or body composition [44, 45]. It is interesting that an inverse correlation ($p < 0.008$) was observed between plasma betaine concentrations and body mass index (BMI) in 531 patients with acute coronary syndrome in New Zealand [46]. In the Hordaland Health study (7074 men and women), plasma betaine concentrations also were inversely associated with BMI and body fat, while plasma choline concentrations were positively associated with these parameters [47].

Cysteine and body weight

As discussed earlier, choline and betaine are important modulators of homocysteine metabolism (Figure 2). Cysteine is formed from homocysteine and there is a growing body of evidence linking cysteine to obesity [48]. Plasma cysteine concentrations are associated with increasing BMI [49] and are highly associated with body fat mass [50]. Mice with the cystathionine β-synthase (CBS) or cystathionase gene deleted (both genes are involved in cysteine synthesis), are leaner than wildtype [48]. Humans with genetic CBS deficiency have lower BMI [48]. In rat adipocytes, cysteine inhibits catecholamine-stimulated lipolysis and stimulates oxidation of glucose and its utilization in de novo lipogenesis with potency similar to insulin [51, 52].

Crosstalk between choline, betaine and energy metabolism may be bidirectional

Activities of several key enzymes in choline metabolism [BHMT, PEMT and choline dehydrogenase (CHDH); catalyzes conversion of choline to betaine [53]] are decreased by insulin and are increased by diabetes in rats [54, 55]. These changes are prevented if streptozotocin-treated rats are given insulin [55].

Potential pathways for crosstalk between choline, betaine and energy metabolism

Many observations strongly support the hypothesis that there is crosstalk between choline/t-carbon metabolism and energy homeostasis but there is a paradox: decreased choline results in increased metabolic rate and increased insulin sensitivity, but increased betaine also results in increased metabolic rate and increased insulin sensitivity. This paradox has previously been reported by others, who examined metabolic syndrome risk markers in plasma [47]. How could this be if choline can be converted to betaine? The differentiation between the effects of these two related metabolites must lie in mechanisms that choline can participate in but betaine cannot. The potential pathways regulating insulin sensitivity and energy metabolism are many, but there are several for which choline and betaine could have differential effects (Figure 1).

Phosphatidylcholine and PPARα

A specific PC species (16:0/18:1) is the endogenous peroxisome proliferator-activated receptor α (PPARα) ligand [56]. PPARα is expressed at high levels in liver where it promotes fatty acid oxidation, ketogenesis, lipid transport, and gluconeogenesis. Activation of PPARα in rodents leads to improvement of insulin sensitivity by multiple mechanisms [57]. However, deletion of PPARα also increases insulin sensitivity [58] perhaps by reducing expression of the mammalian tribbles homolog TRB-3 (an inducer of insulin resistance) [59]. Thus, the literature predicts both increased and decreased insulin sensitivity in mice when PPARα signaling is reduced; perhaps the insulin sensitizing effect is peculiar to the knockout mouse. The effect of PPARα agonists on insulin sensitivity in humans is no more clear [57]. Both choline and betaine can be precursors for PC formation. Betaine is a precursor for $S$-adenosylmethionine (SAM), which can be used to methylate phosphatidylethanolamine to form specific fatty acid species of PC [60]. Choline also is used to form PC by a different mechanism (CDP-choline pathway). These two pathways make PC species with different fatty acid composition [61], and since the endogenous PPARα ligand is a specific fatty acid species of PC, this difference may underlie the differential effects of choline vs. betaine on energy metabolism.

Phosphatidylcholine and SREBP-1

Sterol regulatory element-binding protein 1 (SREBP-1) is encoded for by the gene SREBF-1 which is induced by insulin signaling [62]. SREBP-1 regulates genes of fatty acid, phospholipid, and triacylglycerol biosynthesis,
and also induces multiple genes in 1-carbon metabolism needed to synthesize SAM [63]. In C. elegans (and probably in mammals as well), SREBP-1 is embedded as a transcriptionally inactive precursor protein in the endoplasmic reticulum and in the nuclear envelope where low membrane PC concentrations activate the maturation of nuclear, transcriptionally active SREBP-1 (thereby providing feedback activation of SAM formation) [63]. In liver, basal insulin receptor substrate 2 (IRS-2) expression is controlled via negative feedback of SREBP-1 at an insulin response element on the IRS-2 promoter [64]. Decreased IRS-2 leads to insulin resistance [65]. Thus, it is possible that increased betaine results in increased SAM, increased PC, decreased SREBP-1 activity, increased IRS-2 and, thereby, to increased insulin sensitivity. This hypothesis is supported by the observations that overexpression of human BHMT in mice decreased expression of SREBP-1 protein [66]. The above hypotheses are consistent with the observed insulin sensitizing actions of betaine [38], but are the opposite of what we would predict from experiments that find that choline deficiency and deletion of Pemt resulted in insulin sensitization [32, 36]. Further clarification of the role of choline and in SREBP-1 action is needed to shed new light onto this nutrient-sensitive pathway.

Betaine is an important methyl-group donor that can modify epigenetic regulation of gene expression

In humans, betaine is an important methyl donor needed for the conversion of homocysteine to methionine [67, 68], which is the precursor for S-adenosylmethionine, the most important methyl donor in biochemical reactions (including DNA and histone methylation, important for epigenetic control of gene expression). Thus, betaine (and its precursor choline) could be influencing energy metabolism and insulin sensitivity by modifying epigenetic marks [69–71]. A number of studies suggest that diets high in betaine (and/or other methyl group donors, such as choline and methyl-folate) can alter methylation of differentially methylated regions’ (DMR) and cytosines within CpG rich regions in DNA [72, 73]. Could epigenetic mechanisms be important regulators of energy metabolism? A body of evidence suggests that altered epigenetic regulation of gene expression has a role in the development of type 2 diabetes (T2D) [74, 75]. For example, pancreatic duodenal homeobox 1 (PDX-1), a gene important for pancreatic islet development, has multiple CpG sites in the distal PDX-1 promoter and enhancer regions that are hypermethylated in islets from patients with T2D compared with non-diabetic controls, and this hypermethylation was associated with reduced gene expression [76]. In other studies using pancreatic islet cells from people with TDM, expression of the transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α; encoded by the gene PPARGC1A) was reduced by 90% and the PPARGC1A gene promoter was hypermethylated (two-fold) in diabetic islets compared with non-diabetic islets [77]. This hypermethylation was correlated with reduction in insulin secretion in these patients [77].

Betaine is important for normal mitochondrial function

CHDH (converts choline to betaine) is an inner mitochondrial leaflet protein [53] and the Chdh−/− mouse has grossly abnormal mitochondrial structure and function in sperm and in skeletal muscle [53]. This mitochondrial dysfunction can be partially reversed by betaine treatment [53]. This is relevant to humans, as there is a functional SNP in CHDH (rs12676; G233T) that alters CHDH enzymatic activity [78]; 65% of the population has one copy and 9% have two copies of the minor T allele. Sperm from men who are GT or TT for rs12676 have 40% and 73% lower ATP concentrations reflecting mitochondrial dysfunction, respectively, in their sperm than do sperm from men who are GG [78] (Figure 4). The rs12676 genotype is also associated with grossly dysmorphic mitochondrial structure [78].

Mitochondria are important for the metabolism of acylcarnitines, which are byproducts of lipid or amino acid metabolism. Even-chain acylcarnitine species arise
from incomplete β-oxidation of fatty acids, whereas odd-chain species, such as C3 and C5 are produced during amino acid catabolism [80]. Humans with SNPs in several genes of choline metabolism have increased concentrations of acylcarnitines and ketoacids even when eating a normal diet [30], suggesting mitochondrial dysfunction. Disturbed mitochondrial function alters both energy expenditure and insulin sensitivity [81]. There is substantial evidence that reactive oxygen species (ROS) enhance insulin sensitivity by oxidizing the β-chain of the insulin receptor (enhancing its autophosphorylation) and by oxidizing the protein tyrosine phosphatases protein-tyrosine phosphatase 1B (PTP1B) and phosphatase and tensin homolog (PTEN) [82–84] leading to increased phosphorylation of the insulin receptor and IRS1/2 and thereby increasing insulin signaling [85]. The literature on ROS is complex; ROS may improve insulin sensitivity in some instances (particularly at physiological levels), but the long-term effects of excess ROS on insulin action may cause insulin resistance [86].

Betaine and FGF21

Betaine enhances expression of the metabolic regulator fibroblast growth factor 21 (FGF21) that is expressed in the liver [35, 87]. FGF21 increases glucose uptake [88, 89] and decreases intracellular triacylglycerol content in adipocytes [88]. It lowers plasma glucose and triacylglycerol when administered to diabetic mice [89] and it increases energy expenditure and improves insulin sensitivity in diet-induced obese mice [90, 91]. Mice fed betaine had seven-fold increased Fgf21 expression [35]. Bhmt−/− mice accumulated betaine in many tissues, and also had increased hepatic Fgf21 expression as well as increased circulating FGF21 protein [35]. PPARα is essential for FGF21 induction [92] (as noted earlier, a PC species is an endogenous ligand for PPARα).

Summary

Metabolic crosstalk between choline/1-carbon metabolism and pathways of energy homeostasis is readily observed when studying animal models. Feeding practices for domestic livestock have been influenced by such observations. Epidemiological data suggests that similar crosstalk occurs in humans, but rigorous randomized control studies in humans have not been conducted to prove that this is the case. As obesity and insulin resistance are rapidly increasing in the population, it is important to conduct such human studies which might identify modifiable dietary factors that modulate energy homeostasis.

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