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

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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

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

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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 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]. The large variation in dietary intake of choline and the high prevalence of SNPs that create metabolic inefficiencies in choline suggest that a significant portion of the population may be differentially affected by crosstalk between choline metabolism and energy homeostasis.

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 enhanced 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 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/1-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 PPAR α

A specific PC species (16:0/18:1) is the endogenous peroxisome proliferator-activated receptor α (PPAR α) ligand [56]. PPAR α is expressed at high levels in liver where it promotes fatty acid oxidation, ketogenesis, lipid transport, and gluconeogenesis. Activation of PPAR α in rodents leads to improvement of insulin sensitivity by multiple mechanisms [57]. However, deletion of *PPAR α* 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 PPAR α signaling is reduced; perhaps the insulin sensitizing effect is peculiar to the knockout mouse. The effect of PPAR α 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 PPAR α 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 over-expression 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-1 α ; 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]; 45% 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, 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 oddchain 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 (PTB1B) 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]. PPAR α is essential for *FGF21* induction [92] (as noted earlier, a PC species is an endogenous ligand for PPAR α).

Summary

Metabolic crosstalk between choline/1carbon 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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Biography



Dr. Steven Zeisel is the Kenan Distinguished University Professor in the Department of Nutrition in the Gillings School of Global Public Health at the University of North Carolina (UNC) at Chapel Hill. Dr. Zeisel earned his MD from Harvard Medical School in 1975, was a resident in pediatrics at Yale University from 1975 to 1977, and earned his PhD in nutrition at the Massachusetts Institute of Technology in 1980. He served as chair of the Department of Nutrition at UNC from 1990 to 2005. Dr. Zeisel is the Director of the UNC Nutrition Research Institute and Director of the UNC Nutrition and Obesity Research Center (one of 12 centers of excellence funded by the US NIH). He serves on the Federation of American Societies for Experimental Biology (FASEB) Journal editorial board. He serves as the principal investigator on multiple federally-funded research grants that focus on human requirements for choline and the effects of this nutrient on brain development. He has authored more than 300 scientific publications

References

1. Alberti KG, Zimmet P, Shaw J. The metabolic syndrome – a new worldwide definition. *Lancet*. 2005; 366:1059–1062. [PubMed: 16182882]
2. Sung KC, Jeong WS, Wild SH, Byrne CD. Combined influence of insulin resistance, overweight/obesity, and fatty liver as risk factors for type 2 diabetes. *Diabetes Care*. 2012; 35:717–722. [PubMed: 22338098]
3. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004; 27:1047–1053. [PubMed: 15111519]
4. Institute of Medicine, National Academy of Sciences USA. Dietary reference intakes for folate, thiamin, riboflavin, niacin, vitamin B12, pantothenic acid, biotin, and choline. Vol. Volume 1. Washington, DC: National Academy Press; 1998. Choline; p. 390-422.
5. 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. [PubMed: 16600945]
6. Bidulescu A, Chambless LE, Siega-Riz AM, Zeisel SH, Heiss G. Usual choline and betaine dietary intake and incident coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) study. *BMC Cardiovasc Disord*. 2007; 7:20. [PubMed: 17629908]
7. Bidulescu A, Chambless LE, Siega-Riz AM, Zeisel SH, Heiss G. Repeatability and measurement error in the assessment of choline and betaine dietary intake: the Atherosclerosis Risk in Communities (ARIC) study. *Nutr J*. 2009; 8:14. [PubMed: 19232103]
8. Cho E, Willett WC, Colditz GA, Fuchs CS, Wu K, Chan AT, et al. Dietary choline and betaine and the risk of distal colorectal adenoma in women. *J Natl Cancer Inst*. 2007; 99:1224–1231. [PubMed: 17686825]
9. Gossell-Williams M, Fletcher H, McFarlane-Anderson N, Jacob A, Patel J, Zeisel S. Dietary intake of choline and plasma choline concentrations in pregnant women in Jamaica. *West Indian Med J*. 2005; 54:355–359. [PubMed: 16642650]
10. Craciunescu CN, Johnson AR, Zeisel SH. Dietary choline reverses some, but not all, effects of folate deficiency on neurogenesis and apoptosis in fetal mouse brain. *J Nutr*. 2010; 140:1162–1166. [PubMed: 20392884]
11. Shin W, Yan J, Abratte CM, Vermeylen F, Caudill MA. Choline intake exceeding current dietary recommendations preserves markers of cellular methylation in a genetic subgroup of folate-compromised men. *J Nutr*. 2010; 140:975–980. [PubMed: 20220206]
12. Veenema K, Solis C, Li R, Wang W, Maletz CV, Abratte CM, et al. Adequate intake levels of choline are sufficient for preventing elevations in serum markers of liver dysfunction in Mexican American men but are not optimal for minimizing plasma total homocysteine increases after a methionine load. *Am J Clin Nutr*. 2008; 88:685–692. [PubMed: 18779284]
13. Abratte CM, Wang W, Li R, Moriarty DJ, Caudill MA. Folate intake and the MTHFR C677T genotype influence choline status in young Mexican American women. *J Nutr Biochem*. 2008; 19:158–165. [PubMed: 17588738]
14. Buchman AL, Ament ME, Sohel M, Dubin M, Jenden DJ, Roch M, et al. Choline deficiency causes reversible hepatic abnormalities in patients receiving parenteral nutrition: proof of a human choline requirement: a placebo-controlled trial. *J Parenter Enteral Nutr*. 2001; 25:260–268.
15. Zeisel SH, daCosta K-A, Franklin PD, Alexander EA, Lamont JT, Sheard NF, et al. Choline, an essential nutrient for humans. *FASEB J*. 1991; 5:2093–2098. [PubMed: 2010061]
16. Fischer LM, daCosta K, Kwock L, Stewart P, Lu T-S, Stabler S, et al. Sex and menopausal status influence human dietary requirements for the nutrient choline. *Am J Clin Nutr*. 2007; 85:1275–1285. [PubMed: 17490963]
17. Kohlmeier M, da Costa KA, Fischer LM, Zeisel SH. Genetic variation of folate-mediated one-carbon transfer pathway predicts susceptibility to choline deficiency in humans. *Proc Natl Acad Sci USA*. 2005; 102:16025–16030. [PubMed: 16236726]
18. da Costa KA, Badea M, Fischer LM, Zeisel SH. Elevated serum creatine phosphokinase in choline-deficient humans: mechanistic studies in C2C12 mouse myoblasts. *Am J Clin Nutr*. 2004; 80:163–170. [PubMed: 15213044]

19. Shaw GM, Finnell RH, Blom HJ, Carmichael SL, Vollset SE, Yang W, et al. Choline and risk of neural tube defects in a folate-fortified population. *Epidemiology*. 2009; 20:714–719. [PubMed: 19593156]
20. Shaw GM, Carmichael SL, Laurent C, Rasmussen SA. Maternal nutrient intakes and risk of orofacial clefts. *Epidemiology*. 2006; 17:285–291. [PubMed: 16570024]
21. Shaw GM, Carmichael SL, Yang W, Selvin S, Schaffer DM. Periconceptional dietary intake of choline and betaine and neural tube defects in offspring. *Am J Epidemiol*. 2004; 160:102–109. [PubMed: 15234930]
22. Poly C, Massaro JM, Seshadri S, Wolf PA, Cho E, Krall E, et al. The relation of dietary choline to cognitive performance and white-matter hyperintensity in the Framingham Offspring Cohort. *Am J Clin Nutr*. 2011; 94:1584–1591. [PubMed: 22071706]
23. Nurk E, Refsum H, Bjelland I, Drevon CA, Tell GS, Ueland PM, et al. Plasma free choline, betaine and cognitive performance: the Hordaland Health Study. *Br J Nutr*. 2012:1–9. [PubMed: 22717142]
24. Richman EL, Stampfer MJ, Paciorek A, Broering JM, Carroll PR, Chan JM. Intakes of meat, fish, poultry, and eggs and risk of prostate cancer progression. *Am J Clin Nutr*. 2010; 91:712–721. [PubMed: 20042525]
25. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature*. 2011; 472:57–63. [PubMed: 21475195]
26. da Costa KA, Kozyreva OG, Song J, Galanko JA, Fischer LM, Zeisel SH. Common genetic polymorphisms affect the human requirement for the nutrient choline. *FASEB J*. 2006; 20:1336–1344. [PubMed: 16816108]
27. Resseguie M, Song J, Niculescu MD, da Costa KA, Randall TA, Zeisel SH. Phosphatidylethanolamine N-methyltransferase (PEMT) gene expression is induced by estrogen in human and mouse primary hepatocytes. *FASEB J*. 2007; 21:2622–2632. [PubMed: 17456783]
28. Resseguie ME, da Costa KA, Galanko JA, Patel M, Davis IJ, Zeisel SH. Aberrant estrogen regulation of PEMT results in choline deficiency-associated liver dysfunction. *J Biol Chem*. 2011; 286:1649–1658. [PubMed: 21059658]
29. Ivanov A, Nash-Barboza S, Hinkis S, Caudill MA. Genetic variants in phosphatidylethanolamine N-methyltransferase and methylenetetrahydrofolate dehydrogenase influence biomarkers of choline metabolism when folate intake is restricted. *J Am Diet Assoc*. 2009; 109:313–318. [PubMed: 19167960]
30. Sha W, da Costa KA, Fischer LM, Milburn MV, Lawton KA, Berger A, et al. Metabolomic profiling can predict which humans will develop liver dysfunction when deprived of dietary choline. *FASEB J*. 2010; 24:2962–2975. [PubMed: 20371621]
31. Rizki G, Arnaboldi L, Gabrielli B, Yan J, Lee GS, Ng RK, et al. Mice fed a lipogenic methionine-choline-deficient diet develop hypermetabolism coincident with hepatic suppression of SCD-1. *J Lipid Res*. 2006; 47:2280–2290. [PubMed: 16829692]
32. Raubenheimer PJ, Nyirenda MJ, Walker BR. A choline-deficient diet exacerbates fatty liver but attenuates insulin resistance and glucose intolerance in mice fed a high-fat diet. *Diabetes*. 2006; 55:2015–2020. [PubMed: 16804070]
33. Teng YW, Ellis JM, Coleman RA, Zeisel SH. Mouse betaine-homocysteine S-methyltransferase deficiency reduces body fat via increasing energy expenditure and impairing lipid synthesis and enhancing glucose oxidation in white adipose tissue. *J Biol Chem*. 2012; 287:16187–16198. [PubMed: 22362777]
34. Teng YW, Mehedint MG, Garrow TA, Zeisel SH. Deletion of betaine-homocysteine S-methyltransferase in mice perturbs choline and 1-carbon metabolism, resulting in fatty liver and hepatocellular carcinomas. *J Biol Chem*. 2011; 286:36258–36267. [PubMed: 21878621]
35. Teng YW, Ellis JM, Coleman RA, Zeisel SH. Mouse betaine-homocysteine S-methyltransferase deficiency reduces body fat via increasing energy expenditure and impairing lipid synthesis and enhancing glucose oxidation in white adipose tissue. *J Biol Chem*. 2012; 287:16187–16198. [PubMed: 22362777]

36. Jacobs RL, Zhao Y, Koonen DP, Sletten T, Su B, Lingrell S, et al. Impaired de novo choline synthesis explains why phosphatidylethanolamine N-methyltransferase-deficient mice are protected from diet-induced obesity. *J Biol Chem.* 2010; 285:22403–22413. [PubMed: 20452975]
37. Kathirvel E, Morgan K, Nandgiri G, Sandoval BC, Caudill MA, Bottiglieri T, et al. Betaine improves nonalcoholic fatty liver and associated hepatic insulin resistance: a potential mechanism for hepatoprotection by betaine. *Am J Physiol Gastrointest Liver Physiol.* 2010; 299:G1068–G1077. [PubMed: 20724529]
38. Wang Z, Yao T, Pini M, Zhou Z, Fantuzzi G, Song Z. Betaine improved adipose tissue function in mice fed a high-fat diet: a mechanism for hepatoprotective effect of betaine in nonalcoholic fatty liver disease. *Am J Physiol Gastrointest Liver Physiol.* 2010; 298:G634–G642. [PubMed: 20203061]
39. Eklund M, Bauer E, Wamatu J, Mosenthin R. Potential nutritional and physiological functions of betaine in livestock. *Nutr Res Rev.* 2005; 18:31–48. [PubMed: 19079893]
40. Matthews JO, Southern LL, Higbie AD, Persica MA, Bidner TD. Effects of betaine on growth, carcass characteristics, pork quality, and plasma metabolites of finishing pigs. *J Anim Sci.* 2001; 79:722–728. [PubMed: 11263833]
41. Fernandez-Figares I, Wray-Cahen D, Steele NC, Campbell RG, Hall DD, Virtanen E, et al. Effect of dietary betaine on nutrient utilization and partitioning in the young growing feed-restricted pig. *J Anim Sci.* 2002; 80:421–428. [PubMed: 11881930]
42. Esteve-Garcia E, Mack S. The effect of dl-methionine and betaine on growth performance and carcass characteristics in broilers. *Anim Feed Sci Technol.* 2000; 87:85–93.
43. Schwab U, Torronen A, Toppinen L, Alfthan G, Saarinen M, Aro A, et al. Betaine supplementation decreases plasma homocysteine concentrations but does not affect body weight, body composition, or resting energy expenditure in human subjects. *Am J Clin Nutr.* 2002; 76:961–967. [PubMed: 12399266]
44. Hoffman JR, Ratamess NA, Kang J, Gonzalez AM, Beller NA, Craig SA. Effect of 15 days of betaine ingestion on concentric and eccentric force outputs during isokinetic exercise. *J Strength Cond Res.* 2011; 25:2235–2241. [PubMed: 21747291]
45. Trepanowski JF, Farney TM, McCarthy CG, Schilling BK, Craig SA, Bloomer RJ. The effects of chronic betaine supplementation on exercise performance, skeletal muscle oxygen saturation and associated biochemical parameters in resistance trained men. *J Strength Cond Res.* 2011; 25:3461–3471. [PubMed: 22080324]
46. Lever M, George PM, Atkinson W, Molyneux SL, Elmslie JL, Slow S, et al. Plasma lipids and betaine are related in an acute coronary syndrome cohort. *PLoS ONE.* 2011; 6:e21666. [PubMed: 21747945]
47. Konstantinova SV, Tell GS, Vollset SE, Nygard O, Bleie O, Ueland PM. Divergent associations of plasma choline and betaine with components of metabolic syndrome in middle age and elderly men and women. *J Nutr.* 2008; 138:914–920. [PubMed: 18424601]
48. Elshorbagy AK, Kozich V, Smith AD, Refsum H. Cysteine and obesity: consistency of the evidence across epidemiologic, animal and cellular studies. *Curr Opin Clin Nutr Metab Care.* 2012; 15:49–57. [PubMed: 22108094]
49. El-Khairy L, Vollset SE, Refsum H, Ueland PM. Predictors of change in plasma total cysteine: longitudinal findings from the Hordaland homocysteine study. *Clin Chem.* 2003; 49:113–120. [PubMed: 12507967]
50. Elshorbagy AK, Nurk E, Gjesdal CG, Tell GS, Ueland PM, Nygard O, et al. Homocysteine, cysteine, and body composition in the Hordaland Homocysteine Study: does cysteine link amino acid and lipid metabolism? *Am J Clin Nutr.* 2008; 88:738–746. [PubMed: 18779291]
51. Olefsky JM. Comparison of the effects of insulin and insulin-like agents on different aspects of adipocyte metabolism. *Horm Metab Res.* 1979; 11:209–213. [PubMed: 447201]
52. Czech MP, Lawrence JC Jr, Lynn WS. Evidence for electron transfer reactions involved in the Cu²⁺-dependent thiol activation of fat cell glucose utilization. *J Biol Chem.* 1974; 249:1001–1006. [PubMed: 4814334]

53. Johnson AR, Craciunescu CN, Guo Z, Teng YW, Thresher RJ, Blusztajn JK, et al. Deletion of murine choline dehydrogenase results in diminished sperm motility. *FASEB J*. 2010; 24:2752–2761. [PubMed: 20371614]
54. Ratnam S, Wijekoon EP, Hall B, Garrow TA, Brosnan ME, Brosnan JT. Effects of diabetes and insulin on betaine-homocysteine S-methyltransferase expression in rat liver. *Am J Physiol Endocrinol Metab*. 2006; 290:E933–E939. [PubMed: 16352668]
55. Nieman KM, Hartz CS, Szegedi SS, Garrow TA, Sparks JD, Schalinske KL. Folate status modulates the induction of hepatic glycine N-methyltransferase and homocysteine metabolism in diabetic rats. *Am J Physiol Endocrinol Metab*. 2006; 291:E1235–E1242. [PubMed: 16835399]
56. Chakravarthy MV, Lodhi IJ, Yin L, Malapaka RR, Xu HE, Turk J, et al. Identification of a physiologically relevant endogenous ligand for PPARalpha in liver. *Cell*. 2009; 138:476–488. [PubMed: 19646743]
57. Haluzik MM, Haluzik M. PPAR-alpha and insulin sensitivity. *Physiol Res*. 2006; 55:115–122. [PubMed: 15910175]
58. Tordjman K, Bernal-Mizrachi C, Zeman L, Weng S, Feng C, Zhang F, et al. PPARalpha deficiency reduces insulin resistance and atherosclerosis in apoE-null mice. *J Clin Invest*. 2001; 107:1025–1034. [PubMed: 11306606]
59. Koo SH, Satoh H, Herzig S, Lee CH, Hedrick S, Kulkarni R, et al. PGC-1 promotes insulin resistance in liver through PPAR-alpha-dependent induction of TRB-3. *Nat Med*. 2004; 10:530–534. [PubMed: 15107844]
60. DeLong CJ, Shen YJ, Thomas MJ, Cui Z. Molecular distinction of phosphatidylcholine synthesis between the CDP-choline pathway and phosphatidylethanolamine methylation pathway. *J Biol Chem*. 1999; 274:29683–29688. [PubMed: 10514439]
61. da Costa KA, Sanders LM, Fischer LM, Zeisel SH. Docosahexaenoic acid in plasma phosphatidylcholine may be a potential marker for in vivo phosphatidylethanolamine N-methyltransferase activity in humans. *Am J Clin Nutr*. 2011; 93:968–974. [PubMed: 21411618]
62. Browning JD, Horton JD. Molecular mediators of hepatic steatosis and liver injury. *J Clin Invest*. 2004; 114:147–152. [PubMed: 15254578]
63. Walker AK, Jacobs RL, Watts JL, Rottiers V, Jiang K, Finnegan DM, et al. A conserved SREBP-1/phosphatidylcholine feedback circuit regulates lipogenesis in metazoans. *Cell*. 2011; 147:840–852. [PubMed: 22035958]
64. Tsunekawa S, Demozay D, Briaud I, McCuaig J, Accili D, Stein R, et al. FoxO feedback control of basal IRS-2 expression in pancreatic beta-cells is distinct from that in hepatocytes. *Diabetes*. 2011; 60:2883–2891. [PubMed: 21933986]
65. Valverde AM, Burks DJ, Fabregat I, Fisher TL, Carretero J, White MF, et al. Molecular mechanisms of insulin resistance in IRS-2-deficient hepatocytes. *Diabetes*. 2003; 52:2239–2248. [PubMed: 12941762]
66. Ji C, Shinohara M, Vance D, Than TA, Ookhtens M, Chan C, et al. Effect of transgenic extrahepatic expression of betaine-homocysteine methyltransferase on alcohol or homocysteine-induced fatty liver. *Alcohol Clin Exp Res*. 2008; 32:1049–1058. [PubMed: 18498552]
67. Garrow TA. Purification, kinetic properties, and cDNA cloning of mammalian betaine-homocysteine methyltransferase. *J Biol Chem*. 1996; 271:22831–22838. [PubMed: 8798461]
68. Olthof MR, Verhoef P. Effects of betaine intake on plasma homocysteine concentrations and consequences for health. *Curr Drug Metab*. 2005; 6:15–22. [PubMed: 15720203]
69. Zeisel SH. Importance of methyl donors during reproduction. *Am J Clin Nutr*. 2009; 89:673S–677S. [PubMed: 19116320]
70. Mehedint MG, Craciunescu CN, Zeisel SH. Maternal dietary choline deficiency alters angiogenesis in fetal mouse hippocampus. *Proc Natl Acad Sci USA*. 2010; 107:12834–12839. [PubMed: 20624989]
71. Niculescu MD, Craciunescu CN, Zeisel SH. Dietary choline deficiency alters global and gene-specific DNA methylation in the developing hippocampus of mouse fetal brains. *FASEB J*. 2006; 20:43–49. [PubMed: 16394266]
72. Cooney CA, Dave AA, Wolff GL. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. *J Nutr*. 2002; 132:2393S–2400S. [PubMed: 12163699]

73. Waterland RA, Jirtle RL. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol.* 2003; 23:5293–5300. [PubMed: 12861015]
74. Gilbert ER, Liu D. Epigenetics: the missing link to understanding beta-cell dysfunction in the pathogenesis of type 2 diabetes. *Epigenetics.* 2012; 7:841–852. [PubMed: 22810088]
75. Intine RV, Sarras MP Jr. Metabolic memory and chronic diabetes complications: potential role for epigenetic mechanisms. *Curr Diab Rep.* 2012; 12:551–559. [PubMed: 22760445]
76. Yang BT, Dayeh TA, Volkov PA, Kirkpatrick CL, Malmgren S, Jing X, et al. Increased DNA methylation and decreased expression of PDX-1 in pancreatic islets from patients with type 2 diabetes. *Mol Endocrinol.* 2012; 26:1203–1212. [PubMed: 22570331]
77. Ling C, Del Guerra S, Lupi R, Ronn T, Granhall C, Luthman H, et al. Epigenetic regulation of PPAR γ 1A in human type 2 diabetic islets and effect on insulin secretion. *Diabetologia.* 2008; 51:615–622. [PubMed: 18270681]
78. Johnson A, Lao S, Wang T, Galanko J, Zeisel SH. Choline dehydrogenase polymorphism rs12676 is a functional variation and is associated with changes in human sperm cell function. *PLoS ONE.* 2012; 7:e36047. [PubMed: 22558321]
79. Johnson AR, Lao S, Wang T, Galanko JA, Zeisel SH. Choline dehydrogenase polymorphism rs12676 is a functional variation and is associated with changes in human sperm cell function. *PLoS One.* 2012; 7:e36047. [PubMed: 22558321]
80. Boyle KE, Canham JP, Consitt LA, Zheng D, Koves TR, Gavin TP, et al. A high-fat diet elicits differential responses in genes coordinating oxidative metabolism in skeletal muscle of lean and obese individuals. *J Clin Endocrinol Metab.* 2011; 96:775–781. [PubMed: 21190973]
81. Coletta DK, Mandarino LJ. Mitochondrial dysfunction and insulin resistance from the outside in: extracellular matrix, the cytoskeleton, and mitochondria. *Am J Physiol Endocrinol Metab.* 2011; 301:E749–E755. [PubMed: 21862724]
82. Loh K, Deng H, Fukushima A, Cai X, Boivin B, Galic S, et al. Reactive oxygen species enhance insulin sensitivity. *Cell Metab.* 2009; 10:260–272. [PubMed: 19808019]
83. Mahadev K, Zilbering A, Zhu L, Goldstein BJ. Insulin-stimulated hydrogen peroxide reversibly inhibits protein-tyrosine phosphatase 1b in vivo and enhances the early insulin action cascade. *J Biol Chem.* 2001; 276:21938–21942. [PubMed: 11297536]
84. Mahadev K, Motoshima H, Wu X, Ruddy JM, Arnold RS, Cheng G, et al. The NAD(P)H oxidase homolog Nox4 modulates insulin-stimulated generation of H₂O₂ and plays an integral role in insulin signal transduction. *Mol Cell Biol.* 2004; 24:1844–1854. [PubMed: 14966267]
85. Cheng Z, Tseng Y, White MF. Insulin signaling meets mitochondria in metabolism. *Trends Endocrinol Metab.* 2010; 21:589–598. [PubMed: 20638297]
86. Szendroedi J, Phielix E, Roden M. The role of mitochondria in insulin resistance and type 2 diabetes mellitus. *Nat Rev Endocrinol.* 2012; 8:92–103. [PubMed: 21912398]
87. Nishimura T, Nakatake Y, Konishi M, Itoh N. Identification of a novel FGF, FGF-21, preferentially expressed in the liver. *Biochim Biophys Acta.* 2000; 1492:203–206. [PubMed: 10858549]
88. Andriamampandry C, Massarelli R, Freysz L, Kanfer JN. A rat brain cytosolic N-methyltransferase(s) activity converting phosphorylethanolamine into phosphorylcholine. *Biochem Biophys Res Commun.* 1990; 171:758–763. [PubMed: 2403362]
89. Kharitononkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, et al. FGF-21 as a novel metabolic regulator. *J Clin Invest.* 2005; 115:1627–1635. [PubMed: 15902306]
90. Coskun T, Bina HA, Schneider MA, Dunbar JD, Hu CC, Chen Y, et al. Fibroblast growth factor 21 corrects obesity in mice. *Endocrinology.* 2008; 149:6018–6027. [PubMed: 18687777]
91. Xu J, Lloyd DJ, Hale C, Stanislaus S, Chen M, Sivits G, et al. Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. *Diabetes.* 2009; 58:250–259. [PubMed: 18840786]
92. Inagaki T, Dutchak P, Zhao G, Ding X, Gautron L, Parameswara V, et al. Endocrine regulation of the fasting response by PPAR α -mediated induction of fibroblast growth factor 21. *Cell Metab.* 2007; 5:415–425. [PubMed: 17550777]

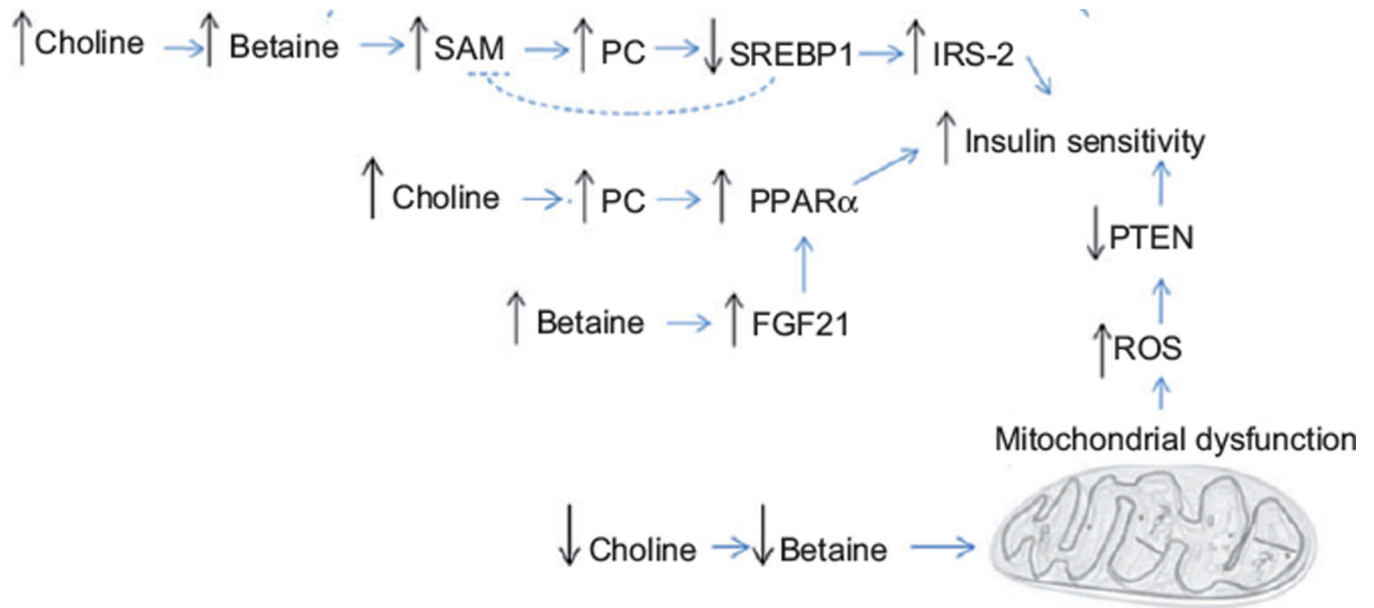


Figure 1. Hypothetical pathways for crosstalk between choline metabolism and insulin sensitivity

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; PPAR α , peroxisome proliferator-activated receptor α ; PTEN, phosphatase and tensin homolog; ROS, reactive oxygen species; SAM, *S*-adenosylmethionine; SREBP1, sterol regulatory element-binding protein 1.

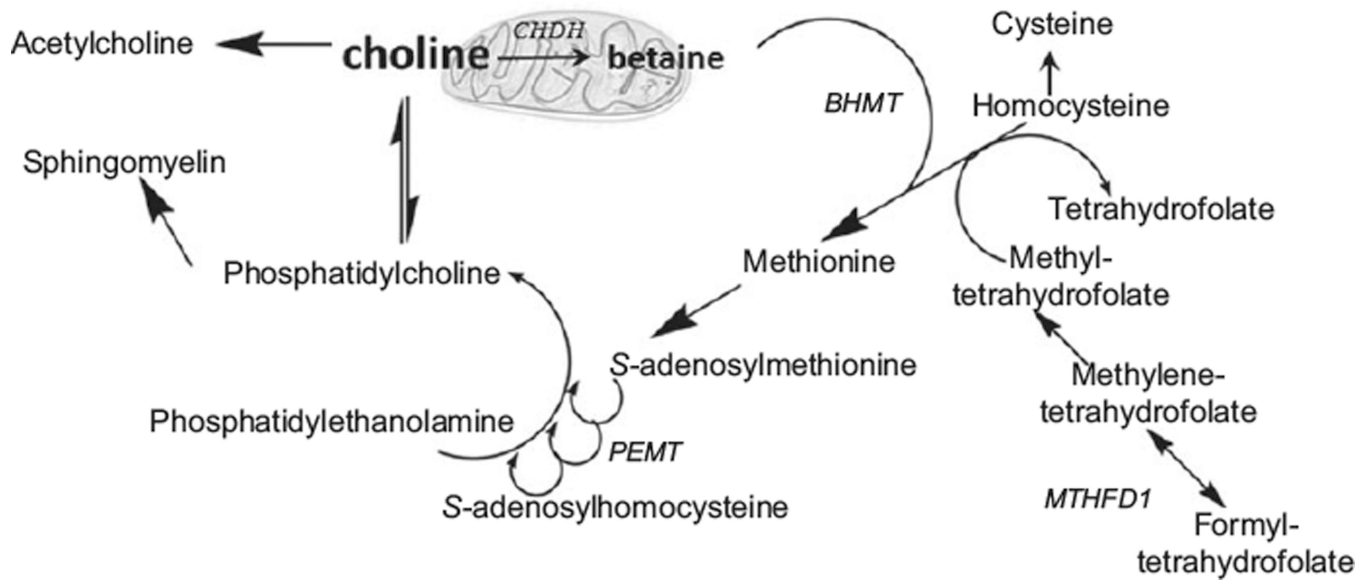


Figure 2. Genes that influence choline metabolism and dietary requirements

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 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.

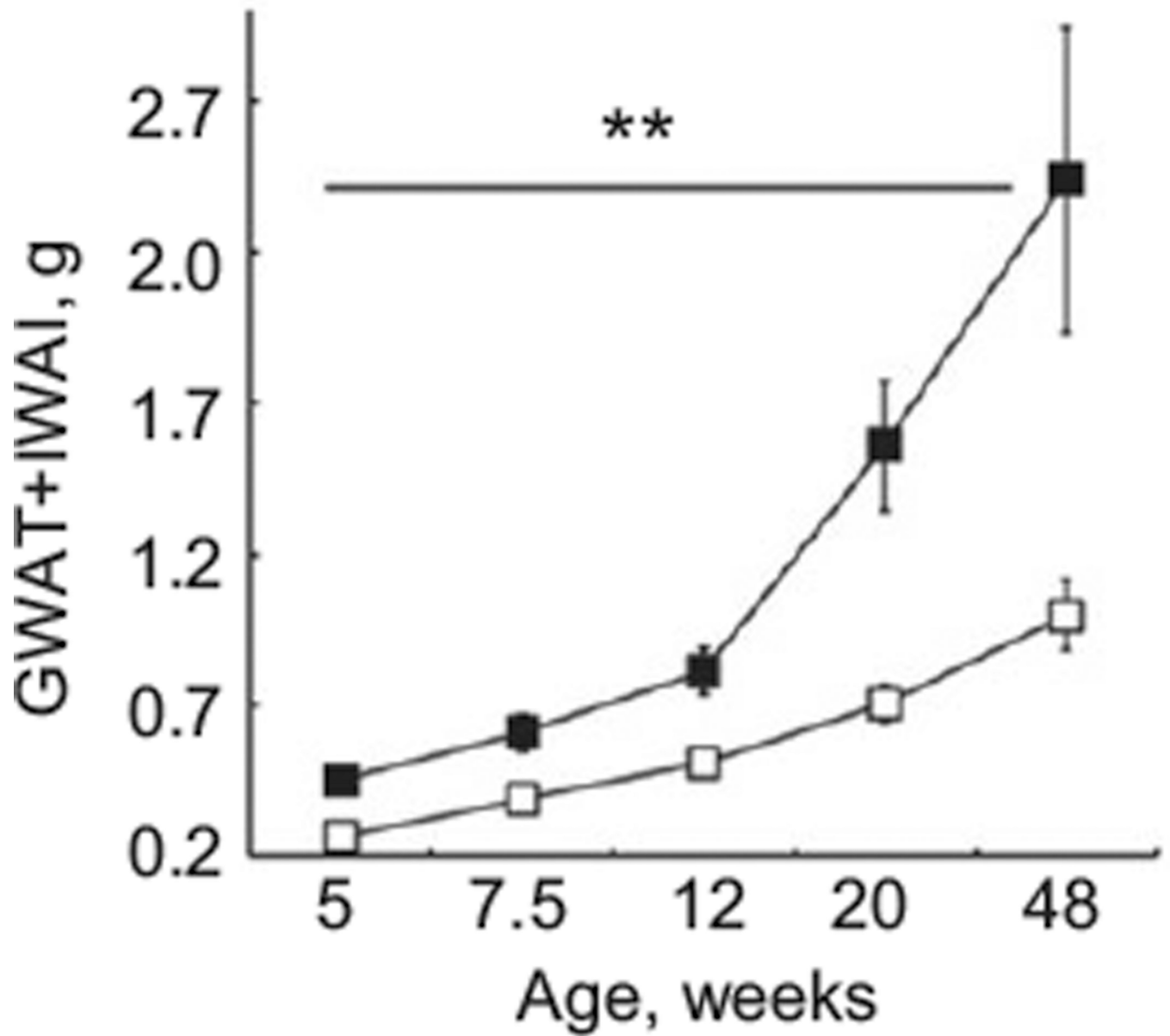


Figure 3. *Bhmt*^{-/-} mice have reduced fat pad weight

GWAT, gonadal white adipose tissue and IWAT, inguinal white adipose tissue were harvested from *Bhmt*^{+/+} (black) and *Bhmt*^{-/-} (white) mice. **p<0.01. From reference [35] with permission.

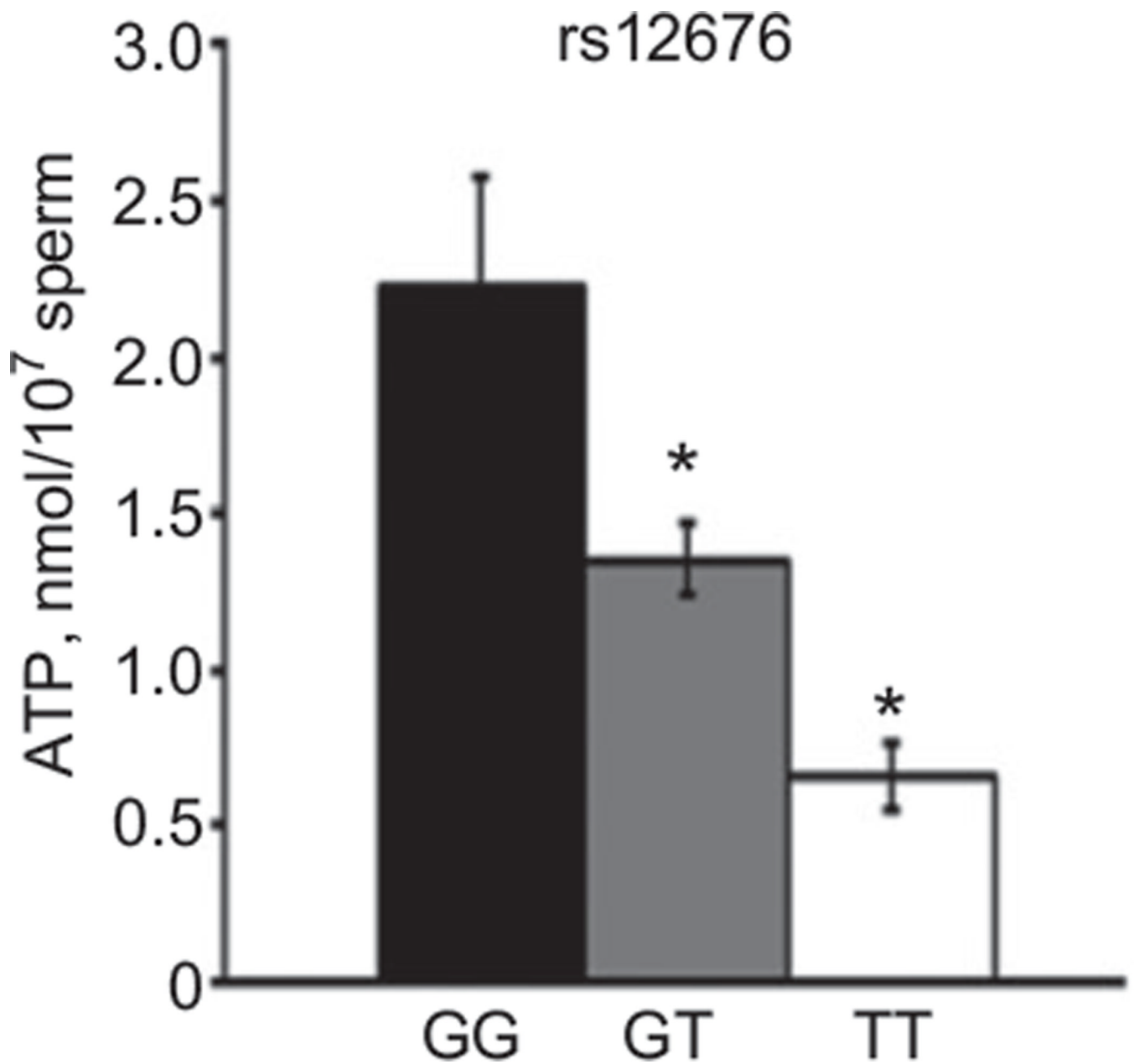


Figure 4. Men with a functional SNP in *CHDH* (rs12676) have decreased ATP concentrations reflecting mitochondrial dysfunction

Men who were heterozygous or homozygous for the rs12676 variant T allele have reduced ATP concentrations in their sperm. N=17 (GG), 18 (GT) and 5 (TT). * indicates difference from GG by ANOVA and Tukey-Kramer HSD, p-value<0.05. From reference [79] with permission.

Table 1

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

Gene	rs number	Base pair and sequence change
MTHFD1	rs2236225	+1958 G → A
PEMT	rs12325817	-744 G → C
CHDH	rs9001	+318 A → C
CHDH	rs12676	+432 G → T

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.