The Journal of Nutrition
Symposium: Systems Genetics in Nutrition and Obesity Research

Nutritional Genomics: Defining the Dietary Requirement and Effects of Choline[1–3]

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Abstract

As it becomes evident that single nucleotide polymorphisms (SNPs) in humans can create metabolic inefficiencies, it is reasonable to ask if such SNPs influence dietary requirements. Epidemiologic studies that examine SNPs relative to risks for diseases are common, but there are few examples of clinically sized nutrition studies that examine how SNPs influence metabolism. Studies on how SNPs influence the dietary requirement for choline provide a model for how we might begin examining the effects of SNPs on nutritional phenotypes using clinically sized studies (clinical nutrigenomics). Most men and postmenopausal women develop liver or muscle dysfunction when deprived of dietary choline. More than one-half of premenopausal women may be resistant to choline deficiency-induced organ dysfunction, because estrogen induces the gene [phosphatidylethanolamine-*N*-methyltransferase (*PEMT*)] that catalyzes endogenous synthesis of phosphatidylcholine, which can subsequently yield choline. Those premenopausal women that do require a dietary source of choline have a SNP in *PEMT*, making them unresponsive to estrogen induction of *PEMT*. It is important to recognize differences in dietary requirements for choline in women, because during pregnancy, maternal dietary choline modulates fetal brain development in rodent models. Because choline metabolism and folate metabolism intersect at the methylation of homocysteine, manipulations that limit folate availability also increase the use of choline as a methyl donor. People with a SNPs in *MTHFD1* (a gene of folate metabolism that controls the use of folate as a methyl donor) are more likely to develop organ dysfunction when deprived of choline; their dietary requirement is increased because of increased need for choline as a methyl donor. J. Nutr. 141: 531–534, 2011.

Introduction

The study of how nutrients interact with genes and how genes influence metabolism, nutrigenomics, is a rapidly developing new discipline within nutrition. Genome-wide association studies (GWAS), in which many thousands of people's genetic variations are associated with a risk for a disease, are at the cutting edge of nutrition epidemiology research. These studies are usually observational and collect limited biological data about the persons studied. As it becomes evident that single nucleotide polymorphisms (SNPs; a nucleotide alteration that occurs in >1% of the population and is inherited) in humans can create metabolic inefficiencies, it is reasonable to ask if such SNPs influence dietary requirements. To date, few studies exist in which fewer numbers of humans have been more intensively characterized by measuring SNPs and nutritionally relevant clinical outcomes (clinical nutrigenomics). Though such studies may eventually enable clinicians to provide personalized nutrition recommendations, in the immediate future, it is this type of study that can help define the role of genetic variation in influencing diet requirements. Currently, nutritionists estimate the average nutrient requirements for a population assuming that the dose-response curve for the effects of a nutrient are normally distributed among the population and thus do not consider that there might be multiple and separate dose-response curves. This can result in recommendations for dietary intake that are hard to achieve by eating foods. Once it is possible to identify the sources of metabolic variation, subgroups that differ in nutrient requirements will be identified, interventions can then be targeted, and dietary recommendations refined.

It is not uncommon in nutrition research to find a nutrient-health association in one study and subsequently not observe this relationship (or even observe an inverse relationship) in another study. In nutrition research studies, when a large variance exists in response to a nutrient, statistical analyses

[1] Published as a supplement to *The Journal of Nutrition*. Presented as part of the symposium entitled "Systems Genetics in Nutrition Research" given at the Experimental Biology 2010 meeting, April 27, 2010, in Anaheim, CA. The symposium was supported by an educational grant from USDA-NIFA, AFRI Award, and sponsored by the Nutrient Gene Interaction RIS and the University of Tennessee Obesity Research Center. The symposium was chaired by Naima Mousstaid-Moussa and cochaired by Brynn Voy. Guest Editor for this symposium publication was Kevin Schalinske. Guest Editor disclosure: Kevin Schalinske had no relationships to disclose.

[2] Supported by the NIH (DK55865) and grants from the NIH to the University of North Carolina Nutrition and Obesity Research Center (DK56350).

[3] S. H. Zeisel, no conflicts of interest; he serves on scientific advisory boards for Hershey, Solae, and Dupont.

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First published online January 26, 2011; doi:10.3945/jn.110.130369.

often argue for a null effect. In part, this problem is due to large variance around the mean in the population studied. This variance is not only due to random biological noise, but also to inclusion of genetically definable subpopulations with widely differing responses to the nutrient. If responders could be differentiated from nonresponders based on nutrigenomic profiling, this statistical noise could be eliminated and the sensitivity (reproducibility) of nutrition research could be greatly increased. This approach was recently reviewed (1,2).

Developing clinical nutrigenomics

The use of genome-wide profiling of common SNPs to identify genetically different subpopulations that have differential risks for disease has become common. For genes that exert small effects on a disease process, a gene variant adds only a small amount of increased risk, often difficult to distinguish from background variation. In GWAS, it is common to measure millions of SNPs in thousands of participants, thereby making a very large number of comparisons and increasing the opportunity for false discovery. For this reason, more stringent definitions of significance are used in GWAS [e.g. require a $P < 5 \times 10^{-7}$ (3)]. Using thousands of participants in a nutrition study is practical if the nutrition effect on phenotype is easily measured (e.g. a simple blood measurement), but studies using this many participants are impossible if assessment of phenotype requires expensive or invasive methods (e.g. MRI or glucose clamps). If the same stringent *P*-values were required in clinically sized nutrition studies, these studies would detect a significant difference only if the effect of the nutrient was very large. However, there are designs for examining the effects of SNPs on nutrient requirements that permit the use of less stringent thresholds for significance of *P*-values than those thresholds used for the GWAS-type design.

The important elements of a clinically sized SNP study have already have been considered by the same scientific panel that suggested the stringent thresholds for *P*-values used in GWAS-type studies (3). Clinically sized studies on SNPs and nutrient metabolism need to select a priori a small number of targeted SNPs based on knowledge of the underlying processes causing the phenotype (e.g. selecting a gene in the metabolic pathway of a nutrient), SNPs for which there is credible laboratory evidence that they alter metabolism of the nutrient, or SNPs that are likely to result in defective protein products (such as nonsynonymous coding SNPs) (3). Because the number of SNP-phenotype comparisons is limited by this study design, correction for false discovery becomes less problematic. By sharpening the focus of the study, it is possible to accept a less rigorous *P*-value; however, creating a credible biological hypothesis post hoc is not acceptable (3). Effect size is still an important issue, for a clinically-sized study to be adequately powered the size of the effect of the SNP on nutrient requirement must be large enough, and consistent enough, to stand out from noise created by individual variation; unknown confounding factors are less likely to produce such large effects (3). Finally, replication of the association between SNP and phenotype in an independent study is important.

The observation of an association between a SNP and a nutritional phenotype or disease risk does not mean that the identified SNPs is the cause of the metabolic inefficiency. A SNP is part of a grouping of SNPs (haplotype) that are inherited together; thus, it could be one of the other linked SNPs that is functionally important. However, a SNP that is related to a nutritional phenotype still can be a very useful biomarker that is predictive of the metabolic inefficiency.

Nutrigenomics also considers transcription factors and epigenetics

Nutrigenomics/nutrigenetics involves more than the study of gene polymorphisms. Other mechanisms can influence gene expression and are relevant to nutrition. Transcription factors are molecules that bind to response elements and then activate or inhibit gene expression, sometimes thereby altering metabolism. An example discussed later is the effect of estrogen (bound to estrogen receptor complex) on the gene for endogenous biosynthesis of phosphatidylcholine, the route for endogenous biosynthesis of choline.

Nutrients also influence the epigenetic marking of DNA, thereby altering gene expression [reviewed in (1,4)]. People all share a great deal of genetic code in common and yet can differ greatly in metabolism and other phenotypes. Some of the variation between individuals is due to genetic differences, as discussed earlier. However, even monozygous twins can be metabolically different despite having identical genetic codes (5). Obviously, metabolic phenotype must be determined by more information than that encoded in DNA sequence alone. In part, it is due to another coding system, the epigenetic code (6). This epigenetic code is a series of marks added to DNA or to the proteins (histones) around which DNA is wrapped. The best understood marks are DNA methylation, but covalent modifications of histones and chromatin, and RNA interference also mediate epigenetic regulation of gene expression (7). An example discussed below is the effect of choline, a major methyl donor, on epigenetic marking of genes.

Clinically sized nutrigenomic studies identifying the dietary requirement for choline

Studies on how SNPs influence the dietary requirement for choline provide a model for how we might begin to examining the effects of SNPs on nutritional phenotypes using clinically sized studies (clinical nutrigenomics).

Choline metabolism has been reviewed in detail elsewhere (8). Choline is used to make acetylcholine (a neurotransmitter) and phospholipids (phosphatidylcholine and sphingomyelin, among the most important), or it is irreversibly oxidized to form betaine (8). Betaine is a methyl donor for the conversion of homocysteine to methionine (8). This is the precursor for the synthesis of *S*-adenosylmethionine, the universal methyl donor needed for methylation of DNA, RNA, and proteins. As a methyl donor, choline's metabolism is highly related to the metabolism of other methyl donors such as methyl-folate and methionine, and manipulations that alter metabolism of any 1 of these 3 result in changes in metabolism of the other 2 methyl donors (8). Thus, an integrated approach is needed when determining the dietary requirement for these 3 nutrients.

A database is available that describes the choline and betaine content of foods (9). Nutritionists have been discouraging intake of many of the foods that are highest in choline, because they are also high in fat and cholesterol (e.g. eggs and liver), and the 2005 NHANES survey found that only a small percent of the population achieves the recommended adequate intake for choline (10). The only source of choline other than diet is from the de novo biosynthesis of phosphatidylcholine (can be converted to choline) catalyzed by phosphatidylethanolamine-*N*-methyltransferase (PEMT) in liver (11). Studies in humans show that dietary choline is required [reviewed in (8)]. The United States has established for choline an adequate intake level (~0.5 g/d) and a tolerable upper intake limit value (3.5 g/d) for adults (12).

The study of the gene-nutrient interactions that modulate the dietary requirement for choline (13–16) provides interesting insights into useful designs for clinically sized nutrigenomic studies. The outcome markers used for this study assessed susceptibility to developing organ (liver and muscle) dysfunction when fed a low-choline diet under controlled conditions. Study participants were fed a standard diet containing a known amount of choline [550 mg·(70 kg·d^{-1}); baseline]. On d 11 participants were switched to a diet containing <50 mg/d choline for up to 42 d. If at some point during the depletion period the participants developed organ dysfunction associated with choline deficiency, they were switched to a diet containing choline until repletion. Most men and postmenopausal women, but only 44% of premenopausal women, fed low-choline diets developed reversible fatty liver (measured by mass resonance spectroscopy) as well as liver and muscle damage (16). This difference in dietary choline requirement for young women occurs, because they have an estrogen-enhanced capacity for producing their own choline; the *PEMT* gene (forms phosphatidylcholine) is induced by estrogen in human hepatocytes, with maximal activation at estrogen concentrations reached at term in pregnancy (17). Thus, capacity for this endogenous source of choline is highest during the period when females need to support fetal development. This is important, because the demand for choline is very high during pregnancy and lactation (18) and, as discussed later, choline is critical for normal fetal development.

Though more than one-half of premenopausal women were resistant to choline deficiency-induced organ dysfunction, those premenopausal women that did require a dietary source of choline had a SNP in *PEMT* (rs12325817; 74% of women in North Carolina had 1 or more variant alleles), making them unresponsive to estrogen induction of *PEMT* (19,20). This common SNP greatly increased the risk for developing organ dysfunction when participants were fed a low-choline diet (OR = 2; $P < 0.00005$, based on 64 women studied) (20). We noted earlier that choline and folate metabolism are highly related. Premenopausal women with a SNP in the gene *MTHFD1* (5,10-methylenetetrahydrofolate dehydrogenase 1; rs2236225) were 15 times more likely to develop signs of choline deficiency ($P < 0.0001$) on the low-choline diet than were the wild types (21). Sixty-three percent of our study population had at least 1 allele for this SNP in 5,10-methylenetetrahydrofolate dehydrogenase, which alters the availability of 5-methyl tetrahydrofolate for homocysteine remethylation, increasing the use of choline-derived methyl groups to remove homocysteine.

Choline, epigenetics, and neural development

Dietary choline (as well as folate) is used to form methionine and thereafter *S*-adenosylmethionine (the methyl donor for DNA and histone methylation). Thus, it is to be expected that diet during pregnancy influences the epigenetic status of the developing fetal brain. In rats and mice, maternal dietary choline intake has an important effect on neurogenesis (22) and angiogenesis (23). A maternal diet low in choline decreased both processes in the fetal hippocampus and increased apoptosis in this area of fetal brain (22,24,25). One of the mechanisms identified that explains this effect was a marked change in the epigenetic marks present in fetal brain (23,26). Global DNA methylation decreased in the neuroepithelial layer of the hippocampus in fetal brains from pregnant rodent dams fed a low-choline diet and, specifically, the gene encoding cyclin-dependent kinase (*Cdkn3*) was hypomethylated in its promoter (26,27). This hypomethylation resulted in increased gene expression and increased downstream signaling that resulted in decreased cell cycling (26), consistent with decreased neurogenesis. Similarly, genes regulating angiogenesis were hypomethylated and overexpressed, resulting in fewer blood vessels in fetal brain (23).

These changes in fetal brain structure that are caused by changing maternal intake of choline during pregnancy have long-lasting effects that alter brain function throughout life, including long-lasting changes in the hippocampal function (memory) of the adult offspring. Choline supplementation of pregnant rats enhanced memory in their offspring and this improvement lasted until they were old animals (28–30). It also enhanced an electrical property of the hippocampus called long-term potentiation (31,32), which is normally positively associated with memory. The offspring from mothers fed a choline-deficient diet manifested opposite outcomes (28,33).

Does this work in rodents apply to humans?

We do not know if these effects on brain development described in rodent models apply to humans, because no comparable studies have yet been performed. One study in humans observed that cord blood choline concentrations were not related to intelligence quotient at 5 y (34), but this study used relatively crude measures of intelligence and cord blood choline is not necessarily a good biomarker for maternal intake of choline.

Despite the dearth of human data on choline and brain development, the similarities between rodent and human hippocampal development are enough that there is reason to be concerned that the 2005 NHANES data suggest that most pregnant women do not consume adequate amounts of choline (10), and case-control studies in California suggest that women eating lower choline diets are at increased risk for giving birth to babies with neural tube defects (35) and cleft palate. In addition, because approximately one-half of the population has gene polymorphisms that affect choline and folate metabolism (20), it is likely that different individuals may have different dietary requirements for choline and may need to pay special attention to choline intake during pregnancy.

Acknowledgments

The sole author had responsibility for all parts of the manuscript.

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