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The Nutrigenetics and Nutrigenomics of the Dietary Requirement for Choline

KAREN D. CORBIN, STEVEN H. ZEISEL

University of North Carolina at Chapel Hill, Nutrition Research Institute, Kannapolis, North Carolina, USA

Abstract

Advances in nutrigenetics and nutrigenomics have been instrumental in demonstrating that nutrient requirements vary among individuals. This is exemplified by studies of the nutrient choline, in which gender, single-nucleotide polymorphisms, estrogen status, and gut microbiome composition have been shown to influence its optimal intake level. Choline is an essential nutrient with a wide range of biological functions, and current studies are aimed at refining our understanding of its requirements and, importantly, on defining the molecular mechanisms that mediate its effects in instances of suboptimal dietary intake. This chapter introduces the reader to challenges in developing individual nutrition recommendations, the biological function of choline, current and future research paradigms to fully understand the consequences of inadequate choline nutrition, and some forward thinking about the potential for individualized nutrition recommendations to become a tangible application for improved health.

I. Introduction

A. Opportunities and Challenges in Nutrigenetics and Nutrigenomics

In the post-genomics era, there is the potential to utilize biological signatures to determine the underlying mechanisms governing metabolic variation and optimal nutrient requirements for individuals. This gives us an unprecedented opportunity to develop tailored interventions that meet an individual's nutritional needs, with the goal of preventing or treating disease. In order to turn this potential into reality, we must first decipher how our genetic code and other biological parameters influence metabolism and thereby nutrient requirements. In addition, we will have to understand how nutrients impact gene expression, metabolite profiles, epigenetic patterns, and protein function; this will necessitate development of computational tools that can be integrated across all these effects of nutrients. Before these questions can be addressed, we must surmount several barriers that now hinder the ability to utilize cutting-edge nutrition science in developing individualized nutrition recommendations.

One challenge is the common misconception that only very large studies can detect the effects of common genetic variants at single bases (single-nucleotide polymorphisms, SNPs) on nutrient demands. This mistaken idea arises because of the belief that the effect size of SNPs on nutrient requirements is very small and because it is common practice to measure every SNP that is easy to measure rather than to select a mechanistically targeted, smaller set of SNPs for analysis. Genome-wide association studies (GWAS) comprehensively address the role of millions of SNPs on phenotypes of interest, but they make many comparisons and

have little power unless tens of thousands of people are genotyped. This is because, to avoid false discoveries, such studies must use very stringent probability values that are impossible to attain in studies of smaller size. The assumption that only large studies can detect metabolically functional SNPs is not true: when scientists limit the number of comparisons made by prospectively focusing on a targeted set of SNPs of interest and when the effect size of the functional SNP is relatively large, clinically sized studies are sufficient to detect SNPs that alter nutrient requirements.¹

Another challenge lies in the increasing complexity of the factors that modulate nutrient–gene interactions. Reductionist approaches to science simplify analyses but often lead to linear thinking (A causes B which causes C). Full understanding of the factors that mediate variation in metabolism and nutrition requirements will necessitate a more integrated approach that recognizes the multifaceted networks of interactions involved. Optimal nutrient intakes are dictated by an individual's capacity to metabolize and utilize ingested nutrients and, in the case of nutrients with endogenous synthesis pathways, nutrition requirements are also highly dependent on the proper function of those pathways. Part of an individual's metabolic capacity is mediated by the inherited genetic code, and many nutrient–gene interactions have been uncovered. Examples range from classic monogenic interactions, such as deficiency in phenylalanine hydroxylase leading to phenylketonuria to the more subtle regulation of gene expression by polyunsaturated fatty acids.²

Although gene–nutrient interactions are highly important for interindividual variability in the requirements and effects of nutrients, further complexity is introduced by the recognition that epigenetic and posttranslational mechanisms are concurrently involved.¹ Because exposure to nutrients during critical developmental windows of time can permanently alter gene expression, nutrition science is presented with a tremendous hurdle, particularly in the context of the multiple nutrients that humans are exposed to differentially throughout a lifetime. From the womb through adulthood, environmental exposures can alter the context within which genetic architecture exerts its effects on metabolism.

B. Clinical-Scale Studies in Nutrigenetics

Some insights into human disease have been gleaned from GWAS, such as the identification of SNPs in the patatin-like phospholipase domain containing protein 3 (*PNPLA3*) gene that are highly associated with fatty liver, liver damage, and liver disease progression and the discovery of several key loci, including the fat mass and obesity associated (*FTO*) gene, that are associated with obesity and diabetes.^{3–6} However, most scientists agree that GWAS have not resulted in as many important gene–nutrition–disease linkages as had been expected when the human genome was first sequenced. Because of the nature of some nutrition research that involves controlled diets and complex phenotyping, it would be very useful if smaller clinical-sized studies could be used to generate data on metabolic individuality.

The size of the study needed to detect nutrient–gene interactions is defined by the design of the study; clinical-sized studies (~100 subjects) are viable if careful attention is given to several factors. First, limit the number of statistical comparisons that will be made, thereby increasing the power of the study (fewer corrections need to be made to avoid false discoveries). Rather than measuring millions of SNPs that are on the commercially available

chips, select SNPs on the basis of knowledge of the underlying metabolic process. For instance, for the nutrients of interest, select genes in pathways responsible for endogenous production, metabolism, and elimination of the nutrients, as well as genes in intersecting pathways. Because there are likely to be many SNPs in each of these genes, further focus can be attained by selecting SNPs that lead to defective protein products, such as SNPs that alter amino acid sequence in regions likely to impact function (e.g., catalytic or targeting domains), or by selecting SNPs in regions of genes that control expression (e.g., near transcription factor binding sites). These are the SNPs that are most likely to have functional effects. In addition, SNPs that are more commonly present in the population can be given priority over rarely occurring SNPs.¹

Precise assessment of gene sequence and expression is not enough; the variance in measurement of phenotype and/or diet exposure must also be minimized. The outcome measure (phenotype) selected should be one that can be accurately measured. Unfortunately, the assessment of diet exposure is often the weakest link, with large potential for errors in diet assessment being the accepted norm.⁷ For this reason, many investigators carefully control diets using monitored-meal paradigms in hospitalized or outpatient volunteers.⁸ It is important to consider diet when conducting studies searching for functional SNPs that influence nutrient requirements and metabolism. It is likely that many such SNPs cause metabolic inefficiencies that can be overcome if dietary intake of the nutrient is high. For example, the common SNP in the methylenetetrahydrofolate reductase (*MTHFR*; rs1801133) gene, which encodes an enzyme important for the remethylation of homocysteine to methionine, results in approximately a 50% reduction in enzyme activity in people homozygous for the variant allele.⁹ This SNP would be important in individuals eating diets low in folate but would be no problem in people with high folate intake. An analysis that did not consider diet intake might miss the effect in the low-folate group when it was averaged across groups eating more folate. With careful consideration of rationale, hypotheses, and study design, it is possible for a clinical-sized study to unravel some of the intricacies that regulate individual nutrition requirements.¹

C. Choline Research: A Platform for Exploring Metabolic Variation

The case study of the effects of genetic variation on dietary choline requirements provides an excellent example of how clinical nutrigenetics/nutrigenomics can be used to understand individual nutrition needs. A comprehensive translational research platform for choline has been instrumental in moving our understanding to a point where it is increasingly evident that optimal choline intake is predictable but varies greatly between individuals. The study of choline gives us a glimpse of the way one can utilize nutrigenetics and nutrigenomics to find individualized, applied solutions to nutrition-related health problems.

II. Choline Biology

A. Choline Functions

Choline is an essential water-soluble nutrient needed for the normal function of all cells. It is metabolized into several compounds that exert a wide range of biological functions including cell signaling, cholinergic neurotransmission, cell membrane structure,

mitochondrial function, cholesterol/lipid transport and metabolism, and methylation reactions.¹⁰ Betaine, phosphatidylcholine, sphingomyelin, and acetylcholine are examples of choline-containing compounds of physiologic relevance (Fig. 1).

- The conversion of choline to betaine is irreversible and occurs in the¹¹ mitochondria. Betaine is a methyl group donor that influences gene expression via epigenetic mechanisms¹² and is an osmolyte used in the glomerulus of the kidney to help reabsorb water.¹³
- Phosphatidylcholine (also called lecithin) is the predominant phospholipid (>50%) in most mammalian membranes and is also important for hepatic lipid packaging and export.¹⁴ Sphingomyelin is a phospholipid needed for membrane and myelin formation. Both phosphatidylcholine and sphingomyelin are sources of second messengers (including diacylglycerol, arachidonic acid, and ceramide) that alter cell function by potentiating signaling cascades.^{15,16}
- Acetylcholine is a neurotransmitter important in brain functions such as memory and mood. It is the neurotransmitter most often used by neurons interfacing between brain and the periphery; the nerves controlling skeletal muscles, heart rate, breathing, sweating, and salivation all use acetylcholine.

For additional details on choline metabolism, the reader is referred to a recent comprehensive review.¹⁰

B. Dietary Choline Requirements

Most of the foods we eat contain varying amounts of choline, choline esters, and betaine. The foods with the greatest abundance of choline are of animal origin, especially egg yolk and liver. Other sources of choline are wheat germ, soy, nuts, and other legumes. Many foods also have lecithin as an additive, usually used for its emulsifying properties, and this provides a significant amount of choline. Details about the choline content of foods are in the most current U.S. Department of Agriculture database (<http://www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/Choline/Choln02.pdf>). Human breast milk is also a good source of free choline and choline esters, and the manufacturers of infant formulas have recently modified the content of choline compounds to levels similar to those in breast milk.

Aside from dietary sources, humans possess a pathway to make choline moiety *de novo*, as part of a phosphatidylcholine molecule, via the phosphatidylethanolamine *N*-methyltransferase (PEMT) pathway.¹⁷ Based on the presence of this endogenous production pathway, for many years choline was classified as a nonessential nutrient. This was the case until 1998 when the U.S. Institute of Medicine (Food and Nutrition Board) established for the first time adequate intake (AI) and tolerable upper intake limit values for choline, based on limited human studies in which dietary choline deficiency was associated with liver disease in humans.¹⁸ Table I lists the AI levels for choline.

The dietary intake of choline has declined in recent years as people have been avoiding foods high in cholesterol (like eggs), which are the richest sources of choline. It is not surprising that the 2005 National Health and Nutrition Examination Survey (NHANES) data

show that only a small percentage of people in the United States, approximately 10%, achieve the recommended dietary intakes for choline.¹⁹ Several studies report that 20–25% of Americans eat one-third to half of the recommended AI for choline (<203 mg/day in the Framingham Heart Study²⁰, <217 mg/day in the Atherosclerosis Risk in Communities Study²¹, <293 mg/day in the Nurses' Health Study²²).

III. Utilizing Nutrigenetics and Nutrigenomics Approaches to Understand Choline Requirements

A. Nutrigenetics of Choline Requirements

One could surmise, based on the availability of an AI level, that the effects of insufficient or excess choline are well understood and that the current AI level is adequate to meet the needs of a broad range of individuals. The research platform for choline has advanced significantly since the establishment of the AI in 1998, and it is now clear that choline requirements are influenced by several factors. This knowledge has been gained through a multifaceted clinical, epidemiological, and basic science approach that has taken advantage of several nutrigenetics and nutrigenomics platforms including genomics, epigenetics, and metabolomics.

In a clinical-sized nutrigenetics study, adult men and women (pre- and postmenopausal) aged 18–70 years were hospitalized and fed a standard diet containing the AI for choline (550 mg/70 kg/day; baseline phase) for 10 days. On day 11, the subjects were placed on a diet containing <50 mg/day of choline for up to 42 days (depletion phase). Blood and urine were collected throughout the study to measure various parameters of dietary choline status, markers of organ dysfunction, and liver fat. If, during the study, functional markers indicated an adverse effect associated with choline deficiency, subjects were switched to a diet containing increasing amounts of choline until repleted (repletion phase). This study demonstrated that 77% of men, 80% of postmenopausal women, and 44% of premenopausal women developed elevated liver fat, increased liver enzymes, or increased muscle enzymes (creatine phosphokinase) when placed on a choline-deficient diet. In addition, 10% of subjects needed 850 mg/day of choline to avoid these same signs of deficiency. Importantly, the symptoms associated with choline deficiency were fully reversed when choline was reintroduced into the diet.^{8,23,24} These were clear indications that choline is indeed a required nutrient and that the requirement is not the same in all people.

Dietary choline requirements are governed by an individual's ability to make choline *de novo* and by the rates of choline utilization. Genes in choline, methionine, and folate metabolism are intertwined in the pathways for choline production and utilization (Fig. 2), and normal function of these genes is required for optimal choline status.

Humans have variation in the sequences for these genes (SNPs) and in the number of copies of these genes (copy number variation). While copy number variation in these genes has not yet been proven to be important, SNPs in these genes strongly influence dietary choline demands.^{8,23,26} One of the SNPs identified as having a role in choline-deficiency-related organ dysfunction is in the promoter region of the *PEMT* gene (rs12325817) and is very

common (the North Carolina, United States, population is 18% homozygous variant [VV], 26% homozygous ancestral alleles [WW]^{8,24}). In women with ancestral alleles, estrogen induces the *PEMT* gene, thereby providing an endogenous source of choline; thus, premenopausal women are relatively resistant to developing organ dysfunction when fed a low-choline diet.^{24,26,27} However, the *PEMT* rs12325817 SNP marks a haplotype with decreased estrogen-responsive induction of *PEMT*.²⁷ Women carriers of the variant allele developed organ dysfunction when fed a low-choline diet (odds ratio=25). Another SNP in the *PEMT* gene (rs7946) is present more often in people with fatty liver. This SNP was functional (altered dietary choline requirements) in individuals also eating a diet low in folate.²⁸ Premenopausal women who are carriers of an SNP in the gene-encoding 5,10-methylenetetrahydrofolate dehydrogenase (*MTHFD1*; rs2236225) are more than 85 times as likely as noncarriers to develop choline-deficiency-induced organ dysfunction. This also is a very common polymorphism (62% of the North Carolina population have one allele, and 11% are homozygous for the variant allele).²³ Another important enzyme in the choline metabolism pathway is choline dehydrogenase (*CHDH*). We observed that an SNP in the *CHDH* gene (rs12676; 37–42% of the population have the variant allele and ~9% are homozygous for the variant²⁹) increased by 20-fold the susceptibility to develop organ dysfunction when premenopausal women were fed low-choline diets.⁸ As studies progress, additional SNPs are being identified that mediate individual choline requirements. The relevance of SNPs on optimal nutrient intakes is different depending on gender and ethnicity and probably on other factors that we do not yet understand.

B. Mouse Models for Studying Choline Requirements

The studies in humans described above identified multiple functional SNPs in choline metabolism. The nature of human studies makes it difficult to assess all of the potential effects of these SNPs. Genetic manipulation of mice is relatively easy, and it is possible to delete genes of interest in mice to determine their functional significance. It is important to realize that the SNPs of interest mark a haplotype, often with multiple SNPs in linkage disequilibrium, and that the actual SNP causing the functional effect may not be the one identified. In addition, patterns of SNPs in multiple functionally related genes likely converge to lead to observed phenotypes. Gene deletions result in a total loss of activity, while gene polymorphisms at worst result in 100% loss of gene function but generally result in intermediate degrees of loss (sometimes they result in a gain of function). With these caveats, genetically manipulated mouse models can be a very useful translation from bedside to bench research. They enhance our ability to design future nutrigenetics studies by allowing us to carefully define relevant mechanisms that can be studied in humans.

The *Pemt* knockout mouse (*Pemt*^{-/-}) seizes and dies when deprived of choline,³⁰ it hypermethylates proteins and DNA,³¹ it has greatly diminished omega-3 fatty acids in membrane phosphatidylcholine,^{32,33} and it has abnormal brain development.^{31,33} Importantly, it also has fatty liver because of the inability to maintain normal concentrations of all choline metabolites,³⁴ making it a useful model for studying mechanisms that might be important for choline-deficiency-mediated fatty liver in humans. The *Chdh* knockout mouse (*Chdh*^{-/-}) is remarkable. In the tissues that express this gene, its deletion resulted in abnormal mitochondrial morphology and function.¹¹ In addition, there was infertility due to

diminished sperm motility in the *Chdh*^{-/-} males secondary to reduced ATP production by mitochondria. Each of the phenotypes discovered in mouse models is now being investigated in humans with the relevant SNPs.

C. Choline, Epigenetics, and Brain Development

A human being has a fixed genetic code but attains some measure of genetic flexibility through epigenetic modifications (usually methylation of DNA or methylation/acetylation of histones associated with DNA), which integrate environmental cues (including diet) with the genome.³⁵ The fetal environment transfers signals to the fetus that reflect the expected environment after birth. These signals lead to a shift in gene expression and modification of biological pathways in a direction that should confer a survival benefit in the expected postnatal environment.³⁶

The DNA methyltransferases, which catalyze the transfer of a methyl group to DNA, all use *S*-adenosylmethionine as the methyl donor. Choline influences DNA methylation because it is a major dietary methyl donor. As shown in Fig. 2, it is at the methylation of homocysteine that the choline, betaine, folate, and methionine metabolic pathways intersect. Homocysteine methylation occurs by two parallel pathways; in the first, vitamins B₁₂ and folic acid are involved in a reaction catalyzed by methionine synthase.³⁷ The alternative pathway for the methylation of homocysteine to form methionine is catalyzed by betaine–homocysteine methyltransferase (BHMT).³⁸ These pathways may be fungible, in that perturbations in one are compensated for by adjustments in the parallel pathway. Rats treated with the antifolate methotrexate had diminished pools of choline metabolites in liver.^{39,40} Rats ingesting a choline-deficient diet had diminished tissue concentrations of methionine and *S*-adenosylmethionine⁴¹ and doubled plasma homocysteine concentrations.⁴² Humans depleted of choline had diminished capacity to methylate homocysteine and developed elevated homocysteine concentrations in plasma after a methionine loading test.⁴³

DNA methylation occurs at cytosine bases that are followed by a guanosine (CpG-sites).⁴⁴ In mammals, most CpG-sites in DNA are methylated (90–98%⁴⁵), but there are specific CpG-rich areas of DNA where most CpGs are not methylated; these are called CpG islands.⁴⁶ It was initially believed that CpG islands that span the 5'-end of the regulatory regions of genes were the only places where DNA methylation occurred, and that when these CpGs were methylated, gene expression was usually suppressed or silenced.^{46,47} We are learning that epigenetic marks are not limited to CpG islands. They are also found in shores of these islands, exons, and intergenic DNA. Methylation may be the default state for genes, and though the purpose for methylation of intragenic DNA is unclear at this time, it may protect against expression of unwanted genes.⁴⁵

Pregnancy and the early postnatal period are important times when choline, via epigenetic mechanisms, has been shown to play a significant role in the development and health outcomes later in life. During pregnancy, there is both a high demand for and an increased production of choline via the PEMT pathway, as the gene is induced by estrogen.^{27,48} In general, this additional production is insufficient to meet choline requirements, and in rodent models choline pools are depleted during pregnancy.⁴⁹ Large amounts of choline are transferred to the fetus across the placenta, and fetal choline concentrations are many fold

higher than adult concentrations.⁵⁰ During critical periods of gestation, low choline availability can lead to poor brain development and long-term cognitive and behavioral impairments in rodents.^{51–53} In addition, several human studies have shown increased incidence of neural tube defects and orofacial cleft defects in infants when pregnant mothers consumed a diet deficient in choline.^{54–56} Interestingly, choline supplementation can improve the behavioral and developmental consequences of maternal alcohol intake on fetal development in rodent models.^{57–60} Choline also protects against seizure-induced memory impairment in rodent models.⁶¹ These data suggest that choline has a protective effect during a suboptimal fetal environment.

One plausible mechanism mediating the role of choline (a methyl donor) in development is via the modification of epigenetic marks that regulate gene expression. In brain and other tissues, a choline/methionine-deficient diet directly altered methylation in CpG islands within several genes.⁶² After feeding pregnant rat dams a choline/methionine-deficient diet, neural progenitor cells of the fetal hippocampus proliferated half as fast compared to fetal brain from mothers fed a control diet. The choline-deficient neural progenitor cells had decreased gene-specific DNA methylation in CpG islands of *Cdkn3*. This gene encodes kinase-associated phosphatase, and when hypomethylated, the gene is overexpressed, resulting in increased phosphatase levels and subsequent activation of the retinoblastoma protein pathway that inhibits cyclin-dependent kinase.^{63,64}

Epigenetic marks on histones (the proteins around which DNA is wound) also regulate gene expression. In mice, maternal choline deficiency during pregnancy altered methylation of lysine residues on histone H3 in fetal neural progenitor cells from gestational day 17 in the areas of the hippocampus where neurogenesis was occurring.⁶⁵ Monomethyl and dimethyl lysine 9 on histone H3 residues are usually associated with silencing of genes, whereas dimethyl and trimethyl lysine 4 on histone H3 are enriched in areas with transcriptionally active chromatin. Thus, choline availability modulates histone methylation and thereby gene expression and proliferation in fetal neural progenitor cells.

Similarly, maternal dietary choline intake modulates angiogenesis, the formation of new blood vessels, in fetal brain.⁶⁶ In mice, maternal diets low in choline were associated with diminished proliferation of endothelial progenitor cells in fetal hippocampus, and the mechanism involved decreased DNA methylation in fetal brain within the promoter of two genes that are important regulators of angiogenesis (vascular endothelial growth factor C, *Vegfc* and angiopoietin 2, *Angpt2*).⁶⁶ This shows that choline's effects on brain development are not limited to neuronal cells, but extend to endothelial cells and blood vessel formation in the fetal hippocampus.

As noted earlier, maternal dietary choline deficiency increases neural tube closure defects in rodent and human fetuses.^{56,67–69} Later in gestation, maternal choline deficiency alters the development of fetal hippocampus by decreasing neural progenitor cell proliferation and by increasing apoptosis and expression of markers of differentiation.^{70–72} The offspring of choline-deficient mothers exhibited insensitivity to long-term potentiation when they were adult animals⁷³ and decremented visuospatial and auditory memory.⁷⁴ More choline (about four times higher than normal) during days 11–17 of gestation in rodents increased

hippocampal progenitor cell proliferation,^{75,76} decreased apoptosis in these cells,^{75,76} enhanced long-term potentiation in the offspring when they were adult animals,^{73,77,78} and enhanced visuospatial and auditory memory by as much as 30% throughout their lifetimes.^{51,53,74,79–82} Indeed, adult rodents decline in memory as they age, and offspring exposed to extra choline *in utero* do not show this “senility.”^{80,81} Thus, choline supplementation during a critical period in pregnancy causes lifelong changes in brain structure and function, probably by changing epigenetic marking.

There are other good examples of how powerful diet can be in changing epigenetic marks. Feeding pregnant pseudoagouti Avy/a mouse dams a choline-, methionine-, and folate-supplemented diet altered epigenetic regulation of agouti expression in their offspring, as indicated by increased methylation of the involved gene and by agouti/black mottling of their coats.^{12,83} In another example,⁸⁴ there was increased DNA methylation of the fetal gene *axin 1 fused* (*Axin1^{Fu}*) after methyl donor supplementation of female mice before and during pregnancy, which reduced by 50% the incidence of tail kinking in *Axin1^{Fu/+}* offspring. It is clear that the dietary manipulation of methyl donors (either deficiency or supplementation) can have a profound impact on gene expression via changes in epigenetic marks.

D. Metabolomics, Gut Microbiome, and Choline Requirements

Metabolomics, the characterization of small-molecule metabolites in a cell or tissue, can give us an overall understanding of the function of biological pathways. This is another scientific discipline that is an important part of nutrigenomics because it allows for the characterization of health status and the monitoring of the efficacy of nutrition interventions. In an effort to define metabolites that could be used as biomarkers to predict which people are at risk for developing choline-deficiency symptoms, a study was developed using targeted and untargeted metabolomics. This study demonstrated that a set of molecules, including choline and folate metabolites, amino acids, and acylcarnitines, could accurately predict which humans would develop fatty liver when placed on a choline-deficient diet.⁸⁵ This study highlighted the value of using metabolomics to define signatures relevant to nutrition requirements.

It is now well accepted that human biology is intricately intertwined with the function of the trillions of bacteria (gut flora or gut microbiome) that inhabit the intestinal tract. The bacterial genome and its active products are participants in host health. In particular, the gut flora play an important role in the breakdown and absorption of many nutrients. This is an important factor to consider in the design and interpretation of nutrigenetics and nutrigenomics research studies because it influences nutritional individuality. In a metabolomics study in mice, it was shown that certain gut bacteria break down choline before it is absorbed, essentially mimicking a choline-deficient scenario. Mice with a gut microbiota that can hypercatabolize choline developed fatty liver and impaired glucose metabolism.⁸⁶ In one of only a limited number of studies related to the gut microbiome in humans, a choline deficient diet led to alterations in gut microbes that correlated with the development of fatty liver. This effect was strengthened when combined with knowledge of genotypes in choline-related genes.⁸⁷

A recent paper characterized metabolomic profiles that could predict risk for cardiovascular disease in mice and humans. In that study, elevated levels of choline, betaine, and trimethylamine-*N*-oxide (a metabolite of choline) were associated with increased macrophage cholesterol and foam cell formation, which are hallmarks of the atherosclerotic process.⁸⁸ Although it is difficult to discern whether alterations in choline metabolites are a cause or consequence of cardiovascular disease, it is certainly reasonable to postulate an important role for choline in cardiovascular health.

This exciting new field holds much promise for giving us a comprehensive understanding of nutrition systems biology, and extensive research is needed to fully understand the role of the gut microbiome in human health. These examples of metabolomics and gut microbiome studies highlight important nutrigenomics tools that can be applied and integrated to develop a more comprehensive picture of the causes and consequences of nutrient inadequacies.

IV. Summary and Future Directions

A. Lessons from Choline

The research platform built to understand the individual nutrition requirements for choline has brought to light several important concepts that should prompt a shift in standard clinical nutrition paradigms. Although nutrition recommendations developed for populations are valuable for helping the majority of people avoid the consequences of nutritional imbalances, there will undoubtedly be a subset of individuals who are unable to reach optimal health through these requirements and, more importantly, who could suffer harm from deficiency or toxicity. Of great importance is the fact that, under certain metabolic backgrounds and depending on exposure, choline could have both positive and negative effects on health outcomes. This paradox is actually quite common in nutrition, where nutrients are found to be beneficial for some and not others.

Rather than looking at outliers or paradoxes as a source of “noise” in our datasets, we should instead see them as the source of better mechanistic insight into the function of a nutrient. By studying the reason for hypo- or hypersensitivity to a nutrient, we can gain valuable information about key functional mechanisms that mediate the nutrient’s biological effects. It is also evident from choline research that better health outcomes are the result of a diet that is well matched both to the environment during fetal development and to an individual’s genotype. This strongly supports the notion that dietary recommendations need to be tailored to developmental needs and biological signatures.

Additional valuable insights from choline research are that a well-designed clinical study can lead to a great deal of information about individual nutrition requirements and that SNPs can have a very large impact on phenotype. Choline research also clearly demonstrates how nutrients directly impact biological pathways on many levels that go beyond changes in transcription of genes, such as epigenetics, organelle function, and metabolic flux.

B. From Research Platforms to Applied Solutions

Despite the great progress in nutrition science since the sequencing of the human genome, we are still not at a point where we can utilize advanced diagnostic tools to prescribe

individual diets based on unique biological signatures. Several important solutions are needed to overcome the hurdles facing nutrigenetics and nutrigenomics. Technology is a key solution in the pursuit of defining nutritional individuality. The development and widespread availability of cutting-edge methodologies to interrogate biological systems is a central application of technology in nutrigenomics. In recent years, many such methods have become accessible to study gene expression, epigenetic marks, genetic variation, metabolite profiles, and protein expression. Sophisticated new bioinformatics tools have been instrumental in managing large nutrition data sets. These advances have been central to the progress in nutrigenomics thus far, and many more advances will be needed to continue this forward motion. Perhaps the most important way to move nutrigenetics and nutrigenomics from an exciting set of ideas into applied reality is the integration of scientific findings into clinical settings so that people can utilize the most current biomedical knowledge to guide their lifestyle choices. In order to implement biomedical science findings into clinical practice, it is clear that nutrition science requires a translational platform where one can seamlessly shift knowledge back and forth from bench to bedside and that utilizes multiple cutting-edge and classical methodologies. It is with this concept of individualized nutrition recommendations, learned in part from our understanding of choline requirements, that we can move toward optimally effective nutrition interventions.

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FIG. 1.
Structures of several important choline-containing molecules.

FIG. 2.

Choline, folate, and homocysteine metabolism are closely interrelated. The pathways for the metabolism of these three nutrients intersect at the formation of methionine from homocysteine. BADH, betaine-aldehyde dehydrogenase; BHMT, betaine-homocysteine methyltransferase; CDP, cytidine diphosphate; ChAT, choline acetyltransferase; CHDH, choline dehydrogenase; CK, choline kinase; CPT, choline phosphotransferase; CT, cytidine triphosphate (CTP):phosphocholine cytidyltransferase; MS, methionine synthase; mTHF, methyltetrahydrofolate; PEMT, phosphatidylethanolamine *N*-methyltransferase; THF, tetrahydrofolate. From Ref. 25, with permission.

TABLE I

ADEQUATE INTAKE (AI) VALUES FOR CHOLINE (MILLIGRAMS PER DAY)

Life stage	Age	Males	Females
Infants	0–6 months	125	125
	7–12 months	150	150
Children	1–3 years	200	200
	4–8 years	250	250
	9–13 years	375	375
Adolescents	14–18 years	550	400
Adults	19 years and older	550	425
Pregnancy	All ages	–	450
Breast feeding	All ages	–	550

Modified from Ref. 18.