The Biochemistry Behind Functional Lab Assessment

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Introduction and Context

Functional medicine clinicians are often early adopters of translational testing, from bench to practice, though RDNs need to ensure the tests they use are evidence-based and supported in peer-reviewed literature. A search in PubMed, Medline, or other source should evaluate the specific test, including the laboratory process and specimen type (blood, urine, fecal), to identify how many studies have utilized the test, how many people were included in the studies, and if a consensus was reached. If no support can be found in peer-reviewed literature, caution should be used. There has been a significant increase in laboratory tests marketed to clinicians and directly to consumers, such as microbiome testing, DNA (SNP) tests, nutritional status, etc. As RDNs it is important to understand how to evaluate the science behind the tests and any recommended therapies clients want to undertake based on the testing.

Laboratory testing includes both direct and functional measurements. A direct laboratory test looks at the actual analyte, such as vitamin D or lactate. A functional marker identifies how well a biochemical pathway is functioning, especially those dependent on nutrient cofactors. For example, methylmalonic acid (MMA) is a functional marker for vitamin B12. Vitamin B12 is a needed nutrient cofactor for the enzyme methylmalonyl-CoA mutase. If vitamin B12 is inadequate, the conversion cannot take place and MMA becomes elevated. Many markers, such as lactate, can be both direct and functional markers. Ideally, a combination of laboratory testing and diet assessment gives the best picture of an individual’s nutrient status.

Some analytes have a “gold-standard” specimen and process, though generally there are several available and it depends on what question you are looking to answer. Urine amino acids can tell you what a person has eaten over the last few days, while plasma amino acids can tell you more about nutritional status. Some specimen types primarily work best for research purposes and cannot be easily shipped. Another important area is ensuring that directions are followed for most accurate results, as reference ranges are set on the specific steps listed in the company’s testing instructions. A reference range on a test report is established in two primary ways. The laboratory can establish the reference range itself using a large group of subjects known to be free of disease, or it can utilize an already established reference range. For markers without an established reference range, the laboratory generally sets the range by testing a healthy population and identifies “normal” as within two standard deviations of the mean. Though practice-based reference ranges have been proposed, they lack evidence and caution should be used.

There are two types of sensitivity and specificity: analytical and clinical. Analytical refers to how well a compound is identified by the laboratory. Clinical pertains to how well a specific compound
Editor’s Notes

This summer not only starts another Academy program year, but it is the 20th anniversary of our DPG and the beginning of a new chapter for The Integrative RDN. As many of you already know, Sarah’s last issue as editor was this past spring. For 17 years and about 70+ issues, Sarah has successfully and gracefully navigated the unpredictable terrain that is producing a newsletter. That kind of commitment, loyalty, and continuity is extremely rare and has been instrumental in the development, growth, and proliferation of not only the newsletter, but DIFM and IFN at large. Thankfully for us, being a parent doesn’t end at 17, so I’ll be looking to Sarah, as will other executive committee members, to be a continual guiding light. Importantly, I want to thank her for entrusting the position to me. With a BA in journalism, past editorial jobs, and a former career in television and music production, this position is a perfect marriage of past and present passions. My nutrition training began with integrative and functional medicine philosophies—it’s the only way I’ve practiced, and it transforms lives. I want to recommit the newsletter to the same principles originally set forth in 1998: to empower members to be leaders in integrative and functional nutrition by providing significant educational value and acting as a major communication tool for the exchange of ideas and experiences.

I would not have accepted this role if not for the experience and steadfast talent of our copy editor, Holly Van Poots, RDN, CSP, FAND and the seasoned editors that have been on the team for many years: Shari Pollack, MPH, RDN, and Dina Ranade, RDN, LD. I’m even more excited about the expertise and enthusiasm of our newer editors: Olivia Dong, Janie Jacoby, Chrissy Barth, Ceci Snyder, Julia Whalen, and Tarah Allen. Much appreciation to Staci Belcher and Anita Davila for always coming up with innovative ways to attract and maintain student interest.

In this issue, we examine the biomarkers and pathways that provide the rationale for functional lab testing. Functional labs have become an important tool for getting to the root cause of disease, practicing preventatively, and personalizing interventions. As the nutrition landscape continually changes, so does the way we test and assess. For comparison, you can go back into the archives to the Fall 2009 newsletter to read our first article on the subject, by the same author, and see how much has changed. We’re also tackling one of the most well-known single nucleotide polymorphisms, methylenetetrahydrofolate reductase, or MTHFR, and all of its biochemical implications. There is always much more to learn, but we hope this serves as primer or a jumping-off point for further education.

As a reminder, the newsletter will be digital only for summer, winter, and spring, with our fall issue in print and digital. To that end, we are currently in the planning stages for winter and spring and are looking for writers and articles! If you have a new way of seeing an old problem, feel like a condition has not been represented, or an issue you feel DIFM RDNs should be educated about, please contact me anytime at jenas_mailbox@yahoo.com. Wishing you all a sunny and relaxing end to summer!

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I’m super excited and honored (and just a little bit nervous) to be writing my first column as chair of our amazing DPG! DIFM is 20 years old this year and has grown by leaps and bounds since 1998. I couldn’t be more thrilled to be taking the reins as we move into another great year of expansion.

As I was preparing for our spring leadership meeting last month, I decided on three words that would serve as sign posts for my leadership year: Expand, Connect, Update—and these words have already begun to serve me and the leadership team. We have so many great things in store for DIFM this year!

For one, we plan to update our newsletter to offer even more IFN articles as we move to more expanded digital offerings. We are updating the website to better integrate all of our amazing member resources like this newsletter, the blog, our network offerings, and educational webinars. This also includes updating the way we communicate with each other through the EML. We have plans to move the Yahoo! EML to a more user-friendly forum on our website where we can create IFN communities and individual practice areas. Those of you seeking connection with other integrative RDNs who share your same interests (whether it be yoga and mind-body modalities or functional lab testing and nutrigenomics), we want you to be able to communicate and share knowledge more easily and effectively—all in one place!

We also plan to create a research library where you can find and share evidence-based resources as well as client and patient-focused handouts for use in your practice.

Speaking of research, I’m thrilled to share that DIFM is partnering with the Academy Foundation to fund the Nutritional Genomics/Personalized Nutrition Evidence Analysis Library (EAL) Project. DIFM’s involvement in an EAL topic and a JAND publication will help bring nutritional genomics into the mainstream nutrition conversation, and DIFM will be leading the way in this effort. The DIFM Executive Committee believes this project aligns with DIFM’s strategic mission, and our contribution will have great impact within the Academy and the profession as a whole.

Did I mention we’re killing it on social media, too? Our active Facebook page has well over 15,000 followers, and our DIFM “brand” is becoming more and more recognizable online. We have a thriving student population and are now the 3rd largest DPG. Integrative and functional nutrition is the future of our profession, and I encourage you to join us on our mission to make it the only way to practice dietetics.

What initiative am I the most excited about this year? Pretty much everything!

Danielle
with clinical disease or symptoms. In a study by Yamamoto et al, researchers evaluated the predictive value of calprotectin, a marker of GI inflammation, to identify relapse in ulcerative colitis patients and found that a cutoff value of 115 μg/g of fecal calprotectin had an 83% sensitivity and 81% specificity in predicting relapses. In clinical terms, the sensitivity is the ability of a test to correctly identify those with the disease (true positive rate), and specificity is the ability of the test to correctly identify those without the disease (true negative rate).

**Integrative & Functional Medical Nutrition Therapy (IFMNT) Radial: Biomarkers and Pathways**

The IFMNT Radial can be used to discuss areas of assessment and includes five key areas: lifestyle, systems, core imbalances, metabolic pathways, and biomarkers. Biomarkers can be laboratory values and include the following groups: Digestion/Absorption, Genomics/SNP, Immune/Inflammation, Metabolism/Macronutrients, Micronutrients, Metabolomics, and Toxins.

**Digestion and Absorption**

Functional assessment of digestion and absorption includes tests related to the gastrointestinal tract and its function. Some of the more common markers and tests include:

- **Assessment of ability to digest and absorb macronutrients**
  - Pancreatic Elastase 1 (PE1): PE1 is a proteolytic enzyme that identifies the function of the exocrine pancreas (not to be confused with the endocrine pancreas). The exocrine pancreas produces 3 types of enzymes: amylase, protease, and lipase. If it is impaired, digestion may be impaired. Exocrine pancreatic insufficiency is identified by a fecal elastase level <200 μg/g stool.¹
  - Fecal fat is an evaluation of fat malabsorption. The gold standard for fat malabsorption is a 3-day quantitative determination of fecal fat. Though it is cumbersome and takes significant dietary preparation, a single fecal assessment is often done as an initial evaluation.²
  - Assessment of intestinal bacteria
    - Breath test (BT): The BT is done to diagnose small intestinal bacterial overgrowth (SIBO), carbohydrate malabsorption, methane-associated constipation, and evaluation of bloating/gas. After a 1- or 2-day preparatory diet, patients drink a sugar solution of glucose or lactulose, then breathe into collection tubes several times over 2 or 3 hours. A 2017 consensus paper found a rise in hydrogen of ≥20 ppm by 90 minutes positive for SIBO; methane levels ≥10 ppm are methane-positive.³
    - Lactulose contains galactose and lactose and is a nondigestible disaccharide that reaches the colon. It is not recommended for individuals who have had allergic reactions to lactulose or who are on galactose/lactose-restricted diets. It should be used with caution in diabetics. Glucose is a monosaccharide which is absorbed in the proximal small bowel.
    - Intestinal permeability: Disruption of the intestinal barrier impairs function and may increase risk of disease.
      - The lactulose-mannitol test evaluates intestinal permeability. It is a urine test following ingestion of a premeasured solution of lactulose and mannitol. The degree of intestinal permeability or malabsorption is reflected in the ratio of the two sugars recovered in a urine sample collected over time.⁴ ⁵
      - Zonulin is a physiologic modulator of intercellular intestinal tight junctions. An increase in zonulin identifies a possible “leaky gut” or permeability and has been related to autoimmune disease, metabolic disorders, heart disease, and gastrointestinal diseases.⁶ ⁷ ⁸
    - Microbiome: A fecal test can evaluate commensal bacteria and/or the intestinal microbiome. The microbiome is a primary player in overall health and is heavily impacted by diet, lifestyle, environment, etc. There are several culture-independent techniques available, such as polymerase chain reaction (PCR), pyrosequencing, etc. The tests are done by several different laboratories. While each are valid assessments, the results are not comparable. Even laboratories that do the same stated processes, such as PCR assessment, are generally not comparable due to a lack of universal standardization.

**Genomics/SNPs**

Nutrigenomics is the study of the interaction of nutrition and genes. Test profiles are often buccal swabs or saliva and packaged to evaluate a specific area such as weight loss, immune function, nutrient absorption, etc. Testing may help to personalize diet treatments. Previously the Food and Drug Administration raised concerns about the validity of the information and the potential for inappropriate medical actions, highlighting the need for RDNs to be aware of the literature on individual SNPs.⁹

**Immune/Inflammation**

Nutrition is related to the immune system in several ways. Nutrients are the substrates for many immune reactions, such as fatty acids and immune cytokines; nutrients can modulate the immune system, such as antioxidants tempering immune responses to inflammation; the immune system reacts to components in foods, such as proteins, toxins, and other bioactive components; and nutrient deficiencies or excesses can lead to increased oxidative stress.¹⁰
Immune Reaction to Foods:
Utilizing laboratory testing to evaluate a patient’s reaction to foods can be controversial. True allergic reactions are IgE reactions, while others are food intolerances or sensitivities. Proposed assessment of non-IgE tests of immune reactions to foods includes:

- Standard celiac assessment includes blood tests of IgA anti-gliadin and tissue transglutaminase (tTG) and is well accepted. The gold standard for celiac diagnosis is an intestinal biopsy.
- IgG testing (ELISA): Total IgG includes IgG1, IgG2, IgG3, and IgG4. IgG testing is controversial. Limited evidence from peer-reviewed research has found correlations with migraines, IBS, IBD.11-14
- Leukocyte Activation Tests (WBC: White Blood Cell): WBC tests—both mediator release and leukocyte activation tests—evaluate changes in WBCs, though they are different processes. Both are also controversial with limited literature.15

Inflammation:
Though acute inflammation is a response to stimuli, a chronic inflammatory state has been identified as an underlying basis in the development of chronic disease that can be identified by markers such as high-sensitivity C-reactive protein (hsCRP) and fibrinogen.16 This low-grade systemic and chronic inflammation has been termed "metaflammation" and is linked to modern environments and lifestyles.17 Evaluation of inflammation can be site-specific, such as using fecal calprotectin to evaluate gastrointestinal inflammation, lipid peroxides to identify fatty acid membrane oxidation, and 8-hydroxy-2-deoxyguanosine to evaluate oxidative damage to DNA. Nutrient deficiencies or excesses can lead to increased oxidative stress and inflammation.10

Metabolic
Metabolic testing includes more conventional laboratory assessments such as cholesterol, glucose, hemoglobin A1c, liver enzymes, etc, and should be evaluated as part of a standard of care. Functional nutrition assessment includes standard of care and a deeper dive into what else may be contributing to health issues.

Macronutrients

Carbohydrates:
Conventional assessment of the body’s response to carbohydrates includes glucose and hemoglobin A1c. Additional early markers of biochemical disruption using metabolomics include elevated alpha-hydroxybutyrate or plasma levels of branched-chain amino acids (BCAA) which have both been found to correlate to early risk of type 2 diabetes and cardiovascular disease.18,19 The composition of the microbiome can also identify the impact of carbohydrate/fiber. Plant-focused diets high in carbohydrates/fiber are associated with greater microbial diversity and higher levels of Prevotella and short chain fatty acids (eg, acetate, propionate, butyrate). Low levels of short chain fatty acids have been associated with poor carbohydrate/fiber intake.20

Protein:
Plasma assessment of individual amino acids may help to identify overall level of protein intake and processing. Plasma assesses longer-term status and is recommended over urine. The table below lists the precursor amino acids and their downstream end products.

Table 1. Individual Amino Acids (IOM)21

<table>
<thead>
<tr>
<th>Precursor Amino Acids</th>
<th>End Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan</td>
<td>Serotonin (melatonin); nicotinic acid (B3)</td>
</tr>
<tr>
<td>Phenylalanine to tyrosine</td>
<td>Catecholamines; thyroid hormones; melanin</td>
</tr>
<tr>
<td>Lysine</td>
<td>Carnitine</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Taurine</td>
</tr>
<tr>
<td>Arginine</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>Glycine</td>
<td>Heme</td>
</tr>
<tr>
<td>Glycine, arginine, methionine</td>
<td>Creatine</td>
</tr>
<tr>
<td>Methionine, glycine, serine</td>
<td>Methyl group metabolism</td>
</tr>
<tr>
<td>Glycine, taurine</td>
<td>Bile acids</td>
</tr>
<tr>
<td>Glutamine, cysteine, glycine</td>
<td>Glutathione</td>
</tr>
<tr>
<td>Glutamate, aspartate, glycine</td>
<td>Nucleic acid bases</td>
</tr>
</tbody>
</table>

Fat:
Evaluation of fatty acids of varying carbon chain lengths and degree of saturation can help to identify impact of diet and individual variability. Patterns of individual fatty acids may help to evaluate function of desaturase and elongase enzymes. For example, in those supplementing with flax, rich in alpha-linolenic acid (ALA), to increase eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), testing fatty acids may identify if and how well the conversion is taking place, since the conversion from ALA is less efficient in some individuals.22 Testing of fatty acids can be done in whole blood, red blood cells (RBC), or plasma. Results can be reported as percent of total or quantitatively as total weight; both are used in the literature, though they cannot be easily compared. Evaluating testing specifics when reviewing research studies is important since differences in methodology and study design may result in discrepancies in conclusions.23

Micronutrients

Micronutrients are common enzyme cofactors and are essential for flow of biochemical pathways. For review of nutritional tests, the Institute of Medicine (IOM) has comprehensive reviews of available laboratory assessment tests for each nutrient. In each nutrient chapter, there is a review of the ways each nutrient can be assessed, followed with the process that was selected and why.24 Even if there is disagreement or if new testing has been introduced, it does provide a good review of a range of laboratory tests and concerns.
### Minerals: Table 2. Laboratory Markers for Minerals

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Criteria Used by IOM to Establish RDAs[^24][^25]</th>
<th>Other Proposed Testing (Not a complete list)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium</td>
<td>A serum magnesium concentration of &lt;0.75 mmol/liter (1.8 mg/dl) is thought to indicate magnesium depletion.</td>
<td>• Serum/plasma magnesium concentration, RBC magnesium, and urinary magnesium excretion appear to be useful biomarkers of magnesium status in the general population.[^26]</td>
</tr>
<tr>
<td>Zinc</td>
<td>Factorial analysis was used to set the Estimated Average Requirement (EAR).</td>
<td>• Physical growth response to zinc supplementation[^25] • While both plasma and serum zinc concentration are used as indicators of zinc status, plasma zinc concentration is preferable.[^27]</td>
</tr>
<tr>
<td>Copper</td>
<td>The primary criterion used to estimate the EAR for copper is a combination of indicators, as no single indicator provides an adequate basis on which to estimate the copper requirement.</td>
<td>• Serum/plasma copper concentration is a reliable indicator of copper deficiency; serum or plasma copper concentration is generally insensitive to detection of copper overload.[^25] Serum copper may fall 30% in deficiency.[^28]</td>
</tr>
<tr>
<td>Selenium</td>
<td>The IOM set the RDA on the amount of selenium needed to maximize synthesis of the selenoprotein glutathione peroxidase.</td>
<td>• Whole blood selenium[^29] • Plasma selenium can be useful to identify selenium status. Normal levels are reflective of diet; at elevated levels, genetics and environmental factors have the biggest impact.[^30] • Dietary selenium and urinary excretion are correlated.[^31]</td>
</tr>
</tbody>
</table>

### Vitamins: Table 3. Laboratory Markers for Vitamins

<table>
<thead>
<tr>
<th>Vitamins</th>
<th>Criteria Used by IOM to Establish RDAs[^24][^25][^30]</th>
<th>Other Proposed Testing (Not a complete list)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>The Estimated Average Requirement (EAR) is based on the assurance of adequate stores of vitamin A. Under controlled feeding conditions, dark adaptation, measured by dark adaptometry, is one of the most sensitive indicators of a change in vitamin A deficiency status.</td>
<td>• Though not feasible, assessment of liver reserves of vitamin A is considered the gold standard because the liver is the major storage organ.[^32] • Serum retinol concentration is a common method used to evaluate vitamin A deficiency and considered a surrogate biochemical measure of liver reserves. Drawbacks are that serum retinol concentrations are controlled until reserves are dangerously low, and serum retinol and retinol binding protein (RBP) concentrations fall during times of infection.[^32]</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Serum 25-hydroxyvitamin D, or 25(OH)D, concentration was used to establish adequacy, though the “ideal” reference range is controversial.</td>
<td>• Serum 25(OH)D is the accepted standard assessment for vitamin D and reflects vitamin D stores. Vitamin D assessment has two different unit types: nmol/L and ng/mL. There is limited consensus on level of deficient, sufficient, or optimal: 30 ng/mL (75 nmol/L) is a proposed cut point of sufficiency.[^33] The Vitamin D Standardization Program (VDSP) has identified liquid chromatography-mass spectrometry (LC-MS) as the preferred reference method for 25(OH)D.[^33]</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>The latest RDA for vitamin E is based on the correlation between hydrogen peroxide-induced erythrocyte lysis and plasma α-tocopherol concentrations.[^34]</td>
<td>• Plasma vitamin E is not a strong indicator in subjects with adequate vitamin E levels, though there is a linear increase in vitamin E–depleted individuals.[^30] • Serum concentrations of α-tocopherol below 20 µmol/L was used as a cut point in a review of NHANES data.[^35]</td>
</tr>
<tr>
<td>B1</td>
<td>The method used to estimate the RDA for thiamin combines erythrocyte transketolase activity (ETK) and urinary thiamin excretion. Thiamin status can be assessed by determining ETK, measuring the concentration of thiamin and its phosphorylated esters in blood or serum, or by measuring urinary thiamin excretion under basal conditions or after thiamin loading.</td>
<td>• Diagnosis of thiamin deficiency is usually based on a favorable response to treatment with thiamin in a patient with symptoms or signs of deficiency.[^30] • The concentration of thiamin diphosphate (TDP) in erythrocytes is a useful index of thiamin status. The transketolase activation (ETK) test is also considered good if it is set at 15–25% as abnormal.[^30] • Urinary branched-chain α-keto acids (organic acids) positively identified subjects with B vitamin–complex deficiency, those with the highest level of urine BCAA were more likely to be B vitamin–deficient.[^36]</td>
</tr>
</tbody>
</table>
B2 Riboflavin
A combination of criteria was used to estimate the RDA for riboflavin, including the erythrocyte glutathione reductase activity coefficient and urinary riboflavin excretion.

Vitamin B6
Plasma 5'-pyridoxal phosphate (PLP) value of at least 20 nmol/L was used to set the RDA. Plasma PLP seems to reflect B6 intake and PLP content in liver; PLP content in the muscle is resistant to B6 depletion.38

B3 Niacin
Urinary excretion of the two major methylated metabolites, N1-methyl-nicotinamide and its 2-pyridone derivative (N1-methyl-2-pyridone-5-carboxamide), was used to set the RDA and has been noted as a reliable and sensitive measure of niacin status.

Folate
The primary indicator used to estimate the RDA for folate was erythrocyte (RBC) folate in conjunction with plasma homocysteine and folate concentrations.

Vitamin B12
The RDA for vitamin B12 was based on the amount needed for the maintenance of hematological status and normal serum vitamin B12 values.

Biotin
Intake data were used to set the AI for biotin. The most useful information concerning indicators of the adequacy of biotin intake arises from clinical observations of patients receiving biotin-free intravenous nutrition, individuals with inborn errors of metabolism, and persons who consume large amounts of raw egg white.

Metabolomics (Organic Acids)
Metabolomics evaluates metabolite profiles to detect which biomarkers or biomarker patterns are associated with an individual, a disease, or a condition. It has been referred to as the specific metabolic “fingerprint.” Metabolomic markers include organic acids, amino acids, fatty acids, etc, in urine or blood. Functional medicine was an early adopter of the concept of metabolomics utilizing it as supportive information, along with the standard of care. Research has generally taken two groups—one with a condition or disease and one without—and compared the metabolites of each group. Adding the additional step of evaluating which biochemical pathways are involved and how they may be related to health and nutrition can help to further identify issues.46 The evaluation of metabolites to identify disease or impaired pathways is continuously being researched. Additional metabolomics resources include the following:
- The Scripps Center for Metabolomics: METLIN
- ExPASy: Metabolomics scientific databases and tools49
- Canada’s Human Metabolome Data Base (HMDB) site for the human metabolome markers50

Toxins
The presence of toxins can be tested in many laboratories. It can be difficult to know when the level of a toxin will have a physiologic impact on a single person, as response to toxins is individual. Nutritional interventions have been proposed
as a key prevention strategy. The impact of toxins is being identified beyond just those that are work-related, and research has found significant impact in seemingly everyday exposures. Two key resources for assessment of toxins include Fourth National Report on Human Exposure to Environmental Chemicals and Centers for Disease Control and Prevention (CDC)'s Agency for Toxic Substance and Disease Registry (ATSDR). ATSDR maintains a comprehensive list of toxins, including a review on preferred testing. The CDC ATSDR ToxFacts sheets address where toxins come from, the clinical indication, how they can be evaluated, and other facts concerning the toxin. The Fourth National Report evaluates toxins assessed by National Health and Nutrition Examination Survey (NHANES) population studies. It gives the geometric mean and selected percentiles of concentrations for the total US population and individual gender, race, and ages from NHANES data. Both are updated regularly.

Conclusion

The ability of RDNs to order laboratory tests is dependent on state regulations or licensure. Some states, such as Georgia, allow RDNs to order independently. When selecting a laboratory, ensure it is licensed and has Clinical Laboratory Improvement Amendments (CLIA) accreditation or College of American Pathologists (CAP) certification. As registered and licensed health professionals, RDNs must ensure standards of care are provided and due diligence is undertaken when utilizing any laboratory test. As the number of laboratories and direct-to-consumer marketing increase, RDNs should be aware of dubious or harmful information claims, and decisions should be based on peer-reviewed literature and not corporate material or individual clinician claims. RDNs are well equipped to understand how to evaluate the science behind laboratory tests and any recommended therapies clients want to undertake based on the testing.

References

46. Wolf B. Biotinidase deficiency: “if you have to have an inherited metabolic disease, this is the one to have”. Genet Med. 2012;14(6):565-575.
Nutrigenetics in Practice: Understanding MTHFR

Lori Taylor, MA, MS, RD, CD, CSO

Lori Taylor is a clinical dietitian with 24 years of experience in patient care, practitioner education, and health care consulting. Lori trained as a biochemist at UC Berkeley in the lab of folate researcher Dr Jesse C Rabinowitz, and worked as a molecular biologist. Lori later earned master’s degrees in education from Stanford University and in nutrition from Bastyr University.

Lori is certified as a Specialist in Oncology by the Commission on Dietetic Registration and has specialized training in functional digestive disorders through the Institute for Functional Medicine. She is trained in sustainable agriculture, holds a Permaculture Design Certificate from the prestigious Regenerative Design Institute in Bolinas, CA, and is a graduate of their Ecology of Leadership program.

In addition, Lori is a small-scale beekeeper and shepherds a burgeoning urban food forest at her home in Coupeville, on Whidbey Island, in Washington State.

In addition to her consulting practice, Save Your Plate, Lori is on the faculty of the graduate program in Integrative and Functional Medicine at Saybrook University and has taught nutrition to the naturopathic medical students at Bastyr University. She is a founding member of Coupeville’s Farm to School program.

Chances are, methylenetetrahydrofolate reductase (MTHFR) is the most common single nucleotide polymorphism (SNP) tested for by functional medicine providers. Which makes sense—MTHFR SNPs are quite common, and the enzyme involved lies at the heart of the folate and methylation cycles.

The folate cycle and methylation are complex enough; however, when combined with the often-conflicting recommendations from conventional or integrative and functional perspectives, it can be difficult to critically evaluate information regarding MTHFR. This article seeks to provide the basics about MTHFR—the enzyme’s function, the common SNPs, their prevalence, metabolic consequences, available testing, and dietary interventions.

Genetic Context

It is important to approach the subject of nutrigenetics with some humility. While we are rapidly learning about the human genome and the epigenetic factors that describe how and when genes are expressed, we still know very little about the consequences of SNPs.

SNPs are remarkably frequent—there is approximately 1 SNP per 300 nucleotides, or about 10 million SNPs in our entire genome, most of them occurring between genes. As most genes carried on autosomal chromosomes occur in pairs, a gene with a SNP is generally only on one chromosome, leaving the other “wild-type” copy to produce the normal protein.

When a SNP occurs within a gene, the one base-pair change transcribes to a change in messenger RNA, and thus a change in the 3-base-pair codon, resulting in a different amino acid chosen by the ribosome to make the protein encoded for by the gene. As amino acid sequence determines protein structure and function, a SNP has the capability to change the function of the enzyme, though possibly not radically. SNPs are typically referred to as variants, as they occur in greater than 1% of the population, while mutations occur in less than 1% of the population.

About MTHFR

The MTHFR gene is one of the more widely studied genes for SNPs, with approximately 43 identified deleterious mutations and 9 common SNPs, two of which occur quite frequently. Because of the MTHFR enzyme’s central location in the folate cycle, changes in its function have the potential to affect a number of metabolic processes.

MTHFR is a key enzyme responsible for reducing 5,10-methylenetetrahydrofolate to 5-methyl-tetrahydrofolate (5-MTHF). 5-MTHF is acted on by methionine synthase and donates its methyl group first to vitamin B12, which then passes the methyl group on to homocysteine to become methionine. Methionine then goes on to become s-adenosyl-methionine (SAM), which is the primary methylating agent in the body, and acts as a co-substrate for a number of methyltransferases (MT). After adenosine triphosphate (ATP), SAM is the most common enzymatic cofactor in humans. If one imagines the methyl group as a football, MTHFR is the center that snaps the methyl group to SAM, the quarterback, who then passes the methyl groups to MTs for localized methylation reactions.

Methylation turns out to be a crucial and common reaction in the body, with 208 identified proteins in the human methyltransferasome, representing 0.9% of all genetic products. Methylation by SAM is key to neural functioning, as it is required for synthesis of all monoamine neurotransmitters. SAM is also used in the synthesis of creatine, carnitine, nucleic acids, phospholipids, and proteins; and methylation reactions can be found in pathways of phase two detoxification, clearance of histamines, hormone biotransformation, and immune cell differentiation.

Methylation is a primary method of epigenetic control and is used to suppress gene expression. Methylation balance is a complex business—globally neither hypomethylation nor hypermethylation are desirable states. In the case of cancer, for example, initiation and progression are marked by shifts in methylation: hypomethylation causing genomic instability through lack of suppression of sequences between genes and hypermethylation silencing tumor suppressor genes.

Optimal methylation status is not yet known.

In addition to activation of folate for methyl transfer, MTHFR also participates in the recycling of biopterin by reducing the BH2 form to the BH4 form. BH4 plays a more crucial role than SAM in neurotransmitter synthesis, being required at three steps in monoamine pathways, whereas SAM is required for two.

MTHFR SNPs

The two most common MTHFR SNPs are C677T and A1298C, meaning at the 677th position, the first SNP has a thymine where a cytosine should be, and at the...
1298th nucleotide in the sequence, the second SNP has a cytosine where an adenine should be. Patients can be heterozygous, with a SNP on one chromosome and a wild-type copy on the other; they can be homozygous with the same SNP in both chromosomes; or they can be compound heterozygous, with a different SNP on each chromosome.

The C677T SNP results in a more thermolabile MTHFR enzyme, which performs less efficiently than the wild type. Heterozygotes have ~60% of normal activity, while homozygotes have about 30%. Both heterozygous and homozygous variants are associated with elevated plasma homocysteine.

The A1298C SNP occurs in the regulatory portion of the enzyme and is not associated with elevated homocysteine, whether heterozygous or homozygous. Heterozygotes have about 50% of normal MTHFR enzyme activity. Individuals who are compound heterozygous (C677T/A1298C) have about 50% of normal MTHFR activity but do show elevated homocysteine, indicating an interaction between the two SNPs. MTHFR SNPs are quite common. C677T heterozygosity varies by ethnic group but is present in 40% to 50% of most populations studied. Homozygosity of C677T is found in 11% of US Caucasians, 15% of US Hispanics, 20% to 26% of Italian populations, and 32% of Mexican populations. The lowest prevalence is found in Black and Asian populations, with a study of Turkish subjects reporting a frequency of 15%. Prevalence data cited in other papers was unable to be confirmed.

Given that half of the US population is heterozygous for MTHFR SNPs, there may be a biological advantage to these SNPs. Elevated 5,10-methylenetetrahydrofolate levels may increase inputs to the thymidine synthase pathway, resulting in improved DNA replication. Mouse studies suggest that the C677T SNP may provide protection against malaria.

**The Perils of Homocysteine**

Homocysteine is produced in all tissues. When MTHFR activity is reduced, less SAM is made, and homocysteine builds up as less can be remethylated into methionine. Early case-control studies linked some MTHFR SNPs to increased disease risk; however, later studies more clearly associate the risk with homocysteine level, folate status, and ethnicity (i.e., the phenotypic expression of the genotype in the patient). Homocysteine not methylated by 5-MTHF can be used in other pathways. It can be remethylated

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**Figure 1.** The schematic overview of homocysteine metabolism and its relationship with folic acid and vitamins. ATP: adenosine triphosphate; AMP: adenosine monophosphate; PPi: pyrophosphate; Pi: orthophosphate; B2/B6/B12: vitamins B2/B6/B12; CoA: coenzyme A; R: acceptor; R-CH3: methylated product; MT: methyltransferases.

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to methionine by betaine-homocysteine s-methyltransferase (BHMT). Homocysteine can also be removed through the transsulfuration pathway, via two vitamin B6-dependent enzymes—cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE). This pathway produces cysteine—an amino acid precursor to coenzyme A, sulfates, proteins, and glutathione.

The folate cycle is found in all cells; however, the other two pathways of homocysteine removal are tissue-specific. BHMT functions only in the kidney, liver, and lens while transsulfuration pathways are limited to the kidney, lens, liver, small intestine, and pancreas. Adipose and brain tissue lack CSE but contain CBS. This leaves the central nervous system vulnerable if MTHFR activity is reduced as it lacks a method to remethylate homocysteine and is unable to remove it otherwise.

Elevated homocysteine is of neurological concern as it has been shown to disrupt the blood-brain barrier. Functionally, high levels of homocysteine can be damaging to all tissues, as they increase oxidative stress, alter redox balance, and attach to proteins, altering their function. Elevated homocysteine is of neurological concern as it has been shown to disrupt the blood-brain barrier. Functionally, high levels of homocysteine can be damaging to all tissues, as they increase oxidative stress, alter redox balance, and attach to proteins, altering their function.

Elevated homocysteine (especially at levels of 15 µmol/L and above) is considered an independent risk factor for cardiovascular, as well as cerebrovascular disease, though it is unclear if it is causative or acts as a biomarker of vascular changes. Similarly, elevated homocysteine is associated with neural tube defects, psychiatric and neurologic disorders, cancer, and some gastrointestinal disorders and may be a marker for aberrant methylation.

Testing

The general recommendation with SNPs is to treat the phenotype, not the genotype. In this case, homocysteine is the most visible measure of the phenotype. Folate, B12, and B6 status all contribute to homocysteine levels. Homocysteine testing should be considered for patients at increased risk of cardiovascular disease and those with psychiatric diagnoses.

There is not consensus on the optimal level of homocysteine. Values over 13 µmol/L are considered elevated, while most lab reports list values 15 µmol/L and above as abnormal. A study of Japanese subjects showed a marked jump in stroke risk when homocysteine levels were above 11 µmol/L. Earlier studies suggested that overall mortality was lowest with levels under 9 µmol/L. The difference of medical opinion on optimal levels is a significant one and is one reason posited for the failure of vitamin supplementation trials in lowering occurrences of myocardial infarction or death, as homocysteine levels may have not been brought low enough to reduce risk.

As part of due diligence, it is also advised to test for methylation cycle cofactors if deficiency is suspected, namely red blood cell folate (which measures long-term folate stores), B12 (noting that there is no gold standard for deficiency), and methylmalonic acid (the functional test for B12).

The American College of Medical Genetics and Genomics does not recommend MTHFR testing due to limited data linking high homocysteine levels to CVD, venous thromboembolism, and recurrent pregnancy loss. The American Heart Association does not support either testing or treatment for MTHFR, nor does the Cleveland Clinic.

Integrative and functional practitioners disagree however, due to a focus on the continuum of health (rather than only hard clinical outcomes), and the status of homocysteine as a marker for possible aberrant methylation, which has multifactorial effects. MTHFR testing is recommended for those with cardiovascular disease, stroke, depression, or venous thromboembolism and a homocysteine level above 13 µmol/L and perhaps for those between 9 and 13 µmol/L.

MTHFR status needs only to be tested once, as genotype does not change. It can be tested through a direct blood draw by physician order (CPT code 81291) or can be included in genome-wide patient-ordered genetic testing, followed by a genetic translation service. The blood draw maintains patient privacy, though it should be ordered as self-pay as few, if any, insurances cover the test.

Dietary Interventions for MTHFR SNPs

Foods:
at the Recommended Dietary Allowance (RDA) of 400 mcg per day, to prevent hypermethylation. For some, treatment with 5-MTHF provokes anxiety.\textsuperscript{29}

It is important to ensure that patients have sufficient B12 and B6 intake. For B12, bioavailability declines with age due to hypochlorhydria. For this reason, the Institute of Medicine recommends that all patients over the age of 50 meet their B12 needs through fortified foods or supplementation.\textsuperscript{30} Contrary to popular practice, B12 does not need to be provided in the methylated form. As previously stated, B12’s methyl group comes from 5-MTHF and is then donated to homocysteine. Additionally, once absorbed by the body, ligands of B12 are cleaved and later reattached so that the body can traffic B12 forms to either the cytosol or the mitochondria.\textsuperscript{31} As for B6, dosages should not be above the upper limit of 100 mcg, which carries a risk of neuropathy and ataxia.\textsuperscript{32} As the MTHFR enzyme requires both riboflavin (B2) and niacin (B3), it would be reasonable to recommend a multivitamin containing 100% RDA of B vitamins.

Probiotic strains may affect folate status. Bifidobacteria are folate-producers, while Lactobacilli are consumers (with the exception of L. plantarum).\textsuperscript{33} Probiotic supplement strains differ, and not all contain Bifidobacteria.

SAM supplementation could theoretically be useful as a way to provide sufficient methylating agents when MTHFR SNPs are present. However, it should be noted that the MTHFR enzyme is allosterically inhibited by SAM.\textsuperscript{34} As MTHFR is also used to regenerate biotinier for neurotransmitter synthesis, supplementation with SAM could theoretically reduce available biotinier and negatively impact neurotransmitter levels.

With follow-up, homocysteine should be redrawn to assess interventions, as well RBC folate, B12, and methylmalonic acid if those levels required treatment. No ideal follow-up laboratory interval has been evaluated; however, if checking RBC folate, then at least 3 to 4 months would be prudent, related to the lifespan of the RBC.

**Humility**

Methylation is complex and involves much more than MTHFR. Twenty-two additional genes have been identified that affect homocysteine levels,\textsuperscript{35} and optimal methylation states have yet to be discovered. Integrative and functional nutrition providers should realize that heterozygous SNPs (except for compound heterozygosity) may not be pathologic nor require treatment. Patients at cardiovascular risk and those with psychiatric diagnoses may benefit from homocysteine testing and, if levels are high, MTHFR testing. Providers should focus on treating the phenotype by normalizing homocysteine levels with foods and conservative, targeted supplementation.

**References**

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100% pomegranate juice is powered by unique polyphenols that early research suggests have promising results for muscle strength recovery.

In a 2011 study, a small set of athletic men drank two servings (about eight ounces per serving) of either pomegranate juice or placebo for about two weeks while following their normal diet and weight training routine[2]. After week one, the men completed specific strength exercises at maximum capacity. They drank an additional serving of pomegranate juice immediately after exercising.

The study found that the men who drank pomegranate juice maintained more of their post-exercise arm strength when compared to the placebo group. A similar trend was seen in the knee though it did not reach statistical significance. Although this research is promising, additional clinical research is needed to establish causation and the potential impact of pomegranate polyphenols on exercise.

[1] Seeram et al., 2008
[2] Trombold et al., 2011

*Not a low calorie food, see nutrition information for sugar and calorie content
Electronic Mailing List (EML) Recent Topics Review

In a discussion related to lower extremity edema in patients who follow a vegan diet, many users mentioned the importance of honoring the clients’ wishes about what type of diet they prefer. It was recommended to do a thorough dietary intake to assess typical diet. It was also mentioned that following a balanced, whole-foods, plant-based diet would be beneficial. Others suggested that renal, thyroid, and cardiac complications could present with lower extremity edema. Conducting nutrient testing and checking red blood cells, fatty acid, and methylation markers along with acid-base balance was recommended by a few users. Other comments were that vitamin C depletion can present with lower extremity edema early on in deficiency and insufficiency. Protein deficiency, specifically albumin, was suggested as a possible cause; if albumin is low, it can cause a water imbalance resulting in edema. It was also noted that the intima of blood vessels requires vitamin K2 to stay supple. Assessing the patient’s K2 status may give insight into vascular health. One user mentioned that if the client is presenting with cold hands, constipation, and edema, there could be an iodine deficiency. Additional suggestions were that perhaps the patient was not getting enough sodium and potassium in the diet, or too much sodium, and noted the importance of checking the balance. Another user emphasized that individualizing and assessing the microenvironment is what the DIFM RDN is all about. In our commitment to being fair and unbiased, rather than identifying individuals, programs, or organizations, discussions regarding certificate programs, testing, and nutrient analysis programs can be found on the Listserv at http://groups.yahoo.com/neo/groups/DIFM_Listserv/info.

What’s New - Journal Reviews and Resources

Inverse Relationship Between Sun Exposure and All-Cause Mortality

In opposition to present recommendations, the more sun exposure, the greater the life expectancy. In this review, a strong correlation was found between limited sun exposure, presumably indicative of low vitamin D levels and all-cause mortality. Observational data from numerous studies showed that low sun exposure increased the risk of cardiovascular disease (CVD) and non-CVD/non-cancer disease. Specifically reviewing the Melanoma in Southern Sweden cohort study of women, the authors found a link between limited sun exposure and venous thromboembolism (VTE) incidence. According to this data, self-reported avoidance of sun exposure increased VTE risk by as much as 30% with a further increase of 50% in winter months. Using the same study data, it was also shown that avoidance of sun exposure led to a 30% increase in the risk of type 2 diabetes development. Another study out of Spain found that sunbed users had a 40% reduced risk for development of endometrial cancer. Another Swedish study with women-only participants, found a reduced risk for all-cause mortality in those who spent vacations sunbathing more than once a year over three decades. This study found that when compared to high sun exposure, those who avoided the sun had a doubled mortality rate; and there was a 40% mortality rate increase for those with moderate exposure. This also showed that avoidance of sun exposure led to a 60% increased risk of death from CVD, with a dose-dependent relationship with moderate and high amounts of sun exposure. The authors found that smokers avoiding sun exposure had similar mortality rates as those with the greatest sun exposure. In conclusion, the authors suggested that sensible sun exposure habits may improve public health.


Upcoming Conferences and Educational Opportunities


September 30, 2018 – October 3, 2018 – 12th Congress of Nutrigenetics/Nutrigenomics. Winnipeg, Manitoba, Canada www.nutritionandgenetics.org


Maternal Caffeine Intake During Pregnancy and Childhood Growth and Overweight: Results from a Large Norwegian Prospective Observational Cohort Study

Caffeine is the world’s most widely consumed stimulant among pregnant women, with coffee and tea being the most common sources of caffeine. Caffeine is readily absorbed into the bloodstream; and during pregnancy, the elimination of caffeine is prolonged. Caffeine can rapidly cross all biological membranes including the blood-brain and placental barriers. This process results in a direct exposure of the fetus to the amounts of caffeine consumed. This study was conducted within the Norwegian Mother and Child Cohort Study (MoBa). A total of 50,943 mothers and children were recruited from 2002 to 2008. Information about average caffeine intake was assessed at mid-pregnancy. After birth, body size was measured at 11 age points from 6 weeks to 8 years. Fetal growth and growth in infancy are important determinants for the development of obesity and long-term cardiometabolic health. Children whose mothers had very high (≥300 mg/day), high (200-299 mg/day), and average (50-199 mg/day) caffeine consumption during pregnancy were 66%, 30%, and 15% more likely to have excessive growth during the first year of life, compared with those whose mothers had low (<50 mg/day) caffeine intake. The findings also tied average to very high prenatal caffeine intake to significantly increased overweight risk at ages 3 and 5, but only very high caffeine consumption was associated with an increased likelihood of children being overweight at age 8. The study concluded that any caffeine consumption during pregnancy is associated with a higher risk of excess infant growth and an increased percentage of children being overweight, mainly at preschool ages. Maternal caffeine intake may modify the overall weight and growth trajectory of children from birth to 8 years. During the process of this study, additional supporting evidence was found to reinforce the current advice to reduce caffeine intake during pregnancy.


This study provides evidence that personalized weight-loss interventions for overweight individuals based on genetic and epigenetic information may be beneficial.


A variety of approaches (nutrigenomics, metabolomics, etc) have been pursued for insights pertaining to the prevention or management of type 2 diabetes. Clear insights have yet to emerge, largely due to the multiple factors involved, including variations in intestinal microflora. More research is still needed, but the authors feel that precision nutrition together with advances in bioinformatics can be helpful.


Although more research is still needed, variations in three zinc-transporter genes (SLC30A3, SLC39A8, and SLC39A14) were found to be significantly associated with blood levels of zinc in the Japanese subjects tested. The authors suggest that these types of variants can be meaningful with regard to zinc deficiencies.

INSIG2 rs7566605 single nucleotide variant and global


A number of variants of the FGF21 gene (eg, rs17108973) were found to alter the affect of high-dose omega-3 fatty acid supplements (3 g/d total of EPA+DHA) on insulin levels and insulin resistance, prompting the authors to suggest that additional research in this area will be helpful for determining individualized nutrition advice.


Various constituents from fruits and vegetables are described with potential preventive properties, with Table 2 indicating some of the molecular mechanisms by which this can occur. Table 3 provides a listing of combinations which produced apparently synergistic effects. (Free PDF found on publisher’s site when accessed on March 20, 2018: https://onlinelibrary.wiley.com/doi/pdf/10.1002/mnfr.201700597.)


Among the Swedish subjects in this observational study, those using modest zinc supplementation (median of 22.4 mg/day total Zn intake) were found to have a significantly reduced risk of
developing type 2 diabetes (T2D) during the median follow-up period of 19 years. Those who carried two copies of the C allele of the rs13266634 variant of the SLC30A8 gene were associated with higher T2D risk, which was further modified by both body mass index and by the zinc-to-iron ratio.


Table 2 provides a listing of adipose tissue-promoting microRNAs (miRNAs) and table 3 a listing of anti-adipogenic microRNAs. Discussion is provided on the effect of miRNAs within various tissues, including pancreas, skeletal muscle, and liver as well as in adipose tissue per se.

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Check out www.NutritionAndGenetics.org to learn more about ISNN membership discount for dietitians, which includes 24/7 database access plus a subscription to the *Journal of Lifestyle Genomics* (formerly named the *Journal of Nutrigenetics and Nutrigenomics*). ISNN’s 12th Congress will occur this Sept 30 – Oct 3 in Winnipeg, Manitoba, Canada: www.ISNN2018.org.
Join us for the DIFM Saturday Symposium, Washington, DC

Extinguish the Flame: Integrative & Functional Approaches to Neuroinflammation, Neurodegeneration, and Cancer
Saturday, October 20, 2018
Walter E. Washington Convention Center
801 Mt Vernon Place NW, Washington, DC 20001

9 AM – 9:45 AM
Registration/Exhibits/Welcome

9:45 AM – 10 AM
Preview of Best Available Evidence: A New Clinical Decision Tool to Guide Critical Thinking presented by Mary Beth Augustine, RDN, CDN, FAND

Strengthen your evidence game with DIFM’s new Best Available Evidence Decision Tool which guides users to follow the evidence wherever it leads, and to critically question the results! See a preview of this DIFM-funded Academy tool.

10 AM – 11:30 AM
Metabolic Drivers of Cancer and Associated Integrative Strategies presented by Lise Alschuler, ND

Cancer is a dynamically complex process with dysregulated metabolism playing a central role. This seminar will provide an overview of the metabolic aberrations that result in the formation of a tumor microenvironment. Armed with this holistic understanding, practitioners will be equipped to identify ways to target the metabolic drivers of cancer. The rationale and role for specific lifestyle, dietary, and botanical interventions will be explored.

11:30 AM
Light Lunch served

Awaiting approval from Academy for 4 CPEs
This Symposium will not be recorded

12:30 PM – 2 PM
Approaches to Treating Neuroinflammation and Neurodegeneration presented by Terry Wahls, MD

In this session, Dr. Wahls will discuss the use of diet to treat MS related symptoms. She uses diet and lifestyle interventions based upon Functional Medicine and Ancestral Health principles to treat a wide variety of neurological, medical and psychiatric disorders. In addition, she conducts clinical trials using diet and lifestyle to treat multiple sclerosis. Dr. Wahls has a personal connection to these interventions, having experienced 7 years of steady decline due to progressive multiple sclerosis, and even spent four years dependent upon a tilt recline wheelchair. Thanks to the power of functional medicine, diet and lifestyle interventions she restored her health and vitality, and now bikes to work.

2:30 PM – 3:30 PM
Tackling Difficult Cases: Where to Begin presented by Robin Foroutan, MS, RDN

Integrative and functional nutrition practitioners aim to “seek beyond the symptom” in order to identify underlying system imbalances that contribute to symptoms and fuel disease states. This often requires a different way of looking at both common and complex health issues. Since many people with complicated health histories seek out integrative and functional medicine, most integrative practitioners are tasked with determining a personalized care plan when underlying causes may not be easily determined. In these most difficult cases, a systems-based approach to analyzing cases can be helpful, especially in determining where to begin.

11:30 AM
Light Lunch served

Awaiting approval from Academy for 4 CPEs
This Symposium will not be recorded
Master Your Diabetes: A Comprehensive, Integrative Approach for Both Type 1 and Type 2 Diabetes

Dr. Mona Morstein, ND, DHANP
Chelsea Green Publishing,
www.chelseagreen.com
2017.543 pp
ISBN-9781603587839

The author, a practicing naturopathic physician specializing in diabetes, dedicates the book “to everyone who has diabetes, everyone who cares for someone with diabetes, and every physician who has diabetic patients.” Quite a large undertaking, and for the most part, a valuable one at that. As an integrative registered dietitian nutritionist (RDN) and certified diabetes educator (CDE), I’ve read a plethora of research articles and books on diabetes mellitus (DM) and have not read a more succinct yet thorough description of the types of DM, its pathophysiology, comprehensive testing and screening, and related predisposing factors. Though the information was not new to me, I found myself quoting and referencing chapters 1 through 4 to my patients; I foresee myself continuing to do so!

The next section of the book explored what the author terms “The Eight Essentials,” her comprehensive integrative protocol for both prediabetes and diabetes: (1) Diet, (2) Exercise, (3) Sleep, (4) Stress Management, (5) Healing the Gut and Microbiome, (6) Environmental Detoxification, (7) Supplementation, and (8) Medications. RDNs and CDEs may recognize several of these as parallels to the American Association of Diabetes Educators’ (AADE) self-care behaviors (healthy eating, being active, monitoring, reducing risks, problem solving, and healthy coping). These chapters are chock-full of definitions, helpful hints, practice pearls for both patients and clinicians (denoted by grey boxes), and bulleted lists, any of which could almost stand alone as a patient self-management handout. The written text merely serves to fully elucidate and explain the highlighted information.

For the clinician, the information on conventional pharmacotherapy—both orals and injectables—and integrative supplementation was exceedingly useful and informative. With respect to the conventional pharmacotherapeutic agents, medication class, description, function, and side effect(s) were readable and easily understandable unlike prescription product information inserts! The tables were quite utilitarian, too and serve as a great reference tool. The calculations and formulas for calculating and subsequently adjusting insulin dosages were likewise of great personal professional value; whether or not such information would be useful to patients is an issue of debate—some of my patients could easily grasp the concepts, but others would be equally intimidated. Though the supplementation chapter was informative, it was not as utilitarian as the pharmacotherapy sections; tables much like those used with the conventional agents summarizing the supplements would have enhanced an otherwise useful chapter.

For $29.95, this book would serve as an excellent reference tool with two exceptions. First, the bibliographic reference citations were alphabetized at the end of the book; there were no superscripted numeric references within the chapter text. The second concern with Master Your Diabetes was the diet and nutrition prescription. The author encouragingly writes, “People have different bodies, minds, families, life schedules, and desires to spend time in the kitchen. All these factors have a huge impact on which diabetic diet a patient is drawn to and wishes to commit to. Everyone needs to find a diabetic diet they resonate with best and want to incorporate into their lives.” Yet, the author only promotes (with the exception of the high-carbohydrate macrobiotic diet) a variety of restrictive very low-carbohydrate (less than 45 grams per day) dietary approaches for all patients—type 1, type 2, pediatric, adolescent, adult, and geriatric—with diabetes. And while such an approach has its merits for a certain subset of the population with diabetes, it can be quite problematic from a nutrient quality, economic, or even psychosocial perspective for others.
Evaluating the Research on Homocysteine and Vitamin B

Niha Zubair, PhD, Clinical Research Scientist

Niha Zubair received her undergraduate degree in Mathematics from William & Mary. Her passion for health and data led her to get her PhD in Nutritional Epidemiology from UNC Chapel Hill. She currently works at Arivale, a scientific wellness program in Seattle, Washington. Prior to Arivale, Niha worked at the Fred Hutchinson Cancer Center as a Staff Scientist conducting research on how genetics, diet, and lifestyle influence colorectal cancer and cardiometabolic diseases.

M any individuals take B-vitamin supplements for lowering levels of homocysteine, an independent cardiovascular risk factor. However, new research is calling into question the wisdom of B-vitamin supplements—not only at high doses but also at doses found in many multivitamins. This suggests that the current methods to lower homocysteine may need to be reevaluated.

About Homocysteine

Homocysteine is an amino acid and a breakdown product of protein metabolism. Studies show that high homocysteine levels are associated with cardiovascular disease, cognitive decline, and depression. Taking vitamin B supplements—particularly folate but also vitamin B12 or vitamin B6—is a well-documented way to lower high homocysteine levels. Although this sounds like it should be beneficial, researchers have observed a “homocysteine paradox”: even though high homocysteine levels are associated with cardiovascular disease, cognitive decline, and depression, and vitamin B supplements lower homocysteine, evidence from multiple studies demonstrates that using B supplements to lower homocysteine does not reduce the actual risk of these diseases or health conditions. The reasons for this paradox are unknown but may relate to poor study design in some of the trials or that homocysteine is not part of the causal pathway for these diseases.

Potential Risks of Vitamin B Supplements

Research demonstrates the use of supplemental B vitamins—even at relatively low doses—may increase the risk of cancer (particularly lung cancer) and mortality. In a 2017 study, men who took vitamin B6 had a 40% increased risk of lung cancer compared to those who did not supplement. Men who took vitamin B12 had a 30% increased risk of lung cancer. Both of these statistics accounted for age, race, education, body mass index, alcohol consumption, and smoking behavior, among other factors. The researchers additionally found that men who are smokers are even more at risk. No increased cancer risk was found in women in this study.

In 2009, an analysis of two clinical trials in patients with ischemic heart disease found those given folic acid plus vitamin B12 had an increased risk of cancer by 21%, cancer death by 38%, and death from any cause by 18% (all of these results were attributable to increased lung cancer incidence). These findings were observed for both men and women regardless of smoking status.

Methyalted vs Nonmethylated B Vitamins

The studies mentioned above were observational and did not differentiate between forms of B vitamins reported by the participants. Many functional medicine practitioners believe that the use of nonmethylated forms invalidates the legitimacy of the studies. In a 2017 paper, Brasky et al suggested a hypothesis that excess supplemental folic acid may disrupt the body’s natural feedback loop. Biochemically, higher levels of methylfolate, along with higher levels of homocysteine, could disrupt the equilibrium of the methionine pathway, which could lead to an increase in methyl donor groups. This in turn could promote changes in DNA methylation and thus aberrant DNA replication and cancer growth. Therefore, it is possible that higher-dose methylfolate could have the same safety concern as nonmethylated forms of folate. Hopefully, long-term studies distinguishing methylated vs nonmethylated B vitamins will be available in the future.

Other Ways to Decrease Homocysteine Levels

The potential harm from B-vitamin supplements coupled with the lack of demonstrated efficacy of homocysteine-lowering on disease endpoints suggests it is no longer judicious to recommend B-vitamin supplements to lower homocysteine. As mentioned previously, it is unclear if elevated homocysteine causes cardiovascular disease, cognitive decline, and depression. Despite this paradox, there could be negative physiological effects of high homocysteine levels. In light of the new information, perhaps whole-food dietary sources of B vitamins, rather than supplements, should be used to lower homocysteine levels. Specifically, increasing dietary folate can help lower homocysteine levels. Foods rich in natural folate include leafy green vegetables, crucifers (like broccoli), and legumes. While taking a supplement might be easier than making room in your diet for these nutrient-dense foods, there are many other health benefits to these foods beyond lowering homocysteine, including fiber, vitamins, and minerals. Recommendations for the average adult (not pregnant or lactating) are for 400 mcg per day of dietary folate—primarily from naturally folate-rich foods, or if need be, from fortified foods. For reference, some foods that naturally contain folate include broccoli (1/2 cup cooked broccoli contains 52 mcg or 13% DV) and some whole grains such as millet (1 cup cooked millet contains 33 mcg of folate or 8% DV).

Vitamin B Supplementation

Vitamin B supplements can be helpful in some situations; however, the concern about supplementation specifically for the purposes of
lowering homocysteine may be warranted. Given the "homocysteine paradox," the potential risk (lung cancer) may outweigh the potential benefit (homocysteine reduction) in light of the lack of evidence that lowering homocysteine with supplements reduces the risk of cardiovascular disease. It should be noted that while the Brasky et al. study showed an association with lung cancer risk and B vitamins when taken as individual supplements, the authors did not find an association with taking multivitamins or consuming B vitamins from dietary sources. Further, the European Prospective Investigations into Cancer and Nutrition (EPIC) study and a large trial in 2010 concluded that higher levels of B6 were associated with a lower risk of lung cancer. Food sources of B6 include brewer’s yeast, bananas, cereal grains, legumes, carrots, spinach, peas, cheese, eggs, fish, and sunflower seeds. Food sources of vitamin B12 are dairy products, eggs, meat, fish, poultry, and shellfish. There are certain health conditions that may warrant vitamin B supplementation. It is well known that adequate levels of folate prior to and during pregnancy are very important for preventing certain birth defects. Because of this reason, the United States fortifies grain products with folate; and prenatal vitamins typically contain B vitamins. Individuals who have had bariatric surgery or have gastrointestinal disorders that impair nutrient absorption may also need a supplement to avoid insufficiency, as would many who have a measured deficiency in blood levels of any of the B vitamins. Finally, a practitioner may have other, more specific and personalized reasons for recommending B vitamin supplements.

While supplementation with B vitamins is often used successfully, research is needed and caution must be used until more conclusive studies are done that more clearly elucidates the relationship between cancer metabolism, smokers, and long term supplementation of B vitamins.

References
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Caffeine Intake Around Gestation: Is the Current Allowance Too Liberal?

Introduction

Caffeine intake recommendations during pregnancy are currently capped at 200 mg/day—about two cups of home-brewed coffee. Research indicates the metabolic pathways for caffeine metabolism change over time, including alterations in enzymatic activity, from conception to the postnatal period and throughout childhood. As much remains to be discovered about the influence of different genes on the metabolism of caffeine and altered caffeine metabolism during gestation, the current recommendations may not account for these key factors. There are specific single nucleotide polymorphisms (SNPs) associated with caffeine metabolism that may contribute to maternal or fetal clearance of caffeine around gestation and to risks like intrauterine growth restriction (IUGR). The primary SNP related to the metabolic rate of caffeine metabolism is rs762551, which research suggests may be also associated with caffeine consumption. The allelic expression of the primary gene related to enzymatic breakdown of caffeine (CYP1A2) may play an even greater role. The combinatorial effects of genetic variation in cytochrome p450 (CYP) gene expression, environmental factors like smoking, inhibitive and inductive substrates, as well as maturational changes in caffeine metabolism pathways suggest that the maternal allowance of 200 mg/day may be too liberal around the gestational period.

Methods

PubMed database was searched using the terms “CYP1A2 and caffeine metabolism,” “rs762551 and caffeine metabolism,” “CYP1A2, caffeine, and pregnancy,” and “rs762551 and pregnancy.” Thirteen studies were selected, including one cohort study, one retrospective study analysis, six case-control studies, and five meta-analyses.

Results

Researchers note eight significant gene loci associated with caffeine metabolism. The primary heterogenous loci is CYP1A2, near the aromatic hydrocarbon receptor (AHR), related to the inductive role of cigarettes on CYP1A2 activity in those with the AA or AC alleles. CYP1A2 as well as CYP1A1 are exogenous CYPs; located in the smooth endoplasmic reticulum, they help breakdown xenobiotics, including caffeine. The average variance in CYP1A2 activity between persons is estimated to be 5- to 15-fold. CYP1A2 has a strong genetic component; in the absence of said prominent environmental modulators, studies suggest upwards of 89% of CYP1A2 activity is heritable. The CYP1A2 enzyme has one primary xenobiotic receptor; more research is warranted to determine the extent that competing substrates induce or inhibit CYP1A2 activity in metabolizing caffeine. Notably, polycyclic aromatic hydrocarbons (eg, cigarette smoking), caffeine, cruciferous vegetables, grilled meat, certain drugs (eg, omeprazole and carbamazepine), and heavy exercise induce CYP1A2. Other drugs—fluvoxamine, quinolone antibiotics, and oral contraceptives—act as inhibitors. One study confirmed that aryl hydrocarbons from smoking induce CYP1A2 by as much as 6.3-fold, and oral contraceptives inhibit up to 33%. These environmental factors are important to consider, in addition to genetics, when assessing maternal caffeine metabolism and potential consequences.

Interestingly, persons who are homozygous AA for CYP1A2 rs762551 (CYP1A2*1F) are faster metabolizers. Those with AC are intermediate, and homozygous CC are the slower metabolizers. Faster metabolizers produce more metabolites at a greater rate—mainly paraxanthine (70-82%), theophylline, and theobromine. Research suggests the higher ratio of paraxanthine to caffeine may create a toxic in-utero environment and increase risk for IUGR. On the other hand, caffeine itself, unmetabolized, seemed to be protective: slower metabolizers were found to have up to a 41% reduction in IUGR risk when controlling for environmental factors. Another study showed no correlation to fetal growth or IUGR, however it lacked in standards of measurement and sampling validity. Maternal rate of caffeine clearance decreases via CYP1A2 suppression as gestation increases, with the half-life changing from 4.5 to 10 to 18 hours from the first to second trimesters and full-term respectively. The totality of negative effects from caffeine consumption and delayed caffeine clearance is unknown. Slower clearance means increased levels of caffeine and its metabolites in the mother, placenta, and fetus. Of important consideration for assessing potential risks are the caffeine metabolic pathways that differ between fetus, neonate, and adult.

Research indicates that transition to hepatic metabolism of caffeine with mature levels of CYP1A2 enzyme does not occur until 55 weeks postconception (4-5 months postnatally). Beforehand, other CYPs are the predominant metabolizers. In neonates, the primary clearance of theophylline (and its precursor, caffeine) is via renal excretion (98% unmetabolized in the urine vs 10% in adults). At 30-40 weeks postconception, about 15% of caffeine is metabolized largely by CYP3A7 per C-8 hydroxylation (vs demethylation) to 1,3,7-trimethyluric acid, and theophylline to 1,3 methyluric acid; C-8 hydroxylation matures by one month postpartum.
Interestingly, neonates can N-methylate theophylline to caffeine, resulting in potentially large amounts of serum caffeine not typically found in children over 6 months old. In addition to cautioning caffeine intake during pregnancy, this is important to consider when dosing theophylline/caffeine to neonates for treatment of sleep apnea.

**Conclusion**

Regarding dietary interventions for rs762551 and CYP1A2 activity, caution should be advised with regards to beverage or food caffeine consumption, limiting intake below 200 mg/day for pregnant mothers in the first 55 weeks postconception. It is possible that this daily allotment is too high towards the end of gestation due to altered caffeine metabolism of the fetus and neonate, as well as an increased maternal half-life of caffeine and genetic variance in metabolism. This includes the propensity of neonates to recycle metabolites to regenerate caffeine in utero, and the ease of transfer of caffeine and theophylline across membranes for tissue deposition, regardless of low lipophilicity. Of additional concern are the phytates in coffee that are known to interfere with iron absorption—a deficiency associated with well-known maternal-fetal risks. As maternal-placental-fetal transfer of caffeine and byproducts readily occurs, and as many women have not undergone genetic testing to determine rs762551 allelic expression, special considerations for serum caffeine concentrations between 30-55 weeks postconception should be made.

Many of the existing studies lack sufficient dietary questionnaires, including quantitative maternal caffeine intake and intakes of CYP1A2-inducing foods like cruciferous vegetables. More research is needed that includes these parameters, controlling for key environmental factors like contraceptive use and smoking. This will help clarify safe caffeine consumption around gestation and better elucidate the impact of rs762551 variance and CYP1A2 activity—the mechanism of which is still unknown.

**References**

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