The Nutrition Assessment of Metabolic and Nutritional Balance

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8.1 Introduction

The field of integrative and functional nutrition is expected, by its very nature, to be progressive and on the cutting edge of new concepts in nutrition. Patients in desperate need of healing seek out reliable, valid, and progressive solutions from their nutritionist. The constant barrage of changing nutritional recommendations should teach integrative and functional nutrition practitioners to hold the principle of nutritional balance at the core while new findings continue to come forth.

Fad diets and supplements will come and go through the decades, and it is important to always question the long-term consequences of every proposed new nutritional concept. Concurrently, nutritionists must keep an open mind for something that may help a patient with a puzzling condition. What may seem extreme and/or inappropriate for one person may be lifesaving for another. Many human genetic diseases, due to defective enzymes, may be ameliorated by high doses of specific nutrients required as cofactors for the enzyme to partially restore activity of that enzyme [1]. Over the lifespan, metabolic and biochemical needs change and nutritional balance should be periodically reassessed and rebalanced [1]. Furthermore, detecting and addressing disease at its earliest stage of biochemical imbalance, rather than waiting for the condition to progress to a diagnosed disease, is a cost effective and physiologically beneficial approach for the patient.

"Biochemical individuality," first proposed by Roger J. Williams in 1947, refers to differing metabolic needs for optimal physiological function and infers that nutritional balance for one person may require different amounts of nutrients than for another person [1]. One of the foundational principles of integrative and functional nutrition is to recognize each person's unique nutritional needs. If practitioners rely on nutritional studies as a guide to establishing optimal nutrient intake for individuals, as opposed to using individualized nutrient assessment, this may pose challenges. For instance, many nutrition studies fail to show a benefit of supplementation due to flawed study design, as investigators often fail to establish baseline nutrient status of study participants to accurately measure outcomes [2]. Testing and retesting may be one of the most helpful tools to keep clients' nutritional status in balance and provide metabolic correction. Integrative and functional nutritionists have a vast array of tools to choose from in this regard.

To assess nutritional imbalance, a clinician can use a nutritional physical exam, conventional and integrative laboratory testing, as well as signs and symptoms. When imbalance is found, such as a zinc deficiency, calling for diet change and supplement intervention, one must then pay attention to the balance of other nutrients that may be negatively affected, such as copper status. Another example of this is the critical role that magnesium plays in the synthesis and metabolism of vitamin D and parathyroid hormone [3].

The key considerations for nutritional balance are as follows:

- The microbiome: use of probiotics and foods in different gastrointestinal conditions
- Fiber: appropriate needs for different diagnoses
- Iodine: finding the right balance and removing antagonistic toxic halogens
- B vitamins: appropriate amounts and forms based on biochemical individuality
- Mineral balance: sodium, potassium, zinc, copper, magnesium, calcium iron
- Vitamin D status: associated requirement for magnesium
- Fat-soluble vitamins: individual requirements
- Omega-6 and Omega-3 fatty acids: ratios, adequate gamma-linolenic acid (GLA), and specialized proresolving mediators (SPM)
- Micronutrient and macronutrient ratios in different disease processes

8.2 The Microbiome

One of the core principles of Integrative and Functional Medicine Nutrition Therapy (IFMNT) is biochemical individuality, and recommendations for the feeding and care of individual microbiome are no exception. Although we are far from fully understanding the microbiome, certain research-guided patterns have begun to emerge. For instance, the conditions of obesity, type II diabetes, and nonalcoholic liver disease are associated with a higher abundance of the phylum Firmicutes compared with *Bacteroides* [4]. In certain disease states, we might find that overgrowth or subclinical bowel infections of normally occurring commensal bacteria, such as the *Klebsiella* microbes, may be associated with autoimmune diseases such as Sjogren's syndrome, ankylosing spondylitis, rheumatoid arthritis, or Crohn's disease [5].

Research suggests that changing the diet to one that is plant-based and high in fiber is a rapid, effective way to cause a beneficial change in our intestinal microbiome [6]. Conversely, for those with irritable bowel syndrome (IBS), small intestinal bacterial overgrowth (SIBO), or inflammatory bowel disease (IBD), this approach may not be appropriate. Foods high in certain types of fiber such as highly fermentable oligosaccharides, monosaccharides, disaccharides, and polyols (FODMAPs) may result in rapid gas production and discomfort for those with IBS and possibly those with SIBO or IBD [7]. Additionally, therapeutic diets for autoimmune disease may consist of grain-free, nut-free, dairy-free, egg-free, and legume-free diets which would make it almost impossible to successfully implement a solely plant-based diet. However, many plant foods can be incorporated into an autoimmune diet to promote a healthy microbiome. Diets need to be individualized in every circumstance.

Stool cultures may show that certain strains of beneficial bacteria, such as *Bifidobacterium*, are low, and a specific supplemental probiotic strain might be indicated. Recurrent infections such as *H. pylori* or *Strep pyogenes* may also indicate specific strains of probiotics to be used.

8.3 Fiber

Fiber intake is critical for gut ecology because of its role as fuel for the microbiome [8]. Many food sources of fiber contain a mix of both soluble and insoluble fiber. Soluble fiber becomes gel-like and is fermented by friendly bacteria in the colon to make short-chain fatty acids (SCFA). Insoluble fiber is not only indigestible but also important for stool bulking and better gut motility. Eating a wide variety of plant foods ensures a wide variety of the different types of fibers including pectin, gum, mucilage, cellulose, hemicellulose, lignin, and soluble fiber. The peels of fruits and vegetables are high in fiber, although all peels may not be edible or palatable.

Beneficial bacteria ferment soluble fiber, which produces short-chain fatty acids (SCFA) that, in turn, serve as a fuel source to colonocytes [8]. The fermentation of soluble dietary fiber by the microbiota produces SCFA that promote increased gut cellular proliferation and differentiation, lower intestinal pH which makes the gut more resistant to pathogens, and regulates inflammation. Fiber improves the health of the gut barrier by promoting tight junctions and increasing the production of mucins that play a role in forming mucus to protect the gut barrier [8].

Butyrate, a SCFA produced by fermentation of fiber, may decrease the risk of colon cancer by inducing apoptosis in tumor cells [9]. The 2011 Colorectal Cancer Report found evidence that for every 10 grams per day increase in fiber intake, there was a 10% decrease in colorectal cancer risk [9]. Fiber aids healthy elimination of toxins through the enterohepatic circulatory system with higher elevation of SCFAs while adding bulk to the stool to assist motility through the gut [8]. In general, consuming a variety of dietary fiber helps promote diversity of gut microbiota, which supports a more robust state of health [8]. A 2017 report of the gut microbiome of the Hadza hunter–gatherers of Tanzania who average 100–150 gm of fiber/day showed much greater microbiota diversity when compared with Americans who consume only about 15 grams of fiber per day [10]. Fiber intake from legumes, fruits, vegetables, and nuts had a protective effect against CVD events, and cereal fiber was associated with a lower risk of stroke and ischemic stroke [11]. Fiber intake is inversely associated with cardiovascular disease, body weight, type II diabetes, some cancers, and chronic diseases [12].

The recommended amount of fiber in the UK is 30 grams per day, while it is 14 grams for every 1000 calories in the United States [12]. The Institute of Medicine recommends 38 grams per day for men aged 50 and younger and 30 grams for males over 50. For women, the recommendation is 25 grams daily for those 50 and younger and 21 grams daily for those over 50 [9]. The amount of fiber recommended for children is the child's age plus 5 grams per day; an 8-year-old child, for instance, is recommended to consume 13 grams of fiber per day. However, Americans average 15 grams of fiber per day, which is very low, and only 3% of the population meets the recommendations for fiber intake [9, 12].

Most plant foods are a mix of soluble and insoluble fiber [13]. Some key sources of insoluble fiber include wheat bran, psyllium, quinoa, nuts–seeds, pine nuts, and flaxseed. Key sources of soluble fiber include beans, oats and oat bran, psyllium, barley, prunes, figs, pears, leafy greens, cauliflower, broccoli, flaxseed, acorn squash, and potatoes. Cozma-Petrut and colleagues suggest a new classification of fiber that includes solubility, fermentability, viscosity, and gel formation [13]. Examples of these categories include insoluble poorly fermented (wheat bran), soluble, nonviscous readily fermented (inulin), soluble, viscous gel-forming readily fermented (beta-glucans), soluble viscous–gel-forming low or non-fermentability (psyllium) [13].

Several studies suggest that the best tolerated and most effective fiber for IBS are those with low fermentability, such as psyllium [13]. For IBS, soluble fiber was found to be better tolerated and possibly helpful compared with insoluble fiber such as wheat bran, which may worsen symptoms [7]. For IBS, an intake of 20–30 grams per day of soluble viscous fibers with low rate of fermentation, such as psyllium and ground flax seeds, starting with a low dose and slowly increasing is recommended, at a rate of not more than 5 grams per week [7]. Psyllium husks and powder should always be mixed in plenty of fluid.

A novel concept regarding fiber intake for those with chronic idiopathic constipation is that these individuals may have worsening symptoms when consuming a high-fiber diet. Ho et al. [14] conducted a prospective longitudinal case study on subjects with chronic idiopathic constipation that showed marked improvement in constipation and bloating in subjects who consumed a low-fiber or no-fiber diet compared with those who stayed on a high-fiber diet. The authors point out that most individuals who visit medical practitioners for chronic constipation are already consuming a very high-fiber diet to self-treat their constipation [14]. Those who improve on a low-fiber diet may have bacteria that are fermenting the fiber which may contribute to bloating and constipation [14]. Fiber creates bulk in the stool that can make it more difficult to expel and can result in straining and fissures [14]. Fiber can slow peristalsis and result in a buildup of gas that becomes difficult to expel [14].

When adding fiber supplements to the diet, nutritionists need to use caution and start with low amounts and increase water intake so as not to cause constipation or a bezoar (intestinal blockage of a solid mass of indigestible material that accumulates in the digestive tract). In addition, if clients complain of severe constipation or of not having had a bowel movement for days or weeks, do not recommend fiber supplements until the constipation has resolved. It may be prudent to recommend they visit a physician to uncover the cause of the protracted constipation. In this author's clinical

experience, prunes, magnesium, vitamin C, flaxseed oil, or the herbal blend triphala, and aloe vera juice can be helpful in these cases, although caution is recommended for the use of aloe vera juice. At times, a physician-prescribed laxative is needed to clear a blockage. The nutritionist can follow this intervention with constipation prevention techniques (e.g., diet, 5-HTP, magnesium, etc.). In addition, referral to an integrative or naturopathic practitioner may help to manage lifelong constipation issues with other interventions such as homeopathics, herbals, acupuncture, and the like.

Notwithstanding all the benefits of fiber, the nutritionist may find it challenging to help patients consume a wide variety of fiber when certain gastrointestinal conditions exist. Judicious use of different fibers will be required for different gastrointestinal disease states and symptomatology. Determining the cause of a person's distress may be helpful in determining which type of fibers will be tolerated. An example is someone with severe IBS. Certain fermentable fiber products may worsen the symptoms. Instead of improving the person's health, these products may cause increased distress and worsen their constipation or diarrhea. Those with celiac disease (CD) might be made worse with oat fiber and certainly with wheat bran. Standard high-fiber foods often recommended such as beans, oats, apples, pears, plums, cashews, and pistachios might worsen constipation in someone with IBS, SIBO, or aggravate cases of IBD. In addition, healthy high-fiber seeds such as chia seeds, hemp seeds, and flax seeds might be damaging to sensitive individuals. Being familiar with FODMAPs, SIBO diets, the specific carbohydrate diet, and a low-residue diet is essential in helping patients with certain GI conditions to recover. Combing the market for suitable fiber supplements can be an arduous task but highly recommended, as one size does not fit all.

In summary, dietary fiber is a critical component to maintaining balance and diversity in gut ecology. Fiber can help normalize gut transit time, promote mucus production, and strengthen the gut barrier. Promoting the proper intake of fiber and water is crucial to improving the health of our clients. For those with IBS or chronic constipation, instead of using highly fermentable fiber grains, flax seeds, chia seeds, or fermentable fiber supplements, clinicians may simply want to recommend a diet that uses FODMAP-compliant fruits, vegetables, and increasing water intake. For these individuals, adding *Bifidobacter* probiotic supplements with the fiber may also be essential. If psyllium is to be added, start with very low doses; this author recommends 1–2 grams/day to start and slowly build up depending on individual tolerance. The herbal blend called triphala, as well as supplemental magnesium citrate, can be helpful with chronic constipation.

8.4 Iodine

Iodine is an essential element needed for thyroid hormone synthesis and fetal neurodevelopment [15]. The World Health Organization (WHO) and the US Institutes of Medicine recommend 90–150 mcg/day of iodine for the general population, 220–250 mcg/day during pregnancy, and 250–290 mcg/day of iodine during lactation [15]. The WHO uses median spot urinary iodine concentrations of 100 mcg/L to represent an intake of 150 mcg/day and 100–190 mcg/L per day to define adequate intake for a nonpregnant population [15]. Excessive iodine intake is classified by the WHO as the median urinary iodine excretion >300 mcg/L for general population and >500 mcg/L for pregnant women [16].

To assess iodine status, a clinician may use clinical manifestations such as thyroid size, combined with detailed history focused on the dietary iodine intake and a history of frequent or current infection, and laboratory testing for serum thyroglobulin, thyroid-stimulating hormone (TSH), and urinary iodine [15]. Urinary iodine reflects recent iodine intake within days, whereas thyroglobulin represents iodine intake over a period of months, and thyroid size assessment represents iodine intake for a period of years [15]. Either spot urine or 24-hour urine collection iodine testing may be used. Spot urinary iodine is more useful for assessing populations rather than the individual patient, as anything less than 10 urine samples in an individual is considered misleading due to diurnal variations [16]. Urine iodine levels also vary greatly in and between individuals based on the amount of iodine consumed in a day and the level of fluids ingested by the individual [15]. Expressing the ratio of urinary iodine to creatinine may be especially useful when estimating 24-hour urinary iodine [15].

Those at risk with iodine-deficient diets include vegans and people who avoid dairy or iodized salt, as well as athletes who experience excessive sweating [15]. Alternatively, people who consume kelp may have excessive iodine intakes [15]. Goiter is thought to be an adaptation to chronic iodine deficiency as low iodine intake leads to reduced thyroid hormone production, which stimulates TSH production from the pituitary gland. Goiter results as increased TSH increases iodine uptake, which stimulates thyroid growth [15]. In adults, the thyroid may develop nodules as it enlarges [15]. For those with overt hypothyroidism due to iodine deficiency, Niwattisaiwong et al. recommend initiating levothyroxine treatment along with iodine supplementation by using iodized salt or a multivitamin containing approximately 150 mcg of iodine [15]. When urine iodine has normalized and goiter has decreased, consider decreasing the thyroid treatment, but reassessing thyroid status in 4–6 weeks after stopping levothyroxine [15]. While mild gestational iodine deficiency does not result in cretinism (severe mental retardation and other neurologic or physical defects), children born to mothers with mild gestational iodine deficiency were found to have reductions in spelling, grammar, and English literacy performance [15].

In the recent past, high-dose supplemental iodine between 12 mg and 50 mg (12,000 mcg– 50,000 mcg) was a common recommendation in the integrative and functional medicine community for preventing breast cancer and helping with subclinical or overt hypothyroidism. While adequate amounts of iodine are required for healthy thyroid function, a need for caution exists if using supplemental iodine. The American

Thyroid Association (ATA) recently released a statement advising against the use of >500 mcg of iodine daily through dietary supplements [16]. Excessive amounts of iodine can be an environmental factor linked to the development of autoimmune thyroiditis and can cause hypothyroidism, hyperthyroidism, cancers, and autoimmune thyroid disease [17]. This can especially be a concern for autoimmune-prone individuals who are at risk of developing thyroid autoantibodies [17]. Iodine causes cytokine and chemokine-mediated lymphocyte infiltration in autoimmune-prone individuals, which is a key element in the production of thyroid autoantibodies [17]. Excess iodine is also thought to produce oxidative stress-related injury to thyrocytes, and active lipid peroxidation can occur after high-dose iodine administration [17].

Excessive iodine, an example of poor nutritional balance, can easily occur and is becoming a more common concern due to high levels of salt iodization, iodine in supplements, and regular consumption of iodine-rich foods [17]. Iodine toxicity has been reported due to overconsumption of seaweed in Asian countries [17]. Drinking water can also be a source of iodine excess in places such as Somalia, Saharawi, and Europe and can occur from the use of water purification systems that contain iodine [17]. In Western countries, dairy can be a source of excessive iodine from the animal feed and equipment-cleaning products used in the dairy industry [17]. Iodized salt may contain excessive or deficient amounts of iodine as diligent monitoring of iodine levels in salt does not always occur [17]. Multivitamin supplements testing in the United States had ranges for iodine between 11 and 610 mcg, and 15 brands had higher iodine levels than what was listed on the label [17]. Pharmaceutical products are another source of excessive iodine as Amiodarone contains 37% iodine; one tablet can contain several hundred times the recommended dose [17]. Contrast agents used for diagnostic radiology can contain hundreds of thousands of times the recommended daily amounts for iodine in one single dose. Transdermal antiseptic cleaners can also be a source of excess iodine for patients and healthcare workers [17]. It can take more than 1 month for the iodine levels in the body to normalize following exposure [17] (See Table 8.1)

To protect thyroid health, one lifestyle recommendation integrative and functional clinicians make is to limit exposure to the halogens consisting of fluoride, chlorine, and bromine. Iodine is a halogen as well, and excessive exposure to fluoride, chlorine, and bromine may result in the thyroid absorbing and storing these halogens, and thus displacing iodine. Halogens have the potential to interfere with the production of thyroid hormone, iodine metabolism, and may contribute to hypothyroidism or thyroid hormone derangement. For example, excessive ingestion of fluoride, even in the presence of adequate dietary iodine intake, may induce thyroid disturbances by interfering with enzymes such as deiodinases that are required for metabolizing thyroxine into its derivative forms [19]. Fluoride may also interfere with iodide transport and displace iodide resulting in accumulation in the thyroid [19]. Halogens are found in flame retardants, dioxins, pesticides, polychlorinated biphenyls, and fluoride. Chlorine is present in swimming pools and chlorinated drinking water [20].

Table 8.1 Tolerable upper intake levels for chronic iodine ingestion

Age	Male	Female	Pregnancy	Lactation
Birth to 1 year	200 mcg	200 mcg		
1–3 years	300 mcg	300 mcg		
9–13	600 mcg	600 mcg		
14–18	900 mcg	900 mcg	900 mcg	900 mcg
≥19	1100 mcg	1100 mcg	1100 mcg	1100 mcg

Based on data from Institute of Medicine (US) Panel on Micronutrients [18]
Note: The American Thyroid Association recently advised against consuming >500 mcg of iodine daily in supplements [16]

Current recommendations point to iodine supplementation between 300 and 500 mcg per day with diet and lifestyle changes to support healthy thyroid function. If the patient has autoimmune thyroiditis, it is best to limit iodine to iodized salt and dietary intake and steer clear of supplemental iodine. Some autoimmune patients seem to do better eliminating iodized salt, but every patient is different, and individual needs should be assessed.

8.5 B Vitamins

The concept of nutritional balance is important with dosing of B vitamin supplements. The B vitamins consist of eight water-soluble vitamins interacting together as coenzymes for a variety of catabolic and anabolic enzyme reactions [21]. Collectively, B vitamins have effects in proper brain function, energy production, DNA and RNA synthesis and repair, genomic and non-genomic methylation, and the synthesis of numerous neurochemicals and signaling molecules [21]. Although water-soluble and generally regarded as nontoxic, over-supplementation of a complex of B vitamins or isolated B vitamins such as B6 or folate is a concern. Conversely, underdosing by adhering strictly to RDA levels for certain B vitamins may leave some portions of the population at risk for insufficiency [21] (See Table 8.2).

Homocysteine, a potentially toxic amino acid, is thought to accumulate when vitamins B12, folate, B6, and/or trimethylglycine (TMG) are insufficient. Elevated homocysteine is theorized to increase oxidative stress, inhibit methylation reactions, increase damage to DNA and dysregulation of its repair, promote atherosclerosis [28], and direct and indirect neurotoxicity, leading to cell death and apoptosis [21]. These processes are thought to lead to the detrimental health conditions seen with elevated homocysteine including accumula-

Table 8.2 Tolerable upper limits of B vitamins

B Vitamin	RDA		Upper limit	Additional comments
B1 thiamine	*RDA for adults* [17]		Not established as no adverse effects have been found with daily doses of 50 mg [22]	Common dose of 50–100 mg for adults unless under special medical conditions exist such as alcoholism, malabsorption, the elderly, HIV/AIDS, or after bariatric surgery [22].
	Male	1.2 mg		
	Females	1.1 mg		
	Pregnancy and lactation	1.4 mg		
B2 riboflavin	Adults 19–50 years [23]		Studies have not shown adverse effects from high riboflavin intakes of 400 mg/day and there is no established UL [23]	Essential component of the two coenzymes FMN and FAD that play major roles in energy production, cellular function, growth and development, and metabolism of fats, drugs, and steroids [23]. FAD is required for the conversion of tryptophan to niacin [23]. FMN is required for the conversion of vitamin B6 to coenzyme pyridoxal 5′-phosphate [23]. Also used in the metabolism of homocysteine. Clinically it may be helpful in higher doses with prevention of migraine headaches [24]. People at risk for deficiency include vegetarians, athletes, pregnant and lactating women and their infants, vegan and dairy-free diet followers, infantile Brown–Vialetto–Van Laere syndrome [23].
	Males	1.3 mg		
	Females	1.1 mg		
	Pregnancy	1.4 mg		
	Lactation	1.6 mg		
B3 niacin	RDA for adults [25]: 16 mg males 14 mg females 18 mg pregnancy 17 mg lactation		35 mg [25]	Temporary flushing of the skin may occur at doses of 100 mg [21]. Nausea, vomiting, diarrhea, and very rarely liver damage have occurred at extended doses of 1 gram of more [21]. Doses of up to 250 mg have been used in Parkinson's disease [21].
B5 pantothenic acid	Adequate intake for adults [25]: 5 mg 6 mg pregnant 7 mg lactation		Not established [25]	Essential coenzyme in the mitochondria for formation of adenosine triphosphate (ATP) along with thiamine, riboflavin and niacin [21].
B6 pyridoxine (pyridoxal-5′-phosphate)	RDA for adults [25]: 1.3 mg 1.7 mg 51+ years 1.9 mg pregnancy 2.0 mg lactation		100 mg [25]	50–100 mg [16]. Essential in the metabolism of homocysteine. Doses over 100 mg have been known to cause neurosensory issues [21].
B9 Folate	RDA for adults [25]: 400 mcg 600 mcg pregnancy 500 mcg lactation		1000 mcg [25]	1000 mcg is the daily upper limit; however, 200–400 mcg is often recommended as research is unclear about supplemental folic acid and cancer [21]. Exceptions include the use of certain medications and higher levels than the upper limit may be indicated in some cases of high homocysteine [21]. High doses of folate can exacerbate the effects of B12 deficiency [21]. Folate is essential in the metabolism of homocysteine [24].
B12	RDA for adults [25]: 2.4 mcg 2.6 mcg pregnancy 2.8 mcg lactation		Not established [25]	Dependent on the individual physiological needs and conditions involving absorption [24]. Essential in the metabolism of homocysteine.
Inositol	No RDA set		Not established	Inositol can be synthesized in the body from glucose. Found in the germ and bran of grains, beans, nuts, seeds, and citrus fruit [26].
Biotin	Adequate intake for adults [25]: 30 mcg 35 mcg lactation		Not established [25]	Biotin, thiamine, and B12 interrelate in the citric acid cycle [16]. Biotin can be deficient genetically susceptible individuals or with dysbiosis. Biotin is formed by gut microbiota [27].

tion of beta-amyloid, hyper-phosphorylation of tau, brain tissue atrophy, compromised cerebrovascular circulation, cardiovascular disease, and compromised cognitive function and dementia [21]. Elevated plasma homocysteine levels can be due to renal insufficiency, deficiencies of folate, vitamin B12, vitamin B6 and vitamin B2 [29]. Plasma homocysteine levels were categorized by the 2009 US National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines on Emerging Biomarkers of Cardiovascular Disease and Stroke as (umol/L): desirable <10, intermediate

>10 to <15, high >15, very high >30 [29]. For biomarkers for B vitamin assessment, see Table 8.3.

Elevated homocysteine and low levels of vitamins B12, B6, and folate have also been associated with bone loss and structural deterioration of bone tissue [35]. Deficiency of vitamin B12 is associated with lower blood levels of osteocalcin and alkaline phosphatase and may point to the activity of osteoblasts and bone metabolism being affected by vitamin B12 status [35].

While it is pertinent to address elevated homocysteine levels, the homocysteine hypothesis has resulted in limited B-vitamin research in other conditions with nutritional supplementation research focused mainly on folic acid and vitamin B12, only occasionally including vitamin B6 [3]. This has resulted in virtually ignoring the importance and interconnectivity of the role of the entire spectrum of B vitamins as essential in maintaining optimal physiological and neurological function [21].

Deficiency of even one B vitamin will negatively affect the ability to generate energy in the cell [21]. For example, the status of folate, B6, and B12 is dependent on levels of flavoproteins derived from riboflavin. Riboflavin is also essential to homocysteine metabolism [21]. Niacin, vitamin B3, serves as a necessary cofactor in the folate–tetrahydrobiopterin and methionine cycles [21]. Prolonged elevated folate status is associated with protected cognitive function only in those with normal B12 status, whereas high folate status exacerbated the detrimental effects of low B12 status [21]. Supplementation with folic acid also contributed to riboflavin deficiency in participants in one study [21].

The active forms of thiamine, riboflavin, niacin, and pantothenic acid are essential coenzymes in the mitochondria to

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Table 8.3 Biomarkers to assess B vitamin status

B1	B2	B3	B6	Folate	B12
Whole blood thiamine pyrophosphate (TPP) available at labcorp. NL 66.5–200 nmol/L. Retrieved from ► https://www.labcorp.com/test-menu/36661/vitamin-bsub1-sub-whole-blood# on 2/17/2019. Urinary amino acids test or organic acids test can also be helpful	Riboflavin status is not routinely measured. Please refer to Riboflavin Fact Sheet for Health Professionals for more information [23]. Urinary organic acids test can be helpful	Most reliable: urinary excretion of niacin's two major methylated metabolites, N1-methyl-nicotinamide and N1-methyl-2-pyridone-5-carboxamide [30]. Levels in adults: Normal niacin status is >17.5 micromol/day of these two metabolites [30]. Low niacin status: Excretion rates between 5.8 and 17.5 micromol/day [30]. Deficient niacin status: urinary-excretion rates are less than 5.8 micromol/day [30]	Direct measurement: plasma pyridoxal 5'phosphate (P5P) >20 nmol/L [31]. This is affected by inflammation markers and albumin concentration [32]. Functional biomarkers may be better assessed by integrative testing	RBC folate is considered to be the most robust marker for long-term folate status. RBC folate for populations should be around 1000–1300 nmol/L. Woolf et al. used >140 ng/mL [31]. WHO recommends <906 nmol/L (>400 µg/L) in women of reproductive age to prevent neural tube defects [29]. Evaluate homocysteine and MMA. If homocysteine is high and MMA is normal, this phenomenon may reflect a folate deficiency [33]. Urinary FIGLU normal: not detected. Elevated levels can represent folate deficiency [34]. This case can be assessed through organic acids testing	Serum B 12 marker. Subclinical deficiency: <200 pmol/L. Low is 150–249 pmol/L. Acute deficiency is <149 pmol/L. This marker has limited diagnostic ability. Serum B12 does not represent cellular B12 levels [33]. Severe B12 deficiency has been documented with normal or high serum levels of B12 [33]. Plasma homocysteine is high at >13 µmol/L, can be used along with the most specific test for functional B12 deficiency which is elevated serum methylmalonic acid at >260–350 nmol/L, (118 pmol/L), or 0.80 µmol/L [33]. Reduced kidney function may affect the clearance of both homocysteine and MMA, thus resulting in higher levels. Another marker to consider in these cases is holo-transcobalamin (holo-TC) [33] Normal holo-TC level is 20–125 pmol/L. Below 20 pmol/L would be correlate with B12 deficiency [33]

Note: Iron deficiency should always be taken into account when assessing folate and B12 status. Iron deficiency causes microcytosis, whereas anemia of folate and B12 can cause macrocytosis [33]. Iron-deficiency anemia can mask macrocytosis and megaloblastic anemia from B12 [33].

make adenosine triphosphate (ATP), the cell's energy currency. Acetyl CoA provides the main substrate for this cycle and relies on pantothenic acid [21]. Thiamine, biotin, and B12 also interrelate in the citric acid cycle and electron transport chain. B vitamins have a fundamental impact on brain function and are actively transported across the blood–brain barrier with the concentration of methyltetrahydrofolate, biotin, and pantothenic acid found in the brain at levels much higher than plasma [21]. B vitamins can generally be consumed in amounts much higher than the RDA and may be necessary for optimal health. To date, only vitamins B6, B12, and folate have an established upper daily limit.

8.5.1 Thiamine (Vitamin B1)

Thiamine plays a role in the synthesis of fatty acids, steroids, nucleic acids, and aromatic acid precursors and in the synthesis of neurotransmitters and bioactive compounds essential for brain function [21]. Thiamine also plays a neuromodulatory role in the acetylcholine neurotransmitter system and can relieve fatigue associated with hypothyroidism [36]. Thiamine can be deficient in grain-free diets and depleted with high intake of alcohol [37]. In a study enrolling young women with adequate thiamine stores but given 50 mg of thiamine or placebo for 2 months, the thiamine-supplemented group reported improved mood as assessed by the Profile of Mood States. The thiamine-treated group also demonstrated improved attention evidenced by faster decision-making on reaction time tasks [21]. Thiamine plays a role in glucose metabolism. Between 17% and 79% of obese patients examined for bariatric surgery were found to be deficient in thiamine, and this may suggest a connection between thiamine status, blood sugar regulation, and obesity. In conditions of fatigue, neurological conditions, or hypothyroidism, higher supplemental doses of thiamine (50–100 mg) may be beneficial [19, 25]. Alcoholism and bariatric surgery will likely require far higher doses delivered under medical supervision.

8.5.2 Riboflavin (Vitamin B2)

Riboflavin is required for the synthesis of two flavoprotein coenzymes, FMN and FAD, which are rate-limiting factors in most cellular enzymatic processes [21]. The flavoproteins are required for the synthesis, conversion, and recycling of niacin, folate, B6, the synthesis of heme proteins, nitric oxide synthesis, P450 enzymes, and proteins involved in electron transfer and oxygen transport and storage. The flavoproteins are also involved in fatty acid metabolism in brain lipids, the absorption and utilization of iron, and the regulation of thyroid hormones. For many reasons, riboflavin deficiency would negatively impact brain function. Clinically, higher doses of riboflavin at 400 mg are helpful with preventing migraine headaches [24]. Recent randomized controlled trials have demonstrated that riboflavin may play a novel role as a modulator of blood pressure, specifically in individuals with the MTHFR 677TT genotype, and can reduce systolic BP by 5–13 mmHg in these genetically at-risk adults [38].

8.5.3 Niacin (Vitamin B3)

Niacin-derived nucleotides, such as nicotinamide adenine dinucleotide (NAD) and NAD phosphate (NADP), are critical for enzymes involved in every aspect of peripheral and brain cell function [21]. Niacin receptors are found in immune cells and adipose tissue as well. Niacin plays a role in neuromodulation of inflammatory cascades and antiatherogenic lipolysis in adipose tissue. People with Parkinson's disease are often low in niacin. One study found this population to benefit from 250 mg niacin supplementation, which resulted in attenuation of the disturbed sleep architecture associated with Parkinson's disease [21].

8.5.4 Pantothenic Acid (B5)

Pantothenic acid is required for the synthesis of coenzyme A (CoA). CoA plays a role in oxidative metabolism and contributes to the structure and function of the brain via its role in the synthesis of cholesterol, amino acids, phospholipids (PLs), and fatty acids. Through the action of CoA, pantothenic acid also helps with the synthesis of neurotransmitters and steroid hormones [21].

8.5.5 Vitamin B6

Vitamin B6 plays an essential role in the folate cycle and amino acid metabolism and is a rate-limiting cofactor in the synthesis of neurotransmitters including dopamine, serotonin, GABA, noradrenaline, and the hormone melatonin [21]. Neurotransmitter synthesis is especially sensitive to B6 deficiency. Even a mild deficiency can result in downregulation of GABA and serotonin synthesis. When GABA is unable to participate in its inhibitory role on neural activity, disordered sleep, behavior changes, cardiovascular function, and loss of hypothalamus–pituitary control of hormone secretion can result.

Low B6 status has been found in oral contraceptive users, smokers, and people with celiac disease, alcoholism, and diabetes [32]. B6 deficiency usually does not occur in isolation, but rather, with other B-vitamin deficiencies. Assessing B6 status can be difficult. Pyridoxal phosphate (PLP), the active form of B6, is often used to assess vitamin B6 by laboratory testing. PLP testing appears to be accurate in healthy individuals and has been found to reflect the vitamin B6 content in the liver and correlate with vitamin B6 dietary intake [32]. However, plasma PLP levels can be affected by albumin concentration, alkaline phosphatase activity, inflammation, and alcohol consumption [32]. Also of concern, low plasma PLP has been found with inflammation such as rheumatoid

arthritis, inflammatory bowel disease, cardiovascular disease, diabetes, deep vein thrombosis, and cancer [32]. Plasma PLP shows an inverse relationship with markers of inflammation such as C-reactive protein and other markers of acute phase reactants [32].

The activated form of B6, pyridoxal-5′-phosphate is downregulated during times of inflammation and therefore may contribute to dementia and cognitive decline due to the essential role it plays in brain glucose regulation, immune function, and gene transcription–expression [21]. Vitamin B6 can be over-supplemented and cause sensory neuropathy that is usually reversible. Evidence from case studies suggests that this can happen with as little as 100 mg per day [19]. Keeping the total amount of B6 to 50–100 mg per day is recommended [21]. If higher doses need to be used, consider this as a short-term intervention of 2–3 months, and a lower dose of around 50 mg should be recommended after this time period. There are, however, clinical trials that have used B6 doses as high as 750 mg over several years with no reports of neuropathy [21]. Individuals with certain single nucleotide polymorphisms in the CBS genes and MTHFR will have different requirements for vitamin B6. Please refer to ▶ Chap. 18 for this discussion.

8

8.5.6 Folate (Vitamin B9)

Folate and B12 are linked due to their complementary roles in the folate and methionine cycles [21]. If B12 is deficient, folate can become trapped as methyltetrahydrofolate, resulting in a functional folate deficiency [21]. Requirements for folate may also differ based on genetics. Either an actual or functional deficiency of folate may hamper DNA stability and repair as well as gene expression and transcription. This, in turn, affects neuronal differentiation and repair, which promotes hippocampal atrophy and demyelination. Ultimately, this compromises the integrity of membrane phospholipids resulting in the impaired action potential of the neuron [21].

A lack of folate may result in decreased synthesis of proteins and nucleotides required for DNA and RNA synthesis. This can have negative ramifications for rapidly dividing tissues, such as fetal development, and can lead to megaloblastic anemia and neuronal dysfunction [21]. Additionally, the folate cycle needs to function effectively to synthesize and recycle tetrahydrobiopterin, which is an essential cofactor for enzymes that convert amino acids to monoamine neurotransmitters such as serotonin, melatonin, dopamine, noradrenaline, adrenaline, and nitric oxide [21].

The upper daily limit for folic acid is set at 1000 mcg/day. This upper level is related to folate's ability to mask vitamin B12 deficiency, resulting in irreversible damage [21]. Detrimental effects of high doses of folic acid are related to high levels of unmetabolized folic acid on normal folate metabolism and immune function [21].

The use of folate comes with caveats. Individuals with MTHFR single nucleotide polymorphisms often require higher doses of folate [39]. Caution is advised in using high doses of folic acid in combination with antifolate medications, such as methotrexate, prescribed for conditions such as rheumatoid arthritis, psoriasis, cancer, bacterial infections, and malaria [21]. In addition, supplemental folate may confer protection against cancer at lower doses but may cause increased risk of cancer at higher doses, but there is no consensus on blood levels of folate that may cause harm.

As many patients turn to vitamins and supplements to enhance energy, relieve fatigue, or generally feel better, it is important to understand the connection between the B vitamins and psychiatric symptomatology. Vitamins B6, B8, and B12 have been shown not only to reduce psychiatric symptoms but also shorten the duration of illness [39]. However, when patients lack a specific genetic enzyme which converts folate–folic acid to its most usable form, L-methylfolate, the neuroprotective, and neuropsychiatric benefits are lost. L-methylfolate allows for the synthesis of the three major neurochemicals—serotonin, norepinephrine, and dopamine—across the blood–brain barrier [39]. Exploring the conversion of folate–folic acid into L-methylfolate and the various polymorphisms of the MTHFR gene while examining the B vitamins associated with the treatment of psychiatric symptoms allows integrative and functional practitioners to treat patients with the appropriate B vitamins [39]. For an in-depth analysis of how to assess folate status, please see ◘ Table 8.2 for biomarker assessment. For a detailed discussion, see Nutragenomics in ▶ Chap. 17.

8.5.7 Vitamin B12

Vitamin B12 is protective against neurological deterioration, and deficiency of B12 is associated with peripheral neuropathy, cognitive impairment, and neurodegenerative disease [33]. Causes of B12 deficiency are largely related to absorption in the GI tract, lack of intrinsic factor, or dietary deficiency such as with vegan diets [33]. Autoimmune pernicious anemia, intestinal surgery such as bariatric surgery, and chronic gastritis from *H. pylori* infections all decrease the release of intrinsic factor which can result in B12 malabsorption. Undiagnosed celiac disease can also result in malabsorption of B12 [33]. Some medications interfere with absorption and metabolism of B12, including metformin. Metformin decreases serum B12, but in some studies, it was shown to decrease plasma methylmalonic acid (MMA) and increase intracellular B12, although there are conflicting reports [33]. Metformin may alter B12 homeostasis and tissue distribution, but the clinical consequences remain to be determined. Proton pump inhibitors and other medications that reduce the production of hydrochloric acid are also associated with B12 deficiency [33]. B12 deficiency can be masked by folate supplementation and can cause severe neuropathy and neurodegeneration. An effective test for B12 sufficiency is MMA in blood or urine. Refer to ◘ Table 8.2 for assessment of B12 levels.

Doses of vitamin B12 that far exceed the RDA are commonly used in the integrative and functional nutrition realm. Anecdotally, nutrition practitioners have found that genetically susceptible individuals with catechol-O-methyltransferase (COMT) single nucleotide polymorphisms have a decreased ability to metabolize catecholamines, and supplementation with the methylcobalamin form of vitamin B12 may aggravate symptoms of anxiety and insomnia. Some of these individuals do better with hydroxocobalamin or adenosylcobalamin. To counter untoward effects when beginning B12 supplementation, start with a lower dose, such as 500 mcg, and slowly titrate dose upward.

The take-home message with B vitamins is to supplement all the B vitamins (not just one) because they work synergistically, even if the exact mechanisms has not been fully elucidated in research.

8.6 Fat-Soluble Vitamins

Fat-soluble vitamins are at risk of deficiency with certain states of malabsorption and malnutrition. When used in supplemental form, these vitamins need to stay in balance with each other, notably vitamin K1–K2 and vitamin D and vitamin D and vitamin A. Vitamin E is best provided in a natural full-spectrum combination as delta, beta, gamma, d-alpha tocopherols and the four tocotrienols, the way it is found in food.

8.6.1 Vitamin D and Vitamin K

One of the risks of using supplemental vitamin D is the increased gastrointestinal absorption of calcium that results in high serum levels of calcium and increased vascular calcification [40]. Theoretically, if vitamin D supplementation is balanced with supplementation of vitamin K1 and K2 in the forms of menaquinone-4 (MK 4) and menaquinone-7 (MK 7), this will decrease the likelihood of soft tissue calcification and of bone fracture [40]. In addition, a few vitamin K-dependent small proteins act to inhibit soft tissue calcification and include osteocalcin (bone Gla protein), matrix Gla protein (MGP), and possibly Gla-rich protein (GRP) [41]. The role of these proteins has been elucidated in studies monitoring the effects of treatment with oral anticoagulants that are known vitamin K antagonists (VKA).

With regard to VKA, all vitamin K-dependent calcification inhibitors will remain uncarboxylated and inactive [41]. Compared with controls, subjects taking anticoagulants had significantly higher calcification of arteries and the aortic valve [41]. Despite previous publications that reported vitamin K1 had no effect on bone mineral density, in a recent study, vitamin K1 deficiency was the strongest predictor of vertebral fractures and vascular calcification in chronic kidney disease. In postmenopausal women, 5 mg of vitamin K1 protected postmenopausal women from bone fractures, despite having no positive effect on bone mineral density [42]. Long-term, three-year supplementation with menaquinone (K2) of 180 mcg in 120 healthy postmenopausal women resulted in decreased arterial stiffness [40].

Vitamin K2 includes several different vitamers, of which MK7 is the most well-known. One study found that plasma concentrations of albumin and vitamin K1 were significant predictors of hip fracture in the general population, whereas MK7 was not [42]. To promote a decreased risk of bone fracture while protecting from vascular calcification, it is likely prudent to include both vitamin K1 and vitamin K2, specifically MK 4 and MK 7, when recommending calcium and vitamin D [42].

8.6.2 Vitamin A and Vitamin D

Vitamins A and D are important synergistic partners due to their shared binding of the nuclear retinoid X receptors (RXR), resulting in synergistic effects of one vitamin with the other. In nature, vitamins A and D are found together in balance such as in cod liver oil, egg yolk, and organ meats. Vitamins A and D function in many systems throughout the body beyond the eyes and bones. Furthermore, vitamins A and D are important as immune and hormone modulators as well as affecting structural forms such as bones, cell membranes, tissues, etc.

The effects of vitamins A and D supplementation on bone mineral density have been controversial. Observational studies have reported an association between higher serum retinol concentration and risk of bone fracture; another study reported that high retinol concentration in the presence of vitamin D deficiency can increase the risk of osteoporosis in menopausal women [42]; another recent study found that higher intake of vitamin A, when combined with sufficient intake of vitamin D, can promote increased bone density [43]. In a Korean population, a higher dietary vitamin A intake does not appear to negatively affect bone mineral density when 25 (OH) D levels are moderate at 50–75 nmol/L [43].

When assessing an individual's vitamins A and D status, it is beneficial to get a baseline of blood vitamin A retinol and vitamin D 25–OH, vitamin D1, 25-OH, and PTH, at minimum, to develop more targeted interventions and improve outcomes. If genomic information is available for vitamin D receptor and BCMO1 genes, genomic differences that influence the ability to establish balance between vitamin A and vitamin D might come to light. This highlights the fact that there are unique requirements for individuals to maintain nutrient balance.

Serum retinol and serum 25(OH)D can both be tested to ensure safe and adequate levels are maintained. Normal levels of serum vitamin A range from 50 to 200 mcg/dL or 1.75–6.98 micromol/L [6]. Interestingly, in nature, vitamins A and D are found together such as in egg yolk, liver, and butter. Overall, keeping the vitamin D level adequate appears to be protective to bones when supplementing vitamin A.

8.6.3 Tocopherols and Tocotrienols

Vitamin E is a family of fat-soluble antioxidants that mainly refers to alpha tocopherol, but naturally includes several tocopherols and tocotrienols [40]. Vegetable oils contain higher amounts of tocopherols, while tocotrienols are found in palm oil [44]. Both tocopherols and tocotrienols have four homologues consisting of alpha, beta, gamma, and delta [44]. Gamma-tocopherol is known mainly for its beneficial function in maintaining cardiovascular health, whereas the tocotrienols have shown more diverse application and protection against cancer, cardiovascular disease, neurodegeneration, oxidative stress, fertility, and immune regulation [44].

Tocotrienols exert different effects. Delta- and gamma-tocotrienols were more potent in cancer studies, while alpha-tocotrienols are more effective with neuroprotection. In a trial, subjects supplemented with gamma- and delta-tocotrienols showed a significant reduction in triglycerides and very low-density lipoprotein with no change in total cholesterol, LDL and HDL cholesterol, whereas the other homologues, alpha and gamma, did not show any effect on lipid profiles [44].

In preclinical cancer studies, tocotrienols have been shown to have antiproliferative, antiangiogenic, proapoptotic, and immune-enhancing properties [44]. The first clinical trial of tocotrienols involved a five-year, placebo-controlled, double-blinded study. Participants showed decreased number of deaths and incidence of recurrence in the tocotrienol group. Gamma-tocopherol showed increased apoptosis in a study on pancreatic ductal neoplasia.

Tocopherols and tocotrienols have neuroprotective properties and were shown to reduce glutamate toxicity, and subsequent damage to neurons and astrocytes and the use of vitamin E for the management of Alzheimer's disease is a topic of interest [45]. In a two-year study, daily supplementation of 400 mg of tocotrienol-rich fraction (TRF) resulted in statistically significant reduction in white matter lesions, compared with placebo group [44].

At this writing, there is no general consensus on recommendations for vitamin E intake as it is dependent on age, lifestyle, fat malabsorption, individual differences in vitamin E metabolism, and interaction with pharmaceuticals, such as blood thinners and statins [40]. Vitamin E may become a prooxidant when administered at high levels, but this effect may be mitigated by the coadministration of other antioxidants, such as vitamin C [40]. There is recent speculation that supplemental tocotrienol (T3) exerts more benefit than supplemental tocopherol and tocopherols should be solely obtained through food sources.

8.7 Minerals

8.7.1 Calcium and Magnesium

Calcium and magnesium are important nutrient partners that require balance, as elevation of one can cause suppression of the other. Integrative and functional medicine clinicians recommend supplementing calcium–magnesium at a ratio of 2:1 to 1:1. Supplemental calcium continues to be controversial as it may be associated with an increased risk for myocardial infarction, stroke, and cardiovascular mortality and an increased risk for kidney stones and soft tissue calcification [46] and more recently continues to be controversial as it may be associated with an increased risk for myocardial infarction, stroke, and cardiovascular mortality and an increased risk for kidney stones and soft tissue calcification [46]. There is growing evidence for the need for calcium supplementation to be in tandem and balanced with magnesium. Integrative and functional nutritionists often see clients with malabsorption, and part of the dietary intervention may include eliminating dairy products, often without recommending supplemental calcium or calcium-rich foods. This may have unintended harmful consequences (See Tables 8.4 and 8.5).

Using celiac disease (CD) as an example, calcium malabsorption and insufficient intakes of calcium may result in hypocalcemia, which produces a compensatory increase in serum levels of parathyroid hormone. This, in turn, leads to increased bone turnover and ultimately bone loss, with bone resorption being faster than new bone formation [47]. It is possible that the malabsorption of calcium is the main contributing factor to secondary hyperparathyroidism seen in CD [48, 49]. In addition, whenever parathyroid hormone is elevated, there is rapid conversion of 25 OH vitamin D to 1,

Table 8.4 High calcium foods to recommend to patients on dairy-free diets

1 cup okra, cooked	150 mg
1 cup spinach, cooked	350 mg
1 cup kale, cooked	94 mg
1 cup broccoli, raw	42 mg
1 cup collard greens, cooked	150 mg
1 cup turnip greens, boiled	190 mg
1 cup bok choy, raw	74 mg
3 oz canned sardines with bones	325 mg
3 oz canned salmon with bones	190 mg
½ cup firm tofu made with calcium sulfate	253 mg
1 cup calcium-fortified milk alternative	300 mg
Calcium-fortified juice	261 mg
½ cup soy beans and white beans	80–90 mg
Luna bar	350 mg
Power bar	300 mg
1 cup calcium-fortified cereal	250–300 mg
2 tablespoons of Tahini or sesame seeds	120–180 mg

Based on data from National Institutes for Health Calcium Fact Sheet for Health Professionals retrieved on 8/14/2017 from ► https://ods.od.nih.gov/factsheets/Calcium-HealthProfessional/

Table 8.5 Recommended daily allowances (RDAs) for calcium intake

Age	Male	Female	Pregnant	Lactating
0–6 months[a]	200 mg	200 mg		
7–12 months[a]	600 mg	600 mg		
1–3 years	700 mg	700 mg		
4–8 years	1000 mg	1000 mg		
9–13 years	1300 mg	1300 mg		
14–18 years	1300 mg	1300 mg	1300 mg	1300 mg
19–50 years	1000 mg	1000 mg	1000 mg	1000 mg
51–70 years	1200 g	1200 mg		

[a]AI (adequate intake) National Institutes for Health Calcium Fact Sheet for Health Professionals retrieved on 8/14/2017 from ► https://ods.od.nih.gov/factsheets/Calcium-HealthProfessional/

25 OH vitamin D (the active form of vitamin D) to enhance calcium absorption [48]. Clinically, you may see a low 25 OH and an elevated 1, 25 OH. Regarding CD, 75% of untreated adult CD patients with overt malabsorption, and about half of those with subclinical CD presenting with minimal symptoms or asymptomatic CD patients will have bone loss. In 2000, the British Society of Gastroenterology published guidelines for treating osteoporosis in CD that includes a daily calcium intake of 1500 mg and vitamin D supplementation [47]. Malabsorption of other micronutrients may also contribute to altered bone metabolism and increased pro-inflammatory cytokines that enhance osteoclastogenesis and bone resorption [49].

In cases where malabsorption is suspected, it may be prudent to request the patient have a parathyroid hormone test. It may be possible that secondary hyperparathyroidism may occur with low, normal, or high serum calcium levels. High serum calcium levels may be the result of long-term calcium malabsorption resulting in secondary hyperparathyroidism. Have clients work with a physician to see if they are a candidate for calcium and vitamin D supplementation. Clinically, it may be safe to assume that individuals at risk for calcium and vitamin D malabsorption include those with CD, inflammatory bowel disease, bariatric surgery (gastric bypass surgery) and other GI surgeries, eating disorders, and the presence of gastrointestinal pathogens [49]. Seeing a pattern of low normal serum protein, low normal albumin, low ferritin, or other markers of iron status, elevated MCV (suggesting deficiencies of folate and vitamin B12) and low normal serum calcium might suggest a pattern of malabsorption. Various medications, such as steroids, proton pump inhibitors, and loop diuretics, also increase the need for calcium supplementation [49]. The overarching recommendation is to dose calcium based on individual needs rather than blanket recommendations for the general population.

Magnesium is often left undiscussed in treating bone health and cardiovascular disease. Low magnesium status may lead to a greater risk of metabolic syndrome, type 2 diabetes, cardiovascular disease, skeletal disorders, chronic obstructive pulmonary disease, depression, decreased cognition, and vitamin D deficiency [3]. Magnesium plays a critical synergistic role in the synthesis and metabolism of parathyroid hormone (PTH), vitamin D-binding protein (VDBP), and three major enzymes that determine 25-OH vitamin D concentrations [3]. Magnesium deficiency leads to decreased levels of 1, 25 $(OH)_2$ D, the active form of vitamin D, and subsequent impaired PTH response [3]. Additionally, a specific form of vitamin D-resistant rickets that is magnesium-dependent exists and only responds to vitamin D therapy when magnesium is concurrently administered [3]. Readers will note other nutrients besides calcium, magnesium, and vitamin D that play a role in bone density include protein, vitamin K, zinc, copper, and boron.

Two small clinical studies on magnesium-deficient patients show that magnesium administered alone is not enough to raise vitamin D levels and that administration of vitamin D3 combined with magnesium infusion resulted in a substantial increase in both 25(OH)D and 1, 25 $(OH)_2$ compared with the infusion of magnesium alone. A recent NHANES study found that a high intake of magnesium was independently and significantly associated with a reduced risk of vitamin D deficiency [3]. Although more studies will elucidate the relationship between magnesium and vitamin D status, it may be prudent for clinicians to correct both vitamin D status and magnesium status concurrently. If clinicians experience vitamin D-resistant patients, the patient may be deficient in magnesium as well. Supplementing with vitamin D and calcium in the presence of suboptimal magnesium intake may be affecting health through interactions between the three nutrients that have not yet been fully explained [3].

Since 1977, the United States has increased both dietary calcium and magnesium intake, but dietary calcium intake has increased at a rate of 2–2.5 times that of dietary magnesium [3]. Some US studies have shown that since 2000, the calcium-to-magnesium ratio has increased ≥3 times and coincides with increasing rates of type 2 diabetes and colorectal cancer [3]. High dietary calcium-to-magnesium ratio has been proposed as an explanation for the striking variation in incidence of postmenopausal breast cancer associated with geographic location [50], the lowest calcium intakes being in Asia where the incidence of breast cancer is much lower compared to North America and northern Europe [50]. An imbalance of the calcium–magnesium intake may lead to irregularities in DNA repair, cell proliferation, differentiation, and carcinogenesis [50].

Reduced magnesium intake may be due to increased intake of refined and processed foods, softening of hard water, chronic alcohol ingestion, and gastrointestinal disorders causing malabsorption and certain medications. In addition, calcium supplementation may accentuate the problem of reduced magnesium intake [50]. Subclinical dietary magnesium deficiency was shown to increase calcium retention, and once calcium is high, magnesium absorption can be significantly depressed. Moderate alcohol consumption is

associated with breast cancer and alcohol exacerbates magnesium deficiency [50].

Higher self-reported dietary and supplemental magnesium intakes were associated with lower levels of coronary artery calcification, which is a sensitive, discriminating measure of subclinical cardiovascular disease [51]. Huang et al. found that consumption of moderate amount of calcium and an adequate amount of magnesium with maintenance of calcium-to-magnesium ratio of 2.0–2.5 are important for reducing cardiovascular risks in older patients with diabetes [52]. Individuals who consume 1000–1200 mg of calcium per day need to increase their magnesium intake to maintain the calcium-to-magnesium ratio of 2.0–2.5 [52].

Traditional advice is to maintain calcium-to-magnesium ratios for optimal health [3], and the authors of a 2007 colorectal neoplasia study suggested the optimal dietary calcium-to-magnesium ratio be <2.8. It is thought that calcium-to-magnesium ratios >2.6–2.8 can have detrimental health effects, and it has also been questioned if a calcium-to-magnesium ratio of <2.0 may also be detrimental [3]. More research is needed to establish a beneficial ratio.

8.7.2 Sodium-to-Potassium Ratio and Hypertension

The effect of sodium-to-potassium (Na–K) ratio on hypertension is perhaps more important than looking separately at the effect of individual sodium or potassium intake on hypertension. Inconsistencies have been found in observational studies around the world showing a relationship between high sodium intake and hypertension, as well as high Na–K ratios and hypertension [53]. The mechanisms of sodium and potassium on blood pressure are multiple. In salt-sensitive individuals, sodium intake results in sodium and water retention and extracellular volume expansion. The extracellular volume expansion results in release of substances that increase heart and blood vessel contraction and affect the renin–aldosterone system [53]. Potassium increases urinary sodium excretion, which lowers serum sodium levels, and is thought to induce vascular smooth muscle relaxation or widening of the blood vessels to lower blood pressure [53].

Potassium is an electrolyte needed for normal cellular function and is easily excreted by healthy functioning kidneys rather than stored in the body [54]. Therefore, humans need a constant supply of potassium through the diet. Adequate intake of fruits and vegetables is a major source of potassium [54]. The average potassium consumption is at 54% of the US recommended intake [54]. Low potassium intake is also correlated with central obesity and metabolic syndrome [54]. A caveat is high potassium intake which is contraindicated in renal disease because of poor excretion and potential for elevated levels.

Potassium supplementation has consistently been shown to lower blood pressure, and low dietary potassium is associated with an increased risk of developing hypertension [55]. The joint effect of high sodium and low potassium intakes may have a greater effect on hypertension than elevated sodium intakes or low potassium intakes alone [53]. In a Korean study by Park et al., dietary intake of both sodium and potassium was evaluated, and the authors showed that an increased ratio of sodium to potassium was correlated with increased prevalence of hypertension [53].

Study subjects who had a lower prevalence of hypertension had a Na–K ratio of 1.21:1, and with increased prevalence of hypertension, there was a Na–K ratio of 2.56:1 [56]. Additionally, blood pressure results had almost a linear dose response to increasing ratio of Na–K [53].The blood pressure lowering effects of potassium were greatest in those with the highest intake of sodium, and the ratio of Na–K had a greater effect on the risk of cardiovascular disease than sodium or potassium alone [53]. Recommending a diet high in fresh fruits and vegetables, including low-fat dairy products, and consuming a whole-food diet, is likely the most effective approach for lowering the Na–K ratio [53]. Caution is advised when looking at sodium-to-potassium ratios as great variability in sodium sensitivity exists between different ethnic groups [53].

8.7.3 Zinc and Copper

Zinc and copper are another example of nutrient partners that need to be metabolically balanced. Excessive elevation of either one can suppress the other, much like the relationships described above.

Zinc is used clinically in supplement form to facilitate wound healing; decrease skin inflammation; support immune function, tissue growth, and maintenance of thyroid function; promote GI tract healing; protect against such ocular diseases as macular degeneration; and promote testosterone balance. Zinc supplementation can induce copper deficiency that may result in serious neurological conditions, hematological abnormalities, and possibly thyroid abnormalities; therefore, zinc needs to be balanced with copper [57, 58]. High tissue–copper levels may be associated with inflammation and certain disease states, such as cancer. "The RDA recommendations for zinc and copper intake are in a ratio of 9:1 [59]". When working with medical conditions, it is important to measure a baseline blood test for both nutrients to determine if supplementation of either is needed. (See Table 8.6 Assessing zinc and copper status.)

Measuring the serum copper to zinc ratio is a helpful parameter in states of disease and inflammation. The normal plasma zinc to serum copper ratio in children and adults is 1:1 [64]. The increment of this ratio of the opposite (copper–zinc) above 2.0 in the elderly usually reflects an inflammatory response or decreased zinc status [65]. High serum copper to zinc ratio has been associated with cardiovascular death, malignancy, and all-cause mortality in the elderly [65]. During the acute phase of various diseases, systemic mechanisms decrease zinc and increase copper. Using the ratio of serum copper to zinc may be more predictive of inflamma-

Table 8.6 Summary of copper and zinc assessment

Copper reference ranges	Zinc reference ranges
Serum free copper: 1.6–2.4 μmol/L or 10–15 μg/dL [60] *Total copper*: 10–22 μmol/L or 63.7–140.12 μg/dL [60] *Serum ceruloplasmin*: 2.83–5.50 μmol/L or 18–35 μg/dL [60] <20 mg/dL ceruloplasmin signifies Wilson's disease along with Kayser–Fleischer rings [61]. Wilson's disease is a genetic defect in copper excretion. *24-hour urine copper*: 0.3–0.8 μmol or 20–50 μg [60] <40 mcg/day [61] >100 mcg/day occurs in Wilson's disease [61] *Liver copper*: 0.3–0.8 μmol/g of tissue or 20–50 μg/g of tissue [60]	*Normal serum zinc*: 60–120 μg/dl [62] *Plasma zinc is deficient if below*: 60 μg/dL [63] *Alkaline phosphatase (ALP) normal range*: 45–115 units/liter (U/L) [62] <45 U/L ALP indicates zinc and/or magnesium deficiency [62] * For reference in assessing low ALP, normal serum magnesium: 1.3–2.5 mEq/L [62]

tion than using other inflammatory biomarkers such as CRP and ESR [65]. Nutritional factors such as increased copper intake and decreased zinc intake are not thought to be the cause of an elevated copper-to-zinc ratio during states of inflammation, but rather, other systemic mechanisms may be the cause. Zinc is carried on albumin, and copper is carried on ceruloplasmin (Cp). Synthesis of these proteins and the regulation of serum copper and zinc occurs mainly at the hepatic level.

During oxidative stress, the albumin-bound zinc in the plasma decreases while labile zinc relocates to peripheral tissues [65]. This labile zinc induces the antioxidant metallothionein (MT). Infections and inflammatory conditions which induce oxidative stress cause copper–ceruloplasmin levels to rise. During inflammation and aging, the cytokines interleukin (IL) 6, IL-1 beta, tumor necrosis factor-alpha, and interferon gamma (IFN-gamma) are known to suppress the synthesis of albumin (i.e., bound to zinc) and increase the synthesis of ceruloplasmin. This mechanism may be protective in response to oxidative stress, infection, and low-grade inflammation. Impaired insulin action also decreases the synthesis of albumin, which may, in turn, decrease plasma levels of zinc.

Following inflammatory stimuli, zinc is redistributed from the plasma to the liver, thymus, and marrow. This may be a mechanism to restrict zinc from being used by invading pathogens, protect the liver and tissues from oxidative stress by producing MT, and manufacture lymphocytes to protect against invading pathogens [65]. Increased levels of copper in the serum may help the antimicrobial function of macrophages. Very low or undetectable levels of zinc are found in infected tissue, whereas copper accumulates at sites of infection where macrophages are present in high amounts [65].

Copper deficiency impairs oxidative phosphorylation, cellular antioxidant defense, collagen and elastin biosynthesis, production of several metalloenzymes such as copper–zinc superoxide dismutase, and affects levels of selenium-dependent glutathione peroxidase [64]. Copper deficiency impairs blood coagulation, has adverse effects on blood pressure and heart function, affects cholesterol and glucose regulation, contributes to neurodegeneration, causes anemia, affects cross-linking of connective tissue, and causes mineralization of the bone, ultimately leading to osteoporosis [66]. Copper deficiency results in optic neuropathy [67]. Anemia that is refractory to iron supplementation is a classic, well-documented sign of chronic copper deficiency [68]. One study in frail elderly men found decreased hematocrit and serum iron levels in subjects who had an elevated serum copper to zinc ratio [68]. Additional symptoms of copper deficiency include leukopenia, neutropenia, decreased superoxide dismutase, decreased ceruloplasmin, increased plasma cholesterol and LDL–HDL ratio, and abnormal cardiac function [64].

Copper intoxication rarely occurs in humans, as it is excreted in the bile [64]. However, elevated copper levels are associated with infections, inflammation, Wilson's disease, trauma, systemic lupus erythematosus (SLE), and autism [64]. Both zinc and molybdenum deficiency may be a risk factor for copper toxicity. Due to its role as a cofactor, copper toxicity has been associated with such neurological diseases as amyotrophic lateral sclerosis, Alzheimer's disease, and Creutzfeldt–Jakob disease [64]. Physiological factors independent of copper intake can affect copper levels. Plasma copper levels are higher in women than men due to estrogen [59]. Infection, inflammation, and estrogen levels increase plasma copper. Corticosteroid and adrenocorticotropic hormone lower copper concentrations.

Copper is bound to the protein ceruloplasmin that is regulated by homeostatic mechanisms [59]. High serum copper concentration with elevated copper-to-ceruloplasmin ratio is indicative of copper excess [59]. In Wilson's disease, a condition of copper excess, free copper is elevated, and ceruloplasmin is lowered [59]. Ceruloplasmin is an acute phase reactant and may be raised in response to hepatic inflammation, pregnancy, estrogen use, or infection [61]. Falsely low ceruloplasmin levels may occur with any protein deficiency state including nephrotic syndrome, malabsorption, protein-losing enteropathy, and malnutritions [61].

A review of methods of assessment of copper status in humans suggests that serum copper seems to be the most accurate biomarker to assess copper status in humans, reflecting changes in status in both depleted and replete individuals [69]. Measuring copper levels may be elusive, but low plasma or serum levels of copper indicate depletion. Hair and urinary copper are not useful indicators of copper status [59]. Total ceruloplasmin protein level is related to copper status, but deficiency reflects changes in highly depleted individuals only [69]. One of the first signs of copper deficiency is a drop in ceruloplasmin. Ceruloplasmin accounts for 90% of total plasma copper [70]. Ceruloplasmin and serum copper are

acute phase reactants and can rise in response to inflammation, even in states of copper deficiency [66].

The dosing range for copper in adults is 2–10 mg/day with monitoring of zinc status during supplementation [59]. In states of copper deficiency, typically 2 mg/day of copper will reverse the hematological abnormalities in the early stages [58].

8.7.4 Assessing Zinc Status

Symptoms of severe zinc deficiency include hypogonadism, dwarfism, growth-retarded infants and children, dermatitis, diarrhea, alopecia, and loss of appetite [64]. More moderate zinc deficiency can result in decreased immune function, increased mortality due to infections, and brain damage in a fetus when the pregnant mother is zinc deficient [64]. Zinc-repletion dosing ranges anywhere from 5 mg/day to 50 mg/day with monitoring of copper status during supplementation [59].

There is currently no specific sensitive biochemical or functional indicator of zinc status [71]. Accurate measures of plasma zinc are complicated by the body's homeostatic control of zinc levels and factors affecting zinc status that are unrelated to nutritional status [71]. Plasma zinc is bound to its carrier protein albumin; therefore, anything that alters albumin levels will alter plasma zinc levels [59]. There is no functional reserve of zinc in the body, and the body's zinc levels are maintained by conservation and tissue redistribution [71].

Cessation of growth in children is an example of severe zinc deficiency [71]. When dietary zinc intake is low, fecal zinc excretion may be reduced by 60%, coupled with increased intestinal zinc absorption. In mild zinc deficiency, plasma zinc can be maintained at the expense of zinc from other tissues [71]. Therefore, plasma zinc is not a reliable measurement of zinc intake or whole-body zinc status [71]. Plasma zinc levels in mildly zinc-deficient growth-retarded children who responded to zinc supplementation were not significantly different from normally developed children, before or after zinc supplementation [71].

Decreased plasma zinc levels may result from stress, infection, inflammation, use of estrogen, oral contraceptives, and corticosteroids. Plasma zinc levels are known to fall 15–20% after a meal [71]. Increased zinc levels may occur from fasting or red blood cell hemolysis [59]. A better alternative to plasma zinc levels might be red blood cell zinc levels, as RBCs contain zinc-dependent proteins. RBCs turn over every 120 days; hence, RBC zinc status would reflect whole-body zinc status over a longer period of time than plasma zinc and is not prone to recent changes in whole-body zinc status [59].

Zinc-containing enzymes are another possible marker for zinc status, and alkaline phosphatase has been studied as a biomarker for zinc status. In a study in Guatemalan children, low serum zinc was associated with low serum albumin and low serum alkaline phosphatase [72]. When assessing ALP, it is important to differentiate if low alkaline phosphatase is due to zinc or magnesium deficiency [62]. When zinc was repleted, the serum zinc increased, as did alkaline phosphatase. Other studies in Guatemalan children have shown an association between serum zinc and alkaline phosphatase [72]. However, it was noted in children and adolescents depending on age, serum alkaline phosphatase levels have a wide variation and therefore should be interpreted cautiously.

8.7.5 Copper-to-Zinc Ratio in Cancer

Functional medicine experience-based practice recommendation in serum copper-to-zinc ratio is 1:1 for optimal health, although this author could not find research to corroborate at this time. When serum copper-to-zinc ratio is being assessed during cancer treatment, serum zinc is used. Numerous studies have found an association between elevated copper-to-zinc ratio and disease severity. Inducing copper deficiency to prevent angiogenesis in cancer has been proposed as an adjunctive treatment for cancer [70]. Currently, it is uncertain if enough research exists to put this into clinical practice. A phase II trial of advanced kidney cancer looked at inducing copper deficiency by using TM, a novel antiangiogenic agent, to chelate copper, with the goal of lowering ceruloplasmin level to 5–15 mg/dl for 90 days [70]. This resulted in disease stabilization rather than a reduction in disease burden. The authors proposed that this may only be useful for a patient with minimal disease.

Diez et al. reported on the ratio of serum copper to zinc in diagnosis of lung cancer and found ratios of 2.34 ± 0.78 in the lung cancer cohort, 1.62 ± 0.23 in patients with benign pulmonary lesions, 1.43 ± 0.29 in patients with benign lung diseases, and 0.188 (not significant) in healthy subjects [73]. A cutoff value of Cu–Zn ratio of 1.72 resulted in sensitivity of 89%, specificity of 84%, positive predictive value of 7%, and negative predictive value of 92% between lung cancer patients and healthy subjects [73]. Lung cancer patients with pretreatment Cu–Zn concentrations equal to or above 2.25 had 24-month survival rates of less than 5%. By contrast, pretreatment Cu–Zn levels below 1.72 were associated with 24-month survival rate of 70% [73].

Functional medicine doctors working in oncology typically strive for a serum Cu–Zn ratio of 1.0, although this author could not find adequate studies supporting this ratio.

Serum Cu–Zn ratio should be interpreted cautiously in cases of suspected malignancy that are accompanied by infections, liver diseases, use of oral contraceptives, or other conditions known to affect copper.

8.8 Fatty Acids and Phospholipids

8.8.1 Fatty Acid and Phospholipid Balance

A balanced ratio of omega-6 to omega-3 fatty acids is important for overall health related to its impact on inflammation, immune balance, obesity, depression, and cardiovascular disease. See also ▶ Chaps. 10 and 11. Omega-6 and omega-3

fatty acids cannot interconvert into one another; they are metabolically and functionally distinct and have important, opposing physiological effects [74]. Alpha-Linolenic acid (omega-3) and linoleic acid (omega-6) are the two essential fatty acids that must be obtained through the diet; they cannot be endogenously formed by humans and other mammals due to a lack of enzymes for desaturation [74, 75].

The balance of omega-6 compared with omega-3 fatty acids influences the metabolic pathway of each of these fatty acids [76]. Omega-6 and omega-3 fatty acids produce different eicosanoid products that result in different effects on inflammation. The need for balance between the eicosanoid families is critical for metabolism involving inflammation, resolving inflammation, structural composition, hormones, and immune functions. The nutritionist should be aware of the balance of the eicosanoids, their downstream metabolites, and the functions of such prostaglandins as PG1, PG2, PG3, and specialized proresolving mediators (SPM).

In the past three decades, because of public health prescriptions for low-fat diets, the intake of overall fat in the Western diet has decreased. However, proinflammatory omega-6 fatty acid consumption has increased, while omega-3 fatty acid consumption has decreased [74]. The ratio of omega-6–omega-3, historically, was 1:1–4:1, and today it is reported to be around 20:1. This change in consumption correlates with the rising epidemic of obesity and inflammatory disorders [74]. Arachidonic acid (AA), an omega-6 fatty acid, is a critical fatty acid for cell membrane structure and resolution of inflammation as it promotes specialized SPM. However, when it is out of balance, excessive AA can become pro-inflammatory. As with all molecules and metabolites throughout metabolism, altered metabolic balance can promote dysfunction.

AA is used as a substrate to make eicosanoids such as prostaglandin E2 and leukotriene B4 [74]. These cytokines, when in excess, are pro-inflammatory and more potent mediators of thrombosis and inflammation than cytokines derived from omega-3 PUFAs. Eicosanoids from AA are biologically active in small amounts, and when present in large amounts, they contribute to the formation of thrombus and atheromas, allergic and inflammatory disorders, and proliferation of cells [74].

EPA and DHA, both omega-3 essential fats, suppress the production of proinflammatory cytokines IL-1B, Il-6, and TNF alpha [75]. EPA inhibits AA synthesis from linoleic acid and competes with AA for enzymatic conversion, resulting in reducing AA conversion to pro-inflammatory molecules [75]. The ingestion of fish or fish oil results in the EPA and DHA partially replacing omega-6 in the cell membranes of platelets, erythrocytes, neutrophils, monocytes, and liver cells [74]. In humans, the cerebral cortex, retina, testis, and sperm are particularly rich in DHA, and DHA is abundant in the brain's structural lipids [74].

During evolution, omega-3 fatty acids were found in almost all foods consumed including meat, fish, wild plants, nuts, and berries [74]. The dietary and agricultural changes over the last 100–150 years have resulted in less omega-3 available in foods and an increased risk for changing the physiological state of the body to a more inflammatory, atherogenic state. The balance of omega-6 to omega-3 can affect gene expression, prostaglandin, and leukotriene metabolism and interleukin-1 production [74]. Modern agriculture and aquaculture have changed the omega-3 fatty acid content for the worse in many foods by replacing them with omega-6 fats.

Depression is associated with low plasma phospholipid and erythrocyte levels of EPA and DHA [75]. It is possible that omega-3 fatty acids aid in mitigating depression by decreasing inflammation systemically. Evidence points to utilizing supplemental omega-3 fatty acids for therapeutic intervention of depression as a monotherapy or in addition to antidepressant medications, although results have been mixed [75]. It has yet to be determined what blood levels of EPA and DHA might be associated with improvement in depression. Therapeutic levels of omega-3 depend on diet, genetic variation in omega-3 fatty acid metabolism, and the rate of absorption and incorporation into biologic systems [75]. In both short- and long-term studies, a fixed daily dose of omega-3 results in a large variation of individual omega-3 blood levels, possibly due to genetic polymorphisms and dietary intake. In a study on supplemental omega-3 fatty acids and depression, researchers found that participants whose depression remitted had higher baseline omega-3 levels than those whose depression did not go into remission [75].

RBC levels are less sensitive to recent intake of omega-3 compared with plasma levels and may provide a more reliable estimate of omega-3 levels over time [75]. A level of 5–6% of omega-3 in RBC may be a reasonable target to provide a therapeutic level. However, those who experienced remission of depression had levels of omega-3 in RBC at 8% with the highest probability of remission at 9–10% [75]. This finding is similar to results of a review on omega-3s to improve cardiovascular outcomes [75]. Overall, looking at individual RBC levels to obtain therapeutic efficacy might be a better way to dose supplemental omega-3 than providing a fixed dose for everyone.

Omega-3 fatty acid-enriched diets improve inflammatory status of metabolic dysfunction and reduction in adipose tissue if kept in balance with other essential fats [76]. Supplementation with omega-3 fatty acids and certain doses of omega −6 fatty acids was shown to help with decreasing body fat mass and hip circumference loss [76]. Dietary interventions optimizing the ratio of omega-6 to omega-3 have shown significant reduction in low-density cholesterol as well. Omega-6 versus omega-3 essential fats have different influences on body fat mass through mechanisms of adipogenesis, lipid homeostasis, brain–gut–adipose tissue axis, and systemic inflammation [74]. In summary, lowering omega-6 and increasing omega-3 may decrease the risk for weight gain and associated inflammation.

Testing RBC fatty acid profiles is helpful to clinicians when assessing fatty acid balance and is important because AA and DHA both have critical but different biological func-

tions. There may be instances where omega-3 fatty acid levels are too high compared with levels of arachidonic acid (AA), which may have untoward consequences. The eicosanoids produced from AA are important for immunity and immune response and serve as mediators and regulators of inflammation [77]. AA is critical for infant growth, brain development, and health. Too much DHA may suppress benefits provided by AA [77]. DHA controls signaling membranes in the photoreceptor, brain, and nervous system, whereas AA has a role in the vasculature and certain aspects of immunity [77]. Animal studies have provided evidence that both preformed DHA and AA are required for optimal cognitive function [77]. More about omega-3 dominance is found in the section on gamma-linolenic acid.

8.8.2 Gamma-Linolenic Acid

Gamma-linolenic acid (GLA) and its downstream metabolite di-homo-gamma-linolenic acid (DGLA) are two omega-6 fatty acids important to any discussion of fatty acids (please see ▶ Chaps. 10 and 11). They are often grouped into the "too much omega-6 statements" without addressing their critical importance in nutritional therapy. GLA is not present in the human diet and is formed from the essential fatty acid cis-linoleic acid [78]. It is often supplemented in the form of borage oil, evening primrose oil (EPO), or black currant seed oil. As a metabolite of the essential fatty acid linoleic acid (LA), GLA, in turn, produces DGLA. DGLA is available as a substrate to produce AA or be directed to form the prostaglandin 1 series (PG1) metabolites. The balance needed between the prostaglandin groups is another critical consideration when assessing an individual's fatty acid balance. For example, autoimmune and skin conditions are dependent on adequate PG1 molecules. Most of the PGs, TXs, and LTs are proinflammatory. AA products generally enhance inflammation as the precursor of 2-series PGs and thromboxanes (TXs) and 4-series leukotrienes (LTs) [79]. Therefore, the balance of AA to DGLA in the body may be a critical factor in regulating inflammatory processes [78].

GLA metabolism in humans is complex as all the cellular compartments metabolize GLA differently [79]. This difference is due to differential expression of PUFA-metabolizing enzymes. GLA supplementation can lead to increased DGLA levels in certain inflammatory cells but can increase both DGLA and AA in circulating lipids [79]. The impact of AA accumulation in the body remains controversial. When GLA is given with omega-3 fatty acids, the conversion of DGLA (from GLA) to AA is inhibited, and the accumulation of serum AA appears to be prevented [79].

Inflammation is the immune system's response to infection and injury and facilitates the removal of the cause of the inflammation (the offending agent) and the restoration of tissue structures and physiological function [80]. Inflammatory prostaglandins derived from AA play a key role in necessary inflammation. Therefore, our goal is not to eliminate inflammation but, rather, keep it in check. Persistent and dysfunctional inflammation may promote cancer, cardiovascular disease, autoimmune disease, and the loss of organ function [80]. The nutritional balance between GLA, DGLA, and AA and their metabolites may be key to maintaining a healthy balance of inflammation in the body.

Deficiency of fatty acids is often due to dietary insufficiency, but excessive supplementation of flax or fish oils leads to omega-3 fatty acid dominance [81]. Potential laboratory findings will reveal high omega-3 fatty acids with low AA fatty acids and elevated EPA–DGLA ratio as well as depressed AA–EPA ratios. This imbalance will produce a lowering of class 2 eicosanoid signals, which may lead to blunting of immune responses [81]. Supplementing omega-3 fatty acids in an individual with a deficiency of n-6 fatty acids can further exacerbate clinical outcomes by competing for desaturase enzymes. Adding GLA-rich evening primrose oil may improve the clinical outcome in these individuals [81]. A diet low in LA can be corrected by supplementing with LA-rich oils and/or correcting for malabsorption.

Another risk of omega-3-dominant conditions is lipid peroxidation of cell membranes and increased risk of oxidative damage in the body that may give way to serious health conditions, including heart and neurological diseases. In general, it is recommended to supplement with antioxidant nutrients when using omega-3 fatty acids to protect against lipid peroxidation and monitor fatty acid profiles and antioxidant nutrients [81].

To prevent an imbalance in fatty acids, consider supplementing the full range. When GLA, DGLA, EPA, and DHA are given together, the overproduction of pro-inflammatory cytokines IL-6 and TNFα is suppressed [78]. The combination of GLA, EPA, and DHA in supplemental form would likely induce a powerful combination of anti-inflammatory and necessary inflammatory metabolites [79]. Common genetic and epigenetic variations affect the rate of conversion of long-chain PUFAs and their metabolites and are strongly related to ethnicity, suggesting that "one size fits all" dietary and supplement recommendations for fatty acids may not be appropriate, with laboratory testing critical [79].

8.8.3 Conjugated Linoleic Acid

Conjugated linoleic acid (CLA) is another fatty acid that may have anti-inflammatory and anti-cancer benefits. CLAs are a series of isomers of LA and chemically do not belong to the omega-6 family; however, they can originate from the endogenous biohydrogenation of LA by gastrointestinal tract bacteria [82]. CLAs have demonstrated a benefit in inflammatory bowel disease (IBD) and colon cancer and may serve as an agonist for peroxisome proliferator-activated receptor gamma (PPARγ) [83]. PPARs are nuclear receptors for endogenous lipid molecules such as prostaglandins or hydroxyl-containing PUFAs [83]. Their main biological function is to sense intracellular nutrient concentrations and regulate gene expression involved in maintaining metabolic and tissue homeostasis. When CLA is used in conjunction

with omega-3 fatty acids, it may prevent or ameliorate IBD in animal models [83]. In addition to activating PPAR γ, CLA can also modulate the production of AA metabolites, which may reduce the production of inflammatory lipid mediators. Several CLA isomers are found naturally in milk, cheese, and ruminant products [83]. Grass-fed beef and fatty milk products from grass-fed beef are good dietary sources of CLA.

8.8.4 Phospholipids

Any discussion on fatty acids must include a discussion on the importance of phospholipids. Phospholipids (PLs) are amphiphilic lipids (containing both hydrophilic and hydrophobic properties) found in all animal and plant cell membranes [84]. PLs are arranged as lipid bilayers with the hydrophilic regions reaching toward the outer surface of the cell membrane and the hydrophobic properties reaching toward the inner membrane compartment. PLs can be glycerophospholipids (GPLs) found in most cell membranes or sphingophospholipids (i.e., sphingomyelin or SPM), commonly found in high quantities in the brain and neural tissues [84]. Cell membranes are also composed of glycolipids and cholesterol and proteins.

GPLs can be extracted from soybeans, egg yolk, milk, or marine organisms such as fish, roe, or krill [84]. GPLs can have different fatty acid compositions. Soybean PL consists of GPLs with a high content of unsaturated FA such as LA (n-6 FA); egg yolks have mainly phosphatidylcholine (PC) as their source of PL and contain unsaturated FA, mainly oleic acid; milk GPLs have both PC and phosphatidylethanolamine (PE) as their main PL classes in addition to SPM, and the FA content consists of both unsaturated and saturated; marine GPL is mainly composed of PC and binds the unsaturated eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [84]. Consuming dietary GPLs with a specific FA composition may alter the FA composition of membrane PLs and affect cellular function such as signaling, transport, and activity of membrane enzymes. The possibility for modulating cell function from specific PLs could contribute to health benefits [84].

A number of possible clinical applications of using phospholipids exists. They involve reducing inflammation; improving lipid profile; increasing production of cytoprotective mucosal PGE2 in the GI tract, which may help with ulcerative colitis or the side effects of using NSAIDs; and improving cognitive function, visual function, and memory [84].

Phosphatidylserine (PS), the best studied GPL for brain performance, may be especially protective against age-related cognitive decline. PS has been shown to revitalize memory, learning, concentration, and vocabulary skills and assist with managing stress hormones and depression [84]. PS also stimulates acetylcholine synthesis, which triggers the release of neurotransmitters. Changes in the composition of cell membranes with age include an increased cholesterol–PL ratio, which may reduce the immunological function of lymphocytes. In a study on rats, lymphocyte number and macrophage phagocytic capacity were significantly improved with soy PC supplementation. PLs (purified extract of PPC from soybeans) have shown a beneficial effect with viral hepatitis and alcohol-induced liver damage. PLs have also been demonstrated to reduce total liver lipid, liver TG, and total cholesterol [84].

Chronic alcohol ingestion depletes PL in brain cell membranes and depletes antioxidant systems from the cell membranes which, in turn, promotes lipid peroxidation in the brain and other cells such as enterocytes [84].

8.8.5 Short-Chain Fatty Acids

Short-chain fatty acids (SCFA) are the major metabolic products formed by anaerobic bacterial fermentation of soluble fiber in the gastrointestinal tract and may be the link between microbiota and host tissues [85]. SCFA include acetate (C_2), propionate (C_3), and butyrate (C_4) [85]. Acetate is utilized for lipogenesis in the liver and as a fuel source once it enters the peripheral circulation; propionate is also used in the liver as a substrate for hepatic gluconeogenesis; butyrate is primarily used as a fuel source for the colonocytes [86]. Besides serving as a fuel source for the intestinal epithelial cells, SCFA also modulate electrolyte and water absorption in the GI tract and regulate the inflammatory process in the GI tract [85]. The concentration of SCFA in the GI tract and blood may predispose to or prevent illnesses such as inflammatory bowel disease (IBD), cancer, and diabetes [85]. SCFA modulate different processes such as cell proliferation and differentiation, hormone secretion of leptin and peptide YY, and immune and inflammatory responses and serve as an energy source for colonocytes, the liver, and muscle [85]. SCFA levels have been found to be lower with increasing age in humans, dogs, and mice [86].

Butyrate is the most widely studied SCFA and is predominantly produced by *Faecalibacterium prausnitzii* (*F. prausnitzii*). Butyrate serves as an energy source for colonocytes and exerting an anti-inflammatory effect in the colon [87]. Studies reported benefit when butyrate was administered for IBD-related lesions and symptoms [87]. A study by Zhang et al. in rats found that oral administration of butyrate resulted in increased percentage of butyric acid, fecal concentration of butyric acid, and overall total SCFA [87]. Studies in mice have suggested that the obese microbiota, which has a higher ratio of *Firmicutes* to *Bacteroidetes* compared with lean microbiota, produce a higher amount of SCFA compared with leaner counterparts, potentially contributing to obesity [86].

The reasons for higher SCFA in obese individuals may be due in part to lower colonic absorption of SCFA, reduced colonic transit time, or increased SCFA production due to differences in dietary intake [86]. The ratio of *Firmicutes* to *Bacteroidetes* in humans is not always higher in obese individuals; thus, the higher SCFA theory in obese individuals is not a consistent finding [86]. A study by Rahat-Rozenbloom et al. comparing obese versus lean subjects suggested that

SCFA production was higher in obese individuals, but this was not due to decreased absorption of SCFA or differences in dietary intake. Rather, this may be due to the differences in the microbiome with the obese microbiome being higher in *Firmicutes* than the lean microbiome [86]. Further research will be critical in finding the important relationships.

Increasing the production of SCFA is largely accomplished by eating a whole-foods diet high in plant foods and fiber. Butter is a natural food source of butyrate. Butter from grass-fed cows would provide the additional benefit of the anti-inflammatory effects of the fatty acid CLA. In cases such as IBD, a high-fiber diet may not be tolerated during an exacerbation of the illness, and this is where the oral or enema administration of butyrate might be of benefit. Working with an experienced integrative and functional medicine practitioner in the use of butyrate is recommended.

8.8.6 Increasing Beneficial Fatty Acids

Increasing omega-3 fatty acid intake might include eating grass-fed beef (also high in CLA), wild-caught fish, eggs from free-range chickens with omega-3 fatty acids in their feed, and supplementing with omega-3 fatty acids. Simopoulos et al. recommend lowering omega-6 by changing vegetable oils from corn, sunflower, safflower, cottonseed, and soybean oils to oils that are high in omega-3, such as flax, perilla, chia, rapeseed, and oils that are high in monounsaturated fats such as olive oil, macadamia nut oil, hazelnut oil, and increasing fish intake to two to three times per week while decreasing meat intake [74]. Processed foods are notoriously high in omega-6; therefore, consuming a whole-food, unprocessed diet is also an important factor in balancing the ratio of omega-6–omega-3 fatty acids. Increasing GLA involves supplementing with a good source of GLA, such as evening primrose oil, black currant seed oil, or borage oil. Increasing CLA can be done through supplemental sources that include a variety of isomers or eating grass-fed beef and whole-fat dairy products from grass-fed beef, such as butter, yogurt, cheese, or milk, and enhancing the microbiome through diet to support the endogenous biohydration of LA. Increasing phospholipids such as choline can occur through eating sunflower seeds, egg yolks, fish, and milk. Sunflower or soy lecithin are both sources of phospholipids (sunflower lecithin is typically recommended over soy lecithin) and dietary supplementation of phosphatidylserine and phosphatidylcholine.

8.8.7 Assessing Erythrocyte Fatty Acid Profiles

Because a "one-size-fits-all" approach to fatty acid supplementation is not optimal for balancing fatty acid nutritional status, testing erythrocyte fatty acid profiles is a helpful tool as a part of nutritional assessment. Assessing fatty acid profiles in packed erythrocytes is the most common procedure and is representative of longer-term dietary intake rather than recent dietary intake as is represented in plasma profiles of fatty acids [81]. Fatty acid analysis can also help pinpoint certain key vitamin and mineral needs. Laboratory profiles of fatty acids include more than 40 analytes that may be evaluated using patterns within families rather than assessing each individual fatty acid [81]. Individual variability of intake, digestion, absorption, and degradation can produce different fatty acid profiles and support the notion of not using a "one-size-fits-all" approach to recommending supplemental fatty acids [81]. The assessments of patterns include general fatty acid deficiency, omega-3 deficiency or excess, omega-6 deficiency or excess, hydrogenated oil toxicity, micronutrient deficiency, metabolic and genetic disorders, and fatty acid ratios and indices [81].

RBC fatty acid profiles can indicate certain micronutrient deficiencies. For example, when biotin is deficient, the ratio of vaccenic acid to palmitoleic acid is significantly lower. Vaccenic acid has large effects on membrane fluidity and inhibition of tumor growth in cell culture [81]. B12 deficiency is associated with abnormal fatty acid synthesis that results in odd-chain fatty acids building up in the lipids of the nervous system. This results in altered myelin integrity and demyelination, leading to impaired nervous system functioning [81]. Moreover, this may in part explain the neuropathy associated with cobalamin deficiency [81].

To further elucidate this metabolic process, the production of fatty acids with odd numbers of carbon atoms is initiated by propionic acid [81]. Propionic acid requires B12 to be converted into succinate; therefore, a deficiency of vitamin B12 results in the accumulation of propionate and a build-up of odd-numbered fatty acids [81]. In animal studies of biotin deficiency, abnormalities from the accumulation of propionate have been shown to occur. These abnormalities result in the buildup of odd-chain fatty acids in the plasma phospholipids, plasma, and liver in experimental animals [81]. Gut bacteria in ruminants also produce high amounts of propionate. Consequently, eating animal and dairy products may result in accumulation of odd-numbered fatty acids [81].

Zinc may be the best-known nutritional deficiency that can show up in a RBC fatty acid analysis. The desaturase enzymes needed for the conversion of fatty acid substrates into products rely on zinc. The delta-6-desaturase enzyme that converts LA to DGLA will be under-functioning in zinc deficiency; thus, an elevated ration of LA–DGLA is a sensitive marker for zinc deficiency [81]. In cases where LA-rich foods are restricted and extra flaxseed oil is used, the ALA–zinc ratio may also be elevated as that pathway requires zinc [81].

Essential fatty acid deficiency will show up as an elevation in mead acid [81]. The production of mead acid rises as EFA intake falls. Mead acid is an omega-9 PUFA produced by repeated desaturation of nonessential fatty acids. Mead acid cannot participate in eicosanoid formation but mimics membrane fluidity action of PUFAs derived from EFA.

When supplemental fatty acids are used, they must be kept from air and light to keep oxidation low. In addition,

these supplements often contain vitamin E to help prevent oxidative damage to the fatty acids [81]. Erythrocyte fatty acid follow-up testing is recommended to be done after 90 days of starting interventions [81].

8.8.8 Hydrogenated Oils

Hydrogenated oils are harmful fats also known as trans-fatty acids. Hopefully, trans-fatty acids will be less of a concern in the United States due to the phasing out of these fats in the food manufacturing industry. Trans-fatty acids are harmful because, although listed as unsaturated fats, they behave like saturated fats and lead to higher cholesterol levels, and they mimic unsaturated fats by binding to desaturase enzymes and interfering with the normal production of necessary substances [81]. Trans-fatty acids have been shown to contribute to the risk of heart disease and cancer [81]. Trans-fatty acids are found in margarine, hydrogenated peanut butter, baked goods, desserts, snack foods, and crackers [81]. The main trans-fatty acid type found in hydrogenated foods is C-18 trans-fatty acids including elaidic acid, trans-vaccenic, and trans-petroselinic, followed by palmitelaidic acid.

Milk products and beef can contain some naturally occurring trans-fatty acids (elaidic acid) formed from the bacteria in the gastrointestinal tract of ruminant animals [81]. Naturally occurring trans-fatty acids are not a health concern [81].

8.8.9 Overall Diet and Macronutrient Distribution

Diet is the core treatment for many diseases, including obesity, hypertension, hyperglycemia, and dyslipidemia. Many interventions are beneficial, including replacing harmful fats with health-promoting fats; increasing fiber; increasing phytonutrient content such as flavonoids, polyphenols, and antioxidants from plant-based foods; reducing salt; and restricting calories [88]. The Mediterranean diet is one of the most studied diets and recommends high consumption of extra virgin olive oil, fruits, vegetables, nuts, seeds, legumes, cereals, moderate fish, poultry, dairy, and red wine and lower consumption of eggs, red meat, processed meat, and processed foods. The Mediterranean diet improves blood pressure, lipid profiles, insulin sensitivity, CRP, oxidative stress, atherosclerotic disease, and cognitive function [88]. The Mediterranean, pescetarian, vegetarian, or vegan diets also offer environmental benefits with decreased greenhouse gas emissions [88]. People who eat an organic, Mediterranean diet may contribute to a reduction in global environmental impact [88]. In terms of lowering of HbA1C, a systematic review found that a low carbohydrate Mediterranean diet and low-fat vegan diet were possibly the most effective [89].

To reemphasize, a one-size-fits-all diet or macronutrient recommendation is not plausible. The percentage of calories from carbohydrate, fat, and protein needs to be determined on an individual basis. Specific conditions such as chronic kidney disease will benefit from a controlled protein intake, and epilepsy can benefit from a very low-carbohydrate, high-fat ketogenic diet. For years, the field of nutrition has been bombarded with claims of various adjustments in macronutrient ratios having benefit. Nutritionists need to take into account individual disease states and give the patient the most sustainable, realistic plan to improve health.

While the ketogenic diet (low-carbohydrate, high-fat) is showing promise for various conditions including epilepsy, cancer, weight loss, type 2 diabetes, metabolic syndrome, dementia, and neurological diseases [90], the ketogenic diet can be difficult for the average person to sustain. The guidance of a ketogenic-trained nutritionist can assist in compliance. This dietary regimen can be a critical intervention to produce a successful outcome. It is important that a ketogenic or modified ketogenic diet regimen be developed for the individual and their medical condition. In this author's opinion, intermittent fasting (IF) and time-restricted feeding (TRF) may prove to have as important cardiometabolic and neurological benefits as the ketogenic diet and may be appropriate for those who cannot sustain a ketogenic diet.

Time-restricted feeding (TRF) may be a feasible alternative to calorie restriction, ketogenic diet, and intermittent fasting when compliance with more restrictive eating regimes is not likely or not recommended. TRF is an eating pattern based around circadian rhythms that occurs within a limited time span (usually 8–12 hours), with a span of 4 hours in between meals with no attention paid to calorie intake [91]. In one study, eight overweight participants consumed their entire caloric intake within a 10–11-hour window. These participants consumed 20% less calories (due to eliminating alcohol and late-night snacks) and lost 4% body weight in 16 weeks that they sustained for 1 year. The participants reported improved sleep and elevated alertness during the day [91]. TRF can impart other benefits as well. Prolonged overnight fasting (>13 hours) correlated with reduced risk of breast cancer [91]. TRF is still being investigated, and more studies are needed to validate its effectiveness.

Correcting nutritional imbalances by designing individualized food and dietary supplementation recommendations are the cornerstone of functional nutrition.

8.9 Summary

Nutrient intake is a contributing factor to living with or without disease. Correcting nutritional imbalances by designing individualized food and dietary supplementation recommendations are the cornerstone of integrative and functional nutrition. When using supplements, always keep balance in mind. Retest lab parameters to ensure clients are staying in balance. Adjust dietary macronutrient ratios according to individual needs instead of using a one-size-fits-all approach (See ► Box 8.1).

Box 8.1 Recommendations for Assessment-Based Interventions

- Using the ingestion–digestion–utilization (IDU) worksheet can help to pinpoint which additional tests need to be ordered to better assess nutritional balance.
- Dietary intake may be assessed to see what nutrients are low in the overall dietary intake.
- A micronutrient test can look at individual nutrients in the red blood cell or plasma and RBC fatty acid status.
- Plasma or urinary amino acid testing can be used to assess amino acid status as it relates to behavioral and neurotransmitter function and overall adequacy of nutritional intake.
- Waist circumference and BMI can be used to determine what macronutrient distribution, and calorie level might be best suited for an individual.
- Nutrition physical exam and conventional lab tests such as a CBC can give an indication of protein status, electrolytes, iron, and vitamin B12 status:
  - HBA1C and fasting insulin can be a guide toward adjusting carbohydrate in the diet.
  - Lipid profiles and RBC fatty acids can be combined together to determine the type and quantity of fat and carbohydrate that needs to be included in the diet.
- Comprehensive stool and digestive analysis can give an indication as to how the person is digesting and absorbing their food and the need for digestive or pancreatic enzymes:
  - This test can also identify any pathogens that might be contributing to malabsorption.
  - The need for hydrochloric can also be determined by assessing symptoms such as gas and bloating right after meals, nail strength, easy hair pluckability, serum protein and albumin as well as looking for any protein fragments in the stool analysis.
  - Fat malabsorption may be assessed by looking at fat in the stool and serum levels of cholesterol and triglycerides that are often low normal in conditions of fat malabsorption.
- Antioxidant status can be assessed by integrative nutritional testing that looks at lipid peroxidation and glutathione status and levels of CoQ10, vitamin E, selenium, and zinc.
  - Simple questioning that can assess the quantity of fruits and vegetables, nuts, and legume servings can also give an indication of the level of antioxidants and phytonutrients the person is consuming.
- Vitamins A and D may be assessed by testing serum retinol levels and vitamin D3 (25, OH).
- Ferritin levels may give a more accurate assessment of iron status than hemoglobin or hematocrit.
- Dietary and laboratory assessment and a nutrition physical exam are key tools for identifying clinical and subclinical nutrient deficiencies that could be derailing an individual's ability to achieve an optimal and more functional state of health:
  - Optimizing nutritional status can be pivotal for helping people turn the corner with their health challenges.

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