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Nutritional deficiencies in patients with thalassemia

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Optimal nutritional status is imperative for growth, development, immune function, and bone health. Patients with thalassemia are known to have poor growth, altered puberty, and immune function as well as reduced bone mineral acquisition. The etiology of these comorbidites is typically ascribed to the toxic effects of transfusion-related iron-overload. Recently, our group and others have observed marked nutritional deficiencies in key fat and water-soluble vitamins as well as important essential minerals. Depressed circulating levels of nutrients have been observed despite seemingly adequate dietary intake. This disconnect between intake and circulating levels suggests that patients with thalassemia may have increased needs for certain nutrients due to either poor nutrient absorption, elevated losses, or increased nutrient turnover. Randomized controlled clinical trials are needed to test the efficacy of nutritional therapies toward improving the overall health in thalassemia, as well as decreasing long-term comorbidities such as reduced bone mass.

Keywords: nutrition; thalassemia; zinc; vitamin D; iron, dietary intake

Nutritional red flags: growth deficits and pubertal delay

Optimal nutritional status is imperative for achieving the genetic potential for growth and pubertal development in children, and poor growth and delayed puberty is considered a red flag to any pediatrician leading to exploration of possible nutrient specific or global nutritional deficiencies.[1] In the adult patient, altered nutritional status may adversely affect immune function, oxidative stress, bone health, and/or may limit the effectiveness of some medications.[2]

Growth failure is common in patients with thalassemia. Various reports suggest the incidence of growth deficits ranges from 25% to 75% depending on thalassemia syndrome and severity of disease.[3–5] Height *Z*-score tends to decrease with age, commonly associated with pubertal deficits.[3,5] However, growth failure is multifactorial in thalassemia, related to chronic anemia, chelation toxicity, and iron-associated endocrinopathies such as hypogonadism, hypothyroidism, and growth hormone deficiency. Ethnicity may also be considered when making generalized comparisons of growth to reference populations; however, ethnic disparity alone does not explain the extent of the deficits observed. Little attention has historically been given to the role nutrition may play to growth failure in thalassemia.

A few reports from investigators outside the United States suggest that suboptimal nutritional status plays a role in growth failure and pubertal development. Fikry and colleagues observed a positive correlation between serum zinc level and linear growth in 46 young Egyptian patients with thalassemia.[6] Filosa and colleagues reported that delays in pubertal development were related to poor overall nutritional status in young Italian males with thalassemia.[7] Others have shown in small groups of toddlers with thalassemia that nutrition support for one month improves markers of immune function and growth.[8,9] Soliman and colleagues went one step further and conducted a randomized nutrition intervention in 30 prepubertal children with transfusion-dependent thalassemia.[10] Of interest, when compared to age and gender matched controls, the children with thalassmia had similar dietary intake at baseline despite marked growth and

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body fat deficits. After increasing caloric intake by 30–50% over an 8-week period, they observed significant improvements in weight, fat stores, albumin and insulin-like growth factor 1 (IGF-1) levels compared to the nonsupplemented Thal group. The improvement in IGF-1 following nutritional therapy supports the notion that a component of the growth failure in Thal is related to nutritional deficiency. Unfortunately, the majority of the published work in this area has summarized observations or interventions in relatively small numbers of subjects primarily conducted outside of North America in low profile journals, therefore has received little attention.

Nutrient intake, requirements, and expenditure

Nutritional adequacy is predicated on a balance between intake and requirements. One explanation for the reduced growth rates in thalassemia despite seemingly adequate caloric intake could be increased caloric expenditure. This is quite possible given the existence of hyperactive bone marrow and increased cardiac output due to chronic anemia. In 1995, Vaisman and colleagues evaluated seven adult patients with thalassemia before and 3 days after red blood cell transfusion.[11] Hemoglobin concentration was negatively associated with energy expenditure. They observed energy expenditure to be 12% higher than expected, which decreased to near normal levels following transfusion, presumably due to a reduction in protein turnover and reduced cardiac output.

In addition to the potential increased requirement for total calories, others have shown that the requirements for specific nutrients may be increased in patients with thalassemia. The clearest example of this is with regard to folate metabolism. Folate, an essential nutrient required for normal erthropoietic activity, has been shown recently to be readily catabolized by ferritin.[12] Thus, in patients with thalassemia with hyperactive erythropoiesis, confounded by iron overload, folate requirements are increased and deficiencies are commonly reported.[13] Increased markers of oxidative stress have been reported in Thal by many investigators most frequently associated with iron overload.[14] This topic will be explored in more detail in the paper presented by Lal and colleagues.[15] Insufficient antioxidant intake has been associated with oxidative stress. In the early 1990s, a group in Thailand was able to reduce circulating glutathione peroxidase in young patients with thalassemia through supplementation with 200 mg of vitamin E for 4–8 weeks.[16] Iron overload may also perturb essential trace element status. There is the potential for sequestration of zinc and copper in the liver of iron-overloaded subjects due to the upregulation of metallothionien by high iron concentrations. Depressed circulating zinc and copper levels[17] have been reported, although there is a paucity of data on hepatic concentration of minerals other than iron in transfusion-dependent patients with thalassemia.

Recently, Claster and colleagues reported the circulating levels of a variety of essential nutrients in a convenience sample of 24 chronically transfused patients with thalassemia (age range: 1.5–31.4 years, ferritin 2089 $\pm$ 1920 ng/mL).[18] They found surprisingly low levels of both water soluble and fat soluble vitamins (Table 1). For a few nutrients, over half of the patients sampled had low levels; these included vitamins A, C, D, and selenium. In fact only a few nutrients did not appear to be reduced, which included serum copper, vitamin B-12, and γ-tocopherol, the supplemental form of vitamin E. Circulating levels of many nutrients tended to decrease with increasing age, that is younger subjects tended to have higher circulating nutrient levels compared to older subjects. Although this level of nutritional deficiency has been shown in other studies to be associated with poor health outcomes, the small sample size and cross-sectional study design in this study by Claster and colleagues precluded a cause and effect type analysis.

As summarized thus far, patients with thalassemia may be at increased risk for nutritional deficiencies due to elevated nutrient requirements. However, nutrient balance is related not only to requirement and output, but input: patients with thalassemia may also have inadequate intake or absorption of nutrients. Our group assessed typical dietary intake in 38 patients with thalassemia (21 female, 17.1 $\pm$ 5.4 years, mean $\pm$ *SD*; 28 transfusion dependent) and compared them to 36 age, gender, and ethnically similar controls (20 females, 17.8 $\pm$ 6.2 years). Each subject completed a 160-question Block©2005, food frequency questionnaire. Questionnaires were analyzed and nutrient inadequacy defined as less than two-thirds the current age and gender specific U.S. Dietary Reference Intake value. Overall kcal, and protein, fat, and cholesterol intake were

Table 1. Circulating nutrient levels in patients with thalassemia

Nutrient	Normal range	Value	% Abnormal
Fat soluble vitamins			
Vitamin A (μg/dL)	38–98	34.6 ± 12.2	52.4%
Vitamin D (ng/mL)	20–100	17.1 ± 8.5	50.0%
α-Tocopherol (mg/dL)	5.7–19.9	7.5 ± 7.5	29.2%
γ-Tocopherol (mg/dL)	<4.3	3.0 ± 5.0	4.2%
Water soluble vitamins			
Thiamin (μg/L)	2.4–11.7	4.1 ± 4.0	37.5%
Vitamin B-6 (ng/mL)	3.3–26	7.0 ± 5.9	34.8%
Vitamin B-12 (pg/mL)	200–1100	528 ± 152	0%
Folate (ng/mL)	>8	11.8 ± 7.7	37.5%
Trace elements			
Copper (μg/dL)	59–118	85.1 ± 15.5	0%
Selenium (μg/dL)	110–160	99.5 ± 20.7	75.0%
Zinc (μg/dL)	65–124	83.0 ± 15.6	8.3%

Adapted from Claster *et al.* (2009).[18]
Mean ± *SD*.
Vitamin D: 25-hydroxy vitamin D.

similar between Thal and control subjects, (kcal: 107 ± 41%, Thal vs. 105 ± 38%, Control, NS; macro nutrient data not shown). The percentage of subjects with inadequate specific nutrient intakes are shown in Figure 1. Across the board, more Thal subjects had inadequate nutrient intake. Vitamin D, folate, calcium, and magnesium were significantly lower in Thal compared to controls ($P = 0.02$). These nutrients are of particular concern as they are critical for optimal bone mineralization and low bone mass is a particularly common comorbidity in thalassemia.[19] The number of dairy servings consumed per day were consistent with the specific nutrient intakes. That is, subjects with thalassemia consumed significantly lower servings of dairy compared to the health controls (1.3 vs. 2.0 servings per day, $P = 0.02$).

From these data it is clear that patients with thalassemia have frequently reported depressed circulating nutrient levels, the potential for increased requirements of specific nutrients and some indication that dietary intake is inadequate. Few studies have explored overall dietary intake in large samples of contemporary patients with thalassemia. The Thalassemia Clinical Research Network recently completed a cross-sectional analysis of dietary intake in nearly 200 patients with a variety of thalassemia syndromes.[20] It is anticipated this group will publish further information on dietary intake and body composition in this large cohort of patients with thalassemia within the year.

Specific micronutrients

Vitamin D: skeletal and extra skeletal effects

Vitamin D deficiency has received much attention in the scientific literature recently.[21] Numerous studies have reported on a variety of chronic disease states are apparently at risk for inadequacy or deficiency. Thalassemia is one of the chronic disease states identified that is at particular risk. Last year we reported that only 18% of a contemporary sample of 361 patients with thalassemia residing in North America had sufficient levels of 25-OH vitamin D (defined as >30 ng/mL).[3] Others have reported similar levels of inadequacy.[18,22,23]

Vitamin D is unique in that it is the only essential nutrient for which we are also able to synthesize in the body. It has been estimated that up to 80% of the vitamin D requirement is synthesized in the skin, whereas the remainder is obtained from dietary sources. There are many factors to consider when it comes to the cutaneous synthesis of vitamin D: geographic latitude, season, altitude, cloud cover, air quality, time of day, as well as an individual's clothing, skin pigmentation, and sun screen use. For

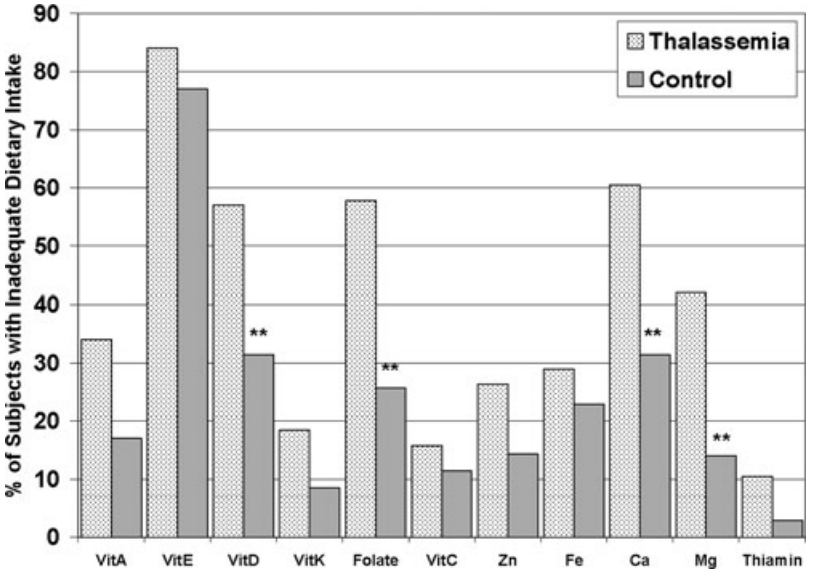

Figure 1. Percentage of subjects with thalassemia ($n = 38$) compared to healthy age and ethnically similar controls ($n = 36$) with inadequate dietary intake of essential nutrients, defined as intake less than two-thirds the recommendation for healthy people. **Denote significant difference between Thal and Controls of $P < 0.02$.

those cities that lie north of 42° latitude, sunlight is too weak to synthesize vitamin D from late October to early March,[24] and for those cities that lie north of 40° latitude, sunlight is too weak to synthesize vitamin D during January and February. Therefore, vitamin D status should be monitored more carefully in those patients with thalassemia who reside in Northern latitudes, are naturally dark skinned, who customarily shroud themselves, or who have limited exposure to sunlight during mid-day hours when the sun is at its highest in the sky, 10:00 am to 2:00 pm. Although dietary intake is not the primary contributor to vitamin D status, it must still be considered as a possible risk factor for deficiency. There are few natural sources of vitamin D in the diet, primarily fatty fish and cod liver oil. As a result, in many countries vitamin D is fortified in the food supply. In the United States, fluid milk is fortified to 100 IU vitamin D per 8 ounces. Therefore, patients with thalassemia who are lactose intolerant or who avoid milk for other reasons may be at greater risk for vitamin D deficiency.

Vitamin D also has unique hormonal functions; its active form, 1,25-OH vitamin D has been shown to have multiple roles in the body not only related to bone health and fracture reduction but also cardiovascular health, immune function, blood pressure, and cancer prevention. A report released this past year thoroughly reviewed the literature that relates to vitamin D and functional outcomes in the U.S. population.[25] In this exhaustive report, a consistent relationship between vitamin D and cardiovascular health was not observed. However, individuals with thalassemia may be at greater risk for vitamin D deficiency and therefore have a greater response to supplementation.

Wood and colleagues found a weak though significant correlation between 25-OH vitamin D levels and left ventricular ejection fraction in patients with thalassemia who were not on vitamin D supplementation, $r^2 = 0.35$.[26] In this small study, all four subjects who had ejection fractions that were considered dysfunctional (LVEF <57%) also had deficient levels of vitamin D. They also observed a correlation between the ratio of 25 to 1,25 hydroxy vitamin D and R2, a level of iron concentration within the heart by magnetic resonance imaging. More recently, Dimitriadou and colleagues reported that PTH was higher in β-thalassemia major patients with increased myocardial iron compared to those with normal levels ($P = 0.017$).[27] Patients with thalassemia and reduced levels of vitamin D have been shown to have a 10-fold greater risk of low bone mass after controlling for age, weight Z-score, and hypogonadism.[19] In addition, those patients with thalassemia who have the vitamin D

receptor Bsml polymorphism, may be further risk for osteoporosis.[28]

With so many subjects at risk for vitamin D insufficiency, how can the medical team tackle this pandemic? Vitamin D is a fat-soluble vitamin, as such, it is stored in the tissues and can be provided in high doses in low frequency. The current guidelines from the National Kidney Foundation for replacement suggest a 50,000 IU supplement be provided every other week for 8 weeks for subjects with 25-OH vitamin D levels less than 15 ng/mL or 50,000 IU every 4 weeks for those with levels <30 ng/mL. At Children's Hospital Oakland, we have observed that many patients continue to have 25-OH vitamin D levels below the optimal range of 30 ng/mL despite a prescribed dose of 1000 IU per day, or 7000 IU per week. Given this, we modified the current guideline to coincide with the thalassemia transfusion regimen. Thirteen patients with transfusion-dependent thalassemia and vitamin D levels $\leq$20 ng/mL were given 50,000 IU of ergocalciferol (D2) orally at time of transfusion for six to eight transfusion cycles or 18–24 weeks. This dosage translates to approximately 1700–2300 IU per day depending upon the frequency of transfusion. We found 25-OH vitamin D increased from baseline in all but one patient, but only reached a level of sufficiency in one patient (defined as >30 ng/mL, Fig. 2). Therefore, this pilot program was successful at improving adherence to the vitamin D supplementation regimen; however, this dosage for patients with low vitamin D levels may be insufficient to boost their levels within a six-month period. Soliman and colleagues suggest a higher dose (100,000 IU/kg with a max of 600,000 IU) or greater frequency (e.g., weekly), may be required to increase circulating levels in many subjects.[23] Vitamin D replacement has been successful in other chronic disease states using similar high-dose, low-frequency supplementation regimens.[29,30]

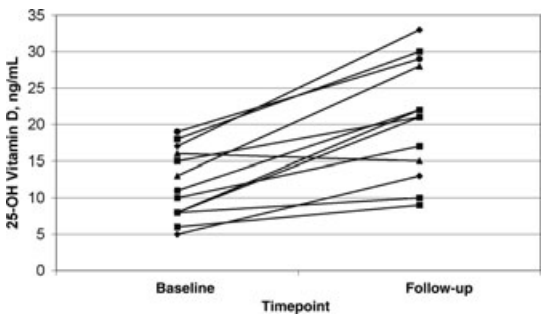

Figure 2. **Response of serum 25-OH vitamin D levels before and after high dose ergocalciferol supplementation in patients with thalassemia.**

Zinc deficiency: growth, immune function, and bone health

Zinc is an essential trace mineral required for cell division, differentiation, and gene expression. It is critical to the function of over 300 enzymes, as such it is important to a myriad of bodily functions including development and maintenance of the immune system, bone health, vitamin A metabolism, and actions of thyroid, insulin, testosterone, and growth hormone.

Zinc is a particularly important mineral to transfused patients with thalassemia because it is similar enough in size and charge to iron, therefore it has the potential to be chelated along with iron in those patients treated for iron overload.

Zinc deficiency has been documented in both transfused and nontransfused patients with thalassemia.[17,31] A 2003 report from Iran found that 80% of adolescent patients with beta thalassemia exhibited poor zinc status defined by depressed plasma zinc.[32] Depletion of circulating zinc may be due in part to the presence of proximal tubular damage[33] and hyperzincuria, as urinary zinc is elevated 4-fold in patients with thalassemia compared to controls.[34] Increases in urinary zinc may be related to the presence or absence of diabetes, a comorbidity also associated with increased zinc losses.[35] Alternatively, chelators used to treat iron overload may also place these patients at risk for zinc deficiency.[35,36] Arcasoy and colleagues conducted a zinc supplementation study in 32 young regularly transfused, nonchelated patients with thalassemia (1–18 years).[37] Twenty-one patients received between 22 and 90 mg Zn/day over a period of 1–7 years, the remaining patients received only their usual transfusion regimen. Although only 50% of the sample were considered growth delayed at study initiation, they observed an increase in height velocity in the zinc compared to the nonsupplemented group ($P <$ 0.01). This result suggests patients within their clinic were experiencing a zinc-limiting growth failure.

Abnormalities of zinc metabolism may also play a role in the pathology of osteoporosis in patients with thalassemia. In 2004, Bekheirnia and colleagues observed that female patients with thalassemia and

severe zinc deficiency had lower lumbar BMD *Z*-scores in comparison to the other females (−3.26 vs. −2.54).[32] Serum zinc was 16.4 μg/dL lower in females with low femoral bone mass compared to those with normal bone mass. More recently, this same group observed a similar relationship between depressed serum zinc and low bone mass in both males and females with thalassemia.[38] Similar to the previous report, low bone mass was common in the 203 adolescent patients studied (50% had *Z*-score <−2.5 in the spine), and low bone mass was strongly associated with low serum zinc. Our group has recently completed a randomized placebo controlled trial of zinc supplementation on bone health in patients with thalassemia (Clinical Trials.gov #NCT00480415). The results from this trial are pending.

Within the last few years, others have explored the relationship between zinc deficiency and diabetes in patients with thalassemia. Dehshal and colleagues measured serum zinc, insulin, and glucose in 70 patients with thalassemia and 69 healthy controls before and during a 1 h oral glucose tolerance test (OGTT).[39] They found that 37% of subjects had depressed serum zinc levels, which were associated with lower fasting and 1-h post-OGTT serum insulin concentrations. Their data support the hypothesis that zinc deficiency might lead to an exacerbation of the inability of the pancreas to secrete sufficient amounts of insulin in response to glucose stimulation in patients with transfusion-dependent thalassemia.

Iron: the dogma and the dilemma

Given the relationship between iron overload and organ dysfunction in Thal, counseling patients to consume a diet low in iron has been part of the standards of care for decades. Typically, a diet that is low in iron rich foods such as red and organ meats, and fortified breakfast cereals is recommended for all patients. However, there is debate regarding the effectiveness of reducing dietary iron consumption for the transfused subject. The amount of iron obtained from just one unit of packed red blood cells (200 mg) far outweighs the amount of iron obtained from a 3 oz steak (5 mg). Typical daily iron accumulation from transfusion-related iron is ∼20 mg/day (2 units every 3 weeks), compared to iron accumulation from an iron rich diet: ∼4 mg (assuming 30% absorption). A low iron diet may decrease the quality of life in some transfusion-dependent patients and/or create a false sense of security; that is, if they decrease their dietary iron intake, they may need to be less diligent with regard to chelator adherence.

For the nontransfusion-dependent patient with thalassemia, reducing iron in the diet is an important part of nutritional counseling as these patients have a tendency to over absorb iron from the intestinal track. Black tea has been shown in one study from 1979 ($n = 6$ subjects) to reduce the absorption of dietary iron from plant based foods up to 95%.[40]

Current nutritional guidelines

Guidelines for monitoring dietary intake and nutritional status in patients with thalassemia have been published either online or as pamphlets by many organizations involved with patients such as the Thalassaemia International Federation (www.thalassaemia.org.cy), Cooley's Anemia Foundation (www.cooleysanemia.com), and the Northern California Comprehensive Thalassemia Center (www.thalassemia.com). Most experts agree that the nutritional status of patients should be evaluated annually by the medical team, which includes a registered dietitian or nutritionist. The adequacy of dietary intake of many important nutrients including calcium, vitamin D, folate, trace minerals (zinc, copper, selenium), and antioxidant vitamins (E and C) should be considered. Annual monitoring of nutritional labs should include albumin, folate, vitamins C, E, zinc, copper, ceruloplasmin, and selenium. Because of the seasonal effects on circulating levels of vitamin D, it is recommended that it be assessed every 6 months (Table 2). Supplementation to the diet is made after investigation of dietary history, and estimated requirements and consideration of growth status, pubertal development and bone health. Typically, a daily multivitamin mineral supplement without iron is suggested for most transfused patients. For nontransfused subjects, folate supplementation is recommended (1 mg daily). Nutritional counseling should be offered to patients with special dietary needs including those with iron-induced diabetes, vegetarians or vegans, lactose intolerant, have numerous food allergies, those who are pregnant, on oral chelation therapy and/or who may be taking bisphosphonate medications.

Summary and future directions

Depressed circulating levels of key fat and water-soluble vitamins as well as important essential

Table 2. Nutrition monitoring recommendations

Growth/development	Sample type	Frequency	Adequacy
Height and weight		Monthly	[a]
Sitting height		Every 3 months	
Calculate growth velocity		Annually	
Puberty: Tanner staging		Every 6 months	
Nutritional laboratory			
Albumin		Annually	3.5–5.0 g/dL
Folate	Serum	Annually	>3 ng/mL
Vitamin D, 25-OH	Serum	Every 6 months	≥ 30 ng/mL
Vitamin C- Ascorbate	Plasma	Annually	>0.4 mg/dL
Vitamin E- α- and γ-tocopherol	Serum	Annually	Age and gender dependent
Zinc	Serum (TE, F)	Annually	≥70 μg/dL
Copper	Serum (TE)	Annually	≥70 μg/dL
Selenium	Serum (TE)	Annually	>45 μg/L
Ceruloplasmin		Annually	>17 mg/dL
Other			
Bone densitometry		Annually, starting at 8 years	Z-score > −2.0

[a]Adequacy of growth is dependent upon the child's genetic potential for growth determined by the mid-parental height index.

TE, All trace elements need to be collected into trace element free vacutainers; F, it is important that these are collected in the fasted state.

For transfused patients, best to draw laboratory values prior to transfusion (e.g., morning of).

May be possible to use red blood cell folate in the non-transfused patient, as it is typically a better indicator of tissue stores than serum folate.

Adapted from Standards of Care Guidelines for Thalassemia, Children's Hospital and Research Center, Oakland, December (2008).

minerals have frequently been observed in patients with thalassemia. There is a paucity of robust information on dietary intake; however, there is increasing evidence that patients with thalassemia have increased needs for certain nutrients owing to either poor nutrient absorption, elevated losses, or increased nutrient turnover. The etiology of frequently reported comorbidites in thalassemia can no longer simply be ascribed to the toxic effects of transfusion-related iron overload. However, more trials are needed to support a causal association between nutritional status and overall health in patients with thalassemia.

Suggested areas of future research for patients with thalassemia include the following:

(1) Noninvasive, potentially anabolic nutritional interventions for improvement of bone density in young patients, for whom bisphosphonate medications may be contraindicated.
(2) Thoughtful investigation of the effect of oral chelation therapy on trace element status (zinc, copper, selenium), including assessment of static as well as functional markers of trace element status and biomarkers of oxidative stress.
(3) Exploration into the role of vitamin D deficiency and risk/benefits of supplementation with vitamin D not only in bone health but also immune function and cardiac disease.
(4) Collaborative, multicenter site studies to explore relationships between circulating levels of nutrients and various comorbidities.
(5) Randomized controlled clinical trials to test the efficacy of individual nutritional therapies towards the improvement of growth, pubertal development, immune function, oxidative damage, and bone health.

Conflicts of interest

The authors declare no conflicts of interest.

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