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Leveraging Bioactives to Support Human Health through the Lifecycle: Scientific Evidence and Regulatory Considerations

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Abstract

The identification of bioactive food components and understanding their role as adjunct therapeutic agents in disease management and prevention has become a significant area of research. Accumulating evidence suggests a link between certain bioactive food components and health outcomes, for example, lutein and zeaxanthin for visual performance and delaying age-related macular degeneration, probiotics for gastrointestinal outcomes related to irritable bowel syndrome or prebiotics for its potential programming of the microbiome in early life to influence later life outcomes.. This rapidly developing science has triggered discussions to determine if public health recommendations can be made on bioactive foods. However, regulatory guidance is necessary to guide the development the science, it's consideration for public health policy and the communication thereof to both healthcare professionals and consumers. This chapter will focus on the clinical and basic science supporting a role for lutein and pre- and probiotics in modulating several aspects of human health, including the gut microbiome through the human lifecycle. Opportunities to translate the science to consumers in a meaningful and accurate way will also be highlighted along with the regulatory landscape to shape the testing, communication and commercialization of these bioactives.

Keywords: bioactives, lutein, zeaxanthin, prebiotics, probiotics, gut microbiome, early life, development, programming, regulatory

1. Introduction

Interest in bioactive foods and ingredients is high among consumers. The 2013 Functional Foods Consumer Survey conducted by the International Food and Information Council showed that of the 1005 participants, 45% said they were very interested and 86% said they were very or somewhat interested in learning more about foods that have benefits beyond basic nutrition.

Although bioactive compounds typically occur in small amounts in foods, they are capable of influencing physiological and cellular activities, and in some cases can modify disease risk. Given the current focus on reducing risk of chronic diseases and programming health outcomes starting early in life, the potential beneficial role of bioactive compounds has garnered significant interest within the scientific community. The Office of Dietary Supplements at the NIH has defined bioactive compounds as constituents in foods or dietary supplements, other than those needed to meet basic human nutritional needs, which are responsible for changes in health status [1].

Over the past three decades, a variety of bioactive compounds have been identified that have potentially important health benefits. These include carotenoids, e.g., lutein; flavonoids, e.g., cocoa polyphenols; plant sterols, e.g., those found in soybeans; n-3 long chain polyunsaturated fatty acids (LCPUFA), e.g., docosahexaenoic acid (DHA); and more recently modulators of the gastrointestinal microbiota, e.g., prebiotics and probiotics. These compounds can act as antioxidants, enzyme inhibitors and inducers, inhibitors of receptor activities, and inducers and inhibitors of gene expression, among other actions [2, 3].

Currently, there are no public health recommendations for bioactive nutrients and bioactive containing foods as part of the everyday diet, including as part of supplementation or medical nutrition. An understanding of the strength of the established and emerging scientific evidence needs to be considered in order to establish such recommendations as well as to drive science-based communication strategies to consumers and healthcare professionals, within the guardrails of the regulatory environments.

Globally, healthcare costs are sky-rocketing and there is increased emphasis on reducing the prevalence of non-communicable diseases, e.g., heart disease, type II diabetes, obesity, and inflammatory bowel diseases. Not surprisingly, in most parts of the world today, the goal of healthcare is focused on disease prevention. Indeed, nutrition has an established role in "preventing, treating, mitigating, or curing a disease" but they are not drugs. However, there are obvious regulatory challenges to clinically testing and communicating the science of nutrients and bioactives to the general population. The FDA has an important role herein to guide the necessary framework that that supports development of the science on bioactives for relevant human health outcomes and its communication. We are certainly not there today.

Our goal with this review is to focus on the strengths and gaps in the science to help scientists, regulators and policymakers initiate dialogue on whether public health recommendations for key bioactive components, such as lutein and zeaxanthin, probiotics and/or prebiotics can be established. This review will focus on the published science supporting the role of these

specific bioactives in influencing both early and later life outcomes. The focus on the lifecycle effect is highly relevant given the accumulating evidence linking early-life nutritional and microbiome exposures and health status or disease risk in later life. In particular, we will focus on: (1) lutein and zeaxanthin as it pertains to visual performance and macular degeneration, and (2) the role of probiotics and prebiotics as they pertain to beneficially modulating the gastrointestinal microflora, gastrointestinal symptom outcomes in the context of irritable bowel syndrome (IBS), as well as early programming effects.

2. Lutein and zeaxanthin—visual function

Lutein, zeaxanthin, and meso-zeaxanthin are the three major carotenoid-based xanthophylls found in the eye. They comprise the macular pigments and give the macula lutea its yellowish color. Humans are unable to synthesize lutein and zeaxanthin and hence rely on their food supply and/or dietary supplements. Meso-xanthin is a metabolite of lutein and also can be absorbed from the diet [4]. Lutein and zeaxanthin are found in highest amounts in green leafy vegetables, egg yolk, corn, citrus, and other fruits [5]. These two macular pigments are highly concentrated in the retina [6]. While they can also be detected in human serum, their levels here are ~2–3 fold lower vs. levels measured in the retina [7]. This preferential localization and concentration of lutein and zeaxanthin suggests a specific uptake and storage mechanism for these xanthophylls in the visual system and highlights their essential role in retinal function [8].

The localization of lutein and zeaxanthin within the retina and their ability to absorb light near 460 nm allows these carotenoids to filter out high energy blue light, typically within the short wavelength spectrum [9, 10]. As a result, lutein and zeaxanthin limits photochemical damage and simultaneously supports visual performance and increases contrast sensitivity [11–13].

In addition to blue light filtration, these macular pigments serve as effective antioxidants, capable of quenching singlet oxygen and triplet state photosensitizers, inhibiting peroxidation of membrane phospholipids, scavenging reactive oxygen species, and reducing lipofuscin formation [14]. Photoreceptors contain chromophores which are vulnerable to damage through oxidation and macular pigments can limit the compromising effects of lipid peroxidation within the retina by quenching reactive oxygen species [14, 15]. Moreover, long chain polyunsaturated fatty acids, especially docosahexaenoic acid (DHA), are also selectively concentrated in the rod outer segments and given its chemical structure, DHA is highly susceptible to lipid peroxidation and cellular damage. As an antioxidant, lutein can return singlet oxygen to the ground state limiting lipid peroxidation. Lutein auto-regenerates in the process and through this way, may work to be a more efficient quencher of singlet oxygen than other antioxidants such as alpha-tocopherol (vitamin E) [16].

Hence, macular pigments support visual function through multiple ways. The filtration of blue light results in reduced chromatic aberration and subsequently improved visual acuity and contrast sensitivity. Lutein and zeaxanthin also reduce discomfort glare and increase visual acuity, photo-stress recovery time, macular function, and neural processing speed. These are further discussed below.

2.1. Glare discomfort and disability glare

Glare discomfort is characterized by photophobia—a phenomenon that occurs when intense light enters the eye and the recipient experiences discomfort. Photosensitivity is an inherent mechanism to protect the eye from high energy wavelengths [17, 18]. Increased sensitivity to shorter wavelengths of light can trigger retinal damage with less energy compared to other wavelengths. Photophobic response studies have shown that subjects with higher macular pigment levels tolerated light better and have less glare [17]. Additionally, small increases in macular pigment were sufficient to increase photophobia thresholds and lessen visual discomfort [19]. These data support that macular pigment supplementation has a role in reducing discomfort associated with glare.

Bright light settings results in scattered light which subsequently causes decreased visual acuity. This phenomenon is commonly referred to as disability glare. Similar to data generated for glare discomfort, it has also been shown that subjects with higher macular pigment levels maintained acuity better than subjects with lower levels when exposed to both bright white light and short wavelength (blue) light. Additionally, lutein and zeaxanthin supplementation improved glare disability under these conditions [20].

2.2. Photo-stress recovery

The time required to recover vision after exposure to a bright light source is called photo-stress recovery. This visual performance parameter describes the time it takes for bleached photopigments to regenerate and it is affected by macular pigments. Similar to the data generated for glare, individuals with higher macular pigment levels had shorter photo-stress recovery time when tested with intense short wavelength and bright white light sources [21]. The mechanism for this benefit of the macular pigment appears to be related to the reduced photoreceptor exposure to short wavelength light in the foveal and parafoveal regions. Recovery time for the subject with the lowest macular pigment levels was twice as long as subjects with the highest macular pigment levels [22]. Moreover, supplementation with lutein and zeaxanthin significantly decreased photo-stress recovery time [20]. More specifically, supplementation with lutein (10 mg/d) and zeaxanthin at a dose of 2 mg/d over 3 months significantly increased serum levels of lutein and zeaxanthin, macular pigment optical density, and improved chromatic contrast and recovery from photo-stress [20].

2.3. Neural processing

It is not surprising that the brain is frequently referred to as the "window to the world" given the intimate relationship between the optical, neurological and physiological mechanisms underlying vision. In addition to the visual system, macular pigments are present in the brain [23, 24]. A reliable and commonly used proxy for macular pigment levels and hence lutein and zeaxanthin levels is macular pigment optical density (MPOD). MPOD correlates with processing speed and cognitive performance in healthy elderly subjects as well as those with mild cognitive impairment [25–27].

Consistent with data generated for visual function, higher macular pigment levels have been linked to improved critical flicker fusion frequency [28–31], higher concentrations in the visual cortex [53], and improvements in electroretinography responses [32, 33]. Bovier et al. found moderate but statistically significant improvements in both MPOD and cognitive function with lutein and zeaxanthin supplementation of young, healthy individuals considered to be at peak cognitive efficiency [34]. These studies suggest that both young, healthy adults and the elderly population can gain cognitive benefits from lutein and zeaxanthin supplementation.

2.4. Age-related macular degeneration (AMD)

Oxidative stress has been identified as a major contributing factor in the pathogenesis of AMD, a disease that is commonly associated with irreversible blindness in older people [35]. Given the selective localization of lutein and zeaxanthin within the retina and their potency as singlet oxygen scavengers to limit oxidative damage, there has been considerable scientific interest to identify if lutein and zeaxanthin can be used as a therapeutic approach to manage AMD.

Observational studies of dietary intakes of lutein and zeaxanthin, generally suggests that high intakes of these carotenoids in the diet are associated with lower risk of AMD. These studies were conducted globally and over multiple years of supplementation [36–40]. In regards to macular pigment levels and the risk of AMD, Bone et al. demonstrated that subjects with AMD had significantly lower levels of macular pigment and those with the highest quartile of lutein/zeaxanthin had a lower risk of having AMD compared those in the lowest quartile [41]. MPOD is positively correlated with dietary intake of lutein and zeaxanthin [31] and their serum levels [42, 43]. The CAREDS study, a prospective cohort analysis of nearly two thousand postmenopausal women, did not find a correlation between MPOD and AMD [44]. Several but not all trials have supported a lower MPOD in eyes with AMD, and several supplementation trials of AMD subjects have demonstrated reduced MPOD in those subjects not receiving supplementation [45–47].

Supplementation trials with lutein and zeaxanthin and reduced risk of AMD have yielded considerably consistent results compared to most other bioactive/nutrient studies as related to measure of MPOD and/or visual acuity. A meta-analysis performed by Liu et al. compared the results of seven randomized, double-blind, placebo-controlled trials, including the LAST, Weigert et al., Ma et al., CARMIS, LUTEGA, CLEAR, and CARMA studies [13, 47–52]. Out of these studies, four reported an increase in visual acuity with supplementation, and the benefit appeared more pronounced in those subjects with early AMD vs. late AMD. This may be due to a greater loss of macular photoreceptors in the late stage of the disease. A stronger effect was noted for studies using higher doses of supplements. Interestingly, a linear association of MPOD and an increase in visual acuity was also measured [53].

Most recently, the Age-Related Eye Disease 2 Study (AREDS2), a 5-year multicenter, double-blinded, placebo-controlled clinical trial involving 4203 participants with intermediate AMD or large drusen in 1 eye and advanced AMD in the fellow eye was completed. Participants were randomized to one of four groups: placebo, lutein (10 mg) and zeaxanthin (2 mg), omega-3 fatty

acids (DHA 350 mg and EPA 650 mg), or a combination of lutein, zeaxanthin, and omega-3 fatty acids. Although the original analysis did not find significant effects from the lutein and zeaxanthin supplementation, a secondary analysis of the effects of xanthophyll supplementation demonstrated reduced AMD progression [54] but did not affect the development of geographic atrophy. Focusing the analyses to eyes with bilateral large drusen at baseline, the comparison of lutein/zeaxanthin vs. β-carotene showed even stronger effects for progression to late AMD and for neovascular AMD.

Collectively, the overall body of evidence supports that structural changes in the retina and improvements in visual acuity can be achieved with lutein and zeaxanthin supplementation. However, additional research is warranted to identify the optimal levels of supplementation in healthy individuals with compromised visual function as well as those with eye disease, e.g., AMD and cataracts, as well the role of early supplementation initiated before disease progression.

2.5. Visual development: role of lutein and zeaxanthin in the prenatal and postnatal periods

Although the placental transfer of carotenoids from mother to child *in utero* has not been directly studied through clinical supplementation trials, there is evidence of the deposition of carotenoids within the eye during the gestational period [14], with ratios of lutein: zeaxanthin: *meso*-zeaxanthin differing from the composition of serum [55]. It is likely that maternal carotenoid status during the gestational period may impact infant macular development, and prenatal supplementation may play a role in maximizing visual development.

Bernstein and colleagues demonstrated an age-dependent increase in MPOD in infants and children, but preterm infants in that cohort did not have measurable MPOD [56]. Interestingly, there were significant correlations between infant MPOD and infant serum zeaxanthin. Additionally, maternal serum zeaxanthin levels correlated with infant MPOD in term infants shortly after birth [57]. This suggests a potential role for maternal nutrition and macular development *in utero* and the opportunity for the mother to increase their lutein and zeaxanthin dietary intakes through food and/or supplements during pregnancy and the breastfeeding period.

The role of lutein in early maturation of the retina is further supported by data from non-human primate studies wherein xanthophyll-free diets resulted in the absence of macular pigmentation, more drusen-like bodies in the retinal pigment epithelial cells (cells that are crucial for nourishment of the retina), increased macular hyperfluorescence, and more retinal abnormalities [58].

Given the promising data on the use of lutein and zeaxanthin to delay macular degeneration, the potential role of these carotenoids in preventing oxidative damage in preterm infants is worthy of further study. There are preliminary data to suggest a potential protective role of carotenoids against oxidative stress during premature life, particularly in cases of retinopathy of prematurity (ROP) [59].

While there is at present no data in humans showing directly that lutein and zeaxanthin influence retinal/visual development, it is highly plausible that these bioactives are important for

visual development given their involvement in three key aspects of the visual system: (1) influence input during a critical/sensitive period of visual development and/or (2) influence maturation and/or (3) protection of the retina during a period when it was particularly vulnerable [60].

2.6. Summary

Of all the carotenoids found in nature, only lutein and zeaxanthin are exclusively found in the retina and selectively concentrated in the macula. These macular pigments have been well documented through epidemiological, observational, and intervention studies to play a promising role in visual performance both in healthy individuals and those with macular degeneration. Preliminary data also suggest a relationship between lutein and zeaxanthin and visual development in infancy. Dietary intakes of lutein and zeaxanthin are dismally low among Americans with most adults and children not consuming intakes clinically demonstrated to be protective for eye health. Strategies need to be identified to increase dietary intake of these relevant bioactive nutrients and create awareness on their essentiality to the health of humans.

3. Probiotics, prebiotics and gastrointestinal microbiome

The gastrointestinal tract is best known for its role in the digestion of food and absorption of nutrients. It has the largest surface area in the body—it is ~9 m in length with a surface area of ~250–400 m², comparable to the size of a tennis court. It hosts a variety of immune cells making it the largest immunological organ in the body and equally interestingly, it contains a similar number of neurons as that found in the spinal cord—so in other words, the gastrointestinal tract has its own nervous system, the enteric nervous system. For this reason, the gastrointestinal tract is frequently referred to as the body's second brain. Additionally, the gastrointestinal tract also houses the greatest number and variety of bacteria in the body. There are 10×s as many bacteria in the gastrointestinal tract (GIT) as there are cells in the body. These bacteria have the unique ability to interact and communicate with the immune cells, intestinal cells, and the neurons in the body to influence digestive health, immune health and overall well-being. Certain lifestyle and environmental factors can influence the balance of the friendly vs. unfriendly bacteria in the gastrointestinal tract including diet, age, medication, stress, travel, and sleep.

At birth, the human gastrointestinal tract is relatively sterile but becomes rapidly colonized with a diverse microbial population comprising tens of trillions of bacteria and hundreds of different species by 3–5 years of age. The density and diversity increases exponentially from the stomach to the colon, where the microbial content is at its highest concentration. Although the phyla *Firmicutes* and *Bacteroidetes* dominate the human gut microbiota, it contains a core microbiome with shared functionality and shared mechanisms of action [61].

The abundance and diversity of the microbiota suggest an important physiological role for this "organ" within the gastrointestinal tract. Herein, this dynamic ecosystem facilitates multiple functions including the digestion of complex carbohydrates; shaping the immune system and modulating immune responses; contributing to the defense against pathogens by the mechanism

of colonization resistance and fermentation of non-digestible carbohydrates. They produce metabolic products including short chain fatty acids such as acetate, propionate, and butyrate. These metabolites serve as a major energy source for intestinal epithelial cells wherein they can influence cell proliferation and differentiation, mucus secretion, intestinal motility, and barrier function; and may also exert anti-inflammatory and antioxidative activity [62].

A shift from a stable intestinal environment occurs when the gut microbiota community is temporarily or permanently altered and is termed "dysbiosis." Factors that may lead to dysbiosis include antibiotics, diet, host immune system, inflammation, and infectious gastroenteritis. Dysbiosis is observed in several gastrointestinal disorders including IBS, and Crohn's disease, and may play a key role in their pathogenesis and possibly in management. Some of the common features of dysbiosis in IBS and Crohn's disease are reduced microbial diversity, lower bifidobacteria, lower bacteroidetes to firmicutes ratio in IBS and in Crohn's disease, and decreased *Faecalibacterium prausnitzii* [63].

Current research efforts have focused on two main approaches to supporting and promoting the stability and diversity of the microbial community within the GIT: (1) offering specific substrates for fermentation by the colonic bacteria (prebiotics); and/or (2) introducing specific bacterial species or strains to the colonic microbiota (probiotics).

Probiotics and prebiotics have been evaluated in a number of clinical trials involving individuals at different stages of the lifecycle, including pregnancy, infants, children and adults and under different health conditions, e.g., infectious diarrhea, antibiotic-associated diarrhea, therapy and prevention of *Clostridium difficile* and other infections, inflammatory bowel disease, IBS, atopic dermatitis and allergic immune outcomes.

In this section, we review the role of probiotic and prebiotics in the context of gastrointestinal health, with a particular focus on IBS. IBS is a chronic functional disorder of the gastrointestinal system. Individuals experience abdominal pain and altered bowel habit, with either predominantly diarrhea (IBS-D), constipation (IBS-C), or both (IBS-M). It has an insidious onset, and frequently does not result in medical care. Irrespective of geography, IBS is a significant health care burden affecting around 11% of the population globally [64]. Recent studies suggest IBS may comprise ~20% of gastroenterology outpatient visits, and thus these statistics highlights the importance of identifying effective therapies to manage their symptoms and improve their quality of life.

Additionally, the role of prebiotics in influencing the gut microbiome composition and activity in early life and the subsequent long term benefits thereof will also be highlighted in this section.

3.1. Probiotics

The term "probiotic" as originally defined by FAO/WHO refers to "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [65]. However, in order to be beneficial, probiotic bacteria must be able to survive along the gastrointestinal tract, to resist gastric acid, bile and pancreatic juice action and to demonstrate functional efficacy [66].

A meta-analysis involving 18 randomized-controlled trials including 1650 patients with IBS was conducted by Moayyedi et al. [67]. Although the review reported considerable heterogeneity among the studies, the analysis reported a preference toward probiotic treatment with statistically significant improvement of individual symptoms such as pain, flatulence and bloating. No side effects were reported and there was no significant differences detected between the various types of probiotics used in the studies, with three studies using *Lactobacillus* (n = 140 subjects), two trials using *Bifidobacterium* (n = 422 subjects), one trial using *Streptococcus* (n = 54 subjects), and four trials using a combination of probiotics (n = 319 subjects). The favorable safety profile reported in this meta-analysis are consistent with the findings of Hungin et al. who also showed several positive effects of probiotics on IBS symptoms and health-related quality of life measures. Their analysis involved 19 studies and 1807 patients [68].

Similar to the findings of Moayyedi et al., Clarke and coworkers also showed that despite significant studies heterogeneity in their analysis of 42 randomized-controlled trials, 34 studies reported beneficial effects on at least one pre-specified endpoint including improvement in abdominal pain/discomfort, improvement on abdominal bloating/distension compared to placebo [69]. Both *Bifidobacteria* and *Lactobacilli* were found effective in ameliorating IBS symptoms, while the beneficial effects of the multispecies lactic acid bacteria preparations, including the multi-strain preparation VSL#3, were less pronounced.

Another systematic review with meta-analysis has been recently published by Didari et al. which focused on a review of 15 studies involving 882 patients with IBS. Not surprising, significant study heterogeneity was observed given differences in the types of bacterial strains used, probiotic dosage, duration of either treatment or follow-up and endpoints/outcome. However, consistent with the other systematic reviews, probiotics were more effective than placebo in reducing abdominal pain after 8 and 10 weeks of treatment. Few adverse events were reported in both probiotics and placebo groups and this meta-analysis reconfirmed the safety profile of probiotic use [70].

3.1.1. Mechanisms of action of probiotics

Probiotics appear to exert their beneficial effects on gastrointestinal healthy through three general mechanisms: antimicrobial effects, mucosal barrier integrity, and immune modulation. Moreover, the important benefits of probiotics is based on their ability to metabolize complex carbohydrates and produce lactic acid and SCFAs such as butyrate [58, 59]. In the context of IBS, there is ample evidence to support the role of probiotics in managing the symptoms of IBS through positive changes in the composition and functionality of the intestinal bacteria, correcting intestinal motility, limiting visceral hypersensitivity, modulating immune responses and benefiting the gut-brain axis [71]. Indeed, more studies need to be conducted to further unravel the mechanisms through which probiotics beneficially influence the symptoms of IBS and thereby further enhanced focused and specific probiotic therapeutic modalities that can also be "personalized" based on an individual's needs.

3.2. Prebiotics

Prebiotics are selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring a benefit on the host. In order for a compound to be classified as a prebiotic, it has to fulfill three criteria: i] resistant to gastric acidity and hydrolysis by mammalian enzymes and gastrointestinal absorption; ii] can be fermented by intestinal microbiota; iii] selectively stimulates the growth and/or activity of the intestinal bacteria associated with health and wellbeing [72].

These non-digestible oligosaccharides, such as fructooligosaccharides (FOS), galactooligosaccharides (GOS), lactulose, and inulin, stimulate and nourish the growth of selective and beneficial gut bacteria, particularly lactobacilli and bifidobacteria [73]. Prebiotics have been clinically tested in a variety of settings for multiple health benefits, including improvement of intestinal function as measured by stool bulking, stool regularity, stool consistency, glucose and lipid metabolism, immune health including allergic outcomes, satiety and appetite regulation, and stimulation of mineral absorption and improvement of bone density. The majority of the studies have focused on inulin and FOS, whereby studies have consistently shown a benefit for overall digestive health, including an increase in the total bacterial mass, growth of beneficial bacteria, reduction in pathogenic bacteria, and production of numerous beneficial bacterial metabolites.

The proceeding paragraphs will highlight two areas of emerging evidence: (1) role of prebiotics in IBS and (2) programming effect of prebiotics when supplemented during the first 1000 days of life.

3.2.1. Prebiotics and IBS

Since IBS is generally categorized by an imbalance of bacteria, the mechanisms through which prebiotics work suggest that they could potentially be used as a therapy, either alone, or in combination with probiotics, to manage IBS and its related symptoms. To date however, there have only been a handful of randomized control trials investigating the effect of prebiotics on IBS. As summarized in the literature, two studies in adults with IBS at doses of 6 g/d of oligofructose and 20 g/d of inulin showed no improvement in symptom or stool output measures. Another trial showed an improvement in composite symptom score with 5 g/d of short-chain FOS in the per-protocol population, but this was not analyzed intention to treat, with a high non-compliance rate and only 50/105 being included in the per protocol analysis. Separately, a 12-week parallel cross-over trial, which used a β-GOS, showed a dose-dependent stimulation of bifidobacteria at 3.5 and 7.0 g/d. Global symptom relief scores were significantly improved in the prebiotic group vs. the placebo, including for flatulence, bloating, and stool consistency [74, 75].

These preliminary data suggest that prebiotics may offer promise as a therapeutic option in the dietary management of IBS but more studies certainly need to be conducted to confirm the benefit of prebiotics for this population, including the optimal type and dose. These factors need to be first addressed prior to prebiotics being considered as therapy option in individuals with IBS.

3.2.2. Gut microbiome in the first 1000 days and the "programming" effects of prebiotics

The first 1000 days of a child's life is now well recognized as a critical timeframe for health into adulthood, wherein nutrition plays a key role. Additionally, a robust link between nutrition and gut microbiota composition with health outcomes has been documented. It is intriguing to consider that events early in life may determine the activity of our gut microbiota for the rest of our life. It is equally fascinating that the gut microbiota in early life can determine our risk of later life heath outcomes.

Colonization of the infant gut contributes to the intestinal homeostasis and mucosal barrier function, that both are essential for our health, at the start of life and apparently also in adulthood. In this regard several studies have demonstrated that the mode of delivery affects the composition of the newborn's microbiota wherein caesarean section birth is associated with a lower total microbial diversity and delayed colonization. Other factors influencing this composition include infant hospitalization and antibiotic use, antibiotic use in the pregnant mother, solid-feeding practices and day care attendance. Alterations of the development of the gut microflora during infancy has been linked to altered immune system development and thus increased risk of allergic immune outcomes, as well as altered metabolic profiles and increased obesity risk [76].

A new exciting development is the role of the gut microbiome as an epigenetic regulator wherein sequencing of DNA methylomes of pregnant women revealed an association between bacterial predominance and epigenetic profiles. Epigenetics comprise genomic modifications that occur due to environmental factors and do not change the nucleotide sequence. In the context of cardiovascular disease and obesity, different methylation status of gene promoters have been correlated with specific gut microbiota signatures, with either *Firmicutes* or *Bacteroidetes* represented as a dominant group. These observations parallel previous studies linking higher levels of *Firmicutes* to obesity. Additionally, an elegant study by Paul HA and colleagues showed that consumption of prebiotics during pregnancy and lactation improves metabolism in diet-induced obese rats and limits the detrimental nutritional programming of offspring associated with maternal obesity. More specifically, there was a reduction in gestational weight gain, increased circulating concentrations of satiety hormones and abundance of *Bifidobacterium* spp. in the gut. These effects were accompanied by an attenuation of increased adiposity in both dams and offspring at weaning [77].

Over the past decade, studies have investigated the effect of specific mixtures of prebiotics, for example short chain GOS + long chain FOS, on the composition of the intestinal microbiota in preterm, term, and weaning infants and have consistently shown that prebiotic supplementation influences early microbial pattern similar that of human milk with an intestinal microbiota dominated by Bifidobacterium and Lactobacillus [78–82].

Studies have also shown that changes in early-life microbial composition by such prebiotics parallels metabolic production of the microbiota, including increased short-chain fatty acid production, lactate and a reduced pH [83, 84]. These favorable metabolic changes induced by prebiotics have been associated with increased colonization resistance to pathogens and this characteristic is supported by in-vitro data [85]. Moreover, the modulation of early-life

microbiota by prebiotics correlates with improved immune system maturation. More specifically dietary supplementation with short chain GOS + long chain FOS has been positively associated with increased production of secretory IgA. Additionally, there are preclinical data supporting the role of such prebiotics in modulating systemic immune responses through direct binding of specific receptors on immune cells and/or through short-chain fatty acid production [86, 87].

Given the accumulating evidence supporting the association between the infant's gut microbiota composition and health in later life, the potential for gut microbe-based modulation including prebiotics, may be a promising approach to improve health during prenatal life, infancy, childhood and thus, later life outcomes.

4. Bioactive foods and the regulatory environment

The functional food components discussed in this chapter can be commercialized under several of the FDA categories that researchers and manufacturers need to consider carefully prior to launch. FDA's authority to regulate a product as a food, supplement, device, or a drug, depend on the product presentation, intended uses, target population, and claims they make about their product. This "intended use" criterion also defines the materials that can be used in the formulation of the product. Together, these dictate the appropriate regulations applicable and regulatory agencies responsible for regulating them. Most important among the claims is whether the product is intended to be used to diagnose, cure, mitigate, treat, or prevent a disease. Although the intended uses of Drugs and Devices may also be applicable, only the dietary regulations are covered in this subsection given the focus on nutritional bioactives.

The FDA regulates claims in four categories [88]:

• Nutrient Content Claims: characterize the amount of nutrients present in the product,

• Health Claims: describe a relation between a nutrient and a disease based on Significant Scientific Agreement (SSA),

• Qualified Health Claims: provide for health claims based on less scientific evidence than SSA standard as long as the claims do not mislead the consumers, and

• Structure/Function Claims: relate the role of a nutrient to the normal structure or function in humans and do not make reference to a disease.

The food labels and messaging are controlled through several federal regulations and agencies such as the Federal Food, Drug, and Cosmetic Act (FFDCA), the Nutrition Labeling and Education Act (NLEA), the FDA, and the Federal Trade Commission (FTC), the false advertising litigations permitted under state laws and section 43(a) of the Lanham Act, and the consumer protection laws in general [89]. The National Advertising Division (NAD) of the Council of Better Business Bureaus, Inc. (CBBB) is another active player in regulating and shaping the food industry communication, including the dietary supplements category, where most of these products are today placed. The NAD is an industry-funded body that reviews nationally disseminated advertising for truth and accuracy [90].

4.1. Dietary supplement

The Dietary Supplement Health and Education Act (DSHEA) of 1994 a "dietary supplement is a product intended for ingestion that contains a "dietary ingredient" intended to add further nutritional value to (supplement) the diet. A "dietary ingredient" may be one, or any combination, of the following substances:

- A vitamin

- A mineral

- An herb or other botanical

- An amino acid

- A dietary substance for use by people to supplement the diet by increasing the total dietary intake

- A concentrate, metabolite, constituent, or extract

Dietary supplements may be found in many forms such as tablets, capsules, softgels, gelcaps, liquids, or powders. Some dietary supplements can help ensure that you get an adequate dietary intake of essential nutrients; others may help you reduce your risk of disease." [91]

Ingredients used in dietary supplements must either demonstrate evidence of use prior to 1994, or that they were used in food in the present form.

4.2. Conventional food

Congress passed the FFDCA in 1938, which grants the FDA the power to ensure that "foods are safe, wholesome, sanitary, and properly labeled." Section 201(f) of the FD&C Act (21 U.S.C. 321(f)) defines a food as "(1) articles used for food or drink for man or other animals, (2) chewing gum, and (3) articles used for components of any such article" and a drug to include "articles (other than food) intended to affect the structure or any function of the body" and "intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease."

4.3. Food for special dietary uses (FSDU)

FSDU are defined as food "(i) used for supplying particular dietary needs which exist by reason of a physical, physiological, pathological or other condition, including but not limited to the conditions of diseases, convalescence, pregnancy, lactation, allergic hypersensitivity to food, underweight, and overweight; (ii) uses for supplying particular dietary needs which exist by reason of age, including but not limited to the ages of infancy and childhood; (iii) uses for supplementing or fortifying the ordinary or usual diet with any vitamin, mineral, or other dietary property. Any such particular use of a food is a special dietary use, regardless of whether such food also purports to be or is represented for general use" [92].

4.4. Medical food

In 1988, with the Orphan Drug Act Amendments, Congress recognized the need to encourage development of "medical foods" for the management of disease and health conditions

and defined "medical food" as "food which is formulated to be consumed or administered enterally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation." (Orphan Drug Act –1988; 21 U.S.C. §360ee(b)(3), 5(b); FFDCA §528)

The FDA later clarified this definition into the five criteria used to define medical food listed below [92]:

(i)   it is a specially formulated and processed product (as opposed to a naturally occurring foodstuff used in its natural state) for the partial or exclusive feeding of a patient by means of oral intake or enteral feeding by tube;

(ii) it is intended for the dietary management of a patient who, because of therapeutic or chronic medical needs, has limited or impaired capacity to ingest, digest, absorb, or metabolize ordinary foodstuffs or certain nutrients, or who has other special medically determined nutrient requirements, the dietary management of which cannot be achieved by the modification of the normal diet alone;

(iii) it provides nutritional support specifically modified for the management of the unique nutrient needs that result from the specific disease or condition, as determined by medical evaluation;

(iv) it is intended to be used under medical supervision; and

(v)   it is intended only for a patient receiving active and ongoing medical supervision wherein the patient requires medical care on a recurring basis for, among other things, instructions on the use of the medical food.

All ingredients used in conventional foods, FSDU, or Medical Foods, must be either Generally Recognized as Safe (GRAS) or pre-approved by the FDA as additives. Further, conventional and FSDU must conform to all nutrient and health claims provisions in 21 CFR Subpart A 101.13 and 101.14 along with the specific requirements for the claims in 21 CFR subpart D (Specific Requirements for Nutrient Content Claims) and subpart E (Specific Requirements for Health Claims). Medical foods must follow many of the same labeling requirements of conventional foods except only Medical Food is exempt from the nutritional and health claims labeling of food [92].

Among the above categories, none is most strife for abuse as the Medical Foods category, primarily because of its claims exemption requirements. This it is also the most controlled as the "FDA considers the statutory definition of medical foods to narrowly constrain" to the definition [93]. The FDA has consistently applied a standard that food are "articles consumed primarily for taste, aroma, or nutritive value" but "used as a drug for some other physiological effect" [94].

The two most important hurdles to overcome in meeting the regulatory requirements for Medical Food are [95]:

(i) Distinctive Nutritional Requirements, and

(ii) Cannot be achieved by the Modification of the Diet Alone.

Nutrient intake requirements assessed by Institute of Medicine (now National Academy of Medicine) are based on population estimates of estimated average requirements. What is interesting to note is that these are based on estimated average intake levels correlated with measure of inadequacy (on the lower end) and risk of adverse events (on a higher end) [96]. The bio-functional molecules covered in this category are currently considered by regulatory agencies as non-essential and therefore ineligible for dietary reference intakes (DRI) estimates. The "nutritive value" today is not a function of DRI, estimated average requirements (EAR) or daily values (DV), but a complex biochemical function derived through genome, epigenetics, nutrigenomics, and the microbiome. In the roundtable and workshop on obesity report by the National Academies of Science [97], the early origins of obesity can be traced to metabolic programming that starts pre-conception and defines the individual's predisposition for a nutrient uptake and metabolism.

Simple biochemical statistics suggest that uptake or utilization of cellular molecules, including nutrients, is multimodal kinetics. For illustration purposes only, the Distinct Nutritional Requirements for an individual can be depicted using a sigmoidal curve nutrient uptake and utilization by the following model (**Figure 1**). In any individual, the individual's diet,
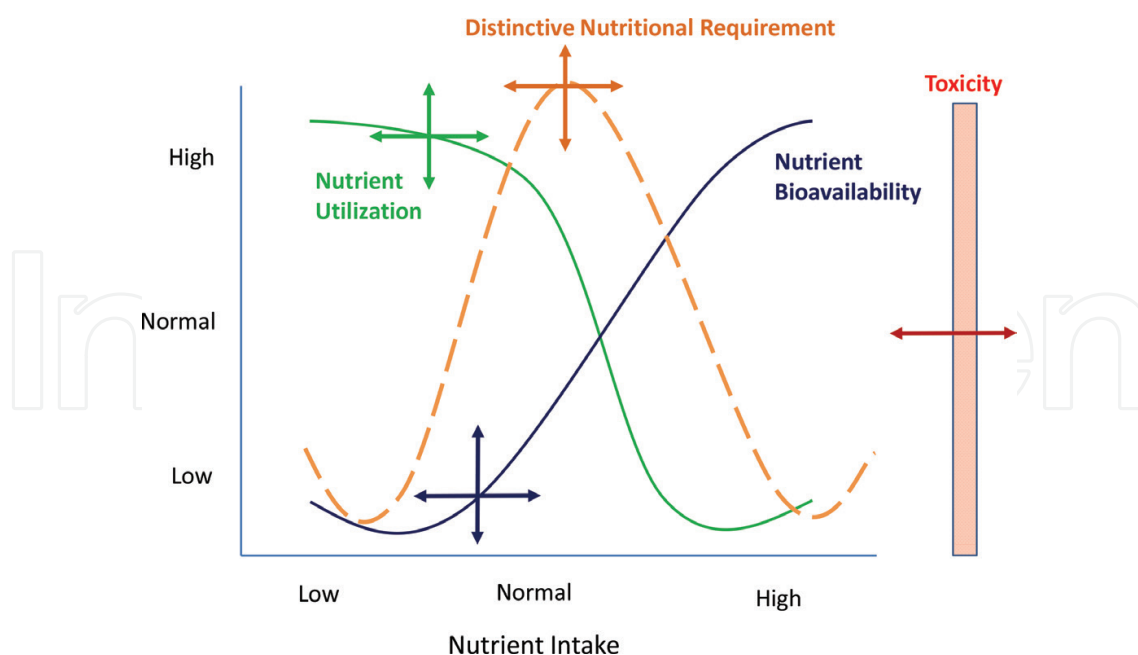

Figure 1. Distinct nutritional requirements for an individual can be depicted using a sigmoidal curve nutrient uptake and utilization of the multimodal kinetics model.

metabolic rate, or any situation-specific external and internal requirements, will dictate the bioavailability curve that in turn will drive the nutrient requirements. Simply because the nutrient shares the cellular network with a pharmacological function should not dictate its classification as a drug. Preponderance of evidence now suggest that a modified view of nutrients as bio-functional components are mandatory and the old way of nutrient intake should give way to the new scientific knowledge. Current FDA regulations that surround definition of Food and Dietary Supplements, not only do not provide consideration for individualistic or disease specific intake (or utilization) of the nutrient, but specifically prohibit such interpretation. The FDA has to acknowledge the complex role of a nutrient in the health and wellbeing of an individual is simply not relatable to the DRI.

In any individual, it is the nutrient bioavailability curve that will dictate the biochemical utilization of that nutrient, all conditions deemed equal. However, depending on the individual's diet, metabolic rate, genetics, epigenetics, or any situation-specific requirements, the availability curve can be right or left shifted. So also, it is not unusual that the nutrient utilization for its cellular or metabolic function can similarly be right or left shifted, again depending on their own cellular availability of nutrient requirements, metabolites concentration, or any other external or internal factors. Rapid net catabolism of body protein occurring in major trauma, burns and sepsis patients have a higher resting energy and protein requirements. In these examples, the utilization curve is right-shifted along the X-axis and without proper balancing of the nutrient bioavailability (and thus intake) curve also right shifted, patients would not recover well. Thus, both the availability and utilization functions can be moved along the Y-axis where a minimum threshold need to be met before the nutrient is available for its cellular functions. Thus, the "distinctive nutrition requirements" for that individual can be the equilibrium function of the two biochemical curves that can also move along the two axes depending on the "conditional" needs of that individual. Similarly, the toxicity function can slide along the X-axis depending on the individual's needs. For example, Lofenalac was specifically formulated for patients with phenylketonuria (PKU) unable to adequately metabolize phenylalanine and is considered FSDU. In this case, the toxicity function would be to the extreme left. This would also be true of other allergic diseases for nutrients where depending on the individual's tolerance to that allergen, the toxicity function can be anywhere along the X-axis. Since the mechanism of action for most nutrients share the same cellular pathway as their pharmacological counterparts, just because the nutrient takes part in that pathway is not a sufficient criteria to qualify as a drug. Hydrolyzed protein epitopes of an allergen are a classic example. Maternal consumption of peanut during pregnancy reduced peanut allergy sensitization in infants born to these mothers [99]. Similarly, maternal serum zeaxanthin levels correlated with infant MPOD in term infants shortly after birth [57] thereby providing opportunity for maternal diet supplementation during pregnancy and the breastfeeding period. Neither of these examples fit the static EAR definition and yet meet all the classic requirements for a nutrient and their role in disease without being a drug. This dynamic nature of the nutrient are necessary and sufficient conditions for "distinctive nutritional requirements."

In the context of bioactives for human health support, the FDA has to take a holistic view of human health where food, drug, and supplements, and alternative therapies, all have a role. The healthcare costs are sky-rocketing and nutrition has an established role in "preventing,

treating, mitigating, or curing a disease" and they are not drugs. Manufacturers should be able to provide functional nutrients to consumers provided the claims are well substantiated. There are obvious challenges to impose drug clinical study design on the substantiation of dietary ingredients since they are not a single chemical entity or are easily achievable by a double blind placebo controlled multi-center trials like drugs [98]. However, a reasonable study design to measure clinical outcomes is still necessary and the FDA should exercise regulatory responsibility to provide the necessary framework that takes into consideration the developing science and the practical limits of the diet. This will considerably help the conscientious industry players as well as to control mavericks trying to circumvent the food category utilization for product placement and claims. When it comes to policy making, Nutrition, Diet, or Food manufacturers are conspicuously absent from the stakeholder list. The Nutrition industry is a necessary partner in the healthcare discussion [100].

5. Concluding remarks: where do we go from here?

There is increasing interest by consumers, researchers, and regulators into the roles that certain bioactive compounds, such as lutein, zeaxanthin, prebiotics and probiotics, can play in health maintenance and promotion, as well as potentially programming health outcomes starting in early life. The state of the science for these bioactives and their benefits to health and wellbeing appear to be sufficiently mature to bring together key stakeholders including policymakers, regulators, and toxicologists, to initiate dialogue on advancing the process for establishing recommended intakes and its communication to the public.

These collaborative dialogues will need to address difficult and controversial questions, e.g., (1) what constitutes sufficient evidence and do we have to adopt an evidence-based medicine model focusing only on randomized controlled trials; (2) should the evidence focus on demonstrating the bioactive is health-promoting or are the study participants performing better than baseline?; (3) availability of reliable and validated biomarkers for both exposure and effect and their relation to health outcomes, especially in vulnerable populations, e.g., pregnancy, postnatal, and early childhood; (4) limited databases for bioactives such as lutein and prebiotics—without such databases in place, intakes of these compounds by groups and populations cannot be evaluated in food consumption surveys; (5) methods to standardize and measure bioactive components.

From a regulatory perspective, the FDA should take a holistic view on the process to regulate and hence commercialize nutritional bioactives. The current health care environment where consumers are taking more ownership for their healthcare starts with laying the foundation where consumers are encouraged to find accurate and reliable information from both the industry and the government. A proper regulatory framework that allows for nutritional benefits of the dietary ingredients and their role in diseases to be conveyed to the consumers will be beneficial for society at large.

Moreover, the process for communicating the science on bioactives to the public including healthcare professionals certainly lags behind commercialization. The accumulating and

promising scientific evidence for lutein, zeaxanthin and pro- and prebiotics, warrants guidance and alignment from key stakeholders on approaches to help educate and communicate the benefits of these bioactives in a manner that is science-based, meaningful, accurate and not misleading. Platforms such continuing medical education programs, webinars, and conference proceedings can be leveraged to disseminate scientific information. This will empower consumers to leverage these self-care strategies in a responsible and compliant manner.

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