Peer-reviewed version available at *Front. Immunol.* **2019**; doi:10.3389/fimmu.2019.01118

Review

Contribution of Dietary PUFAs and Micronutrients in the protection against Food Allergy Development

Tamara Hoppenbrouwers [1], Jelena H. Cvejić Hogervorst [2], Johan Garssen [3,4], Linette E.M. Willemsen [4] and Harry J. Wichers [5*]

[1] Wageningen University Food Quality and Design; tamara.hoppenbrouwers@wur.nl
[2] University of Novi Sad, Faculty of Medicine, Department of Pharmacy; jelena.cvejic@mf.uns.ac.rs
[3] Nutricia Research BV, department immunology, Utrecht; j.garssen@uu.nl
[4] Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University; L.E.M.Willemsen@uu.nl
[5] Wageningen University Food & Biobased Research; harry.wichers@wur.nl

* Correspondence:
harry.wichers@wur.nl; Tel.: +31317480175

Abstract: Specific nutrients including n-3 long chain polyunsaturated fatty acids (LCPUFAs), flavonoids, vitamin A, D and E are considered to possess protective properties on human health by impacting on immunological reactions. An 'inflammation-suppressive' effect appears to be the common denominator of the beneficial effects of most of these dietary components which may protect against the development of chronic immune disorders such as allergy. However, the majority of these promising data are from preclinical studies such as animal disease models, as the majority of clinical studies only indicate associations. PUFAs, especially n-3 LCPUFAs, have been shown to interact with both the sensitization as well as the effector phase in food allergy. However, it should be noted and realised that PUFAs are highly susceptible to lipid oxidation. Flavonoids and fat-soluble vitamins both contain anti-inflammatory properties and are able to act as anti-oxidants as well. Here, we explore the anti-allergic properties of PUFAs, flavonoids and fat-soluble vitamins in order to create an overview and, more importantly, suggest a strategy to target food allergies using these components and combinations thereof. Dietary n-3 LCPUFAs and the above mentioned micronutrients are promising anti-allergy agents capable of influencing the allergic immune response through multiple and different biological pathways.

Keywords: food allergy; PUFA; flavonoid; vitamin A; vitamin D; vitamin E; immune response; anti-inflammatory.

1. Introduction

Allergic reactions, particularly as a result of food allergy, can be life-threatening. The frequency of reported allergies and the severity of allergies in the Western world increases significantly, and is forecasted to affect e.g. 50% of the EU's population by 2025 [1]. Food allergy is one of the most common forms of allergy, with a worldwide increasing prevalence of 5-10% highly dependent on the country [2, 3]. The majority of these allergies are triggered by milk, eggs, nuts, wheat, soy and (shell)fish, of which reactions to milk, eggs and peanuts are the most prevalent in children, while peanuts and (shell)fish are the major triggers of allergic reactions in teenagers and adults [4].

The majority of food allergy is known as a type I allergy, indicating that it is mediated by a relative acute response in which immunoglobin E (IgE) is the pivotal antibody involved. However, also in

absence of allergen specific IgE acute allergic responses may occur upon ingestion of the culprit food. Food contains antigens, which, in case of allergy, are referred to as allergens. Food allergy can be divided into two phases: the sensitization and the effector response.

Allergic sensitization

Allergenic proteins in foods, are recognized by antigen-presenting cells (APCs), of which dendritic cells (DCs) are known to be involved as one of the major cell types aimed at the presentation of antigens to naive T-cells which may either result in tolerance (normal) or Th2 polarized immunity (allergy) and mixed Th2 and Th1 driven allergen specific immune responses in the chronic phase.. DCs reside especially in mucosal tissues of a.o. the nose, lungs, and, for food allergy most important, the oral cavity, intestine and even skin (where DC are referred to as Langerhans cells). After encountering an allergen, the major histocompatibility complex (MHC) class II positive DCs migrate to the draining lymph nodes, where they present the antigen to naïve CD4+ T-cells. Beyond the DC also other antigen presenting cells such as certain macrophages can be involved in the activation of T-cells. In order to activate these T-cells, two signals are needed: the binding of the T-cell receptor (TCR) specifically recognizing the allergenic epitopes presented in connection with the MHCII of the APC, and interaction of the costimulatory molecules CD28, CTLA-1/4 and LFA-1 on the T-cell with B-7 (CD80/CD86) and ICAM-1 on the dendritic cell [5]. Activated CD4+ T-cells, or T-helper (Th) cells, can roughly be divided into four important groups: Th1, Th2, Th17 and Treg. Differentiation into either of these subgroups is regulated by many different factors, of which OX40L expression by DC has been reported to be important in driving Th2 differentiation [6]. While Th1 cells are mainly involved in secreting and activating pro-inflammatory cytokines, Th2 cells play a key role in development of type 1 allergy by driving IgE secretion by plasma cells. Treg cells are able to downregulate the proliferation and activation of both Th1 and Th2 cells. Furthermore, Th1 cell derived mediators (IFN-γ) also downregulate Th2 proliferation. As Th2 cells are activated by IL-4 but also secrete IL-4, this acts as a positive feedback loop. By interacting with B-cells and via the release of IL-4, Th2 cells can activate IgE isotype switching and allergen specific antibody production, resulting in allergic sensitization [7, 8]. Beyond binding to allergic effector cells such as mast cells and basophils, IgE can also bind to DCs, further stimulating the immune response.

Allergic effector response

Upon a second encounter with the allergen, IgE that opsonizes e.g. mast cells and basophils binds the allergen, and FcεR1 receptor crosslinking results in mast cell activation and degranulation and the induction of the allergic effector response. Mast cells release many different components such as histamine, proteases, heparin, leukotrienes, prostaglandins, cytokines and chemokines, which are all involved in the generation of the allergic symptoms by causing redness, swelling and vasodilation. Sometimes this may even lead to an anaphylactic highly acute reaction within minutes.

The development of the gastro-intestinal and systemic immune system is in part driven by the intestinal microbiome. Defective oral tolerance induction may be a consequence of reduced biodiversity of the microbiota composition and inadequate immune maturation. Microbiome disturbances have been reported to be associated with different types of allergies. Changes in (children's) microbiome are claimed to be important in the increase in food allergy cases although it is not completely evident what is cause or consequence [9]. Such microbiome changes can be caused by, among others, an increased intake of fat and processed food, reduced intake of dietary fibres, and fruit and vegetables, overconsumption of junk food and the use of antibiotics during pregnancy and/or in early life. A, probably non-exhaustive, sum-up of factors contributing to increased allergy prevalence are thought to be Caesarean section, exposure to pollution (fine dust) and e.g. smoking behaviour [9]. Therefore, the need for prevention and resolving allergy is becoming of major concern. It has been recognized that nutrition plays an important role in the development, maintenance, and appropriate functioning of the immune system, and consequently in the prevention and management of for example food allergies. Food constituents, such as long-chain

polyunsaturated fatty acids (LCPUFAs), flavonoids and micronutrients, may be capable of influencing the allergic sensitization and/or effector response through multiple biological pathways. In this review we will focus on the potency of LCPUFAs, flavonoids and fat soluble vitamins (A, D, E) in the prevention of food allergy.

2. Long-chain Polyunsaturated Fatty Acids

Polyunsaturated Fatty Acids (PUFAs) are a group of acids that contain more than one double bond in their molecular structure. The most important PUFA groups are omega-3 (n-3) and omega-6 (n-6), which are divided by the placement of the first double bond, which is either between the third and the fourth or the sixth and the seventh carbon of the methyl end (Figure 1). In the n-3 group, α-linolenic acid (ALA, 18:3n-3) is converted, mostly in the liver, into stearidonic acid (SDA), after which the chains are elongated by enzymes into long-chain eicosapentanoic acid (EPA, 20:5n-3), which is converted into docosapentanoic acid (DPA, 22:5n-3) and then docosahexanoic acid (DHA, 22:6n-3). In the n-6 group, linoleic acid (LA, 18:2n-6) is converted into long-chain arachidonic acid (AA, 20:4n-6) (Figure 1). These essential n-3 (LC)PUFA fatty acids can be obtained from fatty fish, such as salmon, tuna, mackerel, herring and sardines and fish oil, and more sustainable sources such as vegetable oil, nuts and seeds, which are rich in n-3 PUFA ALA and algae oil is rich in n-3 LCPUFAs.

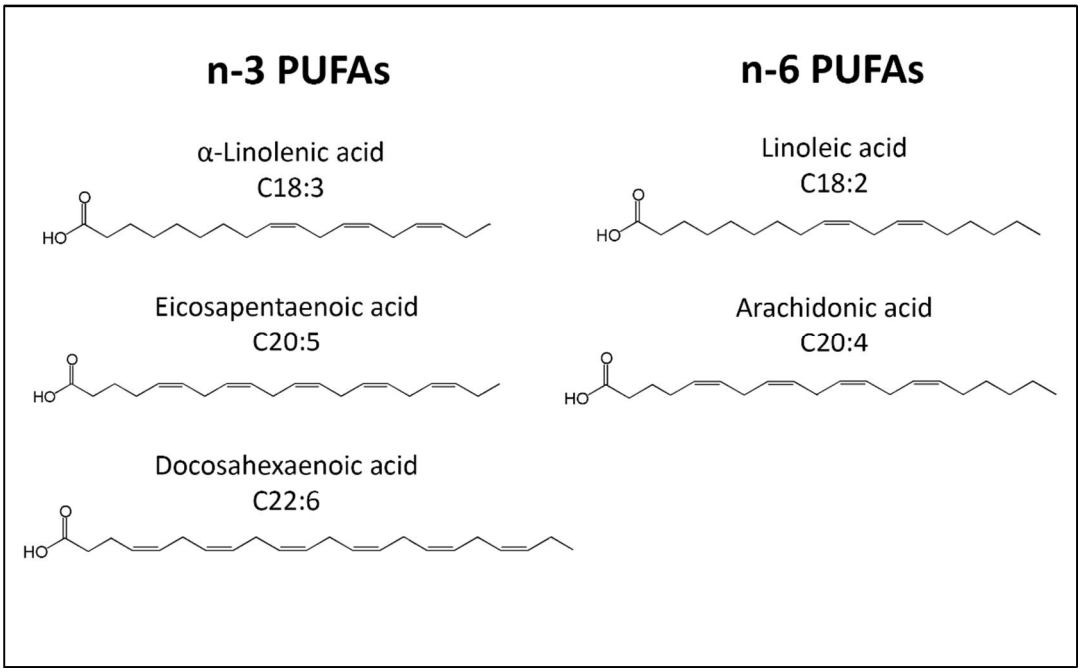


***Figure 1**: Schematic overview of the chemical structures of the n-3 and n-6 PUFAs discussed here.*

As the result of dietary changes over the last decades, the balance between n-3 and n-6 fatty acids was disturbed in favour of n-6. N-6 polyunsaturated fatty acid, present in vegetable oils is increasingly consumed, while the intake of n-3 LCPUFA is, in westernized countries, generally low. Since n-6 fatty acids are associated with pro-inflammatory and n-3 with anti-inflammatory activities, the mentioned imbalance is most likely contributing to the rise of non-communicable diseases, allergies included. Usually recommended consumption of two portions of fatty fish per week corresponds to 200 mg DHA per day [10]. Due to efficient digestion and absorption, approximately more than 95% of ingested fatty acids become biologically available [11].

3. Flavonoids

Flavonoids are one of the most abundant classes of polyphenols in food. These components have the common structure of diphenylpropanes (C6-C3-C6), which is built up of two aromatic rings linked via three carbons, usually forming an oxygenated heterocycle. Common flavonoids are

divided into subclasses such as flavonols, flavones, flavanones, flavan-3-ols (oligomeric and polymeric forms), proanthocyanidins, anthocyanins, and isoflavones (Figure 2).

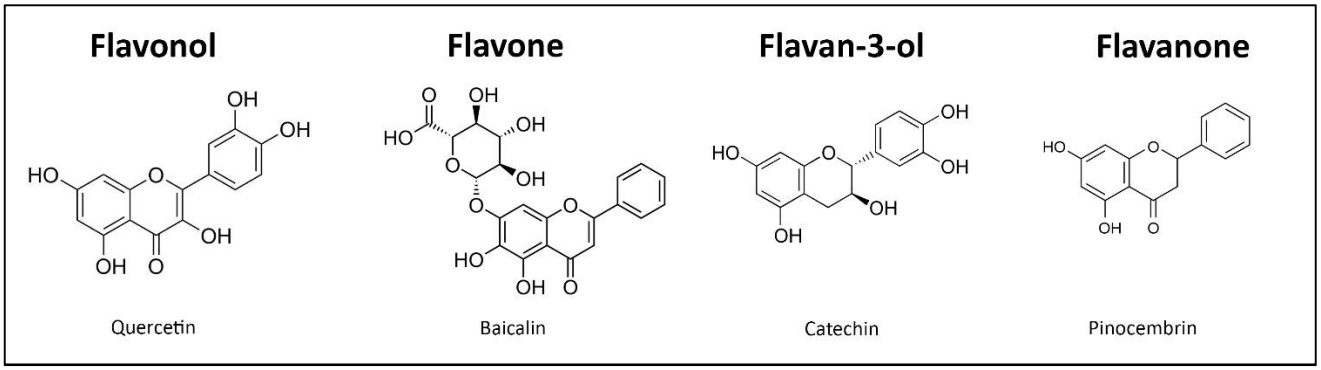


***Figure 2**: Schematic overview of some important flavonoids known in relation to food allergy per subclass, discussed in this paper.*

Out of more than 4000 flavonoids, 900 are present in the human diet. Dietary flavonoids are contained in commonly consumed plant-derived foods, for example fruits, vegetables, herbs, seeds, grains, and certain beverages such as coffee, tea, and wine. Besides these, other popular sources of flavonoids are dietary supplements including green tea (catechins), grape seed (proanthocyanidins), red apple peel and onion (quercetin), soybeans (isoflavones such as genistein), and many others [12]. In food, flavonoids are usually attached to sugars, acids or alcohols [13].

Due to the high variability of flavonoid components and limited clinical trials data, it is still not possible to define recommended and validated daily doses. Recommended consumption of five servings of fruits and vegetables per day corresponds to a flavonoid intake of approximately 150–300 mg/day [14]. Depending on dietary intake this value could vary due to insufficient consumption of fruit and vegetables as in some developed Western countries and India and China or to specific dietary habits such as high daily intake of coffee or tea [14-16].

The high variety in absorption and bioavailability of flavonoids, consequently influencing biological effects, depends primarily on their structural complexity. However, other factors such as amount of flavonoids consumed, food matrix, glycosylation pattern, gut microbiota, and their interactions with receptors and enzymes are considerably contributing to final health properties and possible protective effects of these components [17-19]. The absorption and metabolism of flavonoids in human body has not been elucidated so far. The ability of flavonoids to cross biological membranes is influenced by factors such as size, hydrophobicity, possible glycosylation, and intracellular reactions [20-22]. It has been shown that some components present in food matrix, such as dietary fibres, divalent metals, and viscous and protein-rich meals are likely to cause detrimental effects on flavonoid bioavailability, which could also be modulated by food composition and culinary techniques [17, 19]. Furthermore, it is most likely that changes in the gut microbiota composition contribute to the large inter-individual variations in bioavailability of flavonoids. The general low level of the specific ingested components in plasma is probably due to the formation of metabolites formed by the gut microbiome or the body's tissues [17, 22, 23]. However, flavonoids have been measured in human milk samples, indicating indeed uptake of these components from the diet and even transfer into breast milk [24, 25].

Recent evidence has pointed out the anti-inflammatory ability of flavonoids. Flavonoids may be able to influence multiple biological pathways and immune cell functions in the allergic effector response. Flavonoids are also capable of modulating the mechanisms involved in low-grade inflammation and may as well affect the process of allergic sensitization. Furthermore, the

prominent anti-oxidant ability of these components limits the activity of free radicals included in allergic responses [19, 23, 26, 27].

4. Fat-soluble vitamins

Vitamins and their metabolites have an important control function in the immune homeostasis by influencing both, innate and adaptive immune responses. In this section we will focus on fat-soluble vitamins A, D and E, as they play a pivotal role in immune modulation and are described as important anti-oxidants. The combinations of vitamins with PUFAs will be explored later in this review.

Vitamin A, also known as retinol (Figure 2), is a vitamin obtained from the diet of animal origin as preformed vitamin A (dominantly retinol and retinyl esters) or from plant-derived foods as pro-vitamin A (carotenoids). It has been estimated that preformed vitamin A is efficiently absorbed (70–90%). As the Average Requirement (AR) for vitamin A, 570 µg retinol equivalent (RE)/day for men and 490 µg RE/day for women are recommended by the European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies. Average vitamin A intake ranges between approximately 410-1080 µg RE/day in population < 18 years and between 820-1500 µg RE/day in adults when considering data obtained in nine EU countries [28].
Vitamin A is mostly known for being important in vision, with carrots as its main source. Besides carrots, vitamin A can also be obtained from fish, fruit, eggs, liver and margarine. Furthermore, it is important for normal functioning of the immune system. For example, vitamin A deficiency can cause malfunctioning of T-cells, neutrophils, NK-cells, monocytes/macrophages, Langerhans cells and B-cells, and acts on the production of several cytokines and growth factors, such as TNF-α, IFN-γ and IL-4 [29]. Furthermore, vitamin A metabolite retinoic acid (RA), which is converted by the enzyme retinalaldehyde dehydrogenase (RALDH), has been described to play an important role in priming tolerogenic dendritic cells that generate regulatory T-cells (Treg) and stimulating IL-10 production by these cells [30].

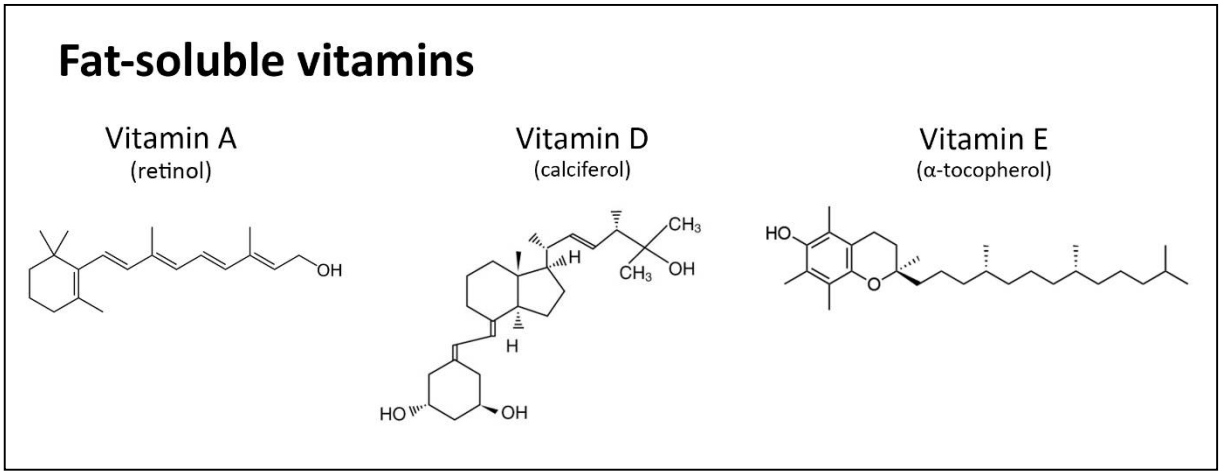


***Figure 3**: Chemical structures of the fat-soluble vitamins discussed here.*

The most important types of vitamin D are D_2 and D_3. For vitamin D_3, the greatest source is endogenous 7-dehydrocholesterol, generated in the liver from cholesterol, which can be converted by solar UVB radiation into vitamin D_3 (cholecalciferol) and further converted in the liver into 25-hydroxyvitamin D (calcidiol). This can either be stored in the liver or converted in the kidney into 1,25-dihydroxyvitamin D_3 (calcitriol), the active vitamin D_3 that can bind the vitamin D receptor (VDR) in cells. Besides endogenous generation of vitamin D_3, vitamin D_3 and D_2 can be obtained via the diet. Vitamin D_3 is present in fatty fish, dairy products and eggs. Vitamin D_2 cannot be produced in animals, but can for example be obtained by eating mushrooms or taking supplements. For

adults, an Adequate Intake (AI) for vitamin D is set at 15 µg/day, considering that at this intake, most of the population will achieve a serum 25(OH)D concentration near or above the target of 50 nmol/L [31]. An effective strategy to prevent vitamin D insufficiency and deficiency is ingestion of foods and supplements containing vitamin D, as well as sensible sun exposure.
Similar to vitamin A, vitamin D can regulate the immune system by enhancing the antimicrobial activity of monocytes and macrophages, reducing antigen presentation by DCs and inhibiting proliferation and differentiation of B-cells and Th1 and Th17 cells, while promoting Th2 cells, thus suggesting a more anti-inflammatory effect [32]. Furthermore, vitamin D_3 has been reported to induce Treg differentiation via instruction of tolerogenic DCs, much like vitamin A, and enhancing IL-10 production and downregulation maturation of DCs [33]. These similarities are not surprising, considering their nuclear receptor complexes (VDR/ retinoid X receptor (RXR) and retinoic acid receptor (RARα)/RXR), required for gene transcription, have been shown to interact [34]. For example, VDR can be repressed by RARα in leukaemia cells [35]. Hence, although it has been suggested that both vitamin A and D could be used to target autoimmune diseases and allergies, the interaction between these vitamins needs to be considered.

Vitamin E (α-tocopherol) is constituted by a trimethylated chromanol ring and a saturated phytyl side chain (Figure 2) and can be found in seeds and oils, such as sunflower oil, almonds and hazelnuts. For adults, an AI for α-tocopherol is set at 13 mg/day for men and 11 mg/day for women. Efficient α-tocopherol absorption requires the presence of fat, and it is estimated that the average α-tocopherol absorption from a usual diet is about 75% [36].
Opposite to vitamins A and D, vitamin E has not been directly linked to immune function, although it has been described to reduce the age-related decay of T-cells by inhibiting PGE_2 production [37]. Furthermore, the most important feature of vitamin E is its anti-oxidant capacity. Of note, what makes vitamin E in particular of great interest is that it is located, alongside with PUFAs, into the membrane of lipid bilayer cells [38], and it this way can scavenge free radicals from PUFA in case of oxidation and protect from membrane damage due to lipid peroxidation.

5. Immunomodulation in allergy

PUFAs

Clinical studies

PUFAs are endowed with immunomodulatory properties. A small number of placebo controlled clinical trials studying the intake of fish oil during pregnancy or lactation report no statistically significant effects or positive effects on allergy development in the offspring. However, supplementation of fish oil starting early during pregnancy and continuing during breastfeeding was shown to reduce allergic sensitisation for food proteins in the offspring [39, 40]. Lower Th2 cytokine levels associated with IL-13 were measured in the plasma of these children [41]. Formula supplemented with AA and DHA, was also shown to prevent allergy development in young children compared to non-supplemented formula milk [42]. From epidemiological studies it is known that allergy is associated with low n-3 LCPUFAs, especially EPA and DHA, and high n-6 LCPUFAs in plasma or serum [43, 44], indicating a protective effect of n-3 LCPUFAs and the importance of aiming for an optimal ratio of n-3 over n-6 LCPUFA for immune development in neonates.

In vivo studies

Fish oils rich in n-3 LCPUFAs DHA and EPA have been found to modulate the function of dendritic cells and to suppress activation of T-cells [45, 46], and even have potential to prevent cow's milk allergy in mice via the generation of Treg [47]. Furthermore, DHA-rich tuna oil was able to modulate the allergic response to whey and peanuts in sensitized mice [48]. In contrast, vegetable oil rich in n-6 PUFA LA has been reported to enhance the allergic reaction to cow's milk by enhancing Th2 cell polarization, increasing mast cell degranulation and the allergic effector response [49]. By contrast, partial replacement of dietary intake of n-6 PUFA LA rich soybean oil with n-3 LCPUFA rich fish oil was found to reduce allergic sensitization and mucosal mast cell

degranulation [48]. This supports the clinical reports where mainly n-3 LCPUFAs have been found to suppress allergic sensitization.

In vitro studies

PUFAs, when supplemented to (immune) cells, are incorporated into the cell membrane, thereby influencing cell properties [50]. For example, when incorporated into the CD4+ T-cell membrane, n-3 PUFAs can inhibit T-cell proliferation *in vitro* and *in vivo* [51-53]. In *in vitro* experiments, PUFAs are usually added in concentrations ranging from 2-100 μM [54-57] to study the effect on macrophages, mast cells, DCs, T-cells, or a combination of the latter two. Despite this variation, most papers report similar results. In macrophages, a downregulation of pro-inflammatory cytokines IL-1β, IL-6, IL-8 and TNF-α [58-60] and Reactive Oxygen Species (ROS) [55, 57] has been reported. The T-cell response instructed by DC is often observed to be downregulated [56], a decrease in secretion of pro-inflammatory cytokines TNF-α and IL-12 and upregulation of Treg cells has been reported [56, 61]. A reduction in histamine and leukotriene B4 levels have been shown when PUFAs (either n-3 alone or in varying ratios to n-6) were supplemented to mast cells [62, 63], while AA was able to activate intracellular ROS production and TNF-α release in mast cells [64]. AA is a component of the cyclooxygenase pathway in mast cells, and can be converted into prostaglandin H_2 (PGH_2). Both PGD_2 and PGE_2 are synthesized from PGH_2, which are important in allergic symptoms by increasing vascular permeability and in maintaining the allergy through the activation of Th2 cells. In contrast, a lack of PGD_2 has been reported to lead to mast cell hyperplasia, as shown in a PGD_2 knockout mice with food allergy [65]. Finally, EPA and/or DHA have been shown to modulate inflammation by binding to several receptors, such as nuclear receptor PPARα/γ and GPR120 [66], and voltage-gated ion channels [67].

Taken together, these studies highlight the ability of n-3 LCPUFAs to reduce the inflammatory response by targeting multiple immune cells. Especially the suppression of Th2 cells and increase in Treg cells, and the decrease in histamine and/or PGD_2 levels suggest that n-3 LCPUFAs may contribute to reduce the risk of allergy development and dampen allergic reactions. Targeting, both DCs/T-cells and mast cells, it can be speculated that n-3 LCPUFAs may have a protective effect in both the sensitization and effector response in food allergy, highlighting the potential of these PUFAs.

Flavonoids

As mentioned, flavonoids have been reported to possess anti-inflammatory properties as well. For example, pycnogenol has been used in a randomized double-blind controlled clinical treatment study, where a positive effect on allergic rhinitis symptoms was observed [68]. Pycnogenol is a French maritime pine bark extract containing a mixture of components (65-75% procyanidins), mostly used for medical purposes, but it can also be obtained from grape seeds and blue grape peels. The most important feature of this extract is its role as a strong anti-oxidant, which can control, for example, oxidative stress and NO production by macrophages [69]. With regards to food allergy, to our knowledge, no clinical trials have been performed using purified flavonoids. However, clinical trials using flavonoids are hard to be controlled, as flavonoids are present in nearly every form of nutrition.

In vivo studies

Flavonoids such as baicalin, quercetin and epicatechin have been tested in rat, mouse and guinea pig models via dietary intervention. All of these components were shown to have anti-allergic properties by preventing allergic sensitization. Effects include the reduction in IgE levels, lowering the amount of effector T-cells, increasing the amount of Treg cells and preventing DC maturation and, therefore, T-cell antigen presentation [70-74]. In contrast, one study also shows that quercetin, in a mouse model for egg allergy, increases Th2 levels and IL-1β and NO levels [75]. Mice received intraperitoneal (i.p.) injections on day 1 and 21 with either 25 or 50 mg/kg quercetin, and an i.p. injection with 20μg Ova. By contrast, the other study which showed the preventive effect of

quercetin used a peanut allergy rat model in which rats were sensitized using intragastric (i.g.) administration of 1 mg CPE and 10 μg cholera toxin, after which they received 50 mg/kg quercetin powder every day via the diet. Differences in effects could, therefore, be subjected to the route of sensitization, or by the way quercetin was administered to the animals. Also, it is commonly known that not all types of allergy, can be targeted in a similar fashion. Therefore, another explanation for the differences might be that quercetin may work beneficially in treating peanut allergies, but not egg allergies.

In vitro studies

Quercetin has been reported to inhibit leukotriene B4 levels in mast cells [76], reduce the gene expression of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-8) [77] and supress inflammation in IgE-mediated intestinal epithelial cell (Caco-2) and rat basophil (RBL-2H3) activation models [78], supporting its potential as an anti-allergy agent. Besides quercetin, many other flavonoids have been shown to also have these properties. Reduction of pro-inflammatory factors (such as TNF-α, IL-6, NO, iNOS, ROS, NF-κβ and PGE_2) has been reported after treatment with pinocembrin [79], nobiletin [80], kaempferol [76, 78], fisetin [77], myricetin [77, 81] and rutin (a combination of flavonoid quercetin and disaccharide rutinose) [77, 82]. Diverse immune cells express multiple types of polyphenol receptors that recognize and allow cellular uptake of polyphenols, consequently activating or suppressing signalling pathways [83]. Interaction of flavonoids with proteins can modulate the process of allergic sensitization to food allergens. Flavonoids are able to bind to the nuclear Ah (aryl hydrocarbon) receptor, thus stimulating protective enzyme activities. After interaction with Toll-Like Receptors (TLRs) on the surface of immune cells, flavonoids are internalized to the cytosol and transferred into the nucleus, where they are attached to the Ah-receptor. Furthermore, the Ah-receptor binds to the Ah-R nuclear translocator, inducing beneficial protective enzymes and cytokines via the Ah response element, leading to upregulation of the anti-inflammatory system and generation of Treg [84, 85].

Beyond their potential support in generation of Treg, flavonoids also have anti-inflammatory effects associated with their anti-oxidant activity and inhibition of enzymes involved in the production of eicosanoids. Therefore, flavonoids have been proposed to be useful in allergy prevention [86-88]. Excessive reactive oxygen or reactive nitrogen species (ROS; RNS) produced in the process of oxidative metabolism as well as other inflammatory stimuli can initiate the inflammatory process resulting in synthesis and secretion of pro-inflammatory cytokines. By interaction with ROS (superoxide O2, hydroxyl radical •OH and H2O2) and RNS, flavonoids can terminate the chain reaction before cell viability is seriously affected and, therefore, they are able to modulate inflammatory processes [89, 90]. Importantly, ROS has been shown to enhance the differentiation to Th2 cells by stimulating the production of IL-4 through the activation of STAT6 and GATA3 as shown in a mouse model [91]. Furthermore, ROS is an important activator of cellular regulation pathways such as NFκβ and MAPK, causing the transcription of pro-inflammatory genes such as TNF-α, IL1β, IL-6 and IL-8. In addition, flavonoids have also been shown to inhibit allergic effector cells such as mast cells known to contribute to allergic symptoms [84]. Certain groups of flavonoids, e.g. flavones and flavonols, are able to inhibit mast cell degranulation by inhibiting Syk at the autophosphorylation level [92, 93]. Syk is an essential signalling molecule in the FcεRI pathway, involved in cell degranulation. Inhibition of Syk has already been shown to reduce allergic asthma in mice [92].

Collectively, flavonoids have been shown to exhibit anti-allergic properties *in vitro* and/ or *in vivo*¸ acting both on the sensitization phase, including inhibition of DC maturation and supporting Treg cell development, and the effector phase, including the inhibition of mast cell degranulation.

Fat-soluble vitamins

Clinical studies

Supplementation of all vitamins has been incorporated into the diets of most households in the

Western Society, especially concerning children, pregnant woman and the elderly. In relation to allergy, children with a history in respiratory allergies have been reported to benefit from vitamin A supplementation [94]. Furthermore, food allergy in children, especially cow's milk allergy, has repeatedly been correlated to low vitamin D levels in plasma and serum [95-99], although one study in egg allergy did not demonstrate any correlation [100]. Vitamin D intake during pregnancy, however, did not correlate with a decrease of allergy in offspring [101], and even an increase has been reported [102]. Interestingly, it has been suggested that vitamin D deficiency is particularly correlated to food allergy in children with certain genotypes [103, 104]. Studies on the effect of vitamin E in food allergy are lacking.

In vivo studies

Vitamin A deficiency caused a break in oral tolerance through abnormally functioning DCs in the gut barrier, therefore inefficient antigen presentation and T-cell activation, which was rescued after vitamin A supplementation to the neonatal mice [105]. Mice with vitamin D deficiency show, in line with clinical data, a positive correlation with allergies [106]. Also in line with a clinical finding, egg allergy has been described to be enhanced by vitamin D deficiency [107], indicating a different mechanism driving this particular phenotype. This study reported a lower occurrence of Treg cells. By contrast, high supplementation of vitamin D in pregnant mice has also been reported to cause allergy due to a shift in the Th1/Th2 cell balance [108]. Other studies suggest that vitamin D is able to control IgE production through the vitamin D receptor in B-cells, therefore modulating allergic reactions [109, 110]. The many contradicting reports regarding vitamin D and its correlation to allergy show that the exact mechanisms behind food allergy and the role of vitamin D in the immune system is still to be elucidated.

In vitro studies

As mentioned before, retinoic acid (RA) can prime DCs to induce Treg cells. In the presence of RA, CD103 expressing DCs enhance the expression of RA converting enzyme RALDH2 which further facilitates the conversion of vitamin A derivate retinal to RA. In the mesenteric lymph nodes CD103 positive tolerogenic DC produce both RA and TGF-β which upregulate the expression of gut-homing integrin $\alpha 4\beta 7$ and CCR9 in T-cells and stimulates Treg cell differentiation [30]. In allergy prevention, generation of IL-10 producing Treg cells is beneficial, as it downregulates the proliferation of Th2 cells.

Similar, vitamin D has also been reported in multiple studies to prime DCs that stimulate Treg differentiation and upregulation of IL-10 [33, 111, 112]. Furthermore, it has been shown to downregulate IL-12, Th1 cell proliferation and DC maturation markers CD40, CD80 and CD86 [112]. However, the mechanism behind this has not been reported so far.

Vitamin E has a principal role in defence against oxidant-induced membrane injury. This lipid soluble component is concentrated in the hydrophobic interior of cell membrane, and donates electron to peroxyl radical, preventing further reactions and consequent cell damage. It has been shown that supplementation with vitamin E decreases lipid peroxidation and superoxide (O_2^-) production by impairing the assembly of nicotinamide adenine dinucleotide phosphate (reduced form) oxidase as well as by decreasing the expression of scavenger receptors (SR-A and CD36). Also, it was observed that high dose vitamin E treatment decreases the release of pro-inflammatory cytokines such as IL-8 [113].

Collectively, fat-soluble vitamins A, D and E may mostly affect the sensitization phase in allergy, by binding influencing the function of DCs, B-cells and T-cells (vitamin A and D) or acting as a strong anti-oxidant (vitamin E) suppressing pro-inflammatory insults induced by oxidative stress.

6. Combinations of components and their effects on allergy management

The possible potential of certain foods to prevent allergy or inflammation has been extensively described. Mostly, PUFAs, flavonoids and micronutrients are suggested to be more effective against allergy when applied together in food. In many cases, in an integral food product, combinations of

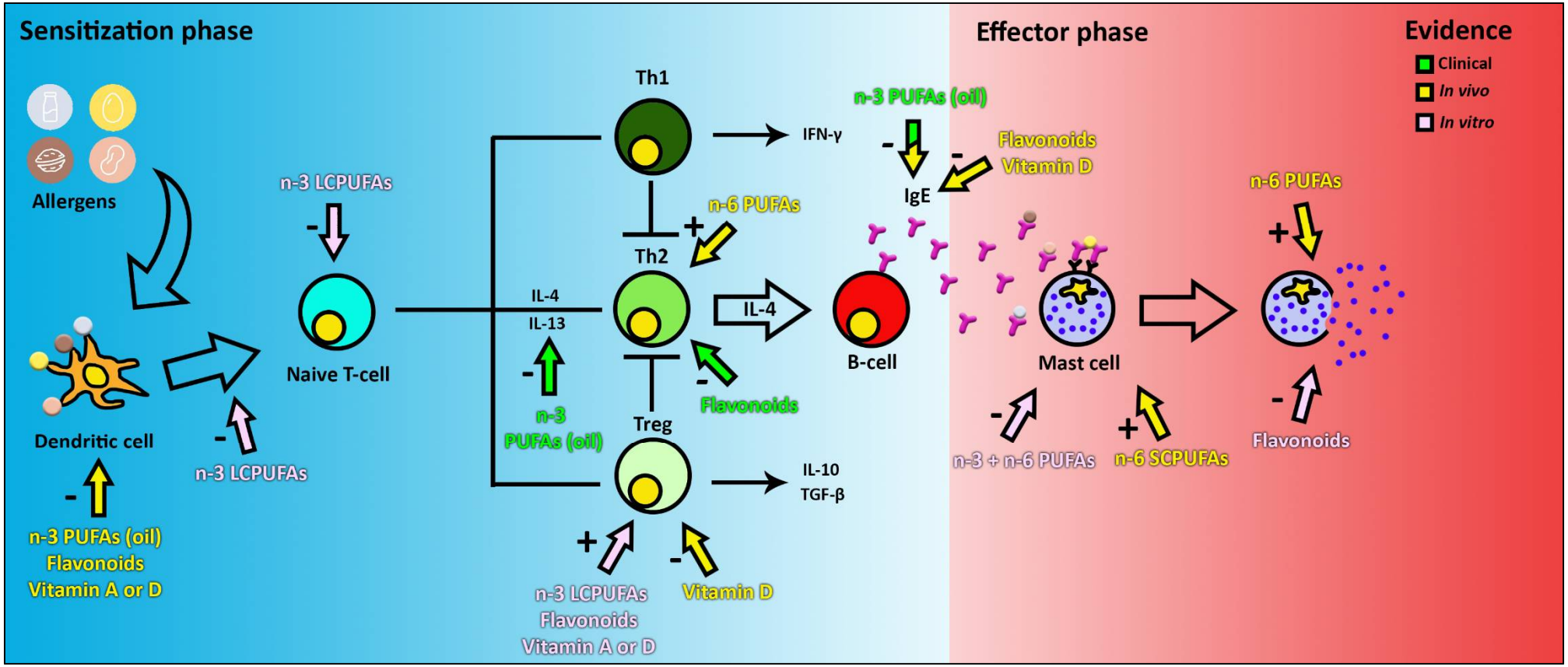
PUFAs, flavonoids and vitamins are present together. Additionally, minerals in food can be important in food allergy prevention as well. For example, food allergy in children has been associated with lower levels of zinc and selenium in their plasma [114, 115]. *In vitro*, zinc and selenium were reported to enhance Treg cell proliferation in allergen-stimulated cells [116] and decrease in PGD_2 levels in mast cells [117]. Mediterranean food is an example of food containing multiple bioactive dietary components and frequently proposed to be beneficial for human health, as they contain fish and olive oil (rich in oleic acids and also contains PUFAs LA and a small amount of ALA), fruits (rich in vitamins and flavonoids) and wine (rich in flavonoids) [118]. Several studies have shown a positive correlation between the Mediterranean diet during pregnancy and a reduction of asthma and rhinitis [119-121]. Another popular source of flavonoids and micronutrients is cocoa from the cacao tree. *In vivo*, it has been shown to have immunomodulatory effects, including a decrease in IgE, TNF-α and IL-10 [122, 123]. Also other common foods such as apple extract (rich in vitamins and flavonoids), rooibos tea and adlay bran (both rich in flavonoids) have been associated with a decrease in pro-inflammatory cytokines and the allergic response, mainly through the lowering of the production of pro-inflammatory cytokines, IL-4, IL-6 and Th2 cells. In addition, walnut polyphenolic components have been described to decrease TNF-α and peripheral blood mononuclear cell (PMBC) proliferation, although it induces a shift towards Th2 cells [124, 125]. It, however, does contain important anti-oxidant properties.

PUFAs alone are highly susceptible to lipid oxidation. Several studies report the protective anti-oxidative effect of different flavonoids on PUFAs [126-129]. Some vitamins have been described to exhibit a similar effect [130-132]. Vitamin E resides in the membrane lipid bilayer cells, like PUFAs [38], leading to membrane stabilization and protection against lipo-oxidation. The importance of this has been highlighted in a review by Raederstorff *et al* [133], stating that the intake of PUFAs is directly linked to the vitamin E requirement. Therefore, they recommend a dose of vitamin E between 12-20 mg/day based on the amount of PUFAs in an average Western diet. Additionally, the use of flavonoids to protect PUFAs from lipid oxidation has been proposed in previous research, where they even introduce PUFA-flavonoid hybrids or conjugates [126, 134].

Conclusion

A summary of the known effects of PUFAs, certain flavonoids and vitamins A, D and E on the allergic sensitization and effector response in food allergy can be found in Figure 4.n-3 LCPUFAs have promising anti-inflammatory and anti-allergic properties, they may act on both the sensitization as well as the effector phase of food allergy and clinical intervention studies have shown some promising allergy protective effects. However, due to their high susceptibility to lipid oxidation, supplementation of n-3 LCPUFA in combination with anti-oxidants, with a preference for vitamin E to scavenge lipid radicals in the cell membrane and thereby protecting against damage caused by lipid peroxidation, is indicated. Flavonoids such as picnogenol or quercetin are also suitable as anti-oxidants, contain anti-inflammatory properties and may be able to induce Treg cell differentiation. The latter is also known for vitamin A and D. Like n-3 LCPUFAs, flavonoids may also act on both the sensitization and effector phase, while for vitamin A and D appropriate levels are required for proper Treg induction to avoid allergic sensitization. Future studies are needed to reveal the food allergy protective properties of combined supplementation with n-3 LCPUFA, certain vitamins and flavonoids for prevention purposes and possibly also for symptom relieve.

441



442

443 ***Figure 4***: *The effect of PUFAs, flavonoids and vitamins on food allergy. The colour of the arrows and text indicate if the evidence is obtained from clinical,* in vivo *or* in vitro *data. The + or –*
444 *indicates if the observed effect is an inhibitory or stimulatory response of a certain cell type. Note that clinical and in vivo arrows indicate the observed end stage effects only, this may not be a*
445 *reflection of the direct effect of PUFAs, flavonoids and vitamins on the on the target cells. Therefore, the components could actually target a cell group earlier in the pathway.*

Funding: This transnational project is part of the ERA-Net SUSFOOD2 with funding provided by national/ regional sources and co-funding by the European Union´s Horizon 2020 research and innovation programme.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. EAACI, *The European Academy of Allergy and Clinical Immunology*. 2015: http://www.eaaci.org/documents/EAACI_Advocacy_Manifesto.pdf.
2. Savage, J. and C.B. Johns, *Food allergy: epidemiology and natural history*. Immunol Allergy Clin North Am, 2015. **35**(1): p. 45-59.
3. Tang, M.L. and R.J. Mullins, *Food allergy: is prevalence increasing?* Intern Med J, 2017. **47**(3): p. 256-261.
4. Panel, N.I.-S.E., et al., *Guidelines for the diagnosis and management of food allergy in the United States: report of the NIAID-sponsored expert panel*. J Allergy Clin Immunol, 2010. **126**(6 Suppl): p. S1-58.
5. Tai, Y., et al., *Molecular Mechanisms of T Cells Activation by Dendritic Cells in Autoimmune Diseases*. Front Pharmacol, 2018. **9**: p. 642.
6. Chu, D.K., et al., *T helper cell IL-4 drives intestinal Th2 priming to oral peanut antigen, under the control of OX40L and independent of innate-like lymphocytes*. Mucosal Immunol, 2014. **7**(6): p. 1395-404.
7. Johnston, L.K., K.B. Chien, and P.J. Bryce, *The immunology of food allergy*. J Immunol, 2014. **192**(6): p. 2529-34.
8. Hayen, S.M., et al., *Novel immunotherapy approaches to food allergy*. Curr Opin Allergy Clin Immunol, 2014. **14**(6): p. 549-56.
9. Aitoro, R., et al., *Gut Microbiota as a Target for Preventive and Therapeutic Intervention against Food Allergy*. Nutrients, 2017. **9**(7).
10. Koletzko, B., et al., *Current information and Asian perspectives on long-chain polyunsaturated fatty acids in pregnancy, lactation, and infancy: systematic review and practice recommendations from an early nutrition academy workshop*. Ann Nutr Metab, 2014. **65**(1): p. 49-80.
11. Calder, P.C., *Functional Roles of Fatty Acids and Their Effects on Human Health*. JPEN J Parenter Enteral Nutr, 2015. **39**(1 Suppl): p. 18S-32S.
12. Cvejić Hogervorst, J., et al., *Beneficial Effects of Polyphenols on Chronic Diseases and Ageing*, in *Polyphenols: Properties, Recovery, and Applications, 1st Edition*, C.M. Galanakis, Editor. 2018, Elsevier, Academic press: Duxford, CB, United Kingdom. p. 69-102.
13. Storniolo, C.E., et al., *Polyphenol fraction of extra virgin olive oil protects against endothelial dysfunction induced by high glucose and free fatty acids through modulation of nitric oxide and endothelin-1*. Redox Biol, 2014. **2**: p. 971-7.

14. Martin, K.R. and C.L. Appel, *Polyphenols as dietary supplements: A double-edged sword.* Nutrition and Dietary Supplements, 2010.
15. Grosso, G., et al., *Estimated dietary intake and major food sources of polyphenols in the Polish arm of the HAPIEE study.* Nutrition, 2014. **30**(11-12): p. 1398-403.
16. Hu, M., *Commentary: bioavailability of flavonoids and polyphenols: call to arms.* Mol Pharm, 2007. **4**(6): p. 803-6.
17. Bohn, T., *Dietary factors affecting polyphenol bioavailability.* Nutr Rev, 2014. **72**(7): p. 429-52.
18. Cvejić Hogervorst, J., et al., *Polyphenols*, in *Nutraceutical and Functional Food Components: Effects of Innovative Processing Techniques*, C.M. Galanakis, Editor. 2017, Elsevier, Academic press.
19. Tresserra-Rimbau, A., R.M. Lamuela-Raventos, and J.J. Moreno, *Polyphenols, food and pharma. Current knowledge and directions for future research.* Biochem Pharmacol, 2018. **156**: p. 186-195.
20. Gugler, R., M. Leschik, and H.J. Dengler, *Disposition of quercetin in man after single oral and intravenous doses.* Eur J Clin Pharmacol, 1975. **9**(2-3): p. 229-34.
21. Camenisch, G., et al., *Estimation of permeability by passive diffusion through Caco-2 cell monolayers using the drugs' lipophilicity and molecular weight.* Eur J Pharm Sci, 1998. **6**(4): p. 317-24.
22. Williamson, G., C.D. Kay, and A. Crozier, *The Bioavailability, Transport, and Bioactivity of Dietary Flavonoids: A Review from a Historical Perspective.* Comprehensive Reviews in Food Science and Food Safety, 2018. **17**(5): p. 1054-1112.
23. Scalbert, A., et al., *Dietary polyphenols and the prevention of diseases.* Crit Rev Food Sci Nutr, 2005. **45**(4): p. 287-306.
24. Song, B.J., Z.E. Jouni, and M.G. Ferruzzi, *Assessment of phytochemical content in human milk during different stages of lactation.* Nutrition, 2013. **29**(1): p. 195-202.
25. Khymenets, O., et al., *Dietary Epicatechin Is Available to Breastfed Infants through Human Breast Milk in the Form of Host and Microbial Metabolites.* J Agric Food Chem, 2016. **64**(26): p. 5354-60.
26. Harborne, J.B. and C.A. Williams, *Advances in flavonoid research since 1992.* Phytochemistry, 2000. **55**(6): p. 481-504.
27. Chirumbolo, S., *Dietary assumption of plant polyphenols and prevention of allergy.* Curr Pharm Des, 2014. **20**(6): p. 811-39.
28. (EFSA), E.F.S.A., *Scientific opinion on Dietary Reference Values for vitamin A.* EFSA Journal, 2015. **13**(3).
29. Semba, R.D., *The role of vitamin A and related retinoids in immune function.* Nutr Rev, 1998. **56**(1 Pt 2): p. S38-48.
30. Bakdash, G., et al., *Retinoic acid primes human dendritic cells to induce gut-homing, IL-10-producing regulatory T cells.* Mucosal Immunol, 2015. **8**(2): p. 265-78.
31. (EFSA), E.F.S.A., *Scientific Opinion on Dietary Reference Values for vitamin D.* EFSA Journal, 2016. **14**(10).
32. Prietl, B., et al., *Vitamin D and immune function.* Nutrients, 2013. **5**(7): p. 2502-21.
33. Bakdash, G., et al., *Vitamin D3 metabolite calcidiol primes human dendritic cells to promote the development of immunomodulatory IL-10-producing T cells.* Vaccine, 2014. **32**(47): p. 6294-302.
34. Schrader, M., et al., *Interaction between retinoic acid and vitamin D signaling pathways.* J Biol Chem, 1993. **268**(24): p. 17830-6.
35. Marchwicka, A., et al., *Regulation of vitamin D receptor expression by retinoic acid receptor alpha in acute myeloid leukemia cells.* J Steroid Biochem Mol Biol, 2016. **159**: p. 121-30.
36. (EFSA), E.F.S.A., *Scientific Opinion on Dietary Reference Values for vitamin E as α-tocopherol.* EFSA Journal, 2015. **13**(7).

37. Wu, D. and S.N. Meydani, *Age-associated changes in immune function: impact of vitamin E intervention and the underlying mechanisms.* Endocr Metab Immune Disord Drug Targets, 2014. **14**(4): p. 283-9.
38. Shaikh, S.R., et al., *N-3 Polyunsaturated Fatty Acids, Lipid Microclusters, and Vitamin E.* Curr Top Membr, 2015. **75**: p. 209-31.
39. Palmer, D.J., et al., *Randomized controlled trial of fish oil supplementation in pregnancy on childhood allergies.* Allergy, 2013. **68**(11): p. 1370-6.
40. Furuhjelm, C., et al., *Fish oil supplementation in pregnancy and lactation may decrease the risk of infant allergy.* Acta Paediatr, 2009. **98**(9): p. 1461-7.
41. Dunstan, J.A., et al., *Maternal fish oil supplementation in pregnancy reduces interleukin-13 levels in cord blood of infants at high risk of atopy.* Clin Exp Allergy, 2003. **33**(4): p. 442-8.
42. Foiles, A.M., et al., *Formula with long-chain polyunsaturated fatty acids reduces incidence of allergy in early childhood.* Pediatr Allergy Immunol, 2016. **27**(2): p. 156-61.
43. Aldamiz-Echevarria, L., et al., *Fatty acid deficiency profile in children with food allergy managed with elimination diets.* Acta Paediatr, 2008. **97**(11): p. 1572-6.
44. Yu, G. and B. Bjorksten, *Polyunsaturated fatty acids in school children in relation to allergy and serum IgE levels.* Pediatr Allergy Immunol, 1998. **9**(3): p. 133-8.
45. Brix, S., et al., *CD4(+) T-cell activation is differentially modulated by bacteria-primed dendritic cells, but is generally down-regulated by n-3 polyunsaturated fatty acids.* Immunology, 2010. **129**(3): p. 338-50.
46. Arrington, J.L., et al., *Dietary n-3 polyunsaturated fatty acids modulate purified murine T-cell subset activation.* Clin Exp Immunol, 2001. **125**(3): p. 499-507.
47. Weatherill, A.R., et al., *Saturated and polyunsaturated fatty acids reciprocally modulate dendritic cell functions mediated through TLR4.* J Immunol, 2005. **174**(9): p. 5390-7.
48. van den Elsen, L.W., et al., *DHA-rich tuna oil effectively suppresses allergic symptoms in mice allergic to whey or peanut.* J Nutr, 2014. **144**(12): p. 1970-6.
49. van den Elsen, L.W., et al., *Increased intake of vegetable oil rich in n-6 PUFA enhances allergic symptoms and prevents oral tolerance induction in whey-allergic mice.* Br J Nutr, 2015. **114**(4): p. 577-85.
50. Calder, P.C., et al., *Incorporation of fatty acids by concanavalin A-stimulated lymphocytes and the effect on fatty acid composition and membrane fluidity.* Biochem J, 1994. **300 (Pt 2)**: p. 509-18.
51. Hou, T.Y., et al., *n-3 polyunsaturated fatty acids suppress CD4(+) T cell proliferation by altering phosphatidylinositol-(4,5)-bisphosphate [PI(4,5)P2] organization.* Biochim Biophys Acta, 2016. **1858**(1): p. 85-96.
52. Anderson, M.J. and K.L. Fritsche, *Dietary polyunsaturated fatty acids modulate in vivo, antigen-driven CD4+ T-cell proliferation in mice.* J Nutr, 2004. **134**(8): p. 1978-83.
53. Zurier, R.B., et al., *Human peripheral blood T lymphocyte proliferation after activation of the T cell receptor: effects of unsaturated fatty acids.* Prostaglandins Leukot Essent Fatty Acids, 1999. **60**(5-6): p. 371-5.
54. Adolph, S., H. Fuhrmann, and J. Schumann, *Unsaturated fatty acids promote the phagocytosis of P. aeruginosa and R. equi by RAW264.7 macrophages.* Curr Microbiol, 2012. **65**(6): p. 649-55.
55. Ambrozova, G., M. Pekarova, and A. Lojek, *Effect of polyunsaturated fatty acids on the reactive oxygen and nitrogen species production by raw 264.7 macrophages.* Eur J Nutr, 2010. **49**(3): p. 133-9.
56. Carlsson, J.A., et al., *The Polyunsaturated Fatty Acids Arachidonic Acid and Docosahexaenoic Acid Induce Mouse Dendritic Cells Maturation but Reduce T-Cell Responses In Vitro.* PLoS One, 2015. **10**(11): p. e0143741.
57. Fuhrmann, H., et al., *Membrane fatty acids, oxidative burst and phagocytosis after enrichment of P388D1 monocyte/macrophages with essential 18-carbon fatty acids.* Ann Nutr Metab, 2007. **51**(2): p. 155-62.

58. Pauls, S.D., et al., *Anti-inflammatory effects of alpha-linolenic acid in M1-like macrophages are associated with enhanced production of oxylipins from alpha-linolenic and linoleic acid.* J Nutr Biochem, 2018. **57**: p. 121-129.

59. Robertson, R.C., et al., *The Anti-Inflammatory Effect of Algae-Derived Lipid Extracts on Lipopolysaccharide (LPS)-Stimulated Human THP-1 Macrophages.* Mar Drugs, 2015. **13**(8): p. 5402-24.

60. Mullen, A., C.E. Loscher, and H.M. Roche, *Anti-inflammatory effects of EPA and DHA are dependent upon time and dose-response elements associated with LPS stimulation in THP-1-derived macrophages.* J Nutr Biochem, 2010. **21**(5): p. 444-50.

61. Wang, H., et al., *Omega-3 polyunsaturated fatty acids affect lipopolysaccharide-induced maturation of dendritic cells through mitogen-activated protein kinases p38.* Nutrition, 2007. **23**(6): p. 474-82.

62. Kuwamori, M., et al., *Effect of dietary n-3/n-6 fatty acid ratio on the total count, fatty acid composition, and histamine and leukotriene concentrations of mast cells in tunica mucosa bronchiorum of type I allergic guinea pig.* Biosci Biotechnol Biochem, 1997. **61**(5): p. 763-7.

63. Ishihara, K., et al., *Inhibition of icosanoid production in MC/9 mouse mast cells by n-3 polyunsaturated fatty acids isolated from edible marine algae.* Biosci Biotechnol Biochem, 1998. **62**(7): p. 1412-5.

64. Nakano, N., et al., *Effects of arachidonic acid analogs on FcepsilonRI-mediated activation of mast cells.* Biochim Biophys Acta, 2005. **1738**(1-3): p. 19-28.

65. Nakamura, T., et al., *PGD2 deficiency exacerbates food antigen-induced mast cell hyperplasia.* Nat Commun, 2015. **6**: p. 7514.

66. Endo, J. and M. Arita, *Cardioprotective mechanism of omega-3 polyunsaturated fatty acids.* J Cardiol, 2016. **67**(1): p. 22-7.

67. Elinder, F. and S.I. Liin, *Actions and Mechanisms of Polyunsaturated Fatty Acids on Voltage-Gated Ion Channels.* Front Physiol, 2017. **8**: p. 43.

68. Wilson, D., et al., *A randomized, double-blind, placebo-controlled exploratory study to evaluate the potential of pycnogenol for improving allergic rhinitis symptoms.* Phytother Res, 2010. **24**(8): p. 1115-9.

69. Packer, L., G. Rimbach, and F. Virgili, *Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (Pinus maritima) bark, pycnogenol.* Free Radic Biol Med, 1999. **27**(5-6): p. 704-24.

70. Yan, X., et al., *Protective effect of baicalin on the small intestine in rats with food allergy.* Life Sci, 2017. **191**: p. 111-114.

71. Shishehbor, F., et al., *Quercetin effectively quells peanut-induced anaphylactic reactions in the peanut sensitized rats.* Iran J Allergy Asthma Immunol, 2010. **9**(1): p. 27-34.

72. Singh, A., et al., *Identification of epicatechin as one of the key bioactive constituents of polyphenol-enriched extracts that demonstrate an anti-allergic effect in a murine model of food allergy.* Br J Nutr, 2014. **112**(3): p. 358-68.

73. Okada, Y., et al., *Dietary resveratrol prevents the development of food allergy in mice.* PLoS One, 2012. **7**(9): p. e44338.

74. Bae, M.J., et al., *Baicalein induces CD4(+)Foxp3(+) T cells and enhances intestinal barrier function in a mouse model of food allergy.* Sci Rep, 2016. **6**: p. 32225.

75. Singh, D., et al., *Quercetin exhibits adjuvant activity by enhancing Th2 immune response in ovalbumin immunized mice.* Biomed Pharmacother, 2017. **90**: p. 354-360.

76. Takasugi, M., et al., *A new method to evaluate anti-allergic effect of food component by measuring leukotriene B4 from a mouse mast cell line.* Cytotechnology, 2018. **70**(1): p. 177-184.

77. Park, H.H., et al., *Flavonoids inhibit histamine release and expression of proinflammatory cytokines in mast cells.* Arch Pharm Res, 2008. **31**(10): p. 1303-11.

78. Lee, E.J., G.E. Ji, and M.K. Sung, *Quercetin and kaempferol suppress immunoglobulin E-mediated allergic inflammation in RBL-2H3 and Caco-2 cells.* Inflamm Res, 2010. **59**(10): p. 847-54.

79. Hanieh, H., et al., *Pinocembrin, a novel histidine decarboxylase inhibitor with anti-allergic potential in in vitro.* Eur J Pharmacol, 2017. **814**: p. 178-186.

80. Hagenlocher, Y., et al., *Citrus peel polymethoxyflavones nobiletin and tangeretin suppress LPS- and IgE-mediated activation of human intestinal mast cells.* Eur J Nutr, 2017. **56**(4): p. 1609-1620.

81. Medeiros, K.C., et al., *Anti-allergic effect of bee pollen phenolic extract and myricetin in ovalbumin-sensitized mice.* J Ethnopharmacol, 2008. **119**(1): p. 41-6.

82. Chen, S., et al., *Naturally occurring polyphenolic antioxidants modulate IgE-mediated mast cell activation.* Immunology, 2000. **100**(4): p. 471-80.

83. Ding, S., H. Jiang, and J. Fang, *Regulation of Immune Function by Polyphenols.* J Immunol Res, 2018. **2018**: p. 1264074.

84. Singh, A., S. Holvoet, and A. Mercenier, *Dietary polyphenols in the prevention and treatment of allergic diseases.* Clin Exp Allergy, 2011. **41**(10): p. 1346-59.

85. Hoensch, H.P. and B. Weigmann, *Regulation of the intestinal immune system by flavonoids and its utility in chronic inflammatory bowel disease.* World J Gastroenterol, 2018. **24**(8): p. 877-881.

86. Lee, O.H., et al., *Pycnogenol(R) inhibits lipid accumulation in 3T3-L1 adipocytes with the modulation of reactive oxygen species (ROS) production associated with antioxidant enzyme responses.* Phytother Res, 2012. **26**(3): p. 403-11.

87. Choi, I.S., et al., *Kaempferol inhibits P. intermedia lipopolysaccharide-induced production of nitric oxide through translational regulation in murine macrophages: critical role of heme oxygenase-1-mediated ROS reduction.* J Periodontol, 2013. **84**(4): p. 545-55.

88. Zhang, M., et al., *Antioxidant properties of quercetin.* Adv Exp Med Biol, 2011. **701**: p. 283-9.

89. Kumar, S. and A.K. Pandey, *Chemistry and biological activities of flavonoids: an overview.* ScientificWorldJournal, 2013. **2013**: p. 162750.

90. Hussain, T., et al., *Oxidative Stress and Inflammation: What Polyphenols Can Do for Us?* Oxid Med Cell Longev, 2016. **2016**: p. 7432797.

91. Belikov, A.V., B. Schraven, and L. Simeoni, *T cells and reactive oxygen species.* J Biomed Sci, 2015. **22**: p. 85.

92. Ramis, I., et al., *A novel inhaled Syk inhibitor blocks mast cell degranulation and early asthmatic response.* Pharmacol Res, 2015. **99**: p. 116-24.

93. Shichijo, M., et al., *Inhibition of syk activity and degranulation of human mast cells by flavonoids.* Biol Pharm Bull, 2003. **26**(12): p. 1685-90.

94. Pinnock, C.B., R.M. Douglas, and N.R. Badcock, *Vitamin A status in children who are prone to respiratory tract infections.* Aust Paediatr J, 1986. **22**(2): p. 95-9.

95. Guo, H., et al., *Correlation between serum vitamin D status and immunological changes in children affected by gastrointestinal food allergy.* Allergol Immunopathol (Madr), 2018. **46**(1): p. 39-44.

96. Rosendahl, J., et al., *A History of Cow's Milk Allergy Is Associated with Lower Vitamin D Status in Schoolchildren.* Horm Res Paediatr, 2017. **88**(3-4): p. 244-250.

97. Silva, C.M., et al., *Do infants with cow's milk protein allergy have inadequate levels of vitamin D?* J Pediatr (Rio J), 2017. **93**(6): p. 632-638.

98. Baek, J.H., et al., *The link between serum vitamin D level, sensitization to food allergens, and the severity of atopic dermatitis in infancy.* J Pediatr, 2014. **165**(4): p. 849-54 e1.

99. Allen, K.J., et al., *Vitamin D insufficiency is associated with challenge-proven food allergy in infants.* J Allergy Clin Immunol, 2013. **131**(4): p. 1109-16, 1116 e1-6.

100. Molloy, J., et al., *Vitamin D insufficiency in the first 6 months of infancy and challenge-proven IgE-mediated food allergy at 1 year of age: a case-cohort study.* Allergy, 2017. **72**(8): p. 1222-1231.
101. Yepes-Nunez, J.J., et al., *World Allergy Organization-McMaster University Guidelines for Allergic Disease Prevention (GLAD-P): Vitamin D.* World Allergy Organ J, 2016. **9**: p. 17.
102. Norizoe, C., et al., *Increased food allergy and vitamin D: randomized, double-blind, placebo-controlled trial.* Pediatr Int, 2014. **56**(1): p. 6-12.
103. Koplin, J.J., et al., *Polymorphisms affecting vitamin D-binding protein modify the relationship between serum vitamin D (25[OH]D3) and food allergy.* J Allergy Clin Immunol, 2016. **137**(2): p. 500-506 e4.
104. Liu, X., et al., *Longitudinal trajectory of vitamin D status from birth to early childhood in the development of food sensitization.* Pediatr Res, 2013. **74**(3): p. 321-6.
105. Turfkruyer, M., et al., *Oral tolerance is inefficient in neonatal mice due to a physiological vitamin A deficiency.* Mucosal Immunol, 2016. **9**(2): p. 479-91.
106. Matsui, T., et al., *Vitamin D deficiency exacerbates sensitization and allergic diarrhea in a murine food allergy model.* Allergol Int, 2018. **67**(2): p. 289-291.
107. Wu, J., et al., *Maternal and early-life vitamin D deficiency enhances allergic reaction in an ovalbumin-sensitized BALB/c mouse model.* Food Nutr Res, 2018. **62**.
108. Chen, W.J., et al., *Effect of vitamin D supplementation during pregnancy on the Th1/Th2 cell balance of rat offspring.* Pharmazie, 2014. **69**(5): p. 385-90.
109. James, J., V. Weaver, and M.T. Cantorna, *Control of Circulating IgE by the Vitamin D Receptor In Vivo Involves B Cell Intrinsic and Extrinsic Mechanisms.* J Immunol, 2017. **198**(3): p. 1164-1171.
110. Hartmann, B., et al., *Targeting the vitamin D receptor inhibits the B cell-dependent allergic immune response.* Allergy, 2011. **66**(4): p. 540-8.
111. van der Aar, A.M., et al., *Vitamin D3 targets epidermal and dermal dendritic cells for induction of distinct regulatory T cells.* J Allergy Clin Immunol, 2011. **127**(6): p. 1532-40 e7.
112. Adorini, L., *Tolerogenic dendritic cells induced by vitamin D receptor ligands enhance regulatory T cells inhibiting autoimmune diabetes.* Ann N Y Acad Sci, 2003. **987**: p. 258-61.
113. Singh, U., S. Devaraj, and I. Jialal, *Vitamin E, oxidative stress, and inflammation.* Annu Rev Nutr, 2005. **25**: p. 151-74.
114. Kamer, B., et al., *Role of selenium and zinc in the pathogenesis of food allergy in infants and young children.* Arch Med Sci, 2012. **8**(6): p. 1083-8.
115. Kalita, B., et al., *[Selenium plasma concentration level in children with food allergy].* Pol Merkur Lekarski, 2001. **10**(60): p. 411-3.
116. Rosenkranz, E., et al., *Zinc enhances the number of regulatory T cells in allergen-stimulated cells from atopic subjects.* Eur J Nutr, 2017. **56**(2): p. 557-567.
117. Safaralizadeh, R., et al., *Influence of selenium on mast cell mediator release.* Biol Trace Elem Res, 2013. **154**(2): p. 299-303.
118. Massaro, M., et al., *Nutraceuticals and prevention of atherosclerosis: focus on omega-3 polyunsaturated fatty acids and Mediterranean diet polyphenols.* Cardiovasc Ther, 2010. **28**(4): p. e13-9.
119. Chatzi, L. and M. Kogevinas, *Prenatal and childhood Mediterranean diet and the development of asthma and allergies in children.* Public Health Nutr, 2009. **12**(9A): p. 1629-34.
120. Chatzi, L., et al., *Protective effect of fruits, vegetables and the Mediterranean diet on asthma and allergies among children in Crete.* Thorax, 2007. **62**(8): p. 677-83.
121. de Batlle, J., et al., *Mediterranean diet is associated with reduced asthma and rhinitis in Mexican children.* Allergy, 2008. **63**(10): p. 1310-6.

122. Abril-Gil, M., et al., *A diet enriched with cocoa prevents IgE synthesis in a rat allergy model.* Pharmacol Res, 2012. **65**(6): p. 603-8.

123. Abril-Gil, M., et al., *Effect of a cocoa-enriched diet on immune response and anaphylaxis in a food allergy model in Brown Norway rats.* J Nutr Biochem, 2016. **27**: p. 317-26.

124. Anderson, K.C. and S.S. Teuber, *Ellagic acid and polyphenolics present in walnut kernels inhibit in vitro human peripheral blood mononuclear cell proliferation and alter cytokine production.* Ann N Y Acad Sci, 2010. **1190**: p. 86-96.

125. Comstock, S.S., L.J. Gershwin, and S.S. Teuber, *Effect of walnut (Juglans regia) polyphenolic compounds on ovalbumin-specific IgE induction in female BALB/c mice.* Ann N Y Acad Sci, 2010. **1190**: p. 58-69.

126. Sun, C.Q., et al., *Biotransformation of Flavonoid Conjugates with Fatty Acids and Evaluations of Their Functionalities.* Front Pharmacol, 2017. **8**: p. 759.

127. Yan, X., et al., *Nutritional value, chemical composition and antioxidant activity of three Tuber species from China.* AMB Express, 2017. **7**(1): p. 136.

128. Valente, T., et al., *A diet enriched in polyphenols and polyunsaturated fatty acids, LMN diet, induces neurogenesis in the subventricular zone and hippocampus of adult mouse brain.* J Alzheimers Dis, 2009. **18**(4): p. 849-65.

129. Kaur, R., et al., *Inhibition of lipid peroxidation by extracts/subfractions of Chickrassy (Chukrasia tabularis A. Juss.).* Naturwissenschaften, 2009. **96**(1): p. 129-33.

130. Keys, S.A. and W.F. Zimmerman, *Antioxidant activity of retinol, glutathione, and taurine in bovine photoreceptor cell membranes.* Exp Eye Res, 1999. **68**(6): p. 693-702.

131. Leskovec, J., et al., *Effects of supplementation with alpha-tocopherol, ascorbic acid, selenium, or their combination in linseed oil-enriched diets on the oxidative status in broilers.* Poult Sci, 2018. **97**(5): p. 1641-1650.

132. Meltzer, H.M., et al., *Supplementary selenium influences the response to fatty acid-induced oxidative stress in humans.* Biol Trace Elem Res, 1997. **60**(1-2): p. 51-68.

133. Raederstorff, D., et al., *Vitamin E function and requirements in relation to PUFA.* Br J Nutr, 2015. **114**(8): p. 1113-22.

134. Tsiailanis, A., et al., *Designing Natural Product Hybrids Bearing Triple Antiplatelet Profile and Evaluating Their Human Plasma Stability.* Methods Mol Biol, 2018. **1824**: p. 371-385.