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Dietary PUFA and flavonoids as deterrents for environmental pollutants

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Abstract

Various nutrients and plant-derived phytochemicals are associated with a reduced risk of many diet-related chronic diseases including cardiovascular disease, cancer, diabetes, arthritis and osteoporosis. A common theme that links many chronic diseases is uncontrolled inflammation. The long-chain (LC) omega-3 polyunsaturated fatty acids (PUFA) and flavonoids are known to possess anti-inflammatory actions in cell cultures, animal models and humans. Minimizing the condition of persistent inflammation has been a primary aim for drug development, but understanding how food components attenuate this process is at the nexus for improving the human condition. The prevalence of environmental toxins such as heavy metals and organics that contribute to diminished levels of antioxidants likely aggravates inflammatory states when intakes of omega-3 PUFA and flavonoids are marginal. Scientists at Purdue University have formed a collaboration to better understand the metabolism and physiology of flavonoids. This new effort is focused on determining how candidate flavonoids and their metabolites affect gene targets of inflammation in cell culture and animal models. The challenge of this research is to understand how LC omega-3 PUFA and flavonoids affect the biology of inflammation. The goal is to determine how nutrients and phytochemicals attenuate chronic inflammation associated with a number of diet-related diseases that occur throughout the life cycle. The experimental approach involves molecular, biochemical and physiological endpoints of aging, cancer, obesity and musculoskeletal diseases. Examples include investigations on the combined effects of PUFA and cyanidins on inflammatory markers in cultures of human cancer cells. The actions of catechins and PUFA on muscle loss and osteopenia are being studied in a rodent model of disuse atrophy to explain how muscle and bone communicate to prevent tissue loss associated with injury, disease and aging. The purpose of this review is to introduce the concept for studying food components that influence inflammation and how LC omega-3 PUFA and flavonoids could be used therapeutically against inflammation that is mediated by environmental pollutants.

Keywords: Omega-3 polyunsaturated fatty acids; Flavonoids; Heavy metals; Inflammation; Oxidative stress; Polychlorinated biphenyls

1. Introduction

Environmental pollutants have been linked to impairment of growth and development, causing damage to the central nervous system and contributing to chronic inflammatory diseases. Many of the environmental pollutants contaminate food supplies, and several examples demonstrate a direct relationship between the levels of these compounds in food and concentrations of the toxicants in animal and human tissues. In a study of the Inuits in the arctic region, exposure to environmental toxicants [organochlorines including polychlorinated biphenyls (PCBs), oxychlordane and dichlorodiphenyldichloroethylene/dichlorodiphenyltrichloroethane and heavy metals such as mercury and lead] was associated with higher incidence of developmental problems in infants. The exposure of pollutants from food and the environment was directly related to the daily consumption of traditional protein-rich foods that originated from over 250 species of wildlife consisting of fish, birds and sea mammals [1]. These wild animal species are known to be the primary environmental pathway for transferring environmental toxicants to the Inuits.

Fish has the highest potential for organic mercury bioaccumulation, and the source and stage of development of the fish consumed are important factors in human mercury acquisition. Aggravating the risk factor exposure is the widespread use of fishmeal in the rations of food-producing

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animals. Mercury can be transferred from the hen to eggs and from the cow to milk and accumulate in the meat of livestock, and farm-reared fish when food animals are fed contaminated fishmeal. Absorbed Hg is either bound to metallothionein or cysteine residues of proteins, whereas lead is preferentially taken up by bones. Lead acquisition and bioaccumulation are influenced by exposure, the metabolic activity in bone and osteoactive factors [2].

More recently, dietary factors have been shown to attenuate the toxicity of the environmental pollutants and, thus, potentially avert the adverse health outcomes in humans. Research suggests that specific food components can either protect against or lessen the toxic effects caused by environmental contaminants. Dietary protein, fiber and trace elements (calcium, iron and zinc) can all influence the extent of cadmium bioavailability and toxicity. Replacing casein with fish protein decreased the toxicity of methylmercury in mice, and selenium has been shown to be protective against mercury-induced toxicity in mice and rats [1,3]. We speculate that foods rich in phytochemicals and omega-3 polyunsaturated fatty acids (PUFA) can decrease the toxicity of and exposure to environmental pollutants and, thus, protect health and reduce disease outcomes associated with toxic chemical insult. A recent publication of Hennig et al. [4] indicates that exposure to persistent organic pollutants, for example, PCB, contributes to the early development of inflammatory diseases such as atherosclerosis. Moreover, chronic inflammation and oxidative stress result in abnormal functioning of vascular endothelium to exacerbate and hasten conditions of atherosclerosis [4,5]. Dietary sources of flavonoids and omega-3 PUFA appear to counter the pollutant-induced proinflammatory conditions by down-regulating prostanoid biosynthesis. Fruits and vegetables containing flavonoids and food sources of omega-3 PUFA are potential safeguards in a healthy diet that potentially reduces the toxic effects of environmental pollutants. The prevention of some of the toxic consequences of these compounds by controlling cytokine-activated chronic inflammation, which leads to long-term disease outcome, can be a benefit of consuming a diet rich in flavonoids and omega-3 PUFA.

2. Omega-3 PUFA, flavonoids and diet-related chronic diseases

Various nutrients and plant-derived phytochemicals are associated with a reduced risk of many diet-related chronic diseases including cardiovascular disease (CVD), cancer, diabetes, arthritis and osteoporosis [6–8]. A diet containing fruits and vegetables as well as fats/oils with appreciable amounts of omega-3 PUFA purportedly improves antioxidant status and restrains chronic inflammation.

Kark et al. [9] found an association of adipose tissue omega-6 PUFA, which are derived primarily from the diet, with acute myocardial infarction (AMI). They observed that very high intakes of linoleic acid did not appear to be associated with an increased risk of nonfatal AMI; however, arachidonic acid (the prostanoid precursor of prostaglandin E_2) increased the risk of AMI. These findings show that the proinflammatory prostanoid precursor is a dietary factor that increases risk for coronary health. The balance between arachidonic acid and long-chain (LC) omega-3 PUFA [eicosapentaenoic acid (EPA, 20:5n3) and docosahexaenoic acid (DHA, 22:6n3)] in cells and tissues is known to directly influence prostanoid biosynthesis, which mediates the inflammatory process. This relationship of PUFA families on chronic disease is discussed below.

2.1. Omega-3 PUFA

Omega-3 PUFA can reduce the progression of atherosclerosis because of their anti-inflammatory properties and effects on atherogenesis in the arterial wall [10]. Burdge and Calder [11] reviewed the metabolism of α-linolenic acid (LNA, 18:3n-3), the efficacy of LNA consumption in elevating the concentrations of EPA and DHA in blood and cellular lipid pools and the protective effect of such dietary interventions against CVD and inflammation and concluded that increased consumption of LNA may be of little benefit in altering EPA+DHA status or in improving health outcomes. This is because of the limited capacity for conversion of LNA to LC omega-3 PUFA and the lack of efficacy in ameliorating CVD risk factors and inflammatory markers in man. Therefore, with regard to averting the toxic effects of environmental pollutants, the focus should be on increasing tissue levels of EPA and DHA.

It is well recognized that the Western diet is deficient in omega-3 fatty acids but has excessive amounts of omega-6 fatty acids compared with the diet on which human beings evolved [12]. Recently, in the United States, the dietary reference intakes (DRIs) provide the first requirement for omega-3 PUFA [13]. The daily adequate intakes for α-linolenic acid (18:3n3) are 1.6 and 1.1 g/day for men and women, respectively. The acceptable macronutrient distribution (AMDR) range is 0.6–1.2% of the daily energy intake (2000–2500 cal/day), and EPA and DHA can supply 10% of the AMDR for n-3 PUFA. The optimal ratio of linoleic acid (omega-6) to LNA is proposed to range from 5 to 10 (the LA DRI is 10 times that of 18:3n3).

In the United States, diet-related chronic illnesses and health problems present a serious threat to public health. The contemporary human genome is conditioned and genetically adapted to the environment where ancestors survived both the fatty acid and macronutrient compositions of the diet. In the current U.S. diet, the ratio of omega-6 to omega-3 PUFA has risen to 10:1 compared to between 2:1 and 3:1 in hunter–gatherer diets predominant in wild animal foods [12]. Increasingly, clinical trials and interventions adopting dietary treatments with nutrition characteristics similar to those found in preindustrial/preagricultural diets have confirmed the beneficial health consequences, confirming that the current Western diet may predispose modern populations to chronic disease [12].

Diets with excessive amounts of omega-6 PUFA and a very high ratio of omega-6/omega-3 PUFA aggravate the pathogenesis of many diseases including CVD, cancer and inflammatory and autoimmune diseases, while diets with large amounts of omega-3 PUFA are suppressive [14]. Clinical studies indicate that LC omega-3 PUFA supplementation showed cardioprotective effects in people with type 2 diabetes without any side effects on glucose control and insulin activity [15,16].

Other potential health benefits of omega-3 PUFA include a greater potential in preventing the establishment of allergic responses early in life. Supplementation of omega-3 PUFA in pregnancy may deter the development of allergic diseases and, possibly, other immunomediated diseases [17]. In patients with multiple sclerosis (MS), PUFA levels and antioxidant status/cellular antioxidant defense are deficient. Both dietary antioxidants and PUFA have the potential to diminish disease symptoms by targeting specific pathomechanisms and supporting recovery in MS patients [18].

Baggio et al. [19] summarized the effect of omega-3 PUFA on the course of experimental and human nephropathies and found that omega-3 PUFA can ameliorate chronic, progressive renal injury by favorably interfering with some stages in renal fibrosis processes, such as mesangial cell activation and proliferation and extracellular matrix protein synthesis. Omega-3 PUFA appear to control some proinflammatory cytokine production, renin and nitric oxide (NO) systems and peroxisome proliferator-activated receptor (PPAR) gene expression.

2.2. Flavonoids and food

Chocolate and other cocoa products are rich sources of plant-derived antioxidant flavonoids that possess favorable physiological effects such as antioxidant activity, vasodilation and blood pressure reduction, inhibition of platelet activity and decreased inflammation, which can be beneficial to cardiovascular health [20]. Multiple lines of evidence from laboratory experiments and randomized trials suggest that flavonoids, when consumed together with chocolate and other cocoa products, are likely protective against coronary heart disease mortality [21]. Substantial evidence support the role that oils containing omega-3 PUFA and flavonoid-rich foods play in reducing cardiovascular mortality. A mechanism of action may be related to the ability of omega-3 PUFA to stabilize vulnerable atherosclerotic plaques and flavonoids to improve endothelial function [22].

Quercetin is a widely found dietary flavonoid that has high concentrations in onions, tea and berries. Castilla et al. [23] reported that supplement-derived polyphenols are quickly absorbed as evidenced by the rapid uptake of quercetin, which reached the maximum plasma concentration within 3 h after red grape juice ingestion in human subjects. In a case–control study that included hemodialysis patients and healthy subjects, the intake of concentrated red grape juice was found to improve the lipoprotein profile and to reduce plasma concentrations of inflammatory biomarkers and oxidized LDL, conferring a beneficial effect in reducing CVD risk factors.

In an in vitro study that evaluated the antioxidant potency of phytoestrogens from food, blochanin A, quercetin, daidzein and genistein, quercetin showed the most potent antioxidant activity, while the others showed a relatively weaker antioxidant activity in an in vitro testing system on LDL [24]. Lekakis et al. [25] examined the effect of acute intake of a red grape polyphenol extract [containing (per gram) 4.32 mg epicatechin, 2.72 mg catechin, 2.07 mg gallic acid, 0.9 mg *trans*-resveratrol, 0.47 mg rutin, 0.42 mg epsilon-viniferin, 0.28 mg *p*-coumaric acid, 0.14 mg ferulic acid and 0.04 mg quercetin] in 30 male patients with coronary heart disease. They found that the grape extract improved endothelial function as indicated by the increases in flow-mediated dilatation of the brachial artery in the patients.

In a rat model with abdominal aortic constriction, feeding a quercetin-supplemented chow (1.5 g/kg chow) reduced hepatic lipid oxidation, attenuated carotid arterial blood pressure and cardiac hypertrophy and reduced aortic thickening, all of which support an antihypertensive and antihypertrophic effect of quercetin in vivo [26].

In a cross-sectional analysis performed in 1286 women and 1005 men in France, flavonoid-rich food consumption was inversely related to systolic blood pressure in women, while no relationship was observed in men. These results indicate that a high consumption of flavonoid-rich foods may prevent CVD in women [27].

Borek [28] summarized the benefits of aged garlic extract (AGE) and concluded that with its rich antioxidant content, AGE could help prevent, alleviate or lower the risk for many chronic diseases linked to oxidative damage, such as cardiovascular and cerebrovascular diseases and dementia. The compounds present in garlic possess the capacity to scavenge oxidants and to increase superoxide dismutase (SOD), catalase, glutathione peroxidase and glutathione levels. These effects appear to inhibit lipid peroxidation and inflammatory prostaglandins.

In a recent review by Rahman and Lowe [29], several studies indicate a health-promoting role of dietary garlic. Epidemiological studies show an inverse correlation between garlic consumption and progression of CVD. A robust finding from in vitro studies confirm the ability of garlic to reduce the parameters associated with CVD, such as elevated level of serum total cholesterol, LDL and platelet aggregation, increased LDL oxidation and hypertension. Garlic has also been shown to inhibit enzymes involved in lipid synthesis, decrease platelet aggregation, prevent lipid peroxidation in erythrocytes and LDL, increase antioxidant status and inhibit angiotensin-converting enzyme. The antioxidant benefits of garlic on lipoproteins appear to occur with short-term supplementation in humans. For example, Lau [30] reported an increased resistance of LDL to oxidation, which is the culprit of endothelial cell dysfunction that eventually causes atherosclerosis, in

subjects given 1.2 g AGE three times a day for 2 weeks in a double-blind, placebo-controlled crossover study.

3. Chronic diseases and uncontrolled inflammation

Inflammation is usually an acute, short-term event that helps the host eliminate invading microorganisms as well as the damaged tissue resulting from infection. This complex process is precisely regulated and self-limiting to prevent extensive damage to the host. When the self-limiting nature of this protective mechanism is inappropriately regulated, it is transformed to a detrimental, chronic state of inflammation. This disease state is characterized by increased release of an array of proinflammatory mediators including cytokines, chemokines, prostaglandins and reactive oxygen species, accompanied by the infiltration of immune cells, particularly mononuclear cells such as macrophages and lymphocytes, to the damaged site. Chronic inflammation is the cause or assisting factor in many chronic disorders including certain types of cancers (liver carcinoma and gastric cancer), CVD (including atherosclerosis), autoimmune diseases (rheumatoid arthritis and systemic lupus erythematosus), Crohn's disease, ankylosing spondylitis, psoriasis and type 2 diabetes [31–34].

Cytokines are major mediators of local, intercellular communications required for an integrated response to a variety of stimuli in immune and inflammatory processes. Numerous cytokines have been identified in diseased tissues across a range of immunomediated inflammatory diseases. Among the cytokines that are present in chronic inflammation sites and in the circulation, tumor necrosis factor α (TNFα) is proposed as a principle and, thus, a primary target for alleviating immunomediated inflammatory diseases. TNFα was identified as a key pathogenic molecule in rheumatoid arthritis as well as in Crohn's disease, ankylosing spondylitis and psoriasis. Recent clinical research using biological therapies for targeting TNFα demonstrates the important common role of TNFα in molecular pathways across a range of chronic immunomediated disease phenotypes [34]. Other major cytokines identified at inflammatory cites include IL-6, IL-1β, interferon-γ and transforming growth factor β [35].

Clinical and epidemiological studies have long been suggesting an association between infectious agents and chronic inflammatory disorders and cancer. Caused by microorganism invasion or the attack of endogenous ligands, the loss of epithelial integrity results in activation of resident inflammatory cells coupled with a failure of normal control mechanisms that limit leukocyte activation, leading to a cascade that induces chronic inflammation and its consequences [33].

Oxidized phospholipids (OxPLs) could be triggers of inflammation in atherosclerosis. Two mechanisms exist to explain OxPL's pathogenic effect in inducing endothelial activation: the c-src pathway and sterol-regulatory element binding protein, as well as induction of cyclooxygenase-2 (COX-2) expression via mechanisms involving PPARγ [32].

Chemokines and their receptors are important factors in the pathogenesis of joint inflammatory disorders. The initial clinical data on chemokine blockade in patients with rheumatoid arthritis suggest that targeting the chemokine and chemokine receptor family could provide a new and promising antirheumatic strategy [36].

The multitude of environmental pollutants contribute to physiological conditions and induce biochemical pathways associated with the metabolism of toxicants that are supporting factors leading to inflammation in tissues and organs. The condition of chronic inflammation resulting from exposure to chemical environmental toxicants might be averted in humans to some degree by a daily diet of flavonoids and omega-3 PUFA.

4. Environmental pollutants/toxicants: health effects and interactions with dietary factors

The prevalence of environmental toxicants such as heavy metals and organics that contribute to diminished levels of antioxidants likely aggravates inflammatory states when intakes of omega-3 PUFA and flavonoids are marginal. Hence, foods rich in omega-3 PUFA and flavonoids can be an important dietary source of anti-inflammatory compounds to reduce the toxic effects of environmental pollutants.

4.1. Polychlorinated biphenyls

4.1.1. Fatty acids attenuate PCBs' toxicity

The LC PUFA (DHA and arachidonic acid) are recognized as essential for proper fetal and infant development, and they appear to be effective in counteracting the toxic effect of PCBs on the eye and the visual process during the early stages [37]. Kakela et al. [38] reported that treatment of a PCB mixture (Aroclor-1242) in mink fed a fish diet high in saturated fat and low in PUFA increased the percentage of hepatic microsomal oleic acid at the expense of omega-3 PUFA with a concomitant drop in liver vitamin E concentration. The change in liver fatty acid composition due to PCB treatment in mink fed a fish diet with a low fat content but with high PUFA and vitamin E content remained small.

Hennig et al. [39] tested the effect of dietary fat (olive oil vs. corn oil) on PCB-induced gene expression changes involved in fatty acid metabolism in LDL receptor-deficient mice and found that genes involved in fatty acid synthesis were reduced in the corn oil group and that lipid transport genes were altered in the olive oil group. These results indicate that the dietary fat source could modulate PCB-induced changes in lipid metabolism in animal tissues.

4.1.2. PCBs affect fatty acid composition of tissues

Treatment with the environmental pollutant PCB (Aroclor-1254) in rats decreased the tissue fatty acid

saturation index (the ratio between saturated and unsaturated fatty acids) in total microsomes and phospholipids of the liver, and the decrease in saturation index of membrane lipids could trigger the induction of free radicals and promote carcinogenesis [40]. PCB (3,3′ ,4,4′ ,5-pentachlorobiphenyl) treatment at 25 mg/kg (one dose) in rats dramatically decreased arachidonic acid concentration by 50%, while oleic and linoleic acids increased significantly in the liver [41]. In mink adipose tissue, Kakela and Hyvarinen [42] reported that the fatty acid composition and the mesenteric lymph nodes responded differently. The phospholipids were more sensitive to PCBs than the triacylglycerols.

Grandjean and Weihe [43] examined the arachidonic acid status and seafood intake in a fishing community that had a high seafood intake and a wide range of PCB exposure. They found that increased PCB exposure was associated with a modest decrease in serum arachidonate content, which plays a key role during the early development of the infant in utero and after birth. In the rat, Bae et al. [44] reported that PCB (Aroclor 1242) treatment stimulated the contraction of longitudinal uterine strips isolated from Gestation Day 10 by activating phospholipase A_2 (PLA_2), mediating arachidonic acid release. This study indicated that PCB could decrease gestational age and increase the risk for spontaneous abortion in women and animals. Similar to PCBs and other organohalogens, Kodavanti and Derr-Yellin [45] reported that polybrominated diphenyl ethers stimulated the release of arachidonic acid by activating PLA_2 in rat cerebellar granule neurons.

Treatment of rat renal tubular cell cultures with PCB (Aroclor 1248) increases the release of oleic acid in a concentration-dependent manner. Studies with two PCB congeners [trichlorobenzene (TCB) and hexachlorobenzene (HCB)] showed that the diorthosubstituted PCB (nonplanar configuration) congener and HCB stimulate the release of oleic acid. While the non-orthosubstituted coplanar congener TCB did not alter the release of oleic acid. None of the PCBs showed a significant increase on the release of palmitic acid from intracellular stores [46].

4.1.3. Certain PUFA intensifies PCB toxicity

Hennig et al. [5] showed that dietary fat linoleic acid increased endothelial dysfunction induced by selected PCBs, presumably by contributing to oxidative stress and as the result of the production of leukotoxins. The imbalance in the cellular oxidant/antioxidant status could trigger oxidative-sensitive transcription factors and turn on or enhance the gene expression for inflammatory cytokines, resulting in intensified inflammatory responses and endothelial cell dysfunction.

Linoleic acid and PCB 77 can cause disruption of endothelial barrier function independently. Linoleic acid metabolites such as leukotoxins and their diol derivatives play a critical role in linoleic-acid-induced endothelial cell dysfunction. Furthermore, the presence of linoleic acid metabolites can contribute to the amplified cytotoxicity of PCB 77 in endothelial cell dysfunction [47].

Diets high in unsaturated fats, especially linoleic-acid-rich triacylglycerols, can alter the cellular lipid environment in a way that contributes to and possibly increases the PCB-mediated dysfunction of vascular endothelial cells and, thus, aggravate the atherogenicity potential of PCBs. The mechanism for the lipid interaction, particularly that between linoleic acid and PCBs, in endothelial cell dysfunction and, possibly, in atherosclerosis is not clear [48].

4.1.4. PCB, lipids and oxidative stress

Tithof et al. [49] showed that Aroclor 1242 stimulated the production of superoxide anion (O^{2-}) via a mechanism involving PLA_2-dependent release of arachidonic acid. Therefore, this PCB activates the first step in prostanoid synthesis that would lead to the production of the proinflammatory prostanoid PGE_2.

Hennig et al. [4] summarized their work on PCBs and concluded that the critical events mediating the PCB-induced dysfunction of inflammatory genes and endothelial cells reside in an increase in cellular oxidative stress and in an imbalance in antioxidant status. The authors further hypothesized that antioxidant nutrients and related bioactive compounds found in fruits and vegetables could down-regulate the signaling pathways involved in inflammatory responses and atherosclerosis as protection from environmental toxic attack to the vascular endothelium.

Senthil Kumar et al. [50] reported that simultaneous administration of vitamins C and E eliminated Aroclor 1254-induced oxidative stress in Sertoli cells of adult male rats. Tharappel et al. [51] reported that feeding rats PCB 77 (3,3′,4,4′-tetrachlorobiphenyl) and PCB 153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) individually or in combination significantly increased the binding activity of NFκB and AP-1 in hepatic nuclear extracts. These nuclear factors are oxidative-stress-responsive transcription factors. In this study, cell proliferation was increased by PCB 77 but not by PCB 153.

Choi et al. [52] found that PCB 104 (2,2′,4,6,6′-pentachlorobiphenyl) increased oxidative stress as evidenced by increased 2′ ,7′ -dichlorofluorscein and rhodamine 123 fluorescence in human vascular endothelial cells in an in vitro study. PCB treatment also greatly elevated the expression of MCP-1, E-selectin and ICAM-1 at both mRNA and protein levels.

Bezdecny et al. [53] tested the effect of PCBs on COX-2 expression in differentiated cultures of HL-60 cells (a human promyelocytic leukemia cell line) and found that COX-2 expression was enhanced by 2,2′ ,4,4′ -tetrachlorobiphenyl, a noncoplanar orthosubstituted PCB congener, compared to 3,3′ ,4,4′ -tetrachlorobiphenyl, a coplanar PCB, which had no effect on COX-2 expression.

Mariussen et al. [54] observed that the cytotoxic effect of Aroclor 1254, which caused cell death in cultured rat cerebellar granule cells, was mediated by increased free

radical formation, as inhibitors of NO and PLA_2 led to a significant reduction of free radical formation and cell death. Treatment of the same cells exposed to PCB by cyclosporin A (a mitochondrial permeability transition pore blocker) and vitamin E also led to a similar result, with increased survival and reduced reactive oxygen species formation.

PCB 77 activates aryl hydrocarbon receptor (AhR) and produces CYP1A1, which leads to oxidative stress and subsequent endothelial cell damage. Dietary flavonoids such as catechins (epigallocatechin gallate) and quercetin can inhibit the toxic effects of environmental contaminants such as PCB at both functional levels of AhR and/or CYP1A1 [55]. PCB (Aroclor 1254) induces oxidative stress in ventral prostate of the rat by decreasing the levels of antioxidant enzymes, which could be effectively reversed by zinc supplementation, presumably via a mechanism of indirect hormonal regulation [56].

4.2. Heavy metals

4.2.1. Heavy metals and PUFA

Guallar et al. [57] reported that toenail mercury level was positively correlated with the risk of myocardial infarction but that adipose tissue DHA concentration was negatively associated in a case–control study (conducted in eight European countries and in Israel) that was composed of 684 men with an initial diagnosis of myocardial infarction and 724 men who were the control subjects. The authors conclude that high mercury content may diminish the cardioprotective effect of fish intake.

Consumption of lead, which has an effect on hepatic fatty acid composition in the chicken, increases the concentration of arachidonic acid and decreases the linoleic acid/arachidonic acid ratio. These findings suggest that lead could increase tissue peroxidation via a relative increase of arachidonic acid and that a decrease in the hepatic ratio of linoleic acid to arachidonic acid could be a specific indication of lead toxicity [58].

Kafer et al. [59] showed that stimulation of HL-60 human leukemia cells by organic lead and tin compounds induced an increase in arachidonic acid release, wherein redistribution occurs within tissue lipid classes. The increase of free arachidonic acid for prostanoid formation was observed prior to cell death in culture. Heavy-metal compounds (i.e., organometals containing lead and tin) stimulate an increase of free arachidonic acid that resulted via liberation of this fatty acid prostanoid precursor from membrane phospholipids by the action of PLA_2 in HL-60 cells [60].

4.2.2. Heavy metals and eicosanoids

Turner et al. [61] showed that heavy-metal ions (Mn^{2+} and Cu^{2+}) inhibited eicosanoid (5-HETE and LTB4) release in ionophore (A23187)-treated human neutrophils. In a male rat model, cadmium (in the form of $CdCl_2$), when dosed at 10 μmol/kg, caused a dramatic elevation of testicular $PGF_{2\alpha}$, and pretreatment with zinc totally abolished this response [62]. Cadmium, an oxidative stressor, was found to exert its cytotoxicity by up-regulating COX-2 gene expression and increase PGE_2 production in mouse HT4 neuronal cell line [63]. The antioxidant *N*-acetylcysteine, a thiol-reducing agent, and the COX-2 inhibitor celecoxib attenuated the cytotoxicity of cadmium in HT4 cells.

4.2.3. Heavy metals and oxidative stress

Howlett and Avery [64] demonstrated in yeast (*Saccharomyces cerevisiae*) enriched with PUFA (linoleic and α-linolenic acids) that copper and cadmium rapidly induced lipid peroxidation, and the metal sensitivity was associated with PUFA enrichment. Changes in cell antioxidant status and membrane fatty acid composition could significantly alter the ability of cells to cope with heavy-metal stress.

A study on serum lipid peroxidase (LPO) level and blood SOD activity in workers with occupational exposure to lead showed that the increase in serum LPO is due not only to the stimulation of lipid peroxidation but also to the inhibition of blood SOD activity resulting from exposure to lead in manufacturing processes [65]. Recent work indicates that transition metals act as catalysts in the oxidative reactions of biological macromolecules, and as a result, the toxicity associated with these metals might be due to oxidative tissue damage. Redox-active metals such as iron, copper and chromium undergo redox cycling, whereas redox-inactive metals such as lead, cadmium and mercury deplete the major antioxidants of a cell (mainly thiol-containing antioxidants and enzymes). Metal-induced oxidative stress in cells can be, in part, responsible for the toxic effects of heavy metals. Preliminary studies to determine the effect of antioxidant supplementation in experimental animals following heavy-metal exposure suggest that antioxidants may play an important role in lessening some hazards of heavy metals [66].

4.2.4. Avoiding heavy-metal intake

The consumption of fish rich in omega-3 PUFA is recommended to decrease the risk of coronary artery disease. Unfortunately, consumption of certain fish such as swordfish and shark and, to a lesser extent, tuna, trout, pike and bass is also a source of potential exposure to toxic heavy metals such as mercury. In one study, several brands of fish oil were examined and shown to have nondetectable (<6 μg/L) to negligible amounts of mercury (10–12 μg/L), which may provide a safer alternative to fish consumption [67].

4.2.5. Controversial view on heavy metals

The environmental pollutants of major significance are heavy metals and persistent organic pollutants. The main exposure is through the ingestion of sea mammals, other foods, drinking water, air and smoking. There is fairly strong evidence that lead is a weak risk factor for high blood pressure even at low levels of exposure. Besides lead, there

is some evidence that environmental pollutants are related to risk for CVD. In view of the fact that these pollutants are found in the traditional diet together with omega-3 PUFA, monounsaturated fatty acids and selenium (which are believed to promote cardiovascular health), there is an indirect link between the pollutants and CVD. Epidemiological evidence from Greenland's arctic population shows that sea mammals are widely consumed for health and other reasons, and because this population has a lax attitude to the potential bioaccumulation of pollutants in these foods, the mortality data do not support the hypothesis that the low rate of ischemic heart disease in Inuits is due to the pollutants in their traditional diet [68]. Another interpretation of the data regarding the relationship between the diet and disease incidence in this population is that the protective actions of LC omega-3 PUFA both promote cardiovascular health and attenuate the toxic effects of environmental pollutants.

5. Approach and models for investigating the effects of flavonoids and omega-3 PUFA on environmental pollutants

Scientists at Purdue University have formed a collaboration to better understand the metabolism and physiology of flavonoids that are purported to benefit health. This new effort is focused on determining how candidate flavonoids and their metabolites affect gene targets of inflammation in cell culture and animal models. The challenge of this research is to understand how LC PUFA (omega-3) and flavonoids affect the biology of inflammation. The relationships were described in a recent publication by Horia and Watkins [69] that demonstrates the complementary actions of DHA and genistein on prostanoid synthesis in MDA-MB-231 human breast cancer cells. The goal of this new collaboration is to determine how nutrients and food components manipulate inflammation that is associated with a number of diet-related diseases that occur throughout the life cycle. The focus on flavonoids is to characterize the bioactivity of these compounds in vivo by measuring their bioaccessibility in vitro.

The experimental approach involves molecular, biochemical and physiological endpoints of musculoskeletal diseases, obesity and aging. Examples include investigations on the combined effects of PUFA and cyanidins on inflammatory markers in cultures of human cancer cells [70]. The actions of catechins and PUFA on muscle loss and osteopenia are being studied in a rodent model of disuse atrophy to explain how muscle and bone communicate to prevent tissue loss that is associated with injury, disease and aging [71].

Absorption and bioavailability of phytochemicals by humans are preferentially investigated in vivo. However, time constraints and cost associated with this type of work have prompted a number of researchers to develop rapid and cost-effective in vitro techniques for estimating bioavailability. In most cases, these in vitro digestion models investigate release from the food matrix and digestive stability of phytochemicals of interest. This set of data leads to insight on a compound's bioaccessibility, which is defined as the fraction of phytochemicals from the food matrix available for uptake by the intestinal mucosa [72]. In vitro digestion models rely on a series of predetermined physiological parameters gained from in vivo studies in humans, including meal size, duration of each digestive phase, pH and gastric and intestinal secretions. Techniques of this kind have been applied successfully for bioaccessibility assessment of vitamins and minerals [73–75], cholesterol [76], carotenoids [77–80], chlorophylls [81,82] and, to a more limited extent, polyphenols [83–85].

Likewise, the Caco-2 cell culture system is a widely utilized model for studying compound intestinal absorption and transepithelial transport of phytochemicals. The Caco-2 is a human epithelial cell isolated from a human colorectal adenocarcinoma [86]. These cells spontaneously differentiate at confluency and exhibit enterocyte-like traits, including the polarized distribution of numerous nutrient transport systems, plasma membrane enzymes and hormone receptors, as well as inducible Phase I and II xenobiotic metabolizing systems [87–89]. The main advantages of using a Caco-2 cellular model include its human origin, its applicability to rapid screen techniques and its record as an effective predictor of human intestinal absorption for passive and, to a certain extent, active drug, nutrient and phytochemical transport [87,90–92]. In combination with in vitro digestion, the Caco-2 intestinal cell culture models allow for effective and rapid screening of factors that influence phytochemical bioavailability prior to more complex, higher-risk and expensive in vivo bioavailability trials. The in vitro and cell culture studies with flavonoids are being coupled with in vivo experiments with rodents to examine the effects on biological targets of inflammation.

6. Conclusions

The purpose of this brief review is to introduce the paradigm for investigating food components that can attenuate the toxic effects of exposure to environmental pollutants. The evidence presented show that the health beneficial actions of omega-3 PUFA and flavonoids are a means to control inflammation. The omega-3 PUFA reduce the formation of PGE_2, a proinflammatory prostanoid. Many of the flavonoids exert an anti-inflammatory action and reduce the formation of cell-damaging free radicals. The aim of future investigations on omega-3 PUFA and flavonoids should be directed at elucidating how these food-derived nutrients and health protectants can reduce the exposure and toxic effects of environmental pollutants. In light of these recent findings, significant efforts must be focused on understanding the bioaccessibility and tissue delivery of flavonoids using appropriate cell culture and in vivo approaches to advance

the research for preventing the consequences of environmental pollutants.

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