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Chemistry behind Vegetarianism

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This review summarizes the effect of a habitual vegetarian diet on clinical complications in relation to chemistry and biochemistry. Omnivores have a significantly higher cluster of cardiovascular risk factors compared with vegetarians, including increased body mass index, waist to hip ratio, blood pressure, plasma total cholesterol (TC), triacylglycerol and LDL-C levels, serum lipoprotein(a) concentration, plasma factor VII activity, ratios of TC/HDL-C, LDL-C/HDL-C and TAG/HDL-C, and serum ferritin levels. Compared with omnivores, vegetarians, especially vegans, have lower serum vitamin B_{12} concentration and n-3 polyunsaturated fatty acid (PUFA) levels in the tissue membrane phospholipids, which are associated with increased collagen and ADP stimulated ex vivo whole blood platelet aggregation, plasma 11-dehydrothromboxane B_2, and homocysteine levels and decreased plasma HDL-C. This may be associated with an increased thrombotic and atherosclerotic risk. It is suggested that vegetarians, especially vegans, should increase their dietary n-3 PUFA and vitamin B_{12} intakes.

KEYWORDS: Vegetarian; vitamin B_{12}; n-3 PUFA; homocysteine; platelet aggregability

INTRODUCTION

Human beings originated in South Africa and then migrated to different parts of the world approximately a hundred thousand years ago. In the course of human evolution, six major genetic clusters have been formed: Africa, Europe, Middle East, Central/South Asia, East Asia and Oceania, and America (*1*). Human beings are very similar in term of our genomes; however, there are significant differences physically and physiologically, such as body weight, height, eye/hair color, skin color, response to drug treatments, dietary intake, and environmental factors. These differences are caused mainly by environmental factors, of which diet is the largest. An unanswered question has always been the difference between omnivores and vegetarians who are descendants of omnivores and vegetarians, respectively, who have both come from the same ancestor in terms of phenotypic variation and biochemistry? Unfortunately, there are no data available on phenotypic variation between omnivores and vegetarians. In this review, I will use available evidence to review the biochemistry behind vegetarianism.

VEGETARIANS AND THEIR DIETS

Vegetarians state that their diet must exclude all animal flesh. There are different varieties of vegetarianism, which exclude or include various foods (*2*) (**Table 1**). Raw veganism includes only fresh and uncooked fruit, nuts, seeds, and vegetables. Fruitarianism permits only fruit, nuts, seeds, and other plant matter that can be gathered without harming the plant. Su vegetarianism (such as Buddhism in China) excludes all animal products as well as vegetables in the *Allium* family such as onion, spring onion, garlic, scallions, and leeks (*3*).

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Strict vegans and Su vegetarians also avoid products that may use animal ingredients not included in their labels or which use animal products in their manufacturing, for example, cheeses that use animal rennet (enzymes from animal stomach lining), gelatin (from animal skin, bones, and connective tissue), some sugars that are whitened with bone char (e.g., cane sugar, but not beet sugar), and alcohol clarified with gelatin or crushed shellfish and sturgeon (*3*).

Some individuals claim themselves to be semivegetarian. However, it is debated by most vegetarian groups because semivegetarian diets include fish and other seafood, and poultry sometimes, whereas it is stated that vegetarians must exclude all animal flesh.

In general, compared with an omnivorous diet, vegetarian diets are rich in fiber, magnesium, Fe^{3+}, folic acid, vitamins C and E, n-6 polyunsaturated fatty acid (PUFA), phytochemicals, and antioxidants but low in total fat, saturated fatty acid (SFA), cholesterol, sodium, zinc, Fe^{2+}, vitamins A, B_{12}, and D, and especially n-3 PUFA (**Table 2**). Low intake of total fat, SFA, and sodium and increased intake of fiber, phytochemicals, and antioxidants in vegetarians is associated with decreased blood pressure and body mass index (BMI). These factors are known to reduce the risk of cardiovascular disease (CVD). However, there is concern over whether vegetarians, and particularly vegans, have an adequate intake of several important nutrients, particularly Fe, Zn, vitamin B_{12}, and n-3 PUFA.

MICRONUTRIENTS STATUS OF VEGETARIANS

Iron, zinc, and vitamin B_{12} are currently the micronutrients of greatest concern when considering the nutritional value of vegetarian diets.

Iron. Iron is an essential trace element for blood formation. Most iron in the human body is found in hemoglobin and

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Table 1. Dietary Composition of Main Types of Vegetarianism

	meat	fish	egg	dairy	honey	*Allium* family
vegan[a]	×	×	×	×	×	√
ovovegetarian	×	×	√	×	√	√
lactovegetarian	×	×	×	√	√	√
ovo-lactovegetarian	×	×	√	√	√	√
raw vegan[b]	×	×	×	×	×	×
Su vegetarian[c]	×	×	×	×	×	×
fruitarian[d]	×	×	×	×	×	×

[a] Excludes all animal flesh and animal products, milk, honey, eggs. May also exclude any products tested on animals, also any clothing from animals. [b] Includes only fresh and uncooked fruit, nuts, seeds, and vegetables, excludes vegetables in the *Allium* family. Vegetables can be cooked only up to a certain temperature. [c] Includes only fruit, nuts, seeds, and other plant matter that can be gathered without harming the plant. [d] Excludes all animal products as well as vegetables in the *Allium* family.

Table 2. Chemical Characteristics of Vegetarian Diet

rich in chemicals	low in chemicals
fiber	vitamin A
vitamin C	vitamin B_{12}
vitamin E	vitamin D
folic acid	zinc
magnesium	iron
n-6 polyunsaturated fatty acid	sodium
carbohydrate	cholesterol
phytochemicals	saturated fatty acids
	n-3 polyunsaturated fatty acid

myoglobin or occurs as part of enzymes in the energy-yielding pathway. Iron deficiency is the most common mineral nutritional deficiency globally, although vegetarians are not more likely to be iron-deficient than omnivores.

Studies reported that vegetarians have iron intakes that are significantly higher than (*4*, *5*) or similar to (*6*−*8*) those of omnivores in different populations. However, vegetarians have a significantly lower serum ferritin concentration than omnivores. Serum ferritin levels did not correlate with dietary iron intake (*5*, *7*, *9*). Ferritin is a storage form of body iron. A small amount of ferritin circulates in plasma, mostly as iron-free apoferritin. Circulating ferritin is in equilibrium with tissue iron stores and, under most circumstances, the concentration of serum ferritin accurately reflects the levels of iron in the tissues. A low serum ferritin concentration is usually diagnostic of iron deficiency. Vegetarians, unlike omnivores, obtain most of their iron from cereals, grains, nuts, seeds, vegetables, fruits, and bakery products, which are nonheme iron sources. Most nonheme iron from vegetarian diets is in the ferric (Fe^{3+}) state, which is soluble at the acidic pH of the stomach but becomes insoluble at the more alkaline pH of the duodenum. Gastric acid converts Fe^{3+} to the Fe^{2+} form when body iron stores are low and by the concomitant ingestion of some dietary components such as ascorbic acid, sugars, and amino acids that form iron chelates to increase iron absorption (*10*). Moreover, bioavailability and absorption of nonheme iron may be inhibited by certain dietary constituents that are abundant in some vegetarian diets, such as oxalates in vegetables and phytates in cereals and legumes (*11*, *12*), tannins in tea and coffee (*13*), and possibly soy protein (*14*). Heme iron comes mainly from seafood and meat, especially red meat, when it is released from the surrounding polypeptide chain. Heme is absorbed intact by the mucosal cell, where the porphyrin ring is split and iron is liberated. It is absorbed more efficiently than nonheme iron and is minimally affected by dietary factors, which probably explains the lower iron status of vegetarians compared with omnivores.

Zinc. Zn is an essential trace mineral that is a constituent of more than 50 different enzymes involved in most metabolic pathways and is important for protein metabolism, cell growth and repair, and immune function (*15*).

Zinc is found in a wide range of foods, including protein foods and plant foods such as legumes, whole grains, nuts, and seeds.

Zn from animal sources is more bioavailable than Zn from plant foods. Protein, insoluble fiber, phytate, and some minerals, for example, Fe, Ca, and P, can reduce Zn absorption. The inhibiting effect of phytate on Zn absorption has been quantified by the phytate/zinc molar ratio. Low zinc bioavailability diets (15% absorption) were listed as high in unrefined cereal grains, with phytate/zinc ratios of >15, and the majority of energy was supplied by high-phytate foods such as sorghum, peanuts, cowpeas, unleavened bread, and unprocessed soybean protein concentrates (*16*). Some food preparation techniques, such as leavening bread, soaking and sprouting beans, grains, and seeds, could reduce binding of zinc by phytate and increase zinc bioavailability (*17*, *18*).

Because there is low zinc bioavailability in vegetarian diets, vegetarians have lower status compared with omnivores. However, intake of zinc density is not significantly different between vegetarians and omnivores (*19*−*21*). There is currently no agreement on the best method to assess zinc status. Despite serum or plasma, hair, and salivary Zn levels being used to assess Zn status, all have shortcomings (*22*). No significant difference has been seen between vegetarians and omnivores on serum/plasma zinc concentrations (*21*, *23*).

Vitamin B_{12}. Vitamin B_{12} is essential for new cell synthesis, blood formation, maintenance of the nervous system, etc. Of the vitamins, B_{12} is the only one containing a mineral (cobalt); it also known as the red vitamin. Seafood, animal meats, eggs, and liver are good sources for vitamin B_{12}. Vitamin B_{12} is not found in plant foods; however, seaweed may contain vitamin B_{12} analogues, which can be counted on as reliable sources of active vitamin B_{12}. Ovo-lactovegetarians may get vitamin B_{12} from eggs and dairy products. Vegans could get some vitamin B_{12} from seaweed, plants, and edible fungi (such as mushrooms) on farms or in the wild, which may be contaminated from bacteria in the soil (*24*). Evidence suggests that vegetarians, especially vegans, who do not take vitamin B_{12} supplements often have abnormally low serum concentrations of vitamin B_{12}. Fortunately, human beings require only tiny amounts of vitamin B_{12}. Because the human body conserves B_{12} and reuses it without destroying the compound, and it can be synthesized by intestinal bacteria, clinical evidence of vitamin B_{12} deficiency is uncommon.

Vegetarians have a lower vitamin B_{12} status compared with omnivores. Serum vitamin B_{12} concentration decreased progressively from the high-meat-eaters group ($n = 18$, >280 g of meat/day) to the moderate-meat-eaters group ($n = 60$, <280 g of meat/day) to ovo-lactovegetarians ($n = 43$) to vegans ($n = 18$) ($p < 0.05$) (*20*). Serum vitamin B_{12} concentration was significantly correlated with plasma homocysteine ($p < 0.05$) in the study subjects from Australia (*25*, *26*). Similar results have also been found in Britain (*27*) and in China (*28*). Chinese vegetarian parents and their preschool children had similar serum vitamin B_{12} and homocysteine concentrations; however, serum vitamin B_{12} was not correlated with plasma homocysteine in the parents, their children, or pooled parents and children (*29*).

Vitamin D. Vitamin D is rare in vegetarian diets; however, it does not seem that ovo-lactovegetarians or vegans are more likely to be deficient than omnivores because human beings can synthesize it. The body's cholesterol can be converted into vitamin D by ultraviolet irradiation from the sun. Fifteen minutes of skin exposure a day during peak hours should be enough for fair-skinned individuals, but those who have darker skin, are older,

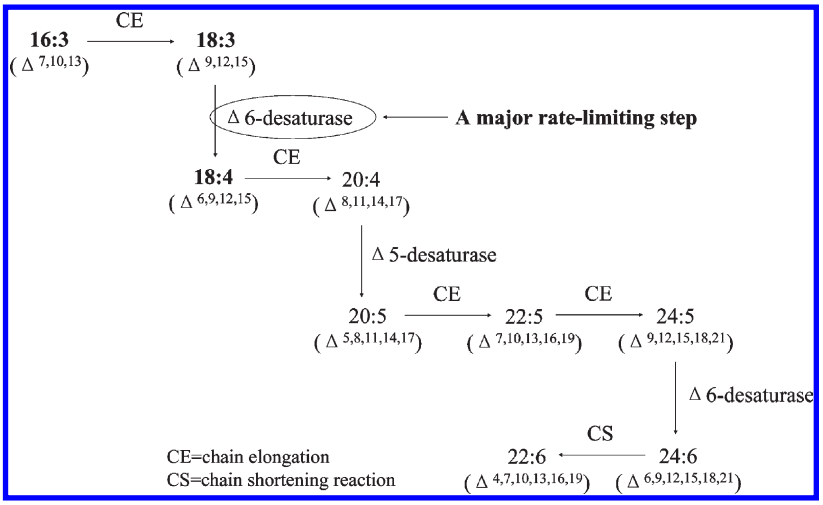

Figure 1. Metabolic pathway of 18:3n-3 to 22:6n-3. CE, chain elongation; CE, chain shortening reaction.

or who live at more northern latitudes might not get enough exposure, especially in the winter (*10*).

MACRONUTRIENTS STATUS OF VEGETARIANS

There is no difference in carbohydrate sources between vegetarian and omnivorous diets. Despite the protein sources being different between the two groups, dietary protein cannot be incorporated into human tissues. When we eat proteins from plant or animal sources, the body must first alter them by breaking them down via denaturation and hydrolysis into amino acids; only then can it rearrange them into specific human body proteins. A balanced vegetarian diet could provide sufficient protein to meet physiological needs. However, this is not the case when it comes to fat and fatty acids.

Fat and Fatty Acids Intake and Status of Vegetarians. The predominant PUFA in the Western diet is linoleic acid (18:2n-6), which is commonly found in vegetable seed oils. This is the parent fatty acid of the n-6 series PUFA, which can be converted in vivo to C20 and C22 n-6 long chain (LC) PUFA. α-Linolenic acid (18:3n-3) is less abundant than 18:2n-6; however, it is also present in vegetable oils and is the precursor of C20 and C22 n-3 LC PUFA. Omnivores can obtain their C20 and C22 n-3 LC PUFA either from dietary 18:3n-3 or directly from the consumption of fish, eggs, or animal products. Ovo-lactovegetarians can gain a limited amount of C20 and C22 n-3 LC PUFA from milk, dairy products, and eggs. In contrast, vegans must rely totally on endogenous synthesis from 18:3n-3 by desaturation and elongation (**Figure 1**). Because animals can convert 18:3n-3 to C20 and C22 n-3 LC PUFA, and plants cannot, there are no C20 and C22 n-3 LC PUFA in plant-based vegan diets. Conversion of 18:3n-3 to 20:5n-3 and 22:6n-3 has relatively poor efficiency because a substantial proportion of the 18:3n-3 is diverted to β-oxidation (*30*).

A major rate-limiting step in 18:3n-3 conversion to 20:5n-3 and other LC n-3 PUFA is considered to be the first Δ6 desaturation. This is demonstrated effectively by consideration of data from studies where 18:4n-3 is fed, which is the product of the Δ6 desaturation of 18:3n-3. The 20:5n-3 level of red blood cell membrane phospholipid (PL) was significantly increased from 0.8 to 1.1% when 32 stroke patients consumed a black currant seed oil (rich in stearidonic acid 18:4n-3 and γ-linolenic acid) supplemented diet (7.5% black currant seed oil, 50% soybean oil, and 42.5% medium-chain triacylglycerols (TAG)) for 3 weeks compared with a 100% soybean oil diet and a 50% soybean oil + 50% medium-chain TAG diet (*31*). In another study, the 20:5n-3 levels of TAG, cholesteryl ester, and PL of guinea pig liver were significantly higher after guinea pigs were fed a diet containing 10% black currant seed oil for 40 days compared with the diet containing 10% walnut oil (18:3n-3 containing) (*32*). A similar result has also been found in rats (*33*). Dietary sources of stearidonic acid (black currant seed oil, alpine currant seed oil, and oil from *Echium* species) might be a viable source of LC n-3 PUFA, particularly for vegetarian groups. Because the formation of 22:6n-3 from 22:5n-3 also requires a Δ6 desaturase, it is likely that none of the intermediate n-3 PUFA (18:3−22:5) will be as effective a source of tissue of 22:6n-3 compared with dietary 22:6n-3 (on a gram for gram basis). In addition, 18:2n-6, a precursor of 20:4n-6, the major dietary PUFA, is a competitive inhibitor of the metabolism of 18:3n-3 to 18:4n-3 (*30*). Furthermore, diets rich in 18:2n-6 decrease the expression of the hepatic Δ6 desaturase compared with fat-free diets, which presumably also reduces the possibility of conversion of 18:3n-3 to 18:4n-3 and 24:5n-3 to 24:6n-3 (*34*).

Is there a decreased n-3 PUFA status in vegetarian populations? Is there any association between n-3 PUFA status and clinical complications? These questions are further explored in the following sections.

n-3 PUFA Status in Vegetarians. One hundred and thirty-nine healthy male subjects aged 20−55 years participated in an observational study. According to subjects' habitual dietary intake (based on the semiquantitative Food Frequency Questionnaire (FFQ)), they were divided into four groups, vegan, ovo-lactovegetarian, moderate meat eater, and high meat eater. The proportion of total n-3 PUFA, 20:5 n-3, 22:5 n-3, and 22:6 n-3 and n-3 to n-6 ratio were significantly lower and the 20:4n-6 to 22:5n-3 ratio was significantly higher in both the ovo-lactovegetarian and the vegan groups than in both the high- and moderate-meat-eater groups in the plasma PL. The proportions of 20:5n-3, 22:5n-3, 22:6n-3, and total n-3 PUFA and ratio of n-3 to n-6 were significantly lower in the vegan group than in the ovo-lactovegetarian and high- and moderate-meat-eater groups in the platelet PL (*35*). In another cross-sectional study, 50 free-living healthy female vegetarians and 24 sex- and age-matched omnivores participated in the study. Compared with the omnivores, the vegetarians had significantly lower concentrations of 20:3n-6, 20:4n-6, 22:5n-6, 20:5n-3, 22:6n-3, total n-6, and n-3 PUFA and ratio of n-3/n-6 PUFA in serum PL (*36*).

A cross-sectional study from the United Kingdom involved 659 male subjects, of which 196 were omnivores, 231 vegetariansm, and 232 vegans. The proportions of 20:5n-3 and 22:6n-6 in plasma were significantly lower in the vegetarians and vegans than in the omnivores ($p < 0.001$), whereas 22:5n-3 was significantly lower in the vegans than in the meat eaters ($p < 0.05$).

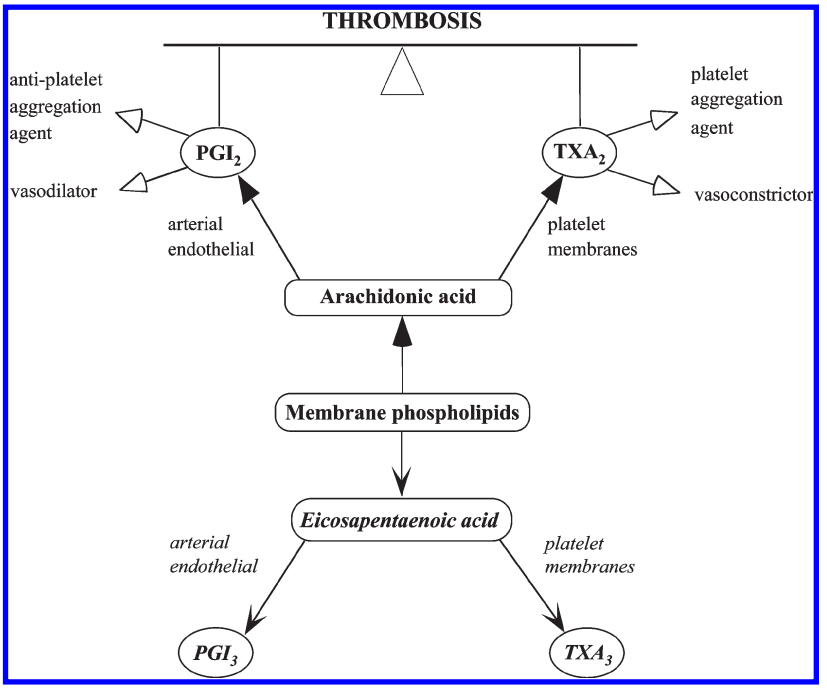

Figure 2. Relationship between thrombosis and metabolites of 20:4n-3 and 20:5n-5.

Proportions of 20:5n-3, 22:5n-3, and 22:6n-6 in plasma were not significantly associated with the duration of time since the subjects became vegetarian or vegan, which ranged from < 1 to > 20 years (*37*).

A recent observational study from Austria involved 98 adult subjects, of which 23 were omnivores, 25 vegetarians, 37 vegans, and 13 semiomnivores. Erythrocyte proportions of 20:5n-3, C22:5n-3, C22:6n-3, and total n-3 fatty acids in sphingo- and phospholipids (SPL), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE) was significantly lower in vegan and vegetarian groups compared with omnivores and semiomnivores (*38*).

Is there any association between decreased n-3 PUFA status and clinical complication in vegetarians?

RELATIONSHIP BETWEEN n-3 PUFA STATUS AND PLATELET FUNCTION IN VEGETARIANS

Collagen- and adenosine-5′-diphosphate (ADP)-stimulated ex vivo whole blood platelet aggregation were significantly higher in both vegetarian and vegan groups than in both high- and moderate-meat-eater groups. The vegan group had a significantly higher mean platelet volume (MPV) than the high- and moderate-meat-eater and ovo-lactovegetarian groups (*35*). Increased MPV in vegans suggests the presence of larger, activated platelets. Evidence from case control studies has indicated that an increased MPV is an independent risk factor for acute myocardial infarction (MI) (*39*) and for acute and/or nonacute cerebral ischemia (*40*). Large platelets, in such cases, have been shown to have increased reactivity. When platelets become activated, they change from their normal resting disk-like structure to assume a spherical shape and their volume increases substantially, leading to the potential for thrombus formation. In a multiple linear regression analysis, after controlling for potential confounding factors such as dietary group, age, exercise, body mass index, and dietary PUFA and saturated fat, cholesterol, carbohydrate, and fiber intake, the MPV was still strongly negatively correlated with platelet PL 20:3n-6 ($p = 0.003$) and 22:5n-3 ($p = 0.001$). The data suggest that 22:5n-3 and 20:3n-6 may play a role in the structural function of the platelet membrane (*41*). This, in conjunction with the increased platelet aggregability, suggests what should be an increased thrombosis tendency in vegans, and in the case of the platelet aggregation is associated with low dietary intake of n-3 PUFA.

Most acute clinical cases of cardiovascular disease are caused by the formation of a thrombus, with platelet aggregation being the initial step in these events (*42*). Increased platelet aggregability is significantly associated with CHD mortality (*43*). Fisher et al. (*44*) reported that there was no significant difference between the vegetarians and the omnivores on ex vivo collagen-, arachidonic acids (AA)-, ADP-, and epinephrine-stimulated platelet aggregation. However, Fisher et al. (*44*) used different methodology from that used by Li et al. (*35*); they used the traditional optical method for platelet aggregation test (using plasma), and they also adjusted the platelet count. It has been reported that dilution of platelet-rich plasma can cause changes in platelet responsiveness (*43*).

In an intervention study, ADP, epinephrin, collagen and arachidonic acid induced platelet aggregation in both maximum percentage or slope were significantly reduced after 10 vegetarians were supplemented with 700 mg/day of each 20:5n-3 and 22:6n-3 for 8 weeks. Both 20:5n-3 and 22:6n-3 were significantly incorporated into plasma lipids (*45*).

Platelet aggregation is initiated by thromboxane A_2 (TXA_2), a potent platelet aggregation agent and vascular contractor, produced from 20:4n-6 in the platelet membrane (*46*, *47*). 20:5n-3 is released from phospholipids of the platelet membrane and acts as a "false" substrate to compete with 20:4n-6 for access to cyclooxygenase and produces an alternative form of thromboxane (TXA_3), which is relatively inactive in promoting platelet aggregation and vasoconstriction (*48*). This situation can lead to a reduced TXA_2 production and thus a lower thrombosis tendency (*49*, *50*). A vegetarian diet with a high n-6 to n-3 PUFA ratio can cause a high tissue n-6 to n-3 PUFA ratio, that is, and increased 20:4n-6 to 20:5n-3 ratio, which may promote production of TXA_2, leading to increased platelet aggregability (*35*) (**Figures 2** and **3**).

PLASMA HOMOCYSTEINE CONCENTRATIONS IN VEGETARIANS

It has been suggested that an increased plasma homocysteine (Hcy) level is an independent risk factor for cardiovascular

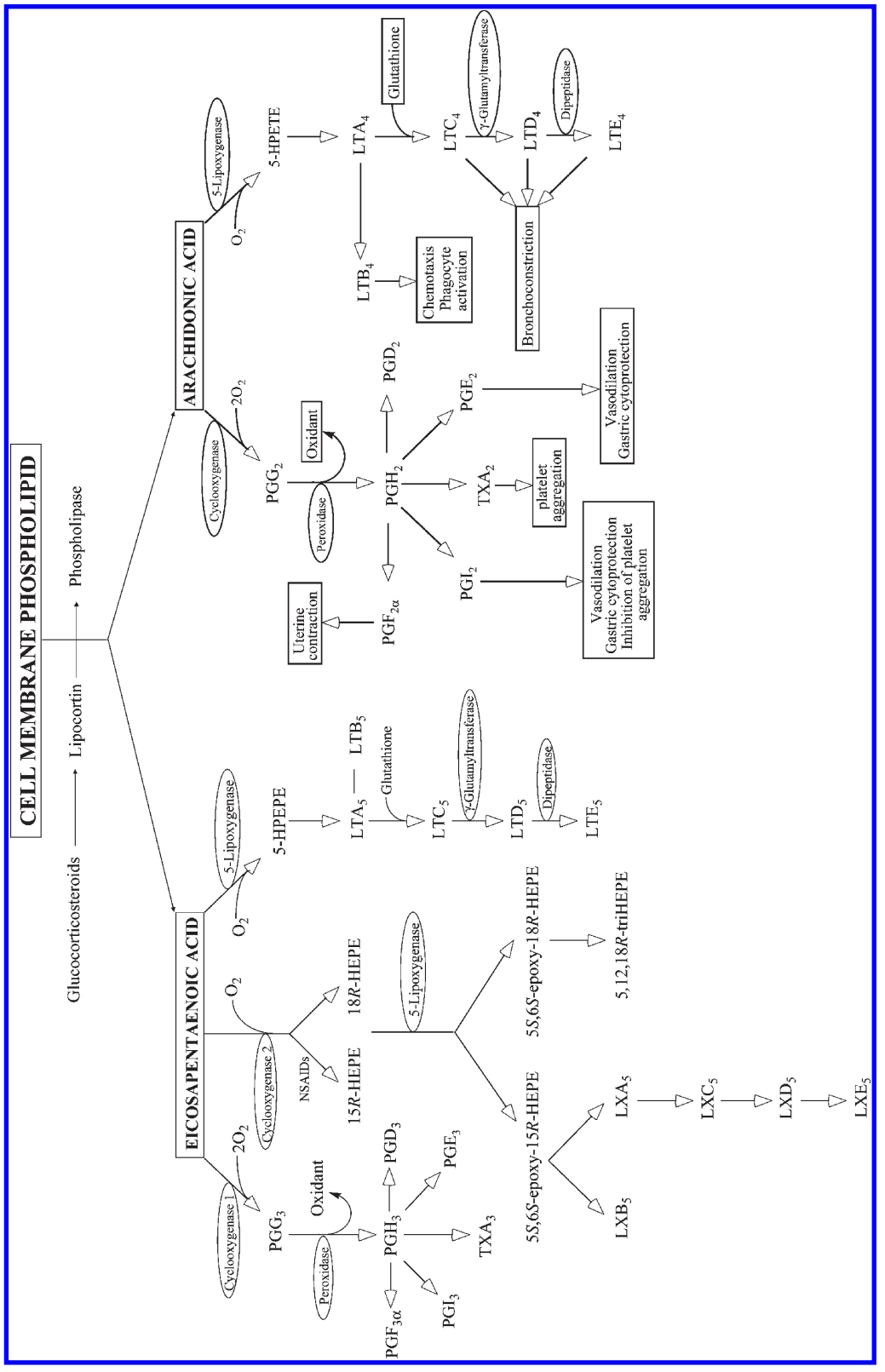

Figure 3. Biosynthetic pathways of 20:4n-6- and 20:5n-3-derived eicosanoids. The binding of a stimulant (e.g., a glucocorticosteroid) to a membrane receptor results in the activation of phospholipase A_2, which cleaves 20:4n-6 and 20:5n-3 from membrane phosphatidylcholine. After they are released from membrane phospholipids, free 20:4n-6 and 20:5n-3 can be metabolized by various enzyme systems to form a range of biologically active eicosanoids. The eicosanoids from 20:5n-3 are generally less potent than the 20:4n-6-derived metabolites that compete with 20:5n-3 for the enzymes. The nature of the products formed depends on the tissue. Abbreviations: PG, prostaglandin; TXA, thromboxane; LT, leukotrienes; LX, lipoxin; HPETE, hydroperoxyeicosatetraenoic acid; HPEPE, hydroperoxyeicosapentaenoic acid; HEPE, hydroxyeicosapentaenoic acid.

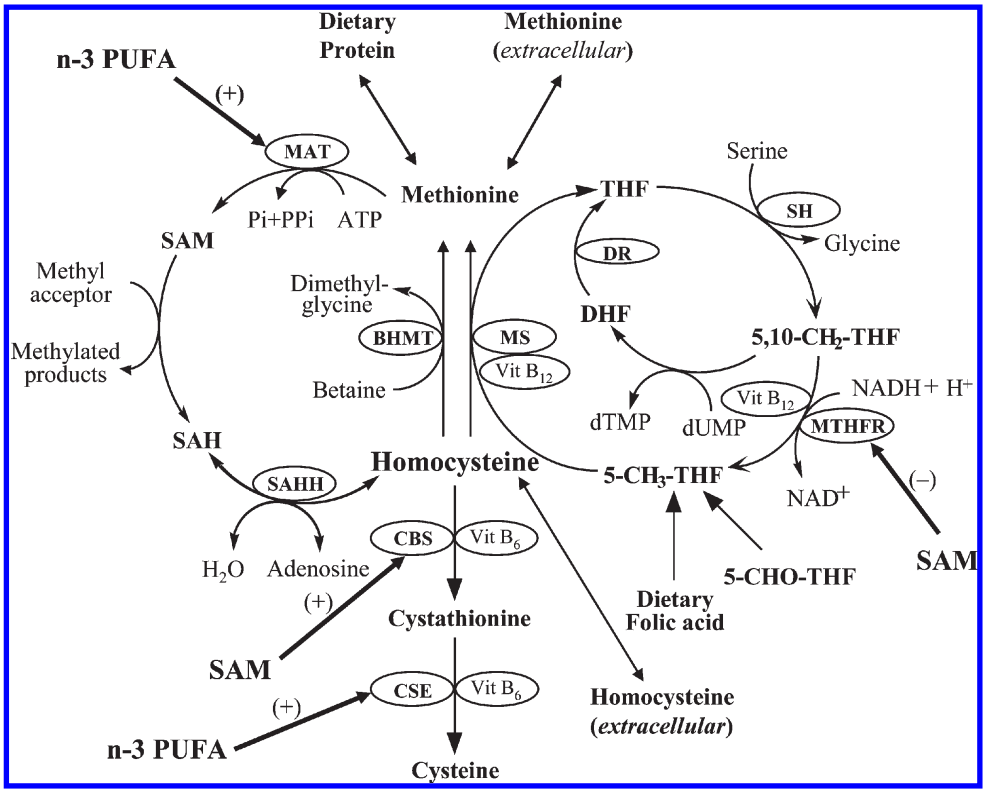

Figure 4. Speculated effect of n-3 PUFA on homocysteine metabolic pathway. Abbreviations: ATP, adenosine triphosphate; 5,10-CH3-THF, 5,10-methylenetetrahydrofolate; 5-CH3-THF, 5-methyltetrahydrofolate; BHMT, betainehomocysteine methyltransferase; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; MAT, methionine adenosyl transferase; MS, methionine synthetase; MTHFR, 5-methyltetrahydrofolate reductase; SAHH, *S*-adenosylhomocysteine hydrolases; SH, serine hydroxymethyl transferase; DR, dihydrofolate reductase; SAM, *S*-adenosylmethione; SAH, *S*-adenosylhomocysteine; THF, tetrahydrofolate; DHF, dihydrofolate; dTMP, 2′-deoxythymidine-5′-monophosphate; dUMP, 2′-deoxyuridine-5′-monophosphate; Pi, orthophosphate; PPi, pyrophosphate.

diseases (*51*−*53*). Hcy is an intermediate metabolite in the metabolism of methionine to cysteine. The normal metabolism of Hcy involves two pathways: remethylation and transsulfuration. Remethylation of Hcy to methionine requires vitamin B_{12} (methylcobalamin form) as a coenzyme for Hcy methyltransferase (methionine synthetase) and *N*5-methyltetrahydrofolate as a methyl donor. The transsulfuration pathway of Hcy to cysteine requires vitamin B_6 as a coenzyme for both cystathionine β-synthase (converts Hcy to cystathionine) and cystathionine lyase (converts cystathionine to cysteine). A lack of dietary vitamin B_{12} and/or folic acid or vitamin B_6 results in elevation of plasma Hcy (*54*) (**Figure 4**).

Vegans and ovo-lactovegetarians had significantly higher mean plasma Hcy levels than omnivores, and plasma Hcy concentration was significantly negatively correlated with serum/plasma vitamin B_{12} concentration (*25*, *28*, *55*, *56*). Plasma Hcy concentration was significantly negatively correlated with plasma phospholipid concentration of PUFA 20:5n-3 ($r = -0.226$, $p = 0.009$), 22:5n-3 ($r = -0.182$, $p = 0.036$), 22:6n-3 ($r = -0.286$, $p = 0.001$), and total n-3 ($r = -0.270$, $p = 0.002$) and the ratio n-3/n-6 PUFA ($r = -0.265$, $p = 0.002$) and significantly positively correlated with 20:4n-6 ($r = 0.180$, $p = 0.037$). In the partial correlation analysis, after controlling for serum vitamin B_{12} and folate concentrations, plasma Hcy was significantly negatively correlated with plasma phospholipid concentration of 22:6n-3 ($r = -0.205$, $p = 0.019$) and total n-3 ($r = -0.182$, $p = 0.038$) and the ratio n-3/n-6 PUFA ($r = -0.174$, $p = 0.048$) (*57*). We have also found a similar result in middle-aged and geriatric hyperlipidemia patients (50 males, 31 females) and 65 healthy subjects (43 males, 22 females). Plasma Hcy demonstrated significant positive correlation with adrenic acid (22:4n-6) ($r = 0.188$, $p = 0.018$) and negative correlation with 22:6n-3 ($r = -0.277$, $p = 0.001$) and the ratio of n-3/n-6 ($r = -0.231$, $p = 0.003$) in sex-, age- and BMI-controlled partial correlation analysis (*58*). Erythrocyte 22:6n-3 levels were significantly negatively correlated with plasma Hcy levels in 49 preeclamptic subjects (*59*) ($p < 0.01$).

Plasma Hcy was significantly decreased in 150 patients with acute myocardial infarction after 1 year of n-3 PUFA treatment (containing 850−882 mg of 20:5n-3 and 22:6n-3) ($p < 0.05$) (*60*), in 81 type 2 diabetic patients after n-3 PUFA supplements (3 g/day) for 2 months (*61*), and in 24 diabetic dyslipidemia patients after 3 months of n-3 PUFA supplementation (3.6 g/day, containing 57.4% of 20:5n-3 and 28.7% of 22:6n-3) with a statin−fibrate combination ($p < 0.01$) (*62*). This may be another beneficial effect of n-3 PUFA.

The mechanism of the n-3 PUFA decrease of plasma Hcy has been explained by the effect of n-3 PUFA on enzyme activity and gene expression involved in Hcy metabolism. Methionine adenosyltransferase (MAT) activity was significantly increased and cystathionine-γ-lyase (CSE) activity was also increased, but statistically insignificant, and mRNA expression of MAT and CSE were significantly up-regulated when Sprague−Dawley rats were fed tuna oil (containing 55% of 22:6n-3) for 8 weeks compared with olive oil (*63*). The resultant increase in *S*-adenosylmethionine (SAM) synthesis by MAT would have also stimulated *S*-adenosylhomocysteine (SAH) production, with the consequential increased methyl transfer to various products. SAM serves primarily as a universal methyl donor to a variety of acceptors (*64*). SAM, as a cosubstrate, can react with a large variety of nucleophilic acceptors by various methyltransferases. Proteins, nucleic acids,

Table 3. Relative Benefits and Risks of Vegetarianism Compared with Omnivore

benefits	risks
BMI	homocysteine
waist/hip ratio	platelet aggregability
blood pressure	mean platelet volume
blood coagulation factor VII activity	iron deficiency anemia
total cholesterol	vitamin B_{12} deficiency anemia (possible for children)
LDL-C	
TAG	

lipids, and xenobiotics can be methylated by these enzymes, changing their mRNA expression, activity, function, or the process in which they are involved (*65*). Increased MAT activity and up-regulated MAT mRNA expression increases cystathionine β-synthase (CBS) activity to provide some protection against the toxic accumulation of Hcy and SAH (*66*, *67*) and also accelerates the permanent removal of Hcy from the methionine cycle by CBS. It has also been shown that increased levels of SAH as an activator up-regulate CBS (*68*) and as an allosteric inhibitor down-regulate methylenetetrahydrofolate reductase (MTHFR), suppress the synthesis of an important substrate (*N*5-methyltetrahydrofolate, 5-CH3-THF) required for remethylation, and promote the initial reaction of transsulfuration (cystathionine synthesis) (*64*, *69*). In CBS-expressing cells, these regulatory functions would expedite permanent removal of Hcy and reduce remethylation with some normalization of one-carbon flow (*67*).

All of the above issues may be associated with an increased thrombotic and atherosclerotic risk in vegetarians, especially vegans. However, meat eaters have a cluster of thrombotic and atherosclerotic risk factors higher than those of both ovo-lacto-vegetarians and vegans. These factors include BMI, waist/hip ratio, blood pressure, coagulation factor VII activity, plasma TC, LDL-C and TAG concentrations, ratios of TC/HDL-C, LDL-C/HDL-C, and TAG/HDL-C, and serum ferritin levels (**Table 3**).

CONCLUSION

On the basis of the present data, it is suggested that vegetarians, especially vegans, could benefit from increased dietary intake of n-3 PUFA and vitamin B_{12} and thus improve the balance ratio of n-3 to n-6 PUFA and vitamin B_{12} status, which may reduce any thrombotic tendency that might increase their generally low risk of cardiovascular disease.

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