CONNECTIVE TISSUE: VASCULAR AND HEMATOLOGICAL (BLOOD) SUPPORT

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ABSTRACT

Connective Tissue (CT) is a ubiquitous component of all major tissues and structures of the body (50% of all body protein is CT), including that of the blood, vascular, muscle, tendon, ligament, fascia, bone, joint, IVD's (intervertebral discs) and skin. Because of its ubiquitous nature, CT is an often overlooked component of any essential nutritional program that may address the structure, and/or function of these tissues. The central role of CT in the health of a virtually all cells, tissues, organs, and organ systems, is discussed. General nutritional CT support strategies, as well as specific CT support strategies that focus on blood, vascular, structural system (eg, muscles, tendons, ligaments, fascia, bone, and joints), integument (skin) and inflammatory and immune mediation will be discussed here and will deal with connective tissue dynamics and dysfunction. An overview of the current scientific understanding and possible options for naturally enhancing the structure and function of CT through the application of these concepts will be discussed in this article, with specific attention on the vascular and hematological systems. (J Chiropr Med 2003;2:25-36)

INTRODUCTION

General overview of Connective Tissue in the blood and vasculature

The concept that root causes of vascular and hematological disorders may be due to underlying connective tissue disorders may be foreign to many readers; however, it is a concept that is gaining support and familiarity. Research and concepts will be presented that will illustrate the dysfunction of connective tissue dynamics, due to underlying nutritional deficiencies, can and do lead to altered vascular and hemodynamics, and histological and functional changes in the blood and vascular system.

The vascular system measures 60,000 miles in length and is the largest organ in the human body. It contains more connective tissue than any other organ or organ

system in the body. The vascular endothelium is the largest endocrine organ and the largest organ in the body, with a 14,000 square foot surface area, the size of 6 1/2 tennis courts in square area. (1) It is a metabolically active organ with endocrine, paracrine, autocrine and intracrine functions. Maintaining the function of the vascular system, and the enormous amount of connective tissue comprised in the blood and vascular system, requires a constant supply and replenishing of key nutrients. Our research indicates the rate limiting nutrients in vascular connective tissue (CT) and health appear to be L-arginine, niacin, L-citrulline, copper, zinc, trace and macro minerals, vitamin C, bioflavonoids, proline, L-lysine, antioxidants, vitamin B-12, folic acid, vitamin B6, taurine, and N-acetyl cysteine (NAC), and endogenous fibrinolytic enzymes. A brief explanation of each and their role in vascular connective tissue health ensues below.

DISCUSSION

L-Arginine and Niacin

L-arginine has many vital functions in the human body. In connection with the cardiovascular system, 1 function is of particular importance: the amino acid arginine can split off a small molecule called nitric oxide (NO) (2). This tiny part of the former arginine molecule has a powerful role in maintaining cardiovascular health. NO relaxes the blood vessel walls and thereby helps to normalize high blood pressure. In addition, NO helps to decrease the adhesion of platelets and thereby has an anti-clogging effect. (3) L-arginine and NADPH are the substrates for nitric-oxide synthase, the enzyme responsible for the production of NO and, is, therefore, essential to maintaining proper vascular function. NADPH is formed from malate and NADP⁺ (nicotinamide adenine dinucleotide phosphate). NADP⁺ is formed from the co-enzyme forms of niacin, or nicotinic acid, which is NAD⁺ (nicotinamide adenine dinucleotide). Familiar to many will be the flushing associated with niacin therapy, as this indicates an increase in vasodilatation. It has been assumed in the past that this reaction was histamine or prostaglandin related, but it may be due to niacin's role in NO production. In animal models, dietary supplementation with L-arginine improves endothelium-dependent dilation, decreases platelet aggre-

gation and monocyte adhesion, and reduces the development of atherosclerosis. (3,4) Administration of L-arginine in humans at doses of 10 grams per day will increase coronary artery diameter, blood flow, reduce angina and improve peripheral blood flow and vascular disease symptoms. (1) The increase in diameter decreases pressure, increases flow, and decreases the coagulability of the blood. (5)

L-Citrulline

Although endogenous L-arginine plays an integral role in hemodynamics, attempts to supplement exogenously with L-arginine have had mixed and limited results. When supplementing to increase arginine, and ultimately NO levels, L-citrulline is an amino acid that may be superior to L-arginine in increasing nitrous oxide levels (vascular derived endothelial relaxation factor). (6) Ironically, L-citrulline appears to be able to increase serum arginine levels better than arginine itself and appears to sustain these levels for a longer period of time. The mechanism for this is thought to be due to the fact that orally ingested arginine is assimilated through the digestive tract and upon entering hepatic circulation, the bulk of it is degraded by the enzyme hepatic arginase. Whereas citrulline is not cleared by the liver; rather, it is taken up by the kidney and other tissues where it is converted to arginine, where it can act locally. (6)

Trace Elements (Copper and Zinc) and Trace and Macro Minerals

Lysyl oxidase (protein-lysine 6-oxidase) is a copper dependent enzyme (cupro-enzyme) that is essential for stabilization of extra-cellular matrixes, specifically the enzymatic cross-linking of collagen and elastin. (7) Lysyl oxidase activity appears to be influenced directly by the amount of dietary copper over a wide physiological range of intakes. Copper deficiency has been associated with vascular lesions that target the vascular lamina and endothelium. (8) The essentiality of copper in vascular connective tissue health has been further shown by observing that the deposition of aortic elastin is restored to near normal values after 48–72 hours of copper repletion. (7)

Zinc is required by over 300 enzymes in the body for optimal activity, and many of these deal with nucleic acid synthesis, protein metabolism and connective tissue repair and maintenance. Furthermore, copper and zinc work synergistically in the body and in large doses the 2 antagonize the absorption and utilization of the

other. Therefore, it is important when taking zinc or copper to take a balanced amount of the other. (1)

An inverse relationship between blood pressure and potassium (K^+), magnesium (Mg^{++}) and calcium (Ca^{++}) has been demonstrated. (9) The association of sodium (Na^+) and blood pressure may be multifactorial, and it may be the relative concentrations of other macro-nutrients in relation to Na^+ that ultimately determine its influence on blood pressure. It has also been noted that higher sodium consumption can actually be associated with lower blood pressure, suggesting that nutritional deficiencies and relative serum levels, or total body stores, of K^+ , Mg^{++} , antioxidants and essential fatty acids, and not excess sodium, cause hypertension. (10) For example, the K^+ to Na^+ ratio should be 5:1 (according to paleolithic studies); however, the hypertensive patient usually consumes a reversed ratio from 1:5 to 1:20 K^+ to Na^+ . This ratio, not the absolute amounts of each mineral, may be the ultimate important aspect when determining blood pressure. Magnesium competes with Na^+ for binding sites on vascular smooth muscle and acts like a calcium channel blocker, increase PGE, and binds cooperatively with K^+ , inducing endothelial vasodilatation and blood pressure reduction. There is a demonstrated and close relationship between Zn^{++} , vitamin D₃, Ca^{++} , Na^{++} , Mg^{++} and K^+ in various hormonal systems that modulate blood pressure. And every single 1 of these trace or macro minerals has been inversely correlated with blood pressure in numerous epidemiological studies. Furthermore, clinical trials using these nutrients individually have shown that on average, supplementation can reduce systolic blood pressure 5 mm Hg and diastolic blood pressure 2.5 mm Hg. (1) Greater effects on blood pressure are expected when these nutrients are taken in combination, in a balanced form that is physiologically bioavailable.

B-Vitamins and Iron

The role of B-vitamins, such as B-12 and folate in macrocytic, megaloblastic and pernicious anemia and iron deficiency in microcytic and hypochromic anemias are well-established. Especially important for final maturation of red blood cells are vitamin B-12 and folic acid. Both these vitamins are essential for the synthesis of DNA. The result of a deficiency on the hematological system is failure of nuclear maturation and division of erythroblastic cells of the bone marrow, which results in slowed proliferation of red blood cells and deformed red blood cells that are increased in size (macrocytic) and fragile. Pernicious anemia is caused by a lack of intrinsic factor or other gastro-intestinal disorders that interfere with folic acid and vitamin B-12 absorption. Iron inad-

equacy negatively effects hematogenesis by limiting the amount of available iron, a necessary component of the heme porphyrin ring of hemoglobin. This causes the RBC to shrink (microcystic) and to have a decreased oxygen carrying capacity, which results in a loss of hue (hypochromic). Although these are the major nutrients involved in heme synthesis, since blood is connective tissue, its formation is still dependent on trace amounts of zinc, copper, amino acids, etc.

Non-Denatured Whey protein isolates

Non-denatured, cold processed whey protein appears to provide dramatic support for healthy hemoglobin and hematocrit levels. It is important to understand that this type of whey protein is different than standard commercially available whey proteins because of the cold temperature extractions process, which does not interfere with the delicate nature of the protein molecules, leaving them more potent and bioavailable. Whey protein 'concentrates' are not whey protein 'isolates.' (11) It is unfortunate that the terminology of the industry evolved as it has, because it is very confusing. After removal of the casein protein, the product is filtered through membranes with the result being a product with anywhere from 35% to 85% protein. This is officially referred to by the dairy industry as whey protein concentrate (WPC). Whey protein isolates are obtained by other processing. Some of the processes employed to obtain whey protein isolate (WPI) are microfiltration, ultrafiltration, spray drying, ion exchange and other elite proprietary processes that are proprietary. (12) The processes employed in manufacturing WPI significantly affect 1) which bioactive proteins are isolated and preserved and 2) the quality of these proteins (ie, the degree of denaturation). WPI are high in bioactive compounds which have broad therapeutic effects. However, concerning the blood and vascular system, one compound of importance seems to be lactoferrin: lactoferrin found in WPI is very similar to transferrin which is found in the blood stream. (13,14) Lactoferrin possesses a number of valuable biological properties, including iron transport. Lactoferrin also has an extremely high affinity for iron and possesses the unique ability to transport iron and release it again at specific receptor cells in the colon. This allows iron to be absorbed (and thus be made available for hemoglobin and red cell production) without having to use high doses or oral iron supplements. Currently, studies are being performed with IMUPlus (a proprietary blend of whey protein isolates) to determine effects on hemoglobin. (15) The preliminary results, especially in cancer patients with depressed hemoglobin and hematocrit are dramatic. The mechanism for improvement may be a

synergistic effect involving a number of non-denatured proteins and through lactoferrin. Preliminary, dramatic results in cancer patients with Stage I, Stage II, Stage III and Stage IV cancer have been demonstrated – sample graphs (from Stage II and Stage IV cancers). (Figures 1, 2)

Vascular Disease

Dr. Matthew Rath, working with the late Dr. Linus Pauling, was one of the first to unequivocally show that vascular disease is the result of insufficient vitamin substrates to maintain and repair the vascular connective tissue and was also the first to provide evidence that by providing these and other key nutrients, vascular disorders, such as atherosclerosis and arteriosclerosis, could be reversed. (16) In one published study, Dr. Rath measured coronary artery calcium deposits in 55 patients by ultrafast CT (the most precise diagnostic technique available today to detect coronary artery disease already in its early stage). The results of this landmark study were published in the *Journal of Applied Nutrition* in 1996. The results of the study showed, "with vitamin therapy the growth of coronary artery deposits can be slowed down during the first half year and essentially stopped within the second half year. Thus, no heart attacks will develop." (16) In some patients, coronary artery calcification was shown to completely reverse. This vitamin program consisted of vitamin C, L-lysine, proline and various other nutrients to support vascular connective tissue. The exciting message brought by this work gives hope to the previous deterministic attitude that these conditions could not be adequately halted or even reversed.

Figure 1. Stage II cancer patient rise in hematocrit

**STAGE IV CANCER PATIENT
RISE IN HEMATOCRIT
(Cold Processed Whey Protein Varied Feedings Per Day)**

Figure 2. Stage IV cancer patient rise in hematocrit

Vitamin C

Vitamin C appears to be one of the most necessary nutrients to maintain optimal vascular health. The mechanism of Vitamin C's relationship to vascular health is its ability to increase the production of collagen, elastin, and other CT proteins in the body. Vitamin C is necessary for the hydroxylation of collagen. (17) Ascorbic acid, an effective reducing agent, maintains prolyl hydroxylase in an active form, probably by keeping its iron atom in the reduced ferrous state. Collagen synthesized in the absence of ascorbic acid is insufficiently hydroxylated and, hence, has a lower melting temperature. This abnormal collagen cannot properly form fibers and thus causes the skin lesions and blood vessel fragility that are so prominent in scurvy (the classical vitamin C deficiency disease). Like with other vitamins, however, we are finding suboptimal levels of vitamins can cause functional changes and subclinical and chronic symptoms and pre-disease states that eventually give rise to dysfunction, dysregulation and disease. Altered expressions of elastin and type II and IV collagen (major components of the endothelial basement membrane) are evident in states of suboptimal vitamin C intake, even as early as a few weeks. (17) There is strong evidence that vascular pathology and dysfunction is primarily the result of suboptimal intakes of vitamin C and key nutrients, and thus has been termed "chronic scurvy" by some leading nutritional researchers. Dr. Rath and Dr. Pauling published studies showing that guinea pigs fed a Vitamin C deficient diet develop vascular changes histologically identical to atherosclerosis and arteriosclerosis. The progression of Vitamin C deficiency to vascular disease might follow these terms:

Chronic vitamin C deficiency → Improper hydroxylation of collagen → Faulty connective tissue production (including that of the vascular endothelium) (18) → in the absence of adequate Vitamin C, the body tries to "plug" the damaged endothelium with cholesterol → lack of antioxidants and the inflammation from the damaged endothelium creates Reactive Oxygen Species (ROS) → ROS oxidize cholesterol and cause endothelial dysfunction → cholesterol gets embedded in the vascular wall, which the immune system attacks → combined with insufficient amounts of antioxidants, the inflammatory processes is further set in motion → cholesterol becomes oxidized, endothelial dysfunction ensues and vascular damage is the result → endothelial dysfunction, hypercoagulability, decreased vascular elasticity → thrombosis, and, or hypertension). (16,19,22,23).

Bioflavonoids

Bioflavonoids were once thought of as "vitamin C like compounds" which works synergistically with vitamin C, however they are unrelated chemically. Proanthocyanidins, anthocyanidins, rutin, hesperidin, quercetin, flavonones, flavanols, and related compounds are examples of bioflavonoids. These compounds are powerful antioxidants that work together with vitamin C and vitamin E in preventing damage to the cardiovascular system by free radicals and play a special role in supporting and stabilizing the integrity of the vascular endothelium. So much so, that bioflavonoids, which were once classified as an essential vitamin, and were called vitamin P, which stood for permeability factor because of their roles in preventing vascular hyper-permeability, but the chemical diversity of the 4,000–5,000 (depending on the source you consult) or so known flavonoids really precludes them from being considered a single vitamin. (24) Although vitamin C and the flavonoids are distinctly different nutrients, like many nutrients, they are more effective when they work together. Bioflavonoids have been shown to bind to elastin, the most important elasticity molecule of connective tissue, and protect elastin molecules against enzymatic degradation. Sources of bioflavonoids include certain herbs, such as ginkgo biloba, Hawthorne berry, bilberry, cherries, grape seeds, citrus plants, onions, and certain trees. They are present in virtually all plants in the pigments that give plants their characteristic colors. Fruits and vegetables are rich sources of flavonoids. The various bioflavonoid compounds found in ginkgo may play a protective role on the vascular and hematological system. Some of these effects include: vasoregulating activity of arteries, capillaries, and veins (increased blood flow); platelet activating factor (PAF) antagonism; thromboxane B₂ antagonism, clotting factor antago-

nism, homeostasis of inflammation and oxidative stress; and prevention of cell membrane damage caused by free radicals. (25–27)

Proline and L-Lysine

The amino acid proline is a major building block of the connective tissue proteins collagen and elastin. One-fourth to one-third of the collagen reinforcement rods, for example, are made up of proline. Therefore, it is easy to understand how important it is, for the optimum stability of blood vessels and the body in general, to get an optimum amount of proline in the diet. Proline is also very important in the process of reversing atherosclerotic deposits. As described previously, lipoproteins are attached to the blood vessel wall via biological adhesive molecules. Proline is a formidable “protective” agent, which can neutralize the stickiness of blood lipids, such as lipoprotein. The therapeutic mechanism is 2-fold. First, proline helps prevent the further build-up of atherosclerotic deposits. Second, proline helps to release already deposited lipids from the blood vessel wall into the blood stream. This can lead to a reversal of fatty acid deposits in the vascular wall. Proline can be synthesized by the body, but the amounts synthesized are frequently too little, particularly in those at an increased risk for cardiovascular disease.

As opposed to proline, lysine is an essential amino acid, which means that the body cannot synthesize this amino acid at all. Lysine has the remarkable property of augmenting collagen synthesis while preventing inappropriate cross-link formation. Lysine, like proline, is an important building block of collagen and of other connective tissue molecules and its intake helps to stabilize the blood vessels and the other organs in the body. The natural amino acids lysine and proline form a protective layer around the lipid molecules. This prevents the deposition of more lipid molecules in the artery wall and also releases lipoprotein molecules that had already been deposited there. The combined intake of lysine and proline with vitamin C is of particular importance for optimum stability of body tissue.

For optimum strength of the collagen molecules, the building blocks lysine and proline need to be biochemically modified to hydroxylysine and hydroxyproline. Vitamin C is the most effective biocatalyst to accomplish this “hydroxylation” reaction, thereby providing optimum strength to the connective tissue.

Antioxidants

An additional mechanism accelerating the development of atherosclerosis, heart attacks and strokes, is biological

oxidation. Free radicals damage the lipoproteins in the bloodstream and also the tissue of the artery walls. By doing so, they further extend the size of atherosclerotic plaques. (28) Furthermore, impairment of endothelial function by homocysteine is reversed by antioxidant vitamins. (29)

Optimal intake of antioxidants, such as vitamin C, magnesium, arginine and co-enzyme Q-10, help support arterial wall elasticity, dilation and relaxation. Oxidative stress may induce hypertension and oxidize LDL cholesterol, resulting in endothelial dysfunction and atherosclerosis. (1,30) Oxidized LDL leads to an increased expression of adhesion molecules on the endothelial cell surface, allowing for and potentiating monocyte infiltration and resulting in endothelial dysfunction. The increases in ROS inactivate nitric oxide (NO), or endothelium-derived relaxing factor, which is the major compound for vasodilatation in the arteries and can be responsible for vascular spasms, coagulation and hypersensitivity. (1)

Endothelial activation and dysfunction, oxidative stress and vascular smooth muscle dysfunction may be some of the first events that trigger essential hypertension, the leading cause of cardiovascular and cerebrovascular complications. (31) Hypertension is a disease of the blood vessel in which the vascular biology is altered, and, or dysfunctional. It appears that many changes in the vascular structure and function precede the onset of hypertension by decades and may underlie its cause. Reductions in arterial compliance with increased arterial stiffness occur before the onset of hypertension. Therefore, nutritional strategies that address vascular structure function and dynamics seem prudent in the prevention of vascular dysregulation/disease and in supporting optimal vascular structure and function.

Oxidative stress with an imbalance between reactive oxygen species (ROS) and the antioxidant defense mechanisms may contribute to the etiology of human hypertension, its initiation, maintenance, pathogenesis, pathophysiology and cardiovascular complications. In hypertensive models, an increase in reactive oxygen species (ROS) precedes the development of hypertension. Antioxidant administration has been shown to ameliorate some forms of hypertension. Oxidative stress has now assumed a major role in the initiation and perpetuation of hypertension. Essential hypertension is not only associated with oxidative stress, but the level of blood pressure is partially related to the level of oxidative stress. Oxidative stress may induce hypertension and oxidize LDL cholesterol, resulting in endothelial dysfunction and atherosclerosis. Oxidized LDL leads to

an increased expression of adhesion molecules on the endothelial cell surface, allowing for and potentiating monocyte infiltration and resulting in endothelial dysfunction. The increases in ROS inactivate nitric oxide (NO), or endothelium-derived relaxing factor, which is the major compound for vasodilatation in the arteries. Studies have demonstrated that hypertensive patients have an impaired endogenous and exogenous antioxidant defense mechanism and in addition, have more oxidative stress with more ROS produced and a greater than normal response to oxidative stress. Although serum ascorbic acid has the highest inverse association, of the antioxidants, with systolic blood pressure, a combination of various antioxidants have additive or synergistic effects in neutralizing ROS, increasing NO and improving endothelial relaxation, ultimately, lowering blood pressure.

Supporting the idea that connective tissue health underlies endothelial structure which gives rise to function, such as hemodynamics, studies have shown that the administration of only 1–2 grams of vitamin C per day can reduce systolic blood pressure 7–11 mm Hg and diastolic blood pressure by 4–6 mm Hg. Furthermore, studies have shown an inverse association between serum ascorbate levels and mean arterial pressure (MAP).

Coenzyme Q-10 is a potent lipid antioxidant, free radical scavenger, co-factor and coenzyme in mitochondrial energy production and oxidative phosphorylation that regenerates vitamin E, C, and A and inhibits the oxidation of LDL, membrane phospholipids, reduces triglycerides and total cholesterol and raises HDL. Studies have documented a deficiency of CoQ10 in 34% of patients with essential hypertension. Supplementation with 100–250 mg per day has demonstrated CoQ10's ability to lower systolic blood pressure an average of 15 mm Hg and Diastolic blood pressure, an average of 10 mm of Hg. In addition, heart rate decreases of an average of 20% are common with CoQ10 administration, showing increased vascular and cardiodynamic efficiency. It is important to note that these effects took time to reach their peak, about 4 weeks on average, and the antihypertensive effect is gone after 2 weeks of discontinuing therapy. Furthermore, studies also support that 50% of patients on antihypertensive drugs, who supplement with therapeutic levels of CoQ10 may be able to stop between 1 and 3 medicines – with both dose and frequency being able to be reduced. Finally, CoQ10 has favorable effects on other cardiovascular risk factors, such as serum lipids and carbohydrate dysmetabolism.

In summary, hypertensives appear to have a triad of increased ROS, an exacerbated response to ROS, and a

decreased antioxidant reserve which is amenable to antioxidant supplementation.

Homocysteine

The role of homocysteine (Hcy) in cardiovascular disease was first discovered over 30 years ago by then Harvard pathologist, Kilmer McCully, MD. (32) He was met with tremendous scorn and ridicule that almost ended his career and it has taken 20 years for his work to gain the respect and prominence it deserves. More than 75 clinical and epidemiological studies have shown a relation between total homocysteine levels and coronary artery disease, peripheral artery disease, stroke, or venous thrombosis. (33) Hcy is an intermediate product of the amino acid methionine and is metabolized by 2 pathways: *re-methylation*, which recreates methionine, and *trans-sulfuration*, which converts homocysteine into taurine. (34–37)

The *re-methylation pathway* transfers a methyl group to Hcy through methylcobalamin, an activated form of B-12 (which receives its methyl group from S-adenosylmethionine (SAdMe) or through 5-methyltetrahydrofolate (5-MTHF), an active form of folic acid) or betaine (Trimethylglycine). (37)

The structure most commonly identified as **vitamin B12** is cyanocobalamin; however, this molecule does not occur naturally, to any extent, in plants, microorganisms or in animals. (38) Although cyanocobalamin (CN-Cbl) and hydroxocobalamin are the supplemental forms of vitamin B-12 most commonly encountered, adenosylcobalamin (AdoCbl) and methylcobalamin (MetCbl) are the primary forms of B12 in the body and are the most metabolically active. (38) Nutritional imbalances, enzyme defects and pre-disease and disease states can lead to a decreased ability to convert cyanocobalamin to one of its active forms. One of the 2 active forms of B-12, **methylcobalamin**, only known biological function in humans is the remethylation of Hcy to methionine via the enzyme methionine synthetase. (38) Methylcobalamin performs its functions in the regeneration of methionine from Hcy by transferring its methyl group to Hcy by acting as a cofactor in the enzyme methionine synthetase. (39) Then, methylcobalamin needs to be regenerated and this requires 5-MTHF (the active form of folic acid). (39) Although absorption rates between B-12 and methylcobalamin appear similar, urinary excretion of methylcobalamin is about 1/3rd that of cyanocobalamin (the non activated form of B-12), possibly indicating greater tissue retention.

5-MTHF (5-methyl-tetra-hydra-folate) is an active form of folic acid and is more stable than folic acid and has a longer half-life in the body. (39) Furthermore, 5-MTHF readily crosses the blood-brain barrier and is slowly cleared, compared to folic acid, which is poorly transported into the brain, and once in the CNS is rapidly cleared. (39) Researchers have identified that up to 51% of individuals may be heterozygous, and 12% may be homozygous for a defect in the gene that encodes for methyltetrahydrofolate reductase (MTHFR), a critical enzyme needed to convert folic acid to its activated forms – a necessary process for Hcy reduction (40,41). Thus, up to 51% of the population is at an increased risk for cardiovascular disease and other complications of high Hcy. Consuming folic acid in its reduced form, therefore, may be desirable, and necessary in many cases.

The *trans-sulfuration pathway* of methionine/homocysteine degradation produces the amino acids cysteine and taurine, and is dependent on adequate levels of vitamin B6 and the hepatic conversion of B6 into its active form, **pyridoxal 5'-phosphate (P5P)**. (37,42) The most common genetic abnormality affecting the trans-sulfuration pathway is a deficiency of the P5P dependent enzyme, cystathione synthase. Fortunately, however, vitamin B-6 supplementation stimulates this enzyme, and when combined with betaine (Trimethylglycine), corrects any resultant hyperhomocystinemia. (37) Again, consuming vitamin B6 in its active form may be most desirable, and necessary in some cases.

Taurine is a unique amino acid because it carries a sulfonic acid group (-SO₃H) instead of a carboxyl group (-CO₂H) involved in homocysteine metabolism. (1) Taurine is widely distributed in foods of animal origin, with the exception of commercial milk and milk products, which contain very low levels. Taurine is biosynthesized from methionine or from cysteine via the trans-sulfuration pathway. As discussed previously, Hcy can be remethylated to form methionine; however, it can also be degraded to form cysteine. (37) This degradation involves a 2-step process resulting in the formation of cystathionine and its subsequent cleavage to cysteine. Both of the enzymes involved require pyridoxal-5'-phosphate as a cofactor, and the committed first step in the degradation of Hcy, cystathionine synthase, also requires **serine**. (43) In humans, defects in both of these enzymatic reactions occur. The absence, or low levels, of cystathionine synthase can also lead to abnormally-high levels of Hcy, especially when remethylation cofactors are also deficient.

Phosphatidylcholine (PC) is a primary component of **lecithin** and is sometimes referred to as pure lecithin. It is the most frequently encountered phospholipid in animals and is structurally related to phosphatidylserine and phosphatidylethanolamine. PC consists of a glycerol backbone that is esterified with fatty acids on carbon atoms 1 and 2, and with a phosphoric acid/choline complex in position 3. Although phosphatidylcholine is usually referred to as if it were a single compound, it is actually a group of related compounds which vary depending upon the fatty acid composition at position C-1 and C-2. Dietary **choline** is derived primarily from PC after absorption by the intestinal mucosa and then metabolized to choline in the liver by the enzyme phospholipase D. Most choline is re-phosphorylated to PC; however, a small amount is carried to the brain via the blood stream, where it is converted to the neurotransmitter acetylcholine. If PC or choline are lacking in the diet they can be synthesized from phosphatidylserine and phosphatidylethanolamine. (Note: phosphatidylserine and phosphatidylethanolamine are inter-convertible by means of decarboxylation reactions which require pyridoxal-5'-phosphate). Synthesis of PC is dependent on the availability of SAME as a methyl donor, since synthesis involves the transfer of methyl groups from 3 SAM molecules to phosphatidylethanolamine in order to generate one molecule of PC.

N-acetyl cysteine (NAC) is a source of sulfhydryl groups that can be utilized in the trans-sulfuration pathway. NAC also induces nitric oxide synthase messenger RNA, transcription and protein expression, resulting in elevated NO levels and endothelial relaxation. Furthermore, studies have documented NAC's ability to lower homocysteine and lipoprotein (a). (1,44,45)

In animal studies, a disturbance in the metabolism of either of these 2 methyl-donor pathways, due to limited availability of either choline, or folates and vitamin B12, has a direct impact on reducing the levels of nutrients in the coexisting pathway since now more of a drain will be placed on the other pathway as a source of methyl groups. For example, rats fed diets deficient in choline and methionine have hepatic folate concentrations half that of controls after 5 weeks. During choline deficiency, hepatic SAME concentrations have also been shown to decrease by as much as 50%. Similarly, THF deficiency results in decreased hepatic total choline levels.

Evidence has mounted that individuals with in the lowest quartiles for folate and vitamin B-12 have significantly elevated levels of homocysteine. (37) And those in the lowest quartile of pyridoxal-5-phosphate (B-6)

also have increased homocysteine levels. (42) Increased serum homocysteine levels are significantly correlated with an increased risk of coronary artery disease (CAD), myocardial infarction, and vascular occlusive disease (45–48). Studies have shown that the odds ratio for CAD increases as plasma homocysteine increases, even within the normal range. As little as a 5 $\mu\text{mol/L}$ increase in plasma homocysteine results in an increase in the odds ratio for CAD by 2.4. In a cohort evaluation of the Framingham Heart Study, serum folate and P5P (vitamin B6) were inversely correlated with homocysteine levels and an increase risk of vascular artery stenosis (32,49,50). Furthermore, lower P5P and vitamin B-12 have been linked with hyperhomocystinemia and significant increased risk of CAD. Elevated Hcy levels have been established as an independent risk factor for deep vein thrombosis and intermittent claudication (IC) as well. (37) A 4-fold increase in peripheral vascular disease has been noted in individuals with hyperhomocystinemia. Up to 50% of patients with peripheral atherosclerotic vascular disease have high homocysteine levels, and up to 100% may have abnormal levels after a methionine load challenge. Stroke patients have significantly elevated homocysteine levels compared to controls and a linear relationship between stroke risk and homocysteine levels has been demonstrated (37,51) Homocysteine (Hcy) harms the vascular endothelium in a variety of ways, impairing the ability of the endothelium to maintain homeostasis. Hcy increases platelet aggregation and thrombosis through enhanced thromboxane synthesis and inactivation of anticoagulant substances. (see Table 1)

Hcy increases oxidative stress by increasing superoxide production. It also increases leukocyte-endothelium interactions. In addition, Hcy down-regulates nitric oxide

(NO) production and acts to increase proliferation of vascular smooth muscle. Well documented in hyperhomocystinemia is impaired endothelium-dependent, flow-mediated vasodilatation. Even moderate elevations of plasma Hcy impair endothelial function by activating coagulation, modifying the adhesive properties of the endothelium, and impairing the vascular responses to L-arginine. (59,60)

All major cardiovascular risk factors are associated with impaired endothelial function, which precedes the appearance of atherosclerosis. A study in *JAMA* demonstrated that by lowering Hcy, a 60% decrease in vascular disease is realized. Studies looking at heart disease and Hcy levels have shown that those with levels in the highest 1/3rd, have 2/3rds of cardiovascular events. (32) Hcy is thought to cause these negative cardiovascular events by directly damaging the endothelium, causing vascular hyperplasia and fibrosis and directly promoting the oxidation of LDL cholesterol and conversion of macrophages into foam cells. All this leads to decreased arterial plasticity and increased peripheral resistance and clotting. An estimated 10–20% of all cardiovascular disease could be eliminated by simply lowering elevated Hcy levels, and studies show that simple key vitamins, such as folate and B12, are quite capable of doing so. These studies show a clear correlations between vitamin status and Hcy levels and that a combination of folate, B6, and B12 can lower Hcy by 50% (61–63,55,64–68).

Antiphospholipid Antibody Syndrome (APS)

Antiphospholipid antibody syndrome (APS) is both a clinical and laboratory phenomena that may impact vascular health and dynamics and may increase the risk of CVA in association with manipulation. Clinical findings include thrombocytopenia, leukopenia, chronic fatigue, fibromyalgia, gulf war syndrome, systemic lupus erythematosus (SLE) neurological complaints, and thrombosis. However, APS can affect any organ in the body and show a broad spectrum of manifestations. (69,70) Laboratory findings include anticardiolipin antibodies, lupus anticoagulants, antiphosphatidylserine antibodies, and anti-B2GPI antibodies. (71) APS has also been highly associated with SLE and a variety of thrombotic manifestations and a higher frequency of stroke. The cause of APS is thought to stem from latent infection (mainly the enveloped viruses) bacteria, fungi, mycoplasma, toxic chemicals, physical trauma, allergens, and hereditary defects in the coagulatory regulatory proteins, prothrombin gene mutation or hyperhomocystinemia.

TABLE 1
RISK FACTORS FOR ELEVATED
HOMOCYSTEINE (52-58,37)

- DECREASE INTAKE OF B-VITAMINS, PARTICULARLY B-6
- COFFEE CONSUMPTION
- SEVERE COMPLICATIONS DURING PREGNANCY, SUCH AS NEUROTUBE DEFECTS
- COGNITIVE DYSFUNCTION
- CANCER
- VASCULAR DISEASE
- SEDENTARY LIFESTYLE
- MALE
- 65 YEARS OF AGE
- DEPRESSION, MOOD OR MENTAL DISTURBANCE
- ALCOHOLISM
- HEART DISEASE
- NIDDM
- MULTIPLE SCLEROSIS
- STROKE
- SMOKER

Exposure and expression of phosphatidylserine (PS) is part of the infectious process that activates the immune system to form antiphospholipid antibodies, whose target is the protective proteins of endothelial cells. Endothelial cells are protected by B2GPI and other proteins, which are a target of antibodies in APS. This protection helps endothelial cells maintain hematological and clotting homeostasis. With the loss of this protective mechanism in place, antiphospholipid antibodies allow coagulation proteins to bind, react and to form thrombin. This can lead to an excess conversion of fibrinogen to fibrin, and, hence, a hypercoagulable state due to immune upregulation – APS is also called immune system activation of coagulation (ISAC). These patients respond well to oral anticoagulant therapy.

Thrombi form when fibrin accumulates in blood vessels. Thrombolytic enzymes are normally generated in the endothelial cells of blood vessels; however, as the body ages, production of these enzymes decline. Furthermore, in APS, this production is diminished, making the blood more prone to coagulation and fibrin accumulation in the vascular endothelium. This may set the stage of a proinflammatory, hypercoagulable state that when the force of a cervical adjustment transmits to the vertebral artery, cause intima tearing, clotting, thrombi and thrombo-emboli. In other words, hypercoagulability due to APS and decreased thrombolytic activity may be an underlying factor in CVA associated with manipulation.

Some of the endogenous and exogenous agents involved in clotting homeostasis are plasmin, heparin, coumarin /warfarin, and T-PA. However, these agents either have limitations, and, or serious side effects. A brief discussion on each ensues: **plasmin** is an endogenous protease that resembles trypsin (a digestive protease), and is the activated form of plasminogen. It is able to digest fibrin fibers; however, its limitation is that it is slowly activated, and therefore, slow acting, and works better to dissolve small clots, rather than larger ones. **Heparin**, is a drug given IV that acts as an anticoagulant by inhibiting thrombin. Heparin is often associated with side effects, such as increased bleeding and hemorrhage. **Coumarin /warfarin** is a drug that works by competing with vitamin K for reactive sites in the enzymatic process for the formation of prothrombin and clotting factors, thereby blocking the clotting action of vitamin K. Another drug used to manipulate this process is **T-PA** (Tissue Plasminogen Activator), which is effective in activating plasminogen to plasmin. Although given as an IV drug, this is also a naturally occurring substance produced endogenously, albeit in much smaller amounts than used as medical therapy.

Streptokinase is another enzyme, derived from beta-hemolytic streptococci, that is used as a medical therapy. In addition to serious side effects and limitations, these medical therapies lack true utility in that to be therapeutic, they must be injected.

There has been a renewed interest in discovering and the possibility of using oral enzymes. Fibrinolytic therapy by oral administration of enzymes was investigated by Sumi and co-workers 10 years ago in an animal model where enteric-coated urokinase (UK) capsules were given to normal and experimental dogs with saphenous vein thrombosis. Previous findings indicated that intravenous (IV) administration did not show any clear thrombolytic effect, but that oral administration enhanced the fibrinolytic activity, serving as a treatment to lyse the thrombi in a mild but maintained way. This supported the idea that oral enzyme agents can and do have systemic fibrinolytic properties. The underlying mechanism of such fibrinolytic therapy by oral administration was then confirmed by basic research to involve absorption of the administered UK across the intestinal tract, and release into the blood of endogenous plasminogen activator which originated from the liver and/or endothelial cells. The enteric coated UK capsules (60,000 U/day for 7 days) also exhibited a clinical efficacy against cerebral thrombi. Moreover, greater results were obtained in double-blind tests at multicenter trials employing a dose of 120,000 U/day for 7 days.

Recently, a natural, enzyme, potent in the oral form, has been discovered, researched and has been found to be a most promising agent not only in reversing clots, but in preventing them and modulating the clotting process in ways that help reestablish homeostasis. (72) This enzyme is called nattokinase, and has been called “the enzyme of enzymes” because it may serve as a non-specific activator of most endogenous enzymes [possibly by cleaving the CH3 terminus of zymogens (pre-made enzymes in their inactive form)]. (73) **Nattokinase** is a serine endopeptidase with a molecular weight of 20,000 Daltons and a point of ionization (pI) of 8.6. This isolated fibrinolytic enzyme, derived from a traditional Japanese food, natto, was discovered by Dr. Hiroyuki Sumi and resembles plasmin. Nattokinase (NK), an enzyme derived from natto—a typical and popular fermented soybean food in Japan derived from vegetable cheese (boiled and fermented soybeans with a special bacterium, *Bacillus subtilis natto*) – shows potent fibrinolytic activity and was discovered by Dr. Hiroyuki Sumi while doing research work at the University of Chicago’s Medical School. (74) Dr. Sumi was conducting research on about 200 kinds of food, includ-

ing several types of liquors, from all over the world in search of natural substance that could dissolve and even prevent blood clots. He found that natto had the highest fibrinolytic activity among all those foods tested. In 1986, Dr. Sumi presented the results of his research in Japan for the first time. Natto has a long cultural history, extending back more than 1,000 years. (75) Traditionally, it was also used as a folk medicine for heart and vascular diseases, to relieve fatigue and as an anti-beriberi agent. Dr. Sumi said of nattokinase, that it "shows a potency matched by no other enzyme." Nattokinase is extremely stable to variations in temperature, and acid and alkali milieu. Currently, Nattokinase has been the subject of 17 research studies, including 2 small human trials. In 1 such study, thrombi were induced in dogs, who were either given Nattokinase or placebo. Angiogram confirmation showed that the dogs who received the enzyme regained normal circulation within 5 hours of treatment, whereas, the placebo group continued to have demonstrable evidence of thrombus occlusion even after 18 hours. (76) In another study done in Japan to compare nattokinase's and plasmin's ability to dissolve thrombus in the carotid arteries of rats, animals treated with the nattokinase regained 62% of their blood flow, whereas, the animals treated plasmin regained 15.8% of their blood flow. (76) While the human body contains several enzymes that promote the creation of blood clots, it only produces 1 that can dissolve clots (plasmin), and the production of that enzyme diminishes as we age. It is interesting to note that oral nattokinase in this study was more effective than plasmin in dissolving thrombi. (77,78) Research indicates that the mechanisms of nattokinase stems from its close resemblance to endogenous plasmin, it appears to be able to dissolve fibrin directly and it may enhance the body's own production of plasmin. Furthermore, nattokinase appears to have ACE (angiotensin converting enzyme) inhibitor activity and in studies has been able to lower systolic blood pressure up to 11% and diastolic pressure up to 9.7%.

Nattokinase produces a prolonged action (unlike anti-thrombin drugs that wear off shortly after IV treatment is discontinued) in 2 ways: it prevents coagulation of blood and it dissolves existing thrombus. Both the efficacy and the prolonged action of NK can be determined by measuring levels of euglobulin fibrinolytic activity (EFA) and fibrin degradation products (FDP), which both become elevated as fibrin is being dissolved. By measuring EFA & FDP levels, activity of NK has been determined to last from 8 to 12 hours. An additional parameter for confirming the action of NK following oral administration is a rise in blood levels of TPA antigen (tissue plasminogen activator), which indicates a

release of TPA from the endothelial cells and/or the liver.

CONCLUSION

Cholesterol, triglycerides, low density lipoproteins (LDL), lipoprotein (a) and other metabolic products are ideal repair factors and their blood levels increase in response to key nutrient deficiencies that lead to an oxidation, weakening and damaging of the artery walls. A chronic weakness of the blood vessel walls increases the demand for production of these repair molecules in the liver. An increased production of cholesterol and other repair factors in the liver increases the levels of these molecules in the bloodstream and, over time, renders them risk factors for cardiovascular disease. Thus, the primary measure for lowering cholesterol and other secondary risk factors in the bloodstream is to stabilize the artery walls by restoring the missing nutritional factors and antioxidants involved in connective tissue production, maintenance and repair, and thereby lower the metabolic demand for increased production of these risk factors inside the body itself. Furthermore, these repair factors themselves damage the endothelium and alter endothelium dynamics and promote an oxidative-inflammatory process. Over time, this reduces the integrity and elasticity of the endothelium with resultant increases in mean arterial pressure, atherosclerotic plaques, stenosis and thrombosis. Key nutritional strategies, aimed at supporting the structure of the endothelium and quenching the inflammation/oxidation, instead of treating the symptoms, are warranted and deserve further investigation.

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