Clinical Laboratory Indicators of Cardiovascular Disease Risk

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Abstract

The focus of this review is to explain the clinical significance of laboratory markers that are not used routinely, as well as standard laboratory tests (triglycerides, cholesterol, HDL, and LDL) to evaluate cardiovascular disease risk. Cardiovascular disease is the number one killer in the United States. According to national statistics, there are over 40 million people (men and women) that suffer with symptoms of heart disease. The current medical interventions in cases of advanced cardiovascular disease are Percutaneous Transluminal Coronary Angioplasty (PCTA) and Coronary Bypass Graft (CABG). The number of performed PCTA and CABG procedures can be drastically reduced if clinically-significant preventive risk markers are used coupled with appropriately designed therapeutics. The clinical significance of the following risk markers will be discussed in this review: Homocysteine, Lipoprotein (a), Fibrinogen, Lipid Peroxide, Anti-oxidative LDL antibody, Triglyceride, Total Cholesterol, LDL, VLDL and HDL. (*Alt Med Rev* 1996;1(3):185-194.

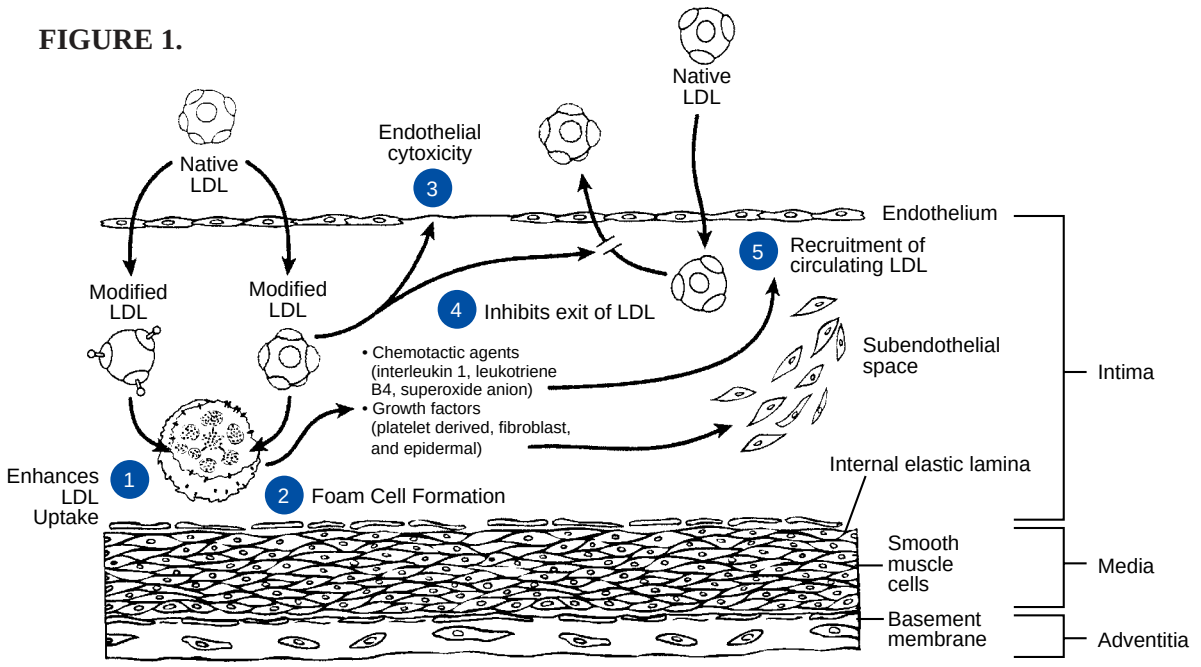
Implications of Lipids in Atherosclerosis

Atheroslcerosis-initiated diseases are one of the major causes of death in the US. Atherosclerosis is a disorder of the arteries that underlies most cardiovascular and cerebrovascular diseases.

The anatomy of an arterial wall shows three layers: adventitia, media, and intima. The outer adventitia consists of loosely interwoven mixture of collagen, elastic fibers, a mixture of smooth muscle cells, and fibroblasts. The middle media mainly consists of smooth muscle cells surrounded by small amounts of collagen and elastic fibers. The inner intima is made of a continuous layer of endothelial cells.

The formation of atherosclerosis is a process, wherein deposits accumulate on the inside of arterial walls resulting in narrowing or clogging the arterial lumen. Intimal layer injury attracts cholesterol deposits, macrophages and calcium salts, which leads to formation of scar-like fibrous tissue forming an atherosclerotic plaque. The occlusion or blocking of the arteries occurs either by large atherosclerotic plaques or thrombosis. Thrombus formation is frequently due to secondary formation of blood clots by platelets blocking the flow of blood. The formation and accumulation of what are known as foam cells in the intima is the hallmark of the early atherosclerotic lesion. Currently it is believed that foam cells are derived from macrophages and smooth muscle cells. The development of the atherosclerotic lesion is promoted by two key blood cells, macrophage and platelets. The macrophage can secrete chemotatic agents (IL-1, superoxide anion, Leukotriene B4) and growth factors (IL-1, Platelet Derived Growth Factor,

FIGURE 1.



Fibroblast Derived Growth Factor, Transforming Growth Factor). (See Figure 1.) These two macrophage-derived groups are probably responsible for the promotion of connective tissue proliferation in the blood vessel during the disease process. The platelets play a smaller role in the formation of a thrombus in response to injury.

Cholesterol

All tissues of the body are capable of synthesizing cholesterol, however, cholesterol is mainly manufactured in the liver and intestines, or obtained through the diet. The normal adult synthesizes about one gram of cholesterol per day, and consumption varies greatly according to individual dietary habits. An increase in the amount of consumed cholesterol can lead to decreases in cholesterol synthesis by the body. Diets that are high in saturated fat provide acetyl-CoA to the liver for synthesis of cholesterol. Insulin and thyroid hormone deficiencies are associated with increases in HMG CoA reductase activity and cholesterol levels. Unoxidized cholesterol is incorporated in every cell membrane as a protective factor against the biological oxidation process. Increased circulating cholesterol can increase the risk of oxidative disease process due to cholesterol's susceptibility to oxidation while in circulation.

Cholesterol is insoluble in water, therefore it is transported in the blood and extracellular fluids conjugatied to proteins, called apolipoproteins. This cholesterol and protein complex is known as a lipoprotein. The Lipoproteins are broadly classified into chylomicrons, very low density lipoprotein (VLDL), low density lipoprotein (LDL), intermediate density lipoprotein (IDL), and high density lipoprotein (HDL), based on their densities. The above groups also vary in their dimensions, cholesterol carrying-capacities, and their function. Total cholesterol values are categorized as: desirable <200 mg/dL, borderline-high 200-239 mg/dL, and high >240mg/dL.[1]

Lipoprotein (a)

Lipoprotein (a) is an atherogenic lipoprotein that resembles LDL cholesterol. Repeated studies demonstrate a correlation with elevated lipoprotein and cardiovascular disease. Survivors of myocardial infarcts have higher Lp (a) than do control groups without history of MI or cardiovascular disease. These studies conclude that higher levels of Lp (a)

serve as a risk factor for coronary heart disease and atherosclerosis. Another interesting correlation is that high levels of Lp (a) occur in patients with cardiovascular disease but without the presence of atherosclerotic lesions. This suggests that Lp (a) could also contribute to the pathogenesis of cardiovascular disease by a mechanism other than atherosclerosis. The common thread of most of these studies pertaining to elevated levels of Lp (a) is that Lipoprotein (a) is an independent risk factor for the development of atherosclerotic disease. Lipoprotein (a) is a heterogeneous family of macromolecular particles consisting of an apolipoprotein (a) molecule joined by disulfide linkage to apolipoprotein B-100 which is solidly anchored in a lipid-rich LDL-like core. This lipoprotein is found in the density range of 1.005 to 1.085 g/ml, and is composed of 27% protein, 65% lipid, and 8% carbohydrate. In several studies Lipoprotein (a) levels were increased in individuals with atherosclerosis, coronary heart disease, and ischemic heart disease. Collective studies demonstrate Lp(a)'s mechanisms of involvement in the atherogenic process. These include inhibition of fibrinolysis, proliferation and migration of smooth muscle cells, uptake of Lp(a)-glycosaminoglycan complex by macrophages, and the formation of an insoluble LDL-Lp(a) complex in the presence of calcium ions. Lp(a) measurements are helpful because they can clarify a potentially important contribution to the measured "LDL-cholesterol" value. In addition, because Lp(a) levels are not lowered by a number of treatments that effectively lower LDL levels, Lp(a) measurements can sometimes reveal why a particular patient may be less responsive to LDL-lowering therapy. There is increasing evidence that high Lp(a) levels (greater than 300mg/L) are associated with an increased risk for Coronary Heart Disease (CHD). The laboratory ranges for Lipoprotein (a) are reported as: normal <20 mg d/L, borderline 20-30 mg/dL, and elevated >30 mg/dL.[2,3]

Very Low Density Lipoproteins

Very low density lipoproteins are synthesized in the liver, and contain high concentrations of triglycerides and moderate concentrations of cholesterol, phospholipids, and apolipoproteins. VLDLs obtain fatty acids from fat stored in adipose tissue, from carbohydrate-derived lipids, and from hydrolysis of lipoprotein triglycerides in liver. Production of VLDL is regulated by the availability of fatty acids, and apo B-100. The availability of apo B-100 is important for the formation of VLDL. The dimensions of VLDL particles are determined by the nature of dietary fatty acids viz., saturated, monounsaturated, or polyunsaturated fatty acids. VLDL concentration is elevated with high carbohydrate diet, and cholesterol content of VLDL increases with a diet that is high in cholesterol. The density of VLDL is similar to IDL in patients with familial hypercholesterolemia or individuals with a high cholesterol diet. The cholesterol-rich VLDL are engulfed by macrophages and endothelial cells, resulting in the formation of macrophage-derived foam cells which are found in atherosclerotic plaques. VLDL particles are smaller than chylomicrons and are rich in triacylglycerides. When excessive amounts of VLDL are present, the plasma is usually turbid.

The value of laboratory analysis of VLDL lies in its ability to identify whether triglyceridemia is caused by excessive carbohydrate consumption. Usually VLDL's represent about 10-15% of the total circulating lipoproteins in a normal healthy individual. Individuals that have a higher consumption of carbohydrate may have an unusually higher induction rate of VLDL synthesis. The breakdown of VLDL leads to the formation of the cholesterol-rich particle LDL. The half-life of

VLDL is 1-3 hours. The normal reference range for VLDL is 1-23 mg/dL. [4-6]

Low Density Lipoproteins

Low density lipoproteins are cholesterol-rich particles with a life span of about 2.5 days. LDL are composed of 10% triglycerides, 23% phospholipids, 45% cholesterol, and 20% proteins made up of 95% Apo-B; LDL formation occurs primarily from the catabolism of VLDL. LDL (which has a density of 1.019-1.063 g/ml) is capable of carrying the highest concentration of cholesterol and phospholipids, hence it is the major transport vehicle carrying cholesterol to the tissues. All cells of the body carry receptors to the apolipoprotein B site of LDL which facilitate the transport of cholesterol to the inside of the cell through a process called pinocytosis. The number of these receptors is regulated by the cholesterol content of the cell, although this receptor-mediated LDL regulation may be bypassed at very high blood cholesterol levels. During these circumstances, LDL enters the cell by a nonspecific endocytic process called "bulk phase pinocytosis". When the plasma level of LDL rises, macrophages which are part of the scavenger system (reticuloendothelial system), degrade the increasing amount of LDL. When the system is overloaded with cholesteryl esters, they are converted into foam cells. In humans, estimates of the proportion of plasma LDL degraded by the LDL receptor system range from 33-66%. The remainder of the degradation is by the reticuloendothelial system (macrophages) and perhaps other mechanisms not yet elucidated. The laboratory ranges for LDL are: desirable <130 mg/dL, borderline 131-160 mg/dL, and high >160.[1,5]

Anti-Oxidative LDL Antibody

Oxidized low density lipoprotein is believed to play a critical role in the development and progression of atherosclerosis. Accumulation of oxidative LDL in macrophages and smooth muscle cells causes foam cell formation, an initial step in the disease process. Recent evidence suggests that autoantibodies against oxidatively-modified LDL can be used as a parameter that consistently mirrors the occurrence of oxidation in vivo. In fact, elevated levels of autoantibodies against oxidative LDL have been detected in the blood stream of patients with coronary artery disease. Recent studies indicate a correlation between autoantibodies against oxidative LDL and the progression of carotid atherosclerosis. Increased serum concentrations of oxidative LDL antibodies have also been described in various disease processes such as pre-eclampsia and SLE. Early stages of atherosclerosis are characterized by penetration into the arterial intima by both T lymphocytes and monocytes. Some of these T lymphocytes show signs of activation, though the mechanisms for such activation is not entirely known. The monocytes develop into macrophages and subsequently in foam cells filled with oxidized-LDL derived lipids. The presence of autoantibodies against oxidative-LDL is correlated with the evolution of atheromatous plaque. Therefore, laboratory assessment of Anti-oxidative LDL antibody can be an early marker of atherosclerotic disease process.[7-20] (See Figure 1.)

High Density Lipoproteins

Known in the medical literature as the "scavenger form" of cholesterol, HDL's complete role in protection against oxidative processes is not fully understood. The plasma half life of HDL is between 3-5 days. Nascent HDL molecules are synthesized in the liver and gut mucosal cells; HDL is also formed from the catabolism of chylomicrons and VLDL. HDL is composed of 2% triglycerides, 30% phospholipids, 18% cholesterol, and 50% proteins, comprised primarily of Apo A-I (65%) and Apo A-II (25%). HDL participates in the regulation of triglyceride catabolism and

cholesterol ester synthesis by providing apo C-II for activation and apo C-III for inhibition of lipoprotein lipase activity. The apolipoprotein content of HDL is dependent on what it is synthesized from. Apo E is the major protein component of nascent HDL, while apo A is the major component of plasma HDL. HDL interacts with the plasma enzyme lecithin cholesterol acyltransferase (LCAT), which esterifies the excess free HDL with fatty acids derived from lecithin, the major phospholipid of plasma. The newly synthesized cholesteryl esters are transformed back to IDL from HDL, while excess cholesterol in the liver is excreted as bile acids. Persons with LCAT deficiency have an accumulation of these cholesterol-ester-deficient particles in plasma. The laboratory ranges for HDL are: low <34 mg/dL, and desirable >35 mg/dL.[5]

Triglycerides

The typical North American diet derives more than 40% of its calories from dietary fat. Fatty acids are equally as efficient as glucose in meeting the energy needs of the body. Triglycerides (also known as neutral fats) consist of long-chain fatty acids, typically palmitic, stearic, and/or oleic acids, bound to glycerol. These lipids, in the form of chylomicrons, initially enter the circulation through the lymphatic system, which eventually dumps them into the aorta via the thoracic duct. Triglycerides are released from chylomicrons by lipoprotein lipase. The free fatty acids enter adipose and liver cells, where they are reesterfied with glycerol and stored. Adipose cells have the capacity to utilize up to 95% of their cell volume to store triglycerides.

Fatty acids are transported by carnitine into the mitochondria, where they are released to generate energy. Fatty acids form acetyl Co enzyme A (acetyl CoA) after a sequence of reactions, collectively known as beta-oxidation. Acetyl CoA reacts with oxaloacetic acid to enter the citric acid, or Kreb's, cycle resulting in the release of ATP. Although there are many links between high triglyceride levels and CHD, our understanding of the risk relationship is poor. Reducing triglyceride levels to prevent CHD has not been generally advocated and is seemingly much less important than reducing LDL-cholesterol levels. Normal reference values for triglycerides are: Male - 40-160 mg/dL, and Female - 35-135 mg/dL.[1,5]

Apo A-1

Apo A-1 constitutes 75% of the total Apo A in HDL. It is synthesized in the liver and intestine and is an activator of the enzyme LCAT(lecithin acyltransferase), which esterifies cholesterol in the plasma. Apo A-1 has demonstrated the ability to activate lipolytic enzymes that can modify lipoprotein composition and structure which, in turn, contributes to the "scavenging" ability of HDL. Increased activity of Apo A-1 is desirable in order to reduce cardiovascular risk. The reference range for Apo A-1 is 98-166 mg/dL, however, this may vary depending upon the laboratory doing the test. [5]

Apo B

Apo B is the major protein (95%) of LDL and also constitutes about 40% of the protein moiety of VLDL and chylomicrons. Apo B has been shown to modulate cholesterol synthesis and degradation. At this time, it appears that it is undesirable to have increased Apo B activity. Recent research has shown that the ratio of serum Apo A-1 to Apo B has a high correlation to the degree of coronary blockage. This correlation is considerably higher than that reported for HDL, LDL, or for the HDL:total cholesterol ratio. Reference ranges for Apo B are 41-124 mg/dL although this may vary depending upon the laboratory doing the test.[1,5]

Homocysteine In Cardiovascular Health

Homocysteine is recognized as an independent risk factor for atherosclerotic vascular disease due to its damaging effects to vascular endothelial cells, resulting in lipid deposition and plaque formation. Damaged endothelial cells attract blood platelets which form clots, in part, as a result of the production of thromboxanes.[21]

Genetic, dietary and lifestyle factors accelerate conversion of methionine to homocysteine, and homocysteine to homocysteine thiolactone. Homocysteine thiolactone reacts with the free amino groups of LDL, causing aggregation and increased uptake of LDL by macrophages, which results in lipid deposition. Homocysteine thiolactone released within the vascular wall from this homocysteine-LDL complex promotes oxidation of cholesterol and unsaturated lipids, platelet aggregation, thrombogenic activity, glycosaminoglycan disruption, fibrosis, intimal injury, and calcification of atherosclerotic plaques. Homocysteine also stimulates proliferation of smooth-muscle cells, a key component in atherosclerosis. Recent studies have shown that even moderately-elevated homocysteine levels are correlated with an increasing risk of myocardial infarction, cardiovascular disease, and stroke.[21,36-42]

The sulfhydryl (thiol) group of homocysteine is a major contributor to atherogenic affects by facilitating the generation of hydrogen peroxide from oxygen. Hydrogen peroxide damages endothelial cells, leading to attenuation of cellular antithrombotic and vasodilatory properties. Endothelial cells secrete an endothelium-derived relaxing factor (EDRF) and other oxides of nitrogen, which react with thiols, forming S-nitrosohomocyteine. These compounds induce vaso-relaxation and platelet inhibition, and suppress hydrogen peroxide generation and subsequent endothelial damage. It has been hypothesized that the inability of endothelial cells to secrete and maintain adequate amounts of EDRF may, in the event of elevated homocysteine levels, lead to endothelial cell damage.

Most patients suffering from premature vascular diseases are heterozygous for homocysteinuria, with the estimated prevalence of the heterozygous state being 10-15% of the population.[22-26] Mild or moderate homocysteinemia can be identified and treated. Plasma homocysteine laboratory reference values are 4-16 nM/L, and levels >200 indicate possible metabolic disease processes.

Vitamin B6, B12 and Folic Acid Deficiencies and Hyperhomocysteinemia

Folic acid deficiency is one of the major causes of elevated homocysteine levels in the homocysteinemic patient. Oral folic acid therapy reduces elevated plasma homocysteine levels in these patients and has also been shown to decrease plasma homocysteine concentrations in normal, non-folate-deficient individuals, which may also significantly reduce future risk of vascular diseases.[27-32]

Cobalamin deficiency can contribute to hyperhomocysteinemia since it is involved, along with folic acid, in the remethylation of homocysteine to methionine. Cobalamin supplementation is only effective in reducing homocysteine levels in cobalamin deficient subjects, and does not compensate for other vitamin deficiencies.[28,30,33,34]

The catabolism of homocysteine through cystathionine synthesis requires pyridoxal 5' phosphate as a coenzyme. As with B12-deficient individuals, administration of high doses of pyridoxine was found to have homocysteine-lowering effects in only a small percentage of homocysteinemic patients, possibly due to the existence of other vitamin deficiencies. [29,33,35]

Lipid Peroxides in Cardiovascular Health

Lipid peroxides are breakdown products of polyunsaturated fatty acids (PUFAs), formed when either free or bound PUFAs are attacked by free radicals. The free radicals pull away an electron from PUFAs during this process. The peroxides react with neighboring PUFAs until they encounter an electron donor, such as alpha-tochopherol (vitamin E) or other antioxidants. This chain reaction of PUFA's can compromise cell membrane integrity, leading to cell death. In addition, when reacted with metal ions such as iron or copper, lipid peroxides may form toxic aldehydes such as malondialdehyde and 4-hydroxynonenal. These aldehydes can attack thiol or amino groups of proteins, injuring the cells. Lipid peroxides have been found in atherosclerotic plaques, in brain tissue damaged by trauma or oxygen deprivation, and body tissues damaged by poisoning by toxins such as carbontetrachloride. PUFAs are susceptible to peroxidation, however, they are also essential for the maintainence of cell membrane integrity. [43-44]

Cholesterol is the least susceptible of all lipids to peroxidation; however, increased levels and/or decreased antioxidant activity can lead to accumulation of oxidized LDL cholesterol in macrophages, leading to foam cell formation and endothelial injury. Endothelial cell cyclooxygenases and lipooxygenases then react with lipids to yield peroxides. Endothelial injury can also facilitate the release of copper and iron ions which decompose peroxides to peroxyl radicals. These radicals attack neighboring lipids, resulting in more peroxide formation. This process can increase production of prostaglandins and leukotrienes, resulting in platelet aggregation. The desirable laboratory value for plasma lipid peroxide is < 4 micromoles/L.

Fibrinogen In Cardiovascular Health

Fibrinogen is one of the major protein moieties in the blood plasma, and is an important factor in clotting. Fibrinogen is a large molecule (400 kD) and its concentration in blood is about 100 to 700 mg/dl. Fibrinogen is formed in the liver, and circulates throughout the body in plasma. During clot formation, fibrinogen is acted upon by thrombin, releasing fibrin monomers, which polymerize to form fibrin threads, resulting in a clot. Fibrinogen strongly affects blood coagulation, blood rheology, and platelet aggregation. It also has direct effects on the vascular wall and is a prominent acute-phase reactant. Elevated fibrinogen, according to the most recent medical literature, is associated with an increased risk of coronary heart disease, stroke, myocardial infarction, and peripheral arterial disease. Therefore, fibrinogen can be an excellent risk marker for cardiovascular disease.

In a prospective study of 333 patients with stable intermittent claudication, an increase in plasma fibrinogen levels of 1 mg/dl of fibrinogen correlated with a nearly 2-fold increase in the probability of death within the next 6 years. These results indicate fibrinogen may not only be involved in the pathogenesis of arterial disease, but may also be the "strongest independent predictor of death" in this clinical setting.[45] Another study revealed that the correlation between fibrinogen and the severity of atherosclerosis supports data suggesting local degradation of cross-linked fibrin is involved in the progression of atherosclerosis.[46]

Risk factors that increase fibrinogen levels include: cigarette smoking, age, race, gender (higher in females), oral contraceptive use, obesity, sedentary lifestyle, diabetes, hypertension, inflammation and stress. Diets rich in omega-3 and omega-6 fatty acids reduce fibrinogen levels. Exercise, weight reduction,

smoking cessation, and stress management are also effective in reducing fibrinogen levels. Plasma fibrinogen in excess of 350 mg/dl is a powerful independent risk factor for infarction of the brain and heart. The reference value for fibrinogen is 200-400 mg/dl.[47-48]

Therapeutic Treatment Considerations

Dietary Factors

When the diet contains an overabundance of meat products, high saturated fat and decreased vegetable content, there is a potential for an increased oxidative challenge, which can cause a greater susceptability to free radical injury, a significant factor in the atherosclerotic disease process.The key is to reverse this pattern by dietary modifications that favor decreased oxidative burden. Therapeutic treatment should include increasing beneficial oils (flax, olive, fish), avoiding margarine due to its high levels of trans fatty acids, increasing dietary consumption of fresh vegetables, and limiting the amount of red meat. A moderate amount of fish, particularly cold water fish, should also be incorporated into the diet, and an adequate caloric intake from protein, carbohydrate, and fat sources should be ensured. Garlic and onions may also be beneficial in reducing atherosclerotic risk. It is also essential to have an adequate amount of dietary fiber.

Antioxidant Defenses

It is essential that causative factors of increased oxidative stress be addressed, e.g., dietary factors, smoking, and excessive alcohol consumption. Antioxidant defense factors should include vitamin A, beta-carotene, vitamin E, vitamin C, all of the B vitamins, amino acids (methionine, dimethylglycine, L-cysteine, L-carnitine and glutamine) zinc, selenium, CoQ10, oligomeric proanthocyanidins, and lipoic acid.

Vascular Integrity and Peripheral Circulation

Improving vascular integrity and peripheral circulation is necessary to ensure overall cardiovascular health. Cardiovascular treatment regimens should include ingredients that improve vascular integrity and peripheral circulation, such as Crataegus oxycantha, Ginkgo biloba,Taraxacum officinale, Uva Ursi, Equisetum arvense, vitamin C, and chondroitin sulfate. Supplementation with vitamin B12, folate, and vitamin B6 have demonstrated therapeutic efficacy in lowering homocysteine levels, which reduces the formation of atherosclerotic plaques.

Exercise

The primary purpose of exercise is to improve cardiovascular function. The target heart rate should first be established and then the exercise regimen designed accordingly. Walking, swimming, biking, hiking, and light weight training can improve cardiovascular function. Consulting a specialist in cardiovascular training may be helpful.

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