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The Pathogenesis of Vascular Disease

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The prevalence of vascular disease has increased in both the United States and Europe since the end of World War II. The pathogenesis of vascular disease has been directly linked to changes in dietary habits and lifestyle practices and the discovery of penicillin by Sir Alexander Fleming in 1928, which led to a reduction in deaths secondary to bacterial infections. Multiple theories have evolved regarding the various factors associated with an increased risk of vascular disease. It is important to realize, however, that the study of the pathogenesis and subsequent treatment of vascular disease requires a "bigger picture" approach rather than consideration of just one or two factors. In this chapter, we review the contributions made by many investigators who have looked at one or more of these issues. We discuss the relationship (Fleming's Unified Theory of Vascular Disease[1]) between these factors (Figure 64.1) and their overall role in the pathogenesis of vascular disease, including coronary artery disease, carotid artery disease, and peripheral vascular disease. We also review the importance and benefit of looking at each of these contributing factors when evaluating and treating an individual with vascular disease.

Sources of Endothelial Injury

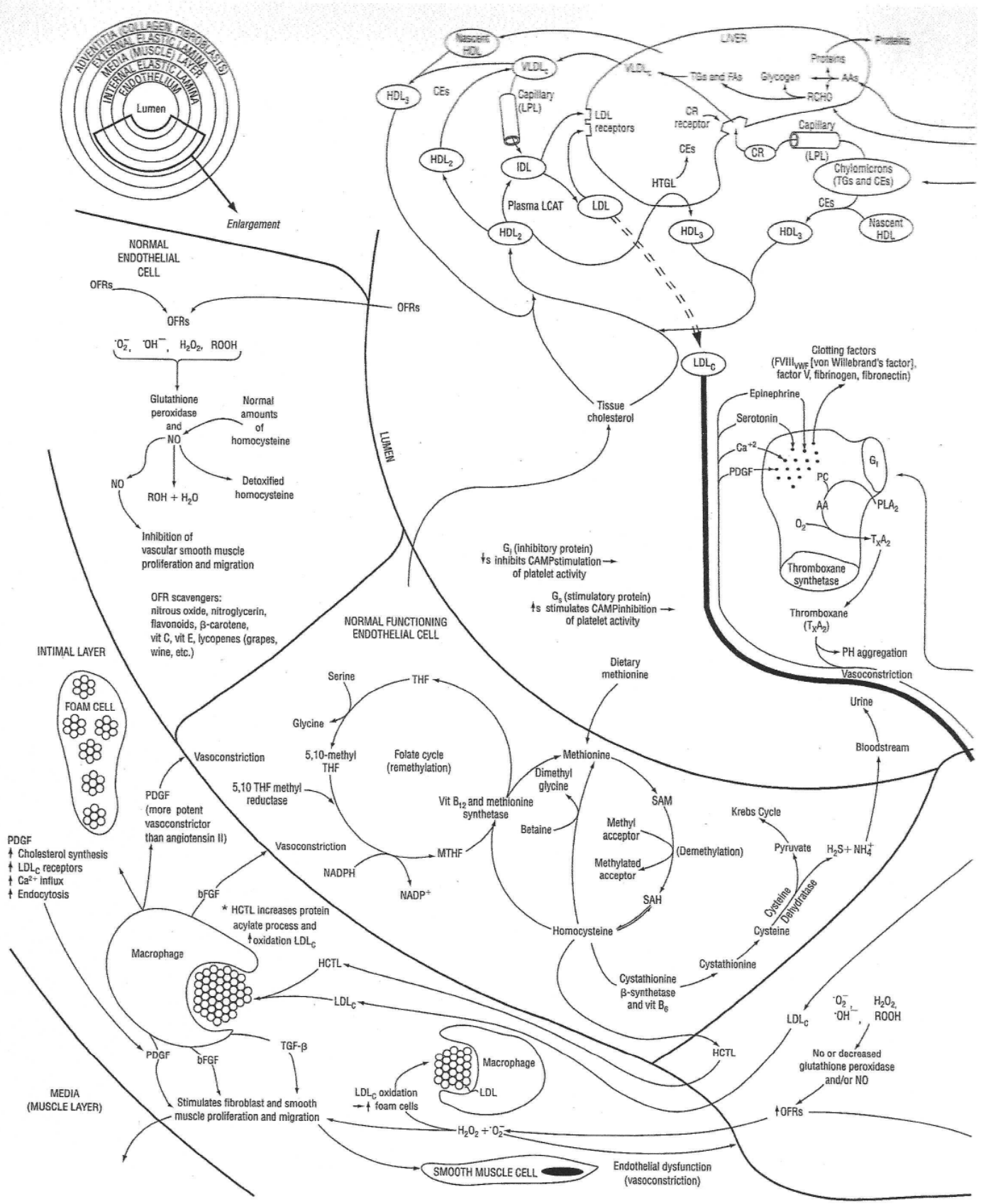
The initiation of vascular disease begins with injury to the endothelial wall. This process can begin as soon as stretching of the endothelium occurs, which is while the child is within the mother's uterus and blood is pulsing through the arteries and veins. This pulsation of blood is necessary for survival, but it initiates the stretching of endothelial cells and the potentiation for injury. Clearly the human organism is designed to deal with this phenomenon or it would be incompatible with life itself. It is also clear from human history that longevity is not related to medical science, in that Muhammad lived for 62 years, Gandhi for 79 years, Buddha for 80 years, and Methuselah for 969 years.

It is now clear that endothelial injury can occur from many causes. Such injuries can be caused by rupture of endothelial plaques after the formation of foam cells. This endothelial rupture may or may not expose collagen or other connective tissue, which can then precipitate the clotting cascade that is discussed later. Other causes include trauma to the blood vessel, free radical (e.g., oxygen) formation, bacterial invasion of the vessel, and formation of a thrombus, with subsequent activation of growth factors, inflammatory mediators, and vasoconstrictors and potential lumen occlusion.

Once the endothelial cells are damaged, the phospholipoproteins that are released include phosphatidylinositol 4,5-biphosphate (PIP_2) and phosphatidylcholine (PC). Both undergo a series of changes and ultimately become a 20-carbon polyunsaturated fat known as arachidonic acid (AA), which is then released from the endothelial cell. Regardless of the cause of endothelial injury, the overall process is as shown in Figures 64.1 and 64.2.

The presence of fatty streaks, which has been documented in children[2] as young as 10 years, gives way to fibroproliferative infiltration by smooth muscle cells from the media. The mechanism for fibroproliferative infiltration is the release of such substances as platelet-derived growth factor (PDGF) and will be discussed later. Once the endothelium is injured or denuded, there is an increased receptiveness to immunoglobulin G and a vasospastic responsive to 5-hydroxytryptamine in the presence of thromboxane A_2 (discussed later). The vasodilative response to 5-hydroxytryptamine is inhibited in the presence of dysfunctional endothelium. The increased uptake of immunoglobulin G[3] is correlated with an increased replication of endothelium necessary to repair and replace damaged endothelium.

The Fleming Unified Theory of Vascular Disease was developed by Dr. Fleming in June 1997.


ADVENTITIA (COLLAGEN, FIBROBLASTS)
EXTERNAL ELASTIC LAMINA
MEDIA (MUSCLE) LAYER
INTERNAL ELASTIC LAMINA
ENDOTHELIUM
Lumen
Enlargement
Nascent HDL
VLDLc
HDL3
CEs
Capillary (LPL)
LDL receptors
IDL
HDL2
Plasma LCAT
LDL
HTGL
LIVER
Proteins
VLDLc
TGs and FAs
Glycogen
AAs
RCHO
CR receptor
CR
Capillary (LPL)
Chylomicrons (TGs and CEs)
Nascent HDL
NORMAL ENDOTHELIAL CELL
OFRs
OFRs
O2-, OH-, H2O2, ROOH
Glutathione peroxidase and NO
Normal amounts of homocysteine
NO
ROH + H2O
Detoxified homocysteine
Inhibition of vascular smooth muscle proliferation and migration
OFR scavengers: nitrous oxide, nitroglycerin, flavonoids, β-carotene, vit C, vit E, lycopenes (grapes, wine, etc.)
LUMEN
Tissue cholesterol
LDLc
Clotting factors (FVIIIVWF [von Willebrand's factor], factor V, fibrinogen, fibronectin)
Epinephrine
Serotonin
Ca+2
PDGF
PC
AA
PLA2
G1
O2
TxA2
Thromboxane synthetase
Thromboxane (TxA2)
PH aggregation
Vasoconstriction
Gi (inhibitory protein) ↓s inhibits CAMPstimulation of platelet activity
Gs (stimulatory protein) ↓s stimulates CAMPinhibition of platelet activity
NORMAL FUNCTIONING ENDOTHELIAL CELL
INTIMAL LAYER
FOAM CELL
Dietary methionine
Serine
THF
Glycine
5,10-methyl THF
Folate cycle (remethylation)
5,10 THF methyl reductase
Vit B12 and methionine synthetase
Methionine
Dimethyl glycine
Betaine
SAM
Methyl acceptor
Methylated acceptor
(Demethylation)
SAH
Homocysteine
Cystathionine β-synthetase and vit B6
Cystathionine
Cysteine
Cysteine Dehydrase
Pyruvate
Krebs Cycle
H2S+ NH4+
Urine
Bloodstream
Vasoconstriction
PDGF (more potent vasoconstrictor than angiotensin II)
PDGF
↑ Cholesterol synthesis
↑ LDLc receptors
↑ Ca2+ influx
↑ Endocytosis
bFGF
* HCTL increases protein acylate process and ↑oxidation LDLc
Vasoconstriction
MTHF
NADPH
NADP+
Macrophage
HCTL
LDLc
TGF-β
PDGF
bFGF
MEDIA (MUSCLE LAYER)
Stimulates fibroblast and smooth muscle proliferation and migration
LDLc oxidation → ↑ foam cells
Macrophage
LDL
H2O2 + O2-
SMOOTH MUSCLE CELL
Endothelial dysfunction (vasoconstriction)
HCTL
LDLc
O2-, OH-, H2O2, ROOH
No or decreased glutathione peroxidase and/or NO
↑OFRs

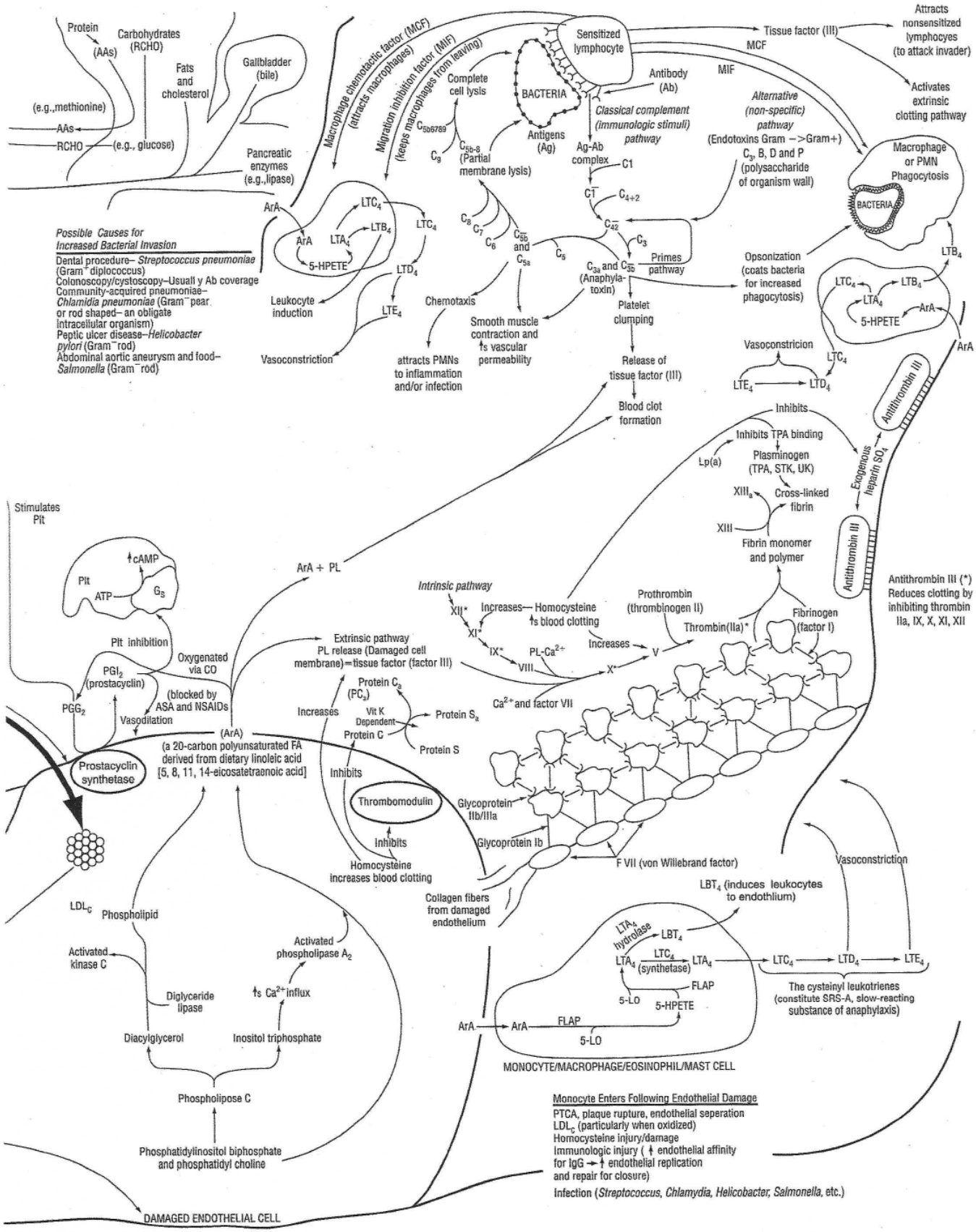

Protein
(AAs)
Carbohydrates
(RCHO)
Fats and cholesterol
Gallbladder (bile)
(e.g.,methionine)
AAs
RCHO
(e.g., glucose)
Pancreatic enzymes (e.g.,lipase)
Macrophage chemotactic factor (MCF) (attracts macrophages)
Migration inhibition factor (MIF) (keeps macrophages from leaving)
Sensitized lymphocyte
Tissue factor (III)
MCF
MIF
Attracts nonsensitized lymphocyes (to attack invader)
Activates extrinsic clotting pathway
Complete cell lysis
BACTERIA
Antibody (Ab)
Classical complement (immunologic stimuli) pathway
Alternative (non-specific) pathway
(Endotoxins Gram −>Gram+)
C3, B, D and P (polysaccharide of organism wall)
Antigens (Ag)
Ag-Ab complex
C1
C9
C5b-8 (Partial membrane lysis)
C5b6789
Macrophage or PMN Phagocytosis
BACTERIA
Possible Causes for Increased Bacterial Invasion
Dental procedure– Streptococcus pneumoniae (Gram+ diplococcus)
Colonoscopy/cystoscopy–Usuall y Ab coverage
Community-acquired pneumoniae–Chlamidia pneumoniae (Gram− pear or rod shaped– an obligate intracellular organism)
Peptic ulcer disease–Helicobacter pylori (Gram− rod)
Abdominal aortic aneurysm and food–Salmonella (Gram− rod)
ArA
LTC4
LTB4
LTA4
5-HPETE
LTD4
LTE4
Leukocyte induction
Vasoconstriction
C8
C7
C6
C5b and C5a
C5
C42
C3
Primes pathway
C3a and C3b (Anaphyla-toxin)
Chemotaxis
attracts PMNs to inflammation and/or infection
Smooth muscle contraction and ↑s vascular permeability
Platelet clumping
Release of tissue factor (III)
Blood clot formation
Opsonization (coats bacteria for increased phagocytosis)
Vasoconstricion
Inhibits
Inhibits TPA binding
Plasminogen (TPA, STK, UK)
Lp(a)
Cross-linked fibrin
XIIIa
XIII
Fibrin monomer and polymer
Antithrombin III
Exogenous heparin SO4
Antithrombin III (*) Reduces clotting by inhibiting thrombin IIa, IX, X, XI, XII
Stimulates Plt
cAMP
Plt
ATP
Gs
Plt inhibition
ArA + PL
Intrinsic pathway
XII*
XI*
IX*
VIII
PL-Ca2+
X*
Increases
Homocysteine ↑s blood clotting
Prothrombin (thrombinogen II)
Thrombin(IIa)*
Fibrinogen (factor I)
V
Extrinsic pathway
PL release (Damaged cell membrane)=tissue factor (factor III)
Ca2+ and factor VII
PGI2 (prostacyclin)
Oxygenated via CO
(blocked by ASA and NSAIDs)
PGG2
Vasodilation
(ArA)
(a 20-carbon polyunsaturated FA derived from dietary linoleic acid [5, 8, 11, 14-eicosatetraenoic acid]
Prostacyclin synthetase
Protein C2 (PC2)
Vit K Dependent
Protein C
Protein S
Protein Sa
Increases
Inhibits
Thrombomodulin
Homocysteine increases blood clotting
Glycoprotein IIb/IIIa
Glycoprotein Ib
F VII (von Willebrand factor)
Collagen fibers from damaged endothelium
Vasoconstriction
LBT4 (induces leukocytes to endothlium)
LTA4 hydrolase
LBT4
LTC4 (synthetase)
LTA4
LTC4
LTD4
LTE4
FLAP
5-LO
5-HPETE
The cysteinyl leukotrienes (constitute SRS-A, slow-reacting substance of anaphylaxis)
ArA
FLAP
5-LO
MONOCYTE/MACROPHAGE/EOSINOPHIL/MAST CELL
LDLc
Phospholipid
Activated kinase C
Activated phospholipase A2
Diglyceride lipase
↑s Ca2+ influx
Diacylglycerol
Inositol triphosphate
Phospholipose C
Phosphatidylinositol biphosphate and phosphatidyl choline
DAMAGED ENDOTHELIAL CELL
Monocyte Enters Following Endothelial Damage
PTCA, plaque rupture, endothelial seperation
LDLc (particularly when oxidized)
Homocysteine injury/damage
Immunologic injury (↑ endothelial affinity for IgG → ↑ endothelial replication and repair for closure)
Infection (Streptococcus, Chlamydia, Helicobacter, Salmonella, etc.)

FIGURE 64.1. The Fleming Unified Theory of Vascular Disease. The interrelatedness of each of the various (eight) groups of factors is shown in this schematic of an artery. The artery represents any artery within the body, including coronary, carotid, and peripheral arteries. See text for details. AA, amino acid; ArA, arachidonic acid; bFGF, basic fibroblastic growth factor; CEs, cholesterol esters; CO, cyclooxygenase; CR, chylomicron remnant; FAs, fatty acids; FLAP, 5-lipoxygenase-activating protein; HCTL, homocysteine thiolactone; 5-HPETE, 5-hydroperoxyeicostetranoic acid; HTGL, hepatic triglyceride lipase; LCAT, lecithin-cholesterol acyltransferase; 5-LO, 5-lipoxygenase; MTHF, 5-methyl tetrahydrofolate; NO, nitrous oxide; OFRs, oxygen free radicals; PDGF, platelet-derived growth factor; Plt, platelets; PL, phospholipoprotein; SAH, *S*-adenosyl homocysteine; SAM, *S*-adenosyl methionine; TGF-β, tissue growth factor β; TGs, triglycerides; THF, tetrahydrofolate.

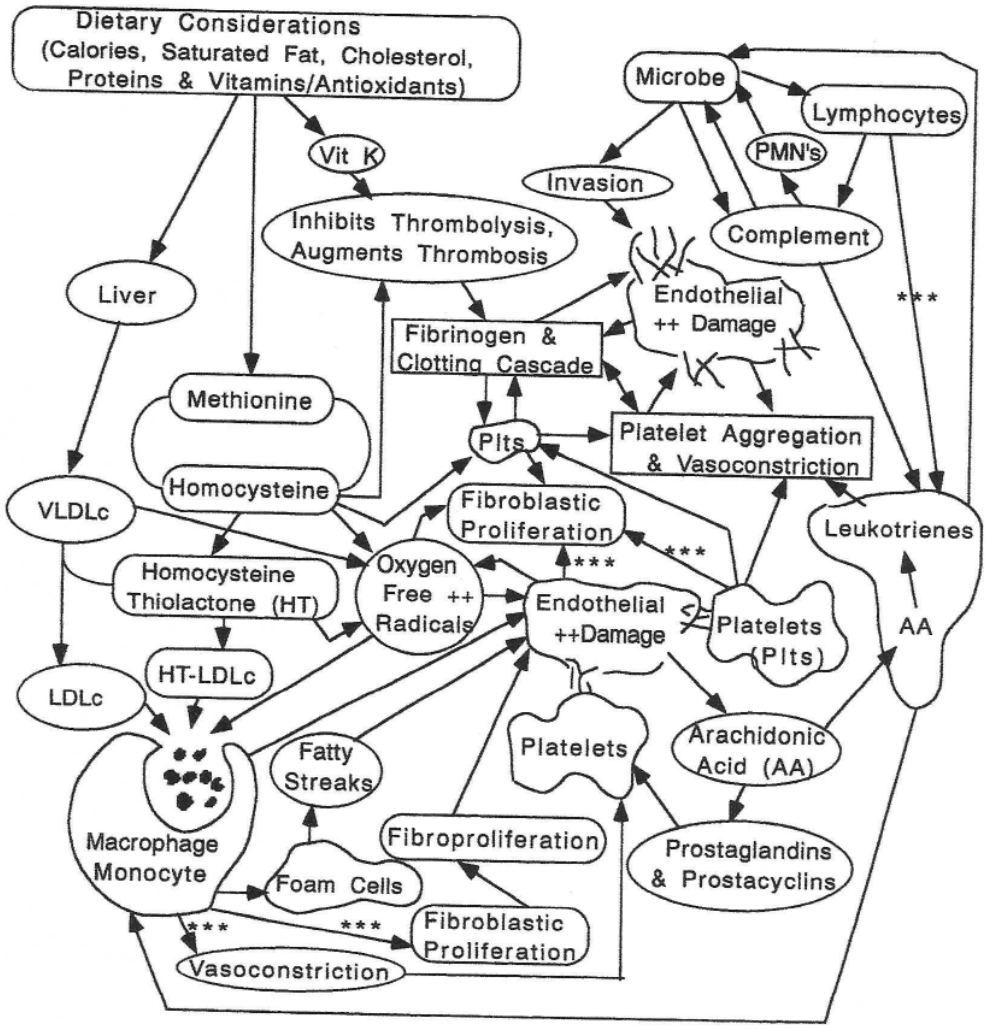


FIGURE 64.2. Simplified model of Fleming's Unified Theory of Vascular Disease. Eight groups of factors are fitted together in the overall explanation of vascular disease. These include dietary concerns, which generate very-low-density lipoprotein cholesterol (VLDLc) and low-density lipoprotein cholesterol (LDLc). These are phagocytized by macrophages along with homocysteine thiolactone ([HT]-LDLc), which results from the metabolic catabolism of the amino acid methionine. Endothelial damage results in activation of prostacyclins, prostaglandins, and leukotrienes. Bacterial invasion precipitates both inflammatory and complement activation as the body makes an effort to remove the offending organisms. The initiation of fibroproliferation results in advanced atherosclerotic plaques that produce further endothelial problems, promoting further disease. ***, growth factors or other chemical mediators; ++, other interactions not shown here (but discussed in text); endothelial damage, endothelial damage and dysfunction. PMNs-polymorphonuclear leukocytes.

Dietary Factors Involved in Vascular Disease: The First Group of Factors

Numerous factors must be considered when looking at the dietary changes[4-6] required to reduce lipids. These changes include five major issues. The first is *caloric consumption.* It is now known that rats that consume twice the recommended number of calories necessary for survival demonstrate a decreased lifespan, with greater health problems, whereas rats that consume 70% of the recommended number of calories live twice as long as the average rat, with fewer health problems. There is no reason to believe this is different for humans.

Regardless of whether consumed calories begin as protein, carbohydrate, fat, or alcohol, excess caloric consumption is stored primarily as triglycerides or fats. This has been shown in the Tarahumara Indians, who have virtually no coronary artery disease despite relatively low levels of high-density lipoprotein cholesterol. When the Tarahumarans were placed on hypercaloric diets, they demonstrated a significant increase in plasma lipids[7] as well as weight. These fats eventually accumulate by way of production of very-low-density lipoprotein cholesterol and, subsequently, low-density lipoprotein cholesterol (LDLc), which is deposited in the subendothelial region of blood vessels. This is an excellent source of fatty acids, which are the primary energy source for organs such as the heart; however, excess accumulation results in monocyte and macrophage phagocytization and the initial development of fatty streaks.

Monocytes exit the lumina of blood vessels and become tissue macrophages that engulf the LDLc in an effort to remove the extracellular material. Eventually this phagocytized LDLc results in the macrophages' becoming foam cells. These macrophages release a number of chemical mediators, including PDGF, which causes vasoconstriction of the blood vessel. PDGF is in fact a more potent vasoconstrictor than angiotensin II. Also released is basic fibroblastic growth factor (bFGF), which enhances fibroblast formation in the medial layer and migration into the subendothelial region; this results in the more advanced stages of atherosclerotic disease (namely, fibroproliferation). The release of basic fibroblastic growth factor[8] from arteries after trauma or intervention (e.g., catheter-induced deendothelialization) increases intimal smooth muscle proliferation along with restenosis. Additionally, TGFb is released, which also promotes fibroproliferation.

Probably more important than any other factor is the role of excessive dietary fat, particularly the *saturated fats.* It has been shown that dietary fat consumption is extremely important for two reasons: (1) fat has 9 calories per gram and (2) saturated fats, which pervade the diets in most industrialized countries today, is artherogenic. The residents of these countries are plagued with vascular disease, cancer, and other related health problems. Regardless of the study considered, successful attempts at reducing cholesterol levels have required simultaneous reductions in dietary saturated fat intake. However, some quantity of dietary fat is necessary (essential fatty acids) for the healthy survival of the human species. The essential fatty acids are polyunsaturated fats (fatty acids) and include linoleic acid (9,12-octadecadienoic), linolenic acid (9,12,15-octadecatrienoic), and AA (5,8,11,14-eicosatetraenoic). AA can be synthesized in the body from linolenic acid. Studies in the 1970s and 1980s saw people lowering fat intake to less than 10% of their total caloric intake. When fat consumption is less than 8% of the daily total caloric intake, significant health issues arise, including immunologic problems.

In the 1940s and 1950s, the ability to preserve food increased as a result of our ability to hydrogenate food. This process of saturating a fat (*trans*-fat) was good for extending the shelf life of food, but not the "shelf life" of people. Saturated fats are particularly problematic, and even a single high-fat meal[9] has been shown to inhibit normal endothelial function by way of oxidative injury. The overall goal, therefore, is not to eliminate all fat from the diet but to reduce the amount of saturated fat and reduce the percentage of calories consumed as fat to approximately 15% of the total caloric intake, assuming the correct amount of calories[4-6] is being eaten daily.

The third dietary factor is *cholesterol intake.* Obviously, the ingestion of cholesterol is of concern because of the relationship between dietary cholesterol and very-low-density lipoprotein cholesterol production by the liver. There is also a relationship[10] between cholesterol levels, heart disease, and systolic blood pressure. However, the major contributing factor would appear to be not the amount of cholesterol in the diet but the amount of saturated fats found in foods that are also relatively high in cholesterol content. Of the cholesterol consumed daily, approximately 10% is absorbed across the gastrointestinal tract. Because the daily American diet includes an average of 250 to 500 mg of cholesterol, 25 to 50 mg are absorbed daily. However, the liver makes an average of 1000 mg of cholesterol daily. This explains why changes in dietary cholesterol intake *alone* may or may not be associated with changes in serum cholesterol levels and, subsequently, with the severity of atherosclerotic disease. This was first emphasized by Ancel Keys, who discussed the benefits of the Mediterranean diet. Dietary changes that do not take into account caloric and saturated fat intake, in addition to cholesterol, may demonstrate initial improvements but are unable to maintain control or reversal of vascular disease. This is the principal problem behind vegetarian diets, when *both* caloric and fat intake are not controlled.

A fourth dietary concern is protein and subsequently *homocysteine,* which is discussed in the next section as an independent risk factor. It is important to note here, however, that elevations in homocysteine result in increased phagocytosis of LDLc and LDLc–homocysteine–thiolactone complexes. This complex results in an increased oxidative load and further endothelial and subendothelial injury.

Finally, the role of *antioxidants* must be taken into consideration. Whether the oxygen free radicals (OFRs) are produced by way of damaged endothelium, excessive homocysteine, or responses to inflammatory or infectious agents, the effect of OFRs is the same. These extremely toxic compounds cause endothelial damage and dysfunction, resulting in vasoconstriction in addition to fibroblastic proliferation, increased phagocytosis of LDLc by macrophages, and further endothelial damage. Therefore, the ingestion of vitamins C and E, carotenoids such as beta carotene and lycopene, flavonoids (found in grape juice), selenium, flaxseed, or soy protein and the use of medications such as nitroglycerin have a positive (e.g., vasodilative) effect by reducing the levels of OFRs.[9,11] Other vitamins such as vitamin K must also be considered because of the effect on the extrinsic clotting pathway, particularly in individuals who take warfarin or related medications.

The Role of Homocysteine: The Second Group of Factors

Homocysteine has been recognized as a risk factor[12–16] for vascular disease ever since researchers looked at the prevalence of elevated homocysteine levels in younger individuals who were thought to have premature coronary artery disease. The enzymatic pathway of homocysteine shows it to be a metabolic product of the essential dietary amino acid methionine. Some endothelial cells have been shown[17] to have less cystathionine β-synthase activity, which could potentially increase their risk of endothelial damage. Excessive accumulation of homocysteine results in several vascular responses. The first is an overwhelming of the endothelial tissue's ability to detoxify the OFRs generated from the homocysteine. The OFRs not only use up the nitrous oxide that would have a vasodilative effect but actually cause endothelial dysfunction and vasoconstriction.

The OFRs also stimulate fibroblast[18,19] and smooth muscle proliferation and migration to the subendothelial region, thereby enhancing the progression of vascular disease. Homocysteine is also metabolized to homocysteine thiolactone,[20,21] which increases the harmful acylate process and oxidative process of LDLc, thereby increasing phagocytosis of LDLc and LDLc–homocysteine–thiolactone complexes by macrophages with the subsequent development of foam cells and fatty streaks.

Homocysteine increases the formation of thrombi and blood clots by interfering with heparin sulfate's activation of antithrombin III and decreasing antithrombin III levels. Homocysteine has also been shown to inhibit thrombomodulin expression, induce the expression of tissue factor, and reduce the binding of tissue plasminogen activator (TPA) to its endothelial receptor. Homocysteine also increases factors XI and V, decreases protein C activation, and increases the expression of thromboxane A_2 (TxA2). Homocysteine subsequently promotes a hypercoagulable state that has been shown to increase the risk for multiple vascular problems,[22–26] including myocardial infarction, cerebrovascular accidents, deep venous thrombosis, and peripheral vascular disease, particularly when coupled with stenotic lesions[27,28] where changes in entry *(a)* and exit *(w)* angles to and from a narrowed vessel will further promote thrombus formation.

The Role of Hyperfibrinogenemia and Hypercoagulability: The Third Group of Factors

Damage to the endothelium of a blood vessel may occur from rupture of a plaque, cracking, fissuring, de-endothelialization (e.g., angioplasty), stretching of endothelial cells (advanced atherosclerosis), inflammatory or immunologic reactions (e.g., lupus anticoagulant), or surgical interventions. Once this has occurred with membrane phospholipid release (phosphatidylinositol 4,5-biphosphate and phosphatidyl choline), the connective tissue components are exposed. Paramount in this process is the release of AA and the exposure of collagen and von Willebrand's factor necessary for initiation of thrombus formation. However, as mentioned previously,[27] narrowed entry *(a)* and exit *(w)* angles also promote thrombus formation.

Once von Willebrand's factor and collagen are exposed, platelets are attracted to the area, where they bind to the damaged subendothelium (von Willebrand's factor–collagen complexes or polymers) and attach by means of platelet glycoproteins (glycoprotein Ib). When there is inadequate release of von Willebrand's factor (type I defect) or defective von Willebrand's factor (type II defect) is released, bleeding disorders (von Willebrand's disease) occur. When the platelets have no glycoprotein Ib (Bernard–Soulier syndrome), bleeding problems also occur. When there are no homeostatic abnormalities, the attachment of platelets to von Willebrand's factor–collagen complex occurs, with subsequent attraction of additional platelets that bind to each other by way of another glycoprotein known as glycopro-

tein IIb/IIIa. This glycoprotein is absent in individuals with Glanzmann's thrombasthenia, who also have bleeding disorders.

As shown in Figures 64.1 and 64.2, the release of AA leads to the production of antagonistic pathways (namely, prostacyclins and prostaglandins) that have opposing effects on platelet activation and vasomotor function. The AA also enters the monocyte/macrophage cells (mononuclear phagocytic/reticuloendothelial system), which is discussed later. The damaged endothelium (extrinsic pathway) also releases tissue thromboplastin (tissue factor), and the simultaneous disruption of blood flow (e.g., narrowed vessel, exposed subendothelium) initiates the intrinsic pathway for blood coagulation. Any factor that increases blood viscosity will also increase the tendency for blood clot formation. Such factors include cancer, inflammatory states, and hyperfibrinogenemia.

The extrinsic and intrinsic pathways converge where prothrombin/thrombinogen (factor II) is converted to thrombin (factor IIa), which is responsible for converting fibrinogen into fibrin monomers and subsequently fibrin polymers. Excessive fibrinogen augments this reaction and promotes thrombus formation. A pronounced effect of homocysteine is the promotion of a hypercoagulable state by increasing certain factors and inhibiting others. Likewise, lipoprotein(a), which has a molecular structure similar to those of LDLc and plasminogen,[29] impairs tissue plasminogen activation of plasminogen while inhibiting the binding of tissue plasminogen activator to the endothelial receptor for tissue plasminogen activator. This last effect is similar to one of the hypercoagulable properties of homocysteine.

One of the frequently unaddressed benefits of exercise is the beneficial impact on clotting factors. It has been demonstrated[30] that lower fibrinogen levels are seen in individuals who exercise regularly. Multiple factors that increase the potential for thrombus formation are reduced with exercise, including thromboxane A_2.[31]

The Role of Antioxidants: The Fourth Group of Factors

OFRs are extremely toxic for all living things. This includes not only the lytic effect on bacteria, which are phagocytized by polymorphonuclear leukocytes and monocytes/macrophages, but also the damage that can occur to the body itself. Multiple enzymatic pathways exist within the body to reduce these toxic products.

OFRs are reduced in endothelial cells (and elsewhere) by means of the enzyme glutathione peroxidase. If this enzyme is missing (as in hyperhomocysteinemia) or overwhelmed, the OFRs accumulate and cause endothelial damage. This oxidative stress has been shown to be associated with coronary artery disease[32–35] as well as cancer,[36] diabetes,[37] and even cardiac failure.[38] Whereas high-density lipoprotein cholesterol serves as a scavenger mechanism for moving LDLc/cholesterol esters, antioxidants serve as free radical scavengers. Unlike high-density lipoprotein cholesterol, antioxidants do not merely move the offending agent around but catabolize the offending molecules.

Increased OFRs not only cause endothelial injury, but they also initiate a series of events including the oxidation of LDLc, which results in an increased propensity for phagocytosis of LDLc by macrophages. Increased levels of homocysteine also result (discussed previously) in the formation of LDLc–homocysteine–thiolactone complexes, which are also phagocytized by macrophages. These macrophages then release several substances (growth factors), to be discussed later. They include PDGF, basic fibroblastic growth factor, and tissue growth factor β. OFRs also cause endothelial dysfunction with subsequent vasoconstriction, which not only decreases blood flow but also enhances the potential for thrombosis.

In the presence of normally functioning endothelium, nitrous oxide, like nitroglycerin, results in inhibition of vascular smooth muscle (vasodilation) and decreases the proliferation and migration of fibroblasts and smooth muscle cells from the media (muscular) layer of the vessel into the subintimal layer. As the levels of OFRs increase, nitrous oxide (NO) is consumed, resulting in enhanced OFR effect without antagonism. Antioxidants have been shown to scavenge the OFRs and reduce the effects of vascular disease[39,40] independent of other risk potentiators or risk factors.

Endothelial and Other Growth Factors: The Fifth Group of Factors

The basic role of the vascular system is to maintain the integrity of blood flow throughout the body. To do this, when damage occurs in one part of the vascular system, the affected area must take action to reduce the overall risk to the rest of the body. An underlying theme to living organisms is the use of a limited number of chemical reactions and mediators to produce various effects throughout the body. These chemical mediators are released from different cells of the body in an effort to carry out their individual tasks and communicate with other cells. Chemical mediators (factors) that have one effect in the vascular system have different effects in other parts of the body. For example, activation of smooth muscles in blood vessel walls results in vasoconstriction, whereas activation of smooth muscles in bronchial endothelium results in bronchospasm, even though the same chemical substances are being released.

When the endothelium is injured, collagen fibers are exposed and tissue factor is released. Both of these events stimulate the formation of blood clotting and fi-

brin development. The damaged endothelium releases AA, which stimulates prostaglandin, prostacyclin, and thromboxane A_2 formation. The AA also activates the leukotriene pathways, which are discussed in greater detail later. Platelets are activated by the formation of a fibrin clot (discussed previously), elevations in homocysteine levels, and prostaglandin synthesis. The platelets release PDGF, epinephrine, and additional thromboxane A_2. The thromboxane A_2 attracts more platelets, and the epinephrine increases platelet aggregation and promotes vasoconstriction. The PDGF, which is also released from macrophages that phagocytize cholesterol, causes vasoconstriction.

Once released, PDGF stimulates fibroblast and smooth muscle proliferation and migration from the media to the intimal layer, where fatty streaks are converted to fibrous plaques.[41,42] As a result of PDGF, macrophages exhibit an increase in LDLc receptors (increased uptake of LDLc) and an increased influx of calcium into the macrophage. The increased phagocytosis of LDLc is associated with an increase in cholesterol synthesis.

Another factor released after endothelial injury is basic fibroblastic growth factor, which is angiogenic and has been found in greater than normal quantities in atherosclerotic vessels[43] and damaged[44] vessels. Although basic fibroblastic growth factor has been shown to be associated with collateralization of blood vessels, it has also been shown[8] to produce vasoconstriction and is released from macrophages. After angioplasty, vasoconstriction routinely occurs[45] in both distal and control regions[46] of the vessel. The three primary mitogenic effects of basic fibroblastic growth factor are smooth muscle and fibroblastic proliferation, as noted previously, and endothelial cell proliferation. Endothelial cell proliferation occurs by means of two mechanisms, the first being a direct effect and the second by upregulation[47,48] of vascular endothelial growth factor, thereby promoting angiogenesis. An additional function for heparin has been postulated[49] based on early research.

Tissue growth factor β is also released from macrophages and stimulates the proliferation and migration of fibroblasts and smooth muscle cells into the intimal layer, further promoting the advancement of fatty streaks to fibrous plaque formation. Other chemical mediators have been shown to be involved in other aspects of vascular disease. These are discussed independently in the following sections.

The Role of Leukotrienes: The Sixth Group of Factors

The release of AA from damaged endothelial cells has been shown to lead to the production of prostaglandins and prostacyclins, which are antagonistic pathways that have added to the understanding of the molecular mechanisms involved in thrombogenesis. Their actual role, although chemically complex, is rather limited in the overall unified theory of vascular disease. More important in the overall regulation and control are the chemical substances known as leukotrienes. These potent chemical mediators have been largely unrecognized until recently. The development of leukotriene inhibitors has resulted in a better understanding of decompression-induced pulmonary injury[50] and treatment options for bronchospastic disease,[51,52] which is the pulmonary equivalent of vasospastic problems.

Once AA is released, it can enter the leukotriene pathway in monocytes/macrophages, eosinophils, or mast cells. Leukotriene production occurs within these cells and gives rise to two pathways free of antagonists. The first is the production of leukotriene B4 (LTB4), which attracts more leukocytes to the region, including the site of damaged endothelium and, as we shall see later, sites of bacterial invasion. The second pathway results in the production of three chemical mediators known as leukotriene C4 (LTC4), leukotriene D4, (LTD4) and leukotriene E4 (LTE4). These three substances are known as cysteinyl leukotrienes and constitute what was formerly called slow-reacting substance of anaphylaxis. Both leukotriene D4 and leukotriene E4 cause vasoconstriction and are activated not only by endothelial injury but also by activated lymphocytes.

The Role of the Complement Cascade (Classic and Alternative Pathways): The Seventh Group of Factors

Although frequently forgotten in the investigation of vascular disease, the complement cascade cannot be ignored. Like all reactions in the body, the role of complement is not limited to certain regions of the body and, as such, should be expected to have a potential role in either the cause of or response to vascular disease. There are two major pathways to the complement system that represent a humoral response to infectious processes. In conjunction with lymphocytes, antibodies, macrophages, and polymorphonuclear leukocytes, these two pathways defend the body and vascular system against foreign invasion. In the next section we discuss the issue of bacterial involvement in vascular disease.

The introduction of bacteria into the vascular space and into the intimal region of a blood vessel would result in activation of the complement pathways as it does elsewhere in the body. Sensitized lymphocytes produce antibody (Ab) to the bacterial antigen (Ag). The antigen–antibody complexes (immunologic stimulus) activate

the first component (C1) of the "classic" pathway, which leads to a series of reactions that produce several important components, including C3b, which results in opsonization (coating of the microbe to optimize phagocytosis), and C3a and C5a, which are anaphylatoxins that result in smooth (vascular) muscle contraction and an increase in vascular permeability. C5a also serves as a chemotactic or attractant for leukocytes. The classic pathway terminates as C5b–C6–C7–C8–C9, which results in bacteriolysis.

The alternative pathway is activated in the presence of endotoxins or shock resulting from the polysaccharide of the microbial wall. This is more prominent for gram-negative organisms but can occur with gram-positive microbes. The alternative pathway is also primed by the C3b produced by way of the classic pathway.

Once the lymphocyte recognizes the microbe and produces an antigen–antibody complex (interaction), the lymphocyte is sensitized. The sensitized lymphocyte releases three major chemical mediators: *transfer factor*, which attracts nonsensitized lymphocytes to the region where they become sensitized; *macrophage chemotactic factor* (MCF), which attracts macrophages to the region for phagocytosis and lysis of microbes (particularly after opsonization); and *migration inhibition factor* (MIF), which keeps the macrophages present and inhibits their leaving.

The Role of Bacterial Involvement: The Eighth Group of Factors

The presence of bacterial infections is not a new problem, and recent work has shown that *Helicobacter pylori* is a major causative agent for gastric ulcers. Atherectomy specimens from coronary plaques in our laboratory and others have demonstrated bacterial agents in some of the lesions. Evidence to date demonstrates microbes not only in coronary plaques[53–56] but also in carotid artery stenosis,[57] which may lead to cerebrovascular accidents. These findings are not surprising, because it has long been recognized that disease in other vascular beds of the body has associated bacterial involvement (e.g., salmonella with abdominal aortic aneurysms).

The bacterial pathogens currently implicated are *Streptococcus pneumoniae, Chlamydia pneumoniae,* and *Helicobacter pylori.* The high prevalence of these bacterial pathogens probably accounts for their detection in atherosclerotic plaques and does not exclude other pathogens. Like rheumatic heart disease and valvular diseases that require prophylactic antibiotic coverage, individuals with vascular disease should be considered for prophylactic antibiotic coverage if there is any question about further vascular injury.

The presence of bacterial invasion in an atheromatous plaque may precipitate further problems by means of an inflammatory reaction, which may include either the complement or leukotriene pathway. The generation of OFRs may bring further problems as a result of the oxidative stress, as discussed previously.

Putting It All Together: The Eight Groups of Factors

To date, research in several different areas of vascular disease has progressed independent of research in other areas. This limitation has allowed investigators to concentrate on specific areas of interest. However, it has also limited our understanding of the interrelatedness (Figure 64.3) of each of the different factors involved.

Significant research has demonstrated the presence of atheromatous plaques in both animal models and human subjects. Diet-induced hypercholesterolemia has demonstrated fatty plaques within 1 to 2 weeks in nonhuman primates. Monocytic involvement is seen early in the process, with the formation of fatty streaks. This has also been seen in children as young as 10 years. We have seen data with progression of disease in adults in as little as 104 days,[58] which suggests that once change has begun, it can progress quite rapidly. These changes are consistent with abrupt changes in coronary blood flow[4,5] that occur too suddenly to be accounted for by changes in LDLc levels alone. Such changes can be accounted for by changes in fibrinogen or viscosity[27,28] and require that these factors also be taken into account. Likewise, improvement in serum lipids has not always been associated with clinical improvement,[59,60] despite reductions in serum lipids. We do know, however, that total serum cholesterol levels should be reduced to below 150 mg/dL, triglycerides to below 150 mg/dL, and LDLc to less than 100 mg/dL to substantially reduce the risk and progression of vascular disease. Despite the frequent testing of cholesterol, this is too often ignored or left untreated.[61]

Several factors must be taken into account when evaluating a person for vascular disease, regardless of whether one is concerned about coronary artery disease, carotid disease, deep venous thrombosis, or other problems. First, you must determine the severity of LDLc and triglyceride levels to determine the load already present, which stimulates fatty streaks, calcium deposition, and fibroproliferation. We know that dietary changes, primarily reduction in calories and the amount of saturated fats, are the primary pivotal points around which reduction of this risk factor occurs. Cholesterol intake, too, must be addressed, but it is more related to foods with high saturated fat and caloric loads. In appropriate indi-

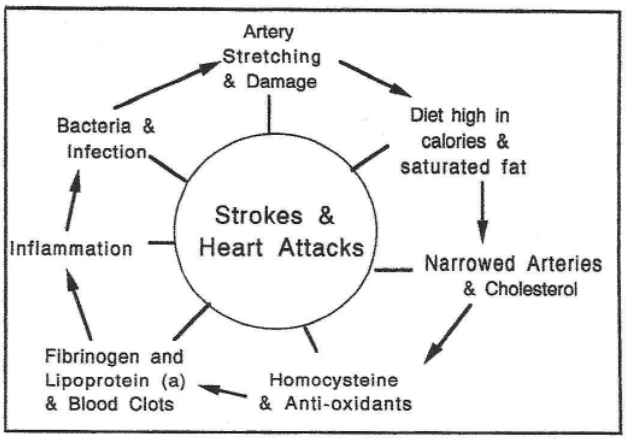


FIGURE 64.3. Damage to an artery from one group of factors may predispose the artery to further damage from other factors.

viduals, medications may in fact be necessary, but their benefit is significantly blunted without the necessary dietary changes. However, cholesterol is not the only detrimental factor affecting blood vessels. To complete the puzzle, the other pieces of the Fleming Unified Theory of Vascular Disease must be considered.

The presence of elevated homocysteine levels would suggest any of a number of other health issues (e.g., chronic renal failure, psoriasis, nitrous oxide and other medications) that need to be addressed directly, in addition to correcting nutritional factors. Treatment includes reducing contributing factors[62] and providing appropriate vitamin supplementation (vitamins B_{12} and B_6 and folate) while monitoring plasma homocysteine levels in an attempt to reduce homocysteine to the normal range of 5 to 15 mmol/L. The oxidative stress that results from hyperhomocysteinemia not only produces endothelial injury but may also be a sign of an already taxed endothelial system. The resultant OFRs along with the homocysteine can promote vasoconstriction, macrophage phagocytosis of LDLc or LDLc–homocysteine–thiolactone complexes, and stimulation and migration of fibroblasts and smooth muscle cells into the subendothelial (intimal) region, where fibrous plaque formation progresses in the presence of fatty streaks. Homocysteine additionally increases the coagulability of blood, which can be particularly problematic in vessels that have stenotic lesions or in individuals who are otherwise predisposed to clotting tendencies. These problems can be addressed nutritionally and pharmacologically but only after they are considered.

Regardless of the etiology of endothelial injury, the release of AA leads to multiple pathways, each of which must be considered, as noted previously. Although research in the arena of prostaglandins and prostacyclins has yielded much useful information, these are pathways that antagonize each other. Attempts to manipulate one has led to the blockage or the unrestrained expression of the other. However, the leukotriene pathway, when activated, has only one noted effect on blood vessels, that being the promotion of vasoconstriction and further inflammatory response. Although helpful in the bleeding scenario, this effect becomes nonproductive in vascular disease, where limitations in blood flow are the issue. Control of these cysteinyl leukotrienes has demonstrated promise in other disease states and suggests great potential for vascular disease. Recent work confirms another component of the Fleming Unified Theory hypothesis: that leukotrienes play a role in diseased coronary arteries. Like nitrous oxide, interleukins produce vasospasm in diseased arteries while leaving no effect on nonatherosclerotic coronary arteries.[63]

A review of most cardiology textbooks reveals the prevalence of cardiovascular disease problems in individuals with inflammatory and connective tissue diseases, whereas there is a paucity of such problems reported for individuals in immunodeficient states. Activation of these inflammatory pathways attracts leukocytes to the region where complement and bacterial involvement may have already been initiated. Several studies have implicated bacterial involvement in individuals who already exhibit vascular problems. Results suggest that the damaged vessel is predisposed to bacterial invasion, which must be considered and treated. Inflammation and damage to the endothelium would appear to be necessary for bacterial involvement to occur. However, once it has occurred, the presence of both complement and cellular responses to the invasion would be no different here than elsewhere in the body, and the importance of this inflammatory and infectious process has only recently been recognized.

The Fleming Unified Theory of Vascular Disease takes information not only from our laboratory but from throughout the world and recognizes the importance of various contributions made by many investigators. It is apparent that each group of factors should somehow be linked together if the studies reported to date are correct. The models shown in Figures 64.2 and 64.3 are simplified versions of how these eight groups of factors fit together. During a patient's initial screening for vascular disease, all eight groups of factors should be checked and treated when abnormal. Periodic reassessment of the patient's response to treatment should include rechecking the abnormal factors as well as diagnostic assessment of change, including nuclear imaging; ultrasound; $\cdot O_2$max evaluation; and, when indicated, angiography. The task before us now is to take this broader working model and apply it to the screening and treatment of vascular disease in each individual.

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