

ATHEROSCLEROSIS – A STORY OF CELLS, CHOLESTEROL, AND CLOTS

John R. Guyton, M.D.

CONTENTS

Introduction

The normal artery

aorta

capillaries

veins

left anterior descending artery

the “widow-maker” plaque

media

adventitia

arterial intima

Progression of atherosclerotic lesions

fatty streak

transitional lesion

fibrous plaque

ruptured plaque

Why the arterial intima is different from other connective tissues

no lymph vessels

Low density lipoproteins (LDL)

retention of LDL

Inflammation and white blood cells

clinical trials of antibiotics

macrophages

C-reactive protein

What happens to cholesterol in atherosclerosis?

cholesterol in the membranes of a cell

cholesterol ester

high density lipoproteins

reverse cholesterol transport

Smooth muscle cells

oxidized cholesterol

fibroproliferation

Endothelial cells

endothelium

blood flow determines artery size

nitric oxide

aerobic exercise

The lipid-rich core in the atherosclerotic plaque

Plaque rupture

Blood clotting, heart attacks, and strokes

heart attacks

strokes

aspirin

clopidogrel (Plavix)

dipyridamole-aspirin combination pills (Aggrenox)

warfarin (Coumadin)

Stabilization of the vulnerable plaque

race between LDL and HDL

balloon catheters

stents

bypass surgery

diet and cholesterol drugs

risk factors

Regression of atherosclerotic lesions

fibrous tissue of a scar

nitric oxide

angiographic regression

colestipol

niacin

lovastatin (Mevacor)

pravastatin (Pravachol)

simvastatin (Zocor)

fluvastatin (Lescol)

atorvastatin (Lipitor)

rosuvastatin (Crestor)

study of lifestyle treatment

dietary saturated fat and cholesterol

Calcium, coronary calcium scans, and chelation therapy

electron beam CT or ultrafast CT

gated CT

the dog that won't hunt

chelation therapy

ATHEROSCLEROSIS – A STORY OF CELLS, CHOLESTEROL, AND CLOTS

Introduction

Atherosclerosis is a disease of arteries that causes more death and disability than any other disease in the industrialized world, more than all types of cancer combined. Atherosclerosis is known by several other names – arteriosclerosis (though technically arteriosclerosis also includes some other rare and minor arterial conditions), hardening of the arteries, cholesterol deposits in the arteries, and arterial blockages. Coronary heart disease is the result of atherosclerosis in the coronary arteries, which supply the heart muscle. We will discuss below how the slow development of coronary atherosclerosis over decades can result in a sudden, severe event – a heart attack – that takes only minutes to happen. The most common cause of strokes is atherosclerosis in an artery supplying the brain.

In this article, we will see how the cells of artery wall, including the lining cells (endothelial cells), special muscle cells (smooth muscle), and inflammatory cells participate in atherosclerosis. Cholesterol carried by lipoproteins in the blood enters the artery wall and builds up in enormous amounts, leading to tissue damage, inflammation, and fibroproliferative scarring. Breakdown of tissue in the inner part of the artery wall sets the stage for blood clots that cause heart attacks and strokes.

Medical research has found opportunities for treatment and prevention. Coronary bypass operations and heart catheterization balloon and stent procedures can relieve the pain of blocked coronary arteries, but these operations and procedures do not prevent future heart attacks very well at all. Instead, we have come to understand that most heart attacks and many strokes can be prevented by medical treatment. Effective treatment includes the right kind of diet and lifestyle, drugs that change cholesterol-carrying lipoproteins, and drugs such as aspirin that partially block blood clotting. This article will try to show how effective medical treatment can be, although we do not have time and space to discuss detailed treatment plans. Reversal of arterial blockage by medical treatment is slow and difficult. However, by removing cholesterol from atherosclerotic plaques, the tendency of the plaques to break down, rupture, and cause clots can be greatly reduced. This may be the reason that heart attacks and strokes can be largely prevented.

Finally, we will look at the interesting way the calcium is involved in atherosclerosis. At this time, calcium does not seem to be as big a player as cells, cholesterol, and clotting. There is no reason to think that chelation therapy works in the treatment of atherosclerosis. Yet calcium does offer the possibility of detecting and measuring coronary atherosclerosis at an early and very treatable stage.

The normal artery

Arteries are essentially high-pressure pipes that carry blood pumped by the heart out to all of the tissues of the body, such as muscle, skin, bone, liver, kidneys, etc. The arterial system resembles a tree with a large main trunk – the **aorta** – and progressively smaller arteries branching out from the aorta. The thick-walled aorta, somewhat less than an inch in diameter, emerges from the top of the heart, gives off branches that supply the head and arms and then curves downward,

running just in front of the spine, to supply the trunk and internal organs, and eventually splits into two large arteries that supply the legs. After many stages of branching, the tiniest arteries become **capillaries** – the smallest blood vessels. In the capillaries, oxygen and nutrients in the blood are delivered to tissues, and waste products are picked up. Capillaries then join together to form tiny veins, which join together many times again to form larger veins. **Veins** are thin-walled, low-pressure blood vessels, which bring blood from the tissues back to the heart.

At the very beginning of the aorta, just beyond the heart valve that separates the pumping chamber of the heart from the aorta, the left and right coronary arteries branch off to supply the heart muscle itself. The left main coronary artery splits almost immediately into two branches, called the left anterior descending coronary artery and the left circumflex coronary artery. Coronary artery disease due to atherosclerosis is often described as 1-, 2-, or 3-vessel disease, depending on whether major blockages are found in various combinations of the right, left anterior descending, and left circumflex coronary arteries and their branches. The most important coronary artery is the **left anterior descending artery**, which supplies the front side of the heart as well as the muscular septum that separates the right and left pumping chambers. Almost all Americans over 50 years of age have some cholesterol deposition and thickening of the inner arterial wall near the beginning of the left anterior descending coronary artery. This thickened, cholesterol-loaded area is an atherosclerotic plaque, sometimes called the **“widow-maker” plaque**.

If we look at a cross-section (a thin, perpendicular slice) of a normal artery under the microscope, 3 tissue layers will be observed in the arterial wall. Most prominent is the middle layer, called the **media**, which consists of tightly packed smooth muscle cells, fibrous tissue proteins such as collagen and elastin, and gel-forming proteoglycans. The inner tissue layer is the intima, which is a much looser structure with fewer cells, less elastin, and considerably more open spaces between tissue components. The outer tissue layer, called the **adventitia**, is also a relatively loose tissue consisting mostly of bundles of collagen along with a few connective tissue cells.

Atherosclerosis is a disease of the **arterial intima** – so it’s worth giving a little more attention to the intima, which is the innermost layer of the arterial wall. The intima, like the other arterial tissue layers, is a type of connective tissue. Connective tissues give form and structure to the body, and keep the organs in place. Examples of connective tissues are the deeper layers of skin, the fascia that separate muscle layers (think of “gristle” in meat), bones, and tendons. Much of the strength in connective tissues comes from fibrous tissue proteins – elastin and various kinds of collagen – which are located between the cells in the tissue and are laid down by the cells. In the arterial intima, scattered smooth muscle cells manufacture the collagen, elastin, and proteoglycans that form the bulk of intimal tissue. When injured, connective tissues form scars, and some features of atherosclerosis are very similar to scar formation.

At the boundary between the arterial intima and flowing blood are found the lining cells of the artery, called endothelial cells. These cells are very long and wide, but have almost no thickness at all (often less than one thousandth of a millimeter), and they are arranged like paving stones on the inner surface of the arterial wall. There are no gaps between the endothelial cells. They appear to be carefully designed to keep blood cells and blood proteins from coming into contact with the underlying connective tissue of the intima. Historically, endothelial cells were

considered to act only as a blood-tissue barrier in blood vessels, but in recent years we have learned that endothelial cells are important for determining arterial tone and size and for causing inflammation. The key role of endothelial cells in atherosclerosis will be described later.

Progression of atherosclerotic lesions

In the normal arterial intima, the stage is already set for the beginning of cholesterol deposition and atherosclerosis. The normal arterial intima is loaded with low density lipoproteins (also called LDL, the “bad cholesterol”). The amount, or concentration, of LDL in the arterial intima is about 10 times higher than in any other connective tissue in the body. We will see below why this is so. Except for this high concentration of LDL, the normal arterial intima looks and acts like most other connective tissues.

Unless special tricks are used to detect LDL in the arterial wall, LDL are not visible even with electron microscopy. The earliest abnormal appearance in the arterial intima comes when the LDL particles change and form deposits of cholesterol and other lipids (lipids are fatty substances) outside of cells. These early cholesterol deposits are not usually visible in ordinary light microscopes, but can be seen when new lipid-stabilizing techniques are used with powerful electron microscopes.

Historically, the first lesion of atherosclerosis (a “lesion” simply means an abnormality in the tissue) is considered to be the **fatty streak**. Cells identified as macrophages become filled with oily cholesterol esters. Some smooth muscle cells also develop the same type of cholesterol ester deposits. If you look at the inner surface of an arterial specimen, fatty streaks appear as flat, yellowish streaks or dots to the naked eye. The larger dots will include hundreds of macrophages filled with cholesterol, many of them just beneath the endothelial surface. Fatty streaks are harmless early lesions, never causing arterial clots or damage.

When a person reaches about age 20, some fatty streaks develop a more troubling kind of cholesterol deposit. This is a kind of cholesterol-loaded membranous debris, which first appears deep in the arterial intima, close to the boundary between intima and media. Solid crystals of pure cholesterol may be found among the membranous debris. Why are these deposits worrisome? Cells sitting close to these deposits begin to die. Too much cholesterol is harmful to cells, and the intense cholesterol deposition is probably the reason for death of nearby cells. The fatty streak with deep cholesterol/membranous debris and early cell dropout is a transitional, or in-between, lesion of atherosclerosis. This **transitional lesion** was first clearly described in 1994 – not very long ago.

It is interesting to note that cells can happily stay alive with enormous amounts of cholesterol ester inside, but not cholesterol itself. However, we haven’t figured out how to shift all the cholesterol into the esterified form to prevent atherosclerosis progression.

It seems likely that cholesterol kills smooth muscle cells and macrophages in atherosclerotic lesions. However, I should mention an alternative idea about how these cells die. The alternative suggestion is that only oxidized cholesterol and other oxidized lipids, but not plain cholesterol, kill cells. We’ll discuss this more later.

There is also a debate about where the membranous debris in the deep intima comes from. I and a few other researchers have suggested that it comes from LDL, more or less directly. Most researchers seem to think that it comes from dead cells, but we think they are weak on the details!

The next routinely identified lesion is the atherosclerotic **fibrous plaque**. The word “plaque,” as we shall use it, means a thickened area on an otherwise smooth surface. An atherosclerotic plaque, therefore, is a thickening of cholesterol-loaded arterial intima. Note that the fatty streak, described above, is a flat and not a thickened lesion. Since the atherosclerotic plaque contains a large amount of fibrous protein, it can also be called a fibrous plaque.

Fibrous plaques contain an increased number of cells and an increased amount of fibrous protein, largely collagen. Almost all fibrous plaques also contain a central, cholesterol-rich core. In the core region, the cells are mostly dead or all dead, and the tissue is weakened because much of the fibrous protein has disappeared.

As time passes, the cholesterol-rich core may expand within an atherosclerotic plaque. The healthy intimal tissue between the core and the endothelial surface, called the fibrous cap, can be eroded from below by the twin processes of cholesterol deposition and cell death. The fibrous cap may become so thin that it finally breaks or ruptures. When this happens, the endothelial surface is also torn apart, and blood inside the artery comes into direct contact with the contents of the cholesterol-rich core. This contact makes the blood rapidly form a clot. The **ruptured plaque** with a blood clot is the final stage of atherosclerosis. By the time it is detected, the unfortunate person may be dead or may have suffered severe heart damage or a stroke.

Why the arterial intima is different from other connective tissues – lack of lymph vessels

Earlier we said that the concentration of LDL in the arterial intima is far higher than it is in any other connective tissue in the body. Other connective tissues contain a separate vascular system – the lymph vessels – that limits the build-up of LDL in the tissue spaces. Lymph vessels are thin tubes that reach into almost every nook and cranny in the connective tissues of the body. They act as sump drains, picking up excess proteins and particles that have leaked out across the capillary endothelial cells into the tissue spaces. The lymph vessels work so well that the concentration of LDL in tissue spaces and in lymph fluid is only one-tenth of the concentration in the blood stream.

The arterial intima, however, possesses **no lymph vessels**. Why not? The answer is not certain, but it probably relates to high pressures within the tissue of the arterial intima. Lymph vessels operate at very low pressure. If lymph vessels attempted to grow into the intima, they would be collapsed and perhaps no lymph would flow. In any case, lymph vessels are found in the adventitial (outside) layer of arteries and partially in the medial (middle) layer, but not in the intima.

LDL are ball-shaped lipid-protein particles, which are tiny compared to cell dimensions and can pass across the arterial endothelial barrier at a slow rate. LDL slowly diffuse throughout the loose structure of the arterial intima. However, when LDL reach the medial layer, they encounter a tightly packed tissue. In the medial layer, spaces between cells and fibers are packed

with a carbohydrate-protein (proteoglycan) meshwork of such fine dimensions that LDL-sized particles cannot enter the tiny pores of the meshwork. Therefore, LDL particles stop at the medial layer. No lymph vessels are available to sump the LDL away. The LDL concentration in the arterial intima rises until it matches the LDL concentration in the bloodstream. This is ten times higher than the LDL concentration in other connective tissues throughout the body.

Low density lipoproteins (LDL) in the arterial intima

A recent major theory of atherosclerosis recognizes that the very high LDL content of the arterial intima sets the stage both for cholesterol build-up and for cell responses of atherosclerosis. It is called the Response to Retention Theory, referring to **retention of LDL as the driving force in atherosclerosis**. Lack of lymph vessels explains much of the retention of LDL, but researchers have also found that collagen, elastin, and proteoglycans – that is, the fibrous matrix between the intimal cells – attract and retain LDL particles. The LDL particles stick to these fibrous tissue components.

The LDL particles enter the arterial intima very slowly across the endothelial cells, and some LDL particles leave the arterial intima very slowly in the same way, crossing the endothelial cell barrier in the reverse direction. However, the slowness of these processes means that LDL particles may remain in the arterial intima for weeks to months. During this time, the particles are subject to chemical attack by various enzymes and even by oxygen. Two bad things happen when LDL particles become chemically altered. First, two or more LDL particles can fuse or coalesce. To understand this, think about what happens when you mix oil and water, then shake the mixture. Initially, you will see small droplets of oil throughout the water, but then the small droplets will coalesce, forming successively larger droplets until all the oil is floating on top. Normal LDL particles are assembled from various lipids and one protein molecule in a way that resists coalescence. In the arterial intima, oxidation and enzymatic attack break the resistance of the LDL particles to coalescence. Large droplets and even “lakes” of oily LDL cholesterol ester lipid begin to form in the intimal tissue spaces. A second bad thing that happens to chemically altered LDL is that they are taken up into inflammatory or scavenger cells called macrophages.

Inflammation and white blood cells in atherosclerosis

Inflammation is a name for the defensive actions taken by various cells of the body to repel foreign invaders such as bacteria and viruses. Some of the same actions help the body to heal wounds, so inflammation has a role in wound healing as well. Finally, inflammatory actions are used to clean up or scavenge damaged protein or lipid molecules, which have been attacked by oxygen, by other reactive chemicals such as glucose, or by enzymes.

As far as we know, atherosclerosis is not caused directly by any bacteria or virus. Certain viruses have occasionally been found in atherosclerotic lesions, but they are thought to be bystanders, not actors. **Clinical trials of antibiotics** have not been effective in improving outcomes in patients with atherosclerosis.

Nevertheless, inflammation plays a major role in atherosclerosis. Certain tissue clean-up, scavenging, and healing aspects of inflammation have been identified in atherosclerotic lesions.

Almost all cells of the body can participate in inflammation, but white blood cells are designed only for inflammation. Two kinds of white blood cells, monocytes and lymphocytes, are attracted into atherosclerotic lesions from the bloodstream. Both of these cell types can be seen in large numbers sticking to the endothelial cells overlying cholesterol deposits. The white blood cells squeeze between the endothelial cells to enter the arterial intima. In the arterial intima, lymphocytes make signaling molecules that can stimulate or activate monocytes, smooth muscle cells, and even endothelial cells, promoting inflammation. However, exactly what “turns on” the lymphocytes in atherosclerosis and how big a part they play are still unknown.

Monocytes have a major role in atherosclerosis, and much has been learned about what they do. Soon after monocytes enter the arterial intima, they become larger, they send out ruffles and extensions, and they develop receptors (or sticky sites) for many of the chemically altered proteins and lipids commonly found in tissues. When these changes occur, the monocytes become **macrophages**, the most important scavenger or clean-up cells in tissues.

What is it in atherosclerotic lesions that needs cleaning up? One obvious answer is damaged, oxidized, disrupted, and coalesced LDL particles. Macrophages engulf and ingest these LDL particles in large numbers. Each macrophage has its own digestive enzymes that further disrupt the LDL, leading to the formation of pure cholesterol ester droplets inside the macrophages. Many macrophages have so many cholesterol ester droplets inside that the cells appear bubbly or foamy in the light microscope. These are called foam cells. More about foam cells below.

One of the most interesting aspects of inflammation in atherosclerosis is the recently discovered relationship between **C-reactive protein** and the risk of heart attacks and stroke. Simply put, by measuring the level of C-reactive protein (often called CRP) in the blood, you can predict a person’s risk of having a heart attack or stroke more accurately than by measuring either LDL or HDL cholesterol. The predictive power of CRP is surprisingly good, about as accurate as that of the ratio of LDL cholesterol divided by HDL cholesterol. Please note that CRP is not considered to cause atherosclerosis. Instead, the liver makes more CRP, whenever molecular signals tell the liver that inflammation is occurring anywhere in the body. This leads to a question: Does a high CRP level in a person with atherosclerosis simply mean that inflammation in the arterial intima is generating molecular signals for the liver? Or alternatively, do some people have a tendency to get tissue inflammation more easily (anywhere in the body), to make more CRP and at the same time to develop more atherosclerosis (that is, inflammation in the arterial wall)? Research studies are trying to answer these questions. In any case, high CRP levels are very predictive of atherosclerosis.

What happens to cholesterol in atherosclerosis?

Cholesterol and other lipids make up the membranes of all cells. The exact amount of **cholesterol in the membranes of a cell** must be carefully regulated, or else the membranes will be too stiff, too leaky, or too tight, and the cell will die.

There are two key facts about cholesterol that will help you understand why cells in an atherosclerotic lesion have such problems with it. First, cholesterol cannot be disassembled, hydrolyzed, or oxidized away by cells, as other molecules such as fats and glucose can be. (Actually cholesterol can be oxidized to a very limited extent, but less than one hundredth of all

the cholesterol in atherosclerosis is oxidized.) Second, all of the cholesterol in a cell will find its way into membranes; it is not stored outside of membranes.

If a cell has too much cholesterol, it can do two things to avoid death. A cell can link cholesterol with a fatty acid by a chemical ester bond, making **cholesterol ester** (sometimes called cholesteryl ester). Cholesterol ester does not get into cell membranes in substantial amounts. Cholesterol ester can be stored away inside the cell, and even in large amounts cholesterol ester does not kill the cell. There is probably some limit on how much cholesterol ester can be stored away, but the entire cell may fill up with it, becoming a foam cell, and even enlarge to a ridiculous size.

The second thing that a cell can do with cholesterol is to ship it out by transferring cholesterol to **high density lipoproteins** (HDL). HDL accept the extra or excess cholesterol from cells. HDL have a very important role to play in the arterial intima, because HDL can carry excess cholesterol from intimal macrophages back across the endothelial cells, into the bloodstream, and eventually back to the liver. This is called "**reverse cholesterol transport.**" The liver is usually able to get rid of any extra cholesterol the body has made.

Atherosclerosis can be viewed as a race between LDL, bringing cholesterol into the arterial intima, and HDL, removing cholesterol from the arterial intima. LDL have a simpler function. LDL cross the endothelial barrier and get trapped in the arterial intima. The cholesterol in LDL is eventually dumped in the intima, and the intimal cells have to deal with it. HDL have a harder job. HDL must cross the endothelial barrier, interact with macrophages and perhaps smooth muscle cells, pick up cholesterol, and carry the cholesterol back across the endothelial barrier. No wonder that atherosclerosis almost always tends to get worse as time goes by; only rarely does atherosclerosis regress and improve.

Smooth muscle cells in atherosclerosis

If not for smooth muscle cells, the intimal and medial layers of the artery wall would not be connective tissues (see 4th paragraph in "The Normal Artery" discussed earlier). Smooth muscle cells differ from the regular, skeletal muscle cells found in the biceps and other muscles around the body. First, smooth muscle cells do not have the striated, or train-track, appearance of skeletal muscle cells. That is why they are called "smooth." More importantly, smooth muscle cells are not as specialized as skeletal muscle cells. In the arterial wall, smooth muscle cells contract and relax, and they also manufacture large amounts of collagen, elastin, and proteoglycans that give extra strength to the artery and give it the characteristics of a connective tissue.

Most connective tissues in the body are built by cells whose primary purpose appears to be manufacturing collagen and other fibrous proteins. These cells are called fibroblasts, and they are the majority of cells found below the skin and in tendons. Why do smooth muscle cells instead of fibroblasts perform this task in the artery wall? The ability of the smooth muscle cell to contract and to relax appears to be important for arteries to grow and develop properly, since the tissue is constantly under tension, because of arterial blood pressure. Furthermore, the smooth muscle cell's intimate connections with fibrous tissue proteins assure that the whole tissue bears the tension properly. And under certain conditions, the artery wall can relax and

gradually expand even in an adult animal. We'll come back to this concept later when we discuss the interaction between endothelial cells and smooth muscle cells in determining arterial diameter.

Two major bad things and one major good thing happen to smooth muscle cells in atherosclerosis: they die (bad), they grow back over dead areas (good), and sometimes they produce fibrous scars that close down the flow of blood in the artery (bad). In addition, smooth muscle cells can participate in inflammation by making signaling molecules that influence the other arterial cells.

The death of smooth muscle cells in the core of atherosclerotic plaques is critical to the weakening of the plaque that leads to rupture and blood clotting. Smooth muscle cells make and maintain the fibrous tissue proteins. With smooth muscle cell death, the ability to repair gaps in the tissue is lost. As mentioned above (see "Progression of Atherosclerotic Lesions" above), smooth muscle cells may die because they take in too much cholesterol. The intense deposition of cholesterol outside of cells may kill nearby cells, as tiny amounts of cholesterol dissolve in water around the deposits and move through the water to nearby cell membranes. Another theory about smooth muscle cell death in atherosclerosis suggests that **oxidized cholesterol** (specifically, hydroperoxy-cholesterol) kills the cells. Hydroperoxy-cholesterol in very small amounts is toxic to cells. Hydroperoxy-cholesterol is formed by a reaction between cholesterol and oxygen dissolved in body fluids. Antioxidants might be able to slow this reaction, whereas antioxidants would likely have no effect on cholesterol-induced smooth muscle cell death.

In atherosclerosis, smooth muscle cells sometimes undergo cell division and proliferate to form new intimal tissue (although smooth muscle cell proliferation is not as prominent as researchers thought 20 years ago). Even more, smooth muscle cells are stimulated to make new collagen. These actions together might be termed "fibroproliferation." Fibroproliferation is both good and bad. As the dead, cholesterol-filled core of the atherosclerotic plaque begins to grow deep in the arterial intima, the smooth muscle cells near the surface begin to undergo fibroproliferation. They form the "fibrous cap" of the atherosclerotic plaque, which sits between the core and the endothelial surface. A strong fibrous cap will keep the plaque from rupturing and thus will prevent a heart attack. In this case, fibroproliferation is a good thing.

On the other hand, excessive **fibroproliferation** can begin to choke off the flow of blood in the artery. Most atherosclerotic plaques in the coronary arteries responsible for coronary chest pain have blocked 70% or more of the area through which blood ordinarily flows – this is called 70% or greater stenosis. In these plaques, excessive collagen usually accounts for the bulk of obstructing intimal tissue. Therefore, researchers have often tried to find ways to prevent fibroproliferation of smooth muscle cells. But this would have the effect of weakening the fibrous cap, perhaps leading to more heart attacks. It is a difficult balancing act. A better strategy may be to prevent, slow, or reverse the development of the cholesterol-rich core, which precedes fibroproliferation in atherosclerotic lesion development (see below).

Endothelial cells in atherosclerosis

Endothelial cells are the inner lining cells of arteries and all blood vessels. The **endothelium** is a term that just means all the endothelial cells. These cells are long, wide, and very flattened, as is

appropriate for lining cells. They are arranged like paving stones on the inner surface of the arterial wall. Endothelial cells are joined at their edges, forming a seal that is almost water-tight. Cells in the flowing blood never come into contact with the underlying connective tissue of the arterial intima, as long as the endothelial surface is intact.

Over the past century and especially in recent decades, researchers have discovered a number of interesting things about endothelial cells. Early on, it was recognized that endothelial cells are unusual in that blood does not clot when in contact with endothelial cells. Blood does clot when it contacts most of the other cell types in the body. The endothelial cell surface resists clotting. In addition, endothelial cells release certain chemicals that inhibit clotting of blood platelets (clotting cells in the blood).

There is a tendency to think that people with atherosclerosis may do better if their endothelial cells very actively resist clotting. That might be the case. However, since most heart attacks result from clots at the site of big, gaping holes in ruptured atherosclerotic plaques, the potential effectiveness of promoting endothelial cell resistance to clotting is unproven.

Endothelial cells not only form a barrier between blood cells and underlying tissue, but also form a barrier between most of the proteins in the blood and the underlying tissue. In particular, this is true for low density lipoproteins (LDL). Because the endothelial cells are an effective barrier for passage of LDL, we used to think that LDL levels in the arterial intima will remain low as long as the endothelial surface is intact. That turns out not to be the case, as explained earlier (“Low density lipoproteins in the arterial intima”). LDL levels in the arterial intima are approximately equal to plasma levels. Nevertheless, because of the endothelial barrier to passage of LDL, the movement of LDL across the endothelium is very slow in both directions (into and out of the intima), and individual LDL particles remain in the intima for very long periods of time.

One of the most fascinating things about endothelial cells is their role in determining the diameter to which arteries will grow. In the 1930s it was observed, by making windows in eggs to look through a microscope at developing chicken embryos, that tiny arteries with a lot of blood flow would grow, while those with sluggish or no blood flow would wither away. It has since been proven that the rate of **blood flow determines the size to which an artery will grow**. This even occurs in adult animals. If a leg is cut off in an accident, then the artery supplying the stump will wither down to a small size. On the other hand, when a surgeon creates a fistula, that is, a direct artery-to-vein connection, in the forearm of a kidney dialysis patient, then the artery supplying the fistula will actually grow in diameter over a number of months. This is due to the very high rate of blood flow in the fistula and its supplying artery.

If the Alaska pipeline could have been made of material as smart as human arteries, then the pipeline company might have laid down only a quarter inch pipe along the entire distance, and the pipe would have grown to an appropriate size and thickness to support thousands of gallons of oil per hour.

How does an artery grow to a certain size depending on its blood flow? The endothelial cells are able to detect the rate of blood flow across their surface. The signal probably starts near the cell surface membrane, where a tethering filament inside the cell binds to the membrane. The frictional force of blood flowing past the cell membrane will be concentrated at the end of the

tethering filament. This appears to open a molecular gate for calcium ions to enter the endothelial cell. Increasing the calcium level inside the cell causes it to start making signaling molecules that are shipped out the lower surface of the endothelial cell. Smooth muscle cells in the arterial wall, both intima and media, relax in response to the signaling molecules. As the smooth muscle cells relax, the artery dilates (widens) just a little bit. The diameter of the artery increases about 5 to 15 percent. This is not much, but it is enough to put the collagen and elastin fibers in the artery wall under considerable tension. Over the course of months the collagen and elastin fibers eventually relax or remodel, allowing the artery diameter eventually to increase as much as 100 percent or more in some circumstances.

It's important to note that when the artery diameter increases, the speed, or velocity, of blood flow slows down. A big pipe can deliver the same amount of blood at a lower velocity than a small pipe. Think about a river. The amount of water flowing down the river is approximately the same for many miles. At wide parts of the river, the flow is slow. At narrow places, the flow is rapid as the water squeezes through the narrow or shallow spots. The same thing happens in blood vessels. After the artery grows, the endothelial cells will detect a reduced, more normal flow velocity that "they are more pleased with." The endothelial cells then turn off the relaxation signaling molecules, and the artery diameter stops growing.

In the early 1980s a blood vessel biologist made the discovery that endothelial cells can send a relaxation signal to smooth muscle cells. It took several more years before the signal was identified as an astonishingly simple molecule, **nitric oxide**, composed of one atom of nitrogen and one atom of oxygen. Pure nitric oxide is a gas, but in the body, nitric oxide is dissolved in body fluids. Nitric oxide is a highly reactive and unstable molecule. Once it is formed, it remains as nitric oxide for only six seconds in body fluids. The smooth muscle relaxation system to nitric oxide is the same system that responds to nitroglycerin, which coronary patients take to relieve anginal chest pain. In the penis, the same system responds to Viagra to produce an erection. (This helps explain why a patient who takes nitroglycerin within 24 hours after taking Viagra can suffer fatal loss of blood pressure.) Nitric oxide is actually made by many different types of cells in the body; it plays a role in inflammation, lung function, and brain function. Over the past 2 decades, nitric oxide has gathered a glittering scientific resumé, somewhat like that of cholesterol!

People at risk for coronary heart disease and stroke (most of us, actually) can make use of the endothelial cell/nitric oxide/smooth muscle relaxation system to improve the long-term clinical outlook. We can rev up nitric oxide production in the coronary arteries simply by exercising. **Aerobic exercise** is required. When air is moving rapidly in and out of the lungs, the heart is also pumping blood rapidly around the body, and the heart itself is receiving increased rates of blood through the coronary arteries. If blood flows rapidly through the coronary arteries, the result is increased nitric oxide production by the coronary endothelial cells. The coronary arteries actually enlarge a little bit as they relax. In animal studies, exercise has produced long-term, structural enlargement of the coronary arteries. If atherosclerotic plaques are developing, then there is more room for them in enlarged coronary arteries! Furthermore, nitric oxide hinders clotting of blood platelets, and it also seems to inhibit atherosclerosis development in ways that are not yet clear. It has been estimated that moderate aerobic exercise (about 2 to 3 hours per week at levels that just noticeably increase breathing, but do not cause breathlessness) may lower the long-term risk of coronary disease by half! Most of the benefit of exercise may occur as

blood flows faster through the coronary arteries, turning on the production of nitric oxide by endothelial cells.

There is yet another aspect to the endothelial cell/nitric oxide/smooth muscle relaxation system that will help us to understand atherosclerosis better. The nitric oxide response to blood flow velocity helps arteries to resist the formation of blockages that cause anginal chest pain. Here is how it happens: When an atherosclerotic plaque develops and an artery just begins to pinch a little tighter, the blood flow speeds up at the site of the plaque, just as water flow speeds up at a narrow spot in a river. But an increase in blood flow velocity will cause endothelial cells at the site to make more nitric oxide and lead to relaxation and enlargement of the artery. The diameter of the channel for blood flow tends to remain unchanged. The artery, therefore, bulges outward, not inward, at the site of an early atherosclerotic plaque. Only advanced atherosclerotic plaques actually lead to inward bulging and pinching off of arterial blood flow. Unfortunately, this means that by the time that a patient actually feels anginal chest pain or has an abnormal exercise stress test due to arterial blockage, coronary atherosclerosis is already far advanced.

The endothelial cell/nitric oxide/smooth muscle relaxation system works a little differently in every person. The capacity of this system to widen the coronary arteries or an artery in the arm can be measured using research techniques. Recently researchers have found that an active arterial relaxation system predicts good outcomes in clinical coronary heart disease. Differences between people in the activity of this system are thought to be partly inherited and partly due to clinical factors. One of the strongest clinical factors is, in fact, an old enemy – high LDL cholesterol. Reducing LDL cholesterol makes arteries relax and widen more effectively.

Nitric oxide production represents the good side of endothelial cell activity in the arterial wall. Endothelial cells, however, can also play a bad role in recruiting inflammatory cells from the blood into the arterial intima. When they are stimulated by pro-inflammatory factors, endothelial cells begin to display “adhesion molecules” on their surface that attach to and bind blood monocytes and lymphocytes (these are the key inflammatory cells recruited from the blood in atherosclerosis). After monocytes and lymphocytes become firmly attached, they squeeze through tight gaps between the endothelial cells to enter the arterial intima. Within the intima, both monocytes and lymphocytes become activated to carry out inflammatory functions. As discussed earlier, inflammation is part of the atherosclerotic process. One way to reduce inflammation may be to inhibit the initial endothelial expression of adhesion molecules.

The lipid-rich core in the atherosclerotic plaque

The core of the atherosclerotic plaque is an area packed with cholesterol, cholesterol esters, and other lipids. The core is often a distinct region in the arterial intima, and it may occupy as much as 70-80% of intimal area. In other places, the core is recognized only as an area of increased lipid deposits. In a developed atherosclerotic core, most of the cells have died and disappeared. It is sometimes called the necrotic core to indicate cell death.

(Cell biologists make a distinction between “necrosis,” which is a rapid, bursting type of cell death, and “apoptosis,” which is a slower, controlled process of cell death. Apoptosis seems to be the way most cells die in atherosclerosis, despite historical use of the term “necrotic core.”)

In addition to cell death, fibrous tissue proteins such as collagen and elastin are eroded away in the atherosclerotic core. The erosion of these proteins is the result of enzymes that lyse, or break apart, the protein chains. The components of the protein chains then dissolve in the tissue fluid and float away. Loss of collagen and elastin leads to serious weakening of the inner arterial wall. Since no living cells are present to make new collagen and elastin, the weakening of the tissue is permanent and progressive.

The lipid-rich core is part of the essential description of atherosclerosis, since the Greek “athero” signifies “gruel” or “thick porridge.” If you cut into an atherosclerotic plaque and then squeeze it, the core contents may be extruded like toothpaste.

It is not certain how the processes of lipid deposition, cell death, and lysis of fibrous tissue work together to produce the atherosclerotic core. This is an important question, since the growth of the core eventually leads to complete breakdown of intimal tissue strength, plaque rupture, and blood clot formation in the artery. There are two main theories about how the core originates and grows.

The first theory states that cells in the plaque, especially macrophages, accumulate large amounts of cholesterol, which they store as oily droplets of cholesterol ester. These cells are then called foam cells. The cells also make and secrete enzymes that digest away the surrounding collagen and elastin. Foam cells can grow to enormous size. The foam cells eventually die, leaving behind lipid deposits in an area with little collagen and elastin.

That’s a neat, fairly simple story. In fact, a great deal has been learned in cell culture dishes about foam cell accumulation of cholesterol, foam cell death, and macrophage production of enzymes. However, certain details about atherosclerotic core development observed in human arteries, rather than culture dishes, argue for a different understanding with less of a role for foam cells. Some of the details are as follows: The smallest, earliest core regions in atherosclerotic lesions are found in the deeper part of the arterial intima, while macrophage foam cells are largely confined to the shallower part of the intima. Intensive lipid deposits are observed within fibers of elastin and within bundles of collagen fibrils, suggesting a direct interaction between these fibers and lipids such as cholesterol. The characteristic lipid deposits of the early atherosclerotic core have a high concentration of cholesterol and a low concentration of cholesterol ester, just the opposite of what one finds in foam cells. Finally, the exact pattern of cholesterol esters found in the atherosclerotic core tends to match the pattern seen in blood lipoproteins (high in cholesterol linoleate), rather than the pattern found in foam cells (high in cholesterol oleate).

These observations lead to a theory of core development that begins with deposition of cholesterol and other lipids in the fibrous tissue matrix between intimal cells. Exactly how this deposition occurs remains unknown. There is a very high content of cholesterol in the lipid deposits. The cholesterol may diffuse through the tissue fluid, perhaps aided by lipoproteins, to the membranes of nearby cells. When the cells acquire an excessive amount of cholesterol in their membranes, the membranes can no longer function properly, and the cells die.

Which of these theories is correct? How does the atherosclerotic core develop? The answer may be that both theories have something to tell us about the atherosclerotic core. Like detectives at

the site of a crime, researchers examining the atherosclerotic core must ask critical questions to evaluate the evidence left behind.

Plaque rupture and ulceration

With the passage of time, an atherosclerotic core that begins as a small cholesterol-rich disruption of tissue deep in the intima will spread upward and outward. In the thickened area that makes up the atherosclerotic plaque, a well-developed atherosclerotic core will occasionally occupy 70% or more of the intimal tissue. As the core spreads upward, it can eventually erode the living tissue very close to the inner surface of the artery, next to flowing blood. The core usually does not reach the surface, but it drastically weakens the intimal tissue. At some moment, the overlying intimal tissue tears or ruptures. When this happens, pressure and flow cause blood to rush into the weakened tissue of the atherosclerotic core. In addition, the cholesterol-laden contents of the core may be expelled into the flowing bloodstream. Contact between the blood and the atherosclerotic core material is a rapid, churning event. The core material quickly causes blood clotting. This is the way that most heart attacks and strokes happen.

Cardiologists and radiologists often inject dyes into arteries to outline the area occupied by flowing blood, revealing atherosclerotic plaques as narrowings in the bloodstream shown by the dye. Sometimes an angiogram shows a plaque with a small outpouching, giving angiographic evidence of a ruptured plaque.

Pathologists have asked what characteristics make atherosclerotic plaques prone to rupture. The key characteristics fall into 3 categories: (1) extensive deposits of cholesterol and other lipids, (2) thin caps of the atherosclerotic lesions overlying the core lipids (these are called “fibrous caps”), and (3) evidence of inflammation in the fibrous caps. The inflammatory component is interesting, because it may offer the possibility of preventing plaque rupture in new ways. The number of macrophages found in the fibrous caps of atherosclerotic plaques is higher in people with many ruptured plaques. (Of course, one can only count macrophages at autopsy, in a person who has already died; this doesn’t help the person being examined!) Furthermore, the macrophages make enzymes that can break down the strong fibrous proteins of the cap, and these enzymes are prominent at sites of plaque rupture. Research efforts are under way to find drugs that might reduce the numbers of macrophages in the fibrous cap or inhibit the macrophage enzymes responsible for dissolving the fibrous proteins of the cap.

Blood clotting, heart attacks, and strokes

Macrophages contribute to the clots that cause heart attacks and strokes in yet another way. Macrophages in and near the atherosclerotic core make a protein called “tissue factor,” which is the primary factor that activates clotting when core material comes into contact with blood.

A tiny blood cell called the platelet, which is much smaller than red blood cells and white blood cells, participates actively in blood clotting. When platelets come in contact with atherosclerotic core material, they quickly change shape, becoming flatter and disk-shaped, and become extraordinarily sticky. If the conditions are right – that is, when tissue factor is around – blood

proteins join with the platelets to make a rapidly growing clot. Most clots that cause **heart attacks** probably happen within a minute after the rupture of an atherosclerotic plaque.

A blood clot that causes a heart attack grows and extends across the width of the coronary artery until it completely blocks blood flow. The person in whom this occurs does not feel any pain until the clot is choking off almost all the blood flow. The heart muscle downstream, which no longer receives the oxygen it needs to continue its rhythmic contraction, sends powerful pain signals to the brain.

Sometimes a blood clot, developing over a ruptured atherosclerotic plaque, does not extend all the way across the coronary artery. If the clot goes only halfway across, the person generally will feel nothing at all. The blockage of blood flow is not enough to cause the heart muscle to cry out in pain. This kind of clot probably happens much more often than complete clots that cause heart attacks. The partial clot is not entirely harmless, however. Over a period of weeks, cells of the arterial grow into and over the clot. This kind of clot eventually becomes part of an enlarged atherosclerotic plaque. The channel for blood flow in the artery is narrowed, and the stage is set for an actual heart attack.

Almost every heart attack is caused by a blood clot forming over a ruptured or ulcerated atherosclerotic plaque. **Strokes** also are caused quite often by ruptured or ulcerated atherosclerotic plaques, but some strokes have causes other than atherosclerosis. For example, some strokes happen because of blood clots that form in the heart chambers and travel in the bloodstream to the brain, and some strokes are due to small blood vessels that burst and bleed into the brain. Atherosclerosis is not the cause of these strokes. One of the key jobs of the physician is to figure out whether a stroke has been caused by atherosclerosis or something else.

About 7 out of 10 strokes, nevertheless, are caused by blood clots that form over a ruptured or ulcerated atherosclerotic plaque. Usually the plaque responsible for a stroke is found in one of the large carotid arteries in the neck. You can feel the pulse of the right or left carotid artery by placing your finger in a location midway between your Adam's apple and the back of your jaw. The carotid artery has an inner diameter about 3 to 4 times larger than the diameter of a major coronary artery. When a blood clot develops over a ruptured atherosclerotic plaque in the carotid artery, the clot usually does not grow big enough to extend entirely across the large diameter of the artery. Instead, a clot may develop, only to break away from the plaque and be swept downstream by the flowing blood. The clot, which can now be called an "embolus" because it is thrown off the arterial wall, enters the smaller and smaller branches of the carotid artery within the brain. Finally it stops when it is wedged into an arterial branch smaller than the size of the embolus. Blood flow in this arterial branch stops, and tissue death occurs in the part of the brain supplied by the arterial branch.

When a plaque ruptures and exposes flowing blood to the tissue factor from the plaque core, clotting of the blood is stimulated very strongly and quickly. Is it possible to protect against the stimulation of clotting? In other words, can the blood clots be prevented, even if plaque ruptures continue to happen? The answer is that approximately one out of every 4 or 5 heart attacks and strokes can be readily prevented by **aspirin**, which partially blocks the clotting of blood. Aspirin does not prevent plaque rupture, but can block clotting enough that blood continues to flow, or enough that no embolus is produced. Under some circumstances, a health care provider will

choose to prescribe somewhat more powerful clotting inhibitors such as **clopidogrel (Plavix)**, **dipyridamole-aspirin combination pills (Aggrenox)**, or **warfarin (Coumadin)**. Whether aspirin should be used along with these prescription medications is a decision for the health care provider to make.

It is possible to completely block blood clotting, but such a strategy would have terrible consequences. Blood clotting is necessary to prevent bleeding when blood vessels are cut or stretched too far. Bleeding that occurs internally in stomach or intestines, or in the brain, can be fatal. (One type of rat poison simply causes excessive bleeding that kills the rats.) Thus there is a limit to what can be done for prevention of heart attacks and strokes by blocking blood clotting.

Stabilization of the vulnerable plaque

Doctors are beginning to talk about a new way of preventing heart attacks and strokes that can be used along with agents like aspirin that partially block blood clotting. The new strategy is called stabilization of the vulnerable plaque. A vulnerable plaque is one that is close to the point of rupturing. Our knowledge is improving on how vulnerable plaques can be stabilized and ruptures prevented, even in people who have widespread and severe atherosclerosis. The most important method appears to be lipid control – that is, reducing LDL and probably increasing HDL. One should remember that cholesterol, the major chemical found in the plaque core, can actually be removed from atherosclerotic tissue by HDL. There is a **race between LDL carrying cholesterol into the atherosclerotic core and HDL removing cholesterol from the core**. The outcome depends upon the balance of these two processes.

An exciting research study recently reported from Seattle suggested that it might be possible to remove most of the cholesterol from atherosclerotic plaques by intensive lipid management. The research group at the University of Washington in Seattle had maintained 60 patients with coronary heart disease for 10 years on good diets as well as drugs that lower LDL and raise HDL levels in blood (the drugs were simvastatin [Zocor], niacin, and colestipol [Colestid] used in combination). Eight of these patients were randomly selected to have a new magnetic resonance imaging (MRI) procedure performed. The new MRI technique allowed the researchers to “see” the lipid and fibrous components of atherosclerosis in the carotid arteries, almost as if they were pathologists looking through a microscope. In cross-section views of the arteries, eight matched control patients (who had not received lipid treatment) showed a lipid core occupying 17% of the area. In the 8 intensively treated patients, the lipid core averaged only 1% of the area. Thus it appears that the lipid core of the atherosclerotic plaques, presumably similar to that in the control patients 10 years ago, may have been almost entirely removed by the intensive lipid treatment.

Heart catheterizations are very often performed with the intention of determining how many blockages exist in the coronary arteries and how severe the blockages are. Almost all such blockages are due to atherosclerotic plaques that narrow the channel for blood flow in the arteries. Very severe blockages (ones that narrow the channel by 70% or more) can be treated by **balloon catheters** that expand the channel, or by balloon catheters followed by wire mesh “**stents**” that keep the channel expanded, or by **bypass surgery** that inserts new blood vessels to re-establish good blood flow downstream of the blocked places. All of these heart catheterization procedures and bypass surgeries would not be needed if it were possible to shrink the atherosclerotic plaques by using – for example – cholesterol drugs. Can this be done by

using diet and drugs instead of catheters and surgeries? Unfortunately, the answer is usually no. Some patients with blocked coronary arteries causing cardiac chest pain (called “angina”) can improve with diet and drugs, but usually they do not improve very much. The very best diet and drug regimens might improve coronary artery blockage by, on average, 1% to 2% (we’ll discuss this later). This is not a very meaningful percentage improvement when the blockages causing anginal chest pain are 70% to 99% blockages.

As research studies were performed to measure the effect of **diet and cholesterol drugs** on coronary artery blockages, a startling and important discovery was made. Although, as stated above, the degree of blockage changed very little, the number of heart attacks and heart-related deaths in the patients dropped dramatically. In a few studies, the number of heart attacks, heart-related deaths, and episodes of rapidly worsening chest pain dropped by 70% to 80%. In many studies, these “cardiac events” dropped by 20% to 40%. These results were far out of proportion to the tiny 1% to 2% improvements in the heart blockages. What was going on?

The answer seems to be as follows: Diet and cholesterol drugs do not change the overall size of atherosclerotic plaques very much at all, so that the blockages improve very little. However, diet and cholesterol drugs have some kind of effect on plaques to make them much less prone to rupture. As stated at the beginning of this section, the “vulnerable plaques are stabilized.”

Experiments in animals have helped us to understand what happens when vulnerable, rupture-prone plaques are stabilized. You may recall that macrophages in the fibrous cap of atherosclerotic plaques play an important role in plaque rupture. Macrophages make enzymes that break down the fibrous proteins of the arterial wall. These macrophages also form many large foam cells that can almost totally occupy parts of the fibrous cap. In animal experiments, when LDL levels in the blood are lowered and/or when HDL levels are raised, the foam cells lose their cholesterol, and eventually the foam cells and most of the macrophages disappear from the atherosclerotic plaque. What is left behind is strong fibrous tissue that is not prone to rupture. This improvement in the atherosclerotic plaque can be accomplished within one to three months in animal experiments. It is thought that the same things happen in human atherosclerotic plaques, when LDL is lowered, HDL is raised, or both. It is possible to stabilize the vulnerable plaque, even when the overall size of the plaque changes very little. Certainly this is good news, since almost all heart attacks, most strokes, and most cardiovascular deaths appear to happen when vulnerable plaques rupture.

The take-home message is this: When a person is experiencing severe anginal chest pain due to coronary artery blockages, that person usually needs a heart catheterization. A balloon procedure to stretch open a blocked artery may then be performed, often with placement of one or more stents, or alternatively, coronary bypass surgery may be performed. These procedures in the catheterization laboratory or in the operating room do a good job of relieving anginal chest pain. However, these procedures usually don’t reduce the risk of subsequent heart attacks, strokes, and heart-related or vascular deaths very much. That is a job for diet, lifestyle, and drug treatment, which can drop the risk of sudden atherosclerotic events by half or more. Treatment of atherosclerosis with diet, lifestyle, and drugs changes the risk factors such as LDL, HDL, blood pressure, and smoking. In addition, drugs that partially block blood clotting, such as aspirin, can help to prevent heart attacks, strokes, and cardiovascular deaths. So, getting your arteries opened up with balloons and stents, or getting your arteries bypassed surgically does not

prevent heart attacks, strokes, and deaths very well. A great deal more can be done to prevent these events by improving risk factors and by partially blocking blood clotting.

Regression of atherosclerotic lesions – slow and uncommon

Doctors, nurses, and other health care providers who treat people needing cardiovascular prevention are often asked if it is possible to “make the atherosclerotic plaques go away.” The technical term for making the plaque go away is regression of the atherosclerotic plaque. Thus far, all the attempts to demonstrate atherosclerotic regression have shown how difficult it is. Regression does occur in some cases, but it is slow and it is not very common.

In the previous section, some exciting new research was mentioned, suggesting that it might be possible to remove most of the cholesterol from atherosclerotic plaques. If that is so, then why doesn't the plaque just go away? The answer is that the bulk of an atherosclerotic plaque is made of fibrous tissue, similar to the **fibrous tissue of a scar**. In fact, at the microscopic level, most of the atherosclerotic plaque looks just like the tissue in a scar. Scar tissue doesn't disappear very easily. Furthermore, when cholesterol is removed from the atherosclerotic core, it might be replaced and filled in by more scarring or fibrous tissue. So the plaque tends to stay, even when cholesterol is removed.

At this time, we don't know exactly how similar an atherosclerotic plaque is to a skin scar. A scar in the skin usually results from sudden cuts or tearing of the skin. The skin scar might shrink away or even disappear within a few months after the injury. A “scar” in the inner wall of an artery – that is, a plaque – results from years or decades of damage from cholesterol and inflammation. Does this mean that the arterial scar would require years to decades to shrink away? We don't know the answer. But consider this: if the skin were continually damaged every day, the skin scar would not shrink away. Instead it would probably continue to grow. In most people, this is exactly the situation that the arterial scar faces. It's difficult to remove the damaging factors from the artery wall, especially LDL cholesterol. Yet with new understanding of appropriate diet and lifestyle, as well as new drugs, it has become possible in recent years to greatly reduce the damaging factors (which are the same risk factors already discussed). As more powerful programs of risk reduction are used by motivated patients, we may yet see meaningful atherosclerosis regression.

Atherosclerosis regression, or shrinkage of the arterial scar, is very difficult, but the good news is that it usually is not necessary. The coronary blockages can be treated by balloons, stents, and/or bypasses. The risk of heart attack and stroke can be greatly reduced by removing cholesterol from the vulnerable plaque.

There is one more way that a narrowed artery might improve with time. In the earlier section on endothelial cells, it was mentioned that endothelial cells produce **nitric oxide** and other chemical signals that cause smooth muscle cells to relax. Over time, the pinched arterial wall can relax and stretch, and the channel for blood flow can enlarge. This is a slow process, and it works better if LDL cholesterol in the blood is reduced. Raising HDL cholesterol may also promote the endothelial/nitric oxide/smooth muscle system, according to a recent research study. Therefore, even if the atherosclerotic plaque does not shrink at all, the entire arterial wall may stretch to

allow blood to flow better past the site of blockage. This improvement may depend on lowering LDL and possibly raising HDL cholesterol.

Thus a partially blocked artery could improve, in theory, either by shrinkage of the atherosclerotic plaque or by stretching of the entire arterial wall. When researchers have performed heart catheterizations to look for improvement in blockages, they cannot say which of these two mechanisms might explain the improvement. X-ray dyes outline the channel for blood flow, but cannot determine what is happening in the artery wall. Any improvement in the channel for blood flow is called **angiographic regression**.

Keeping in mind that angiographic regression does not necessarily mean shrinkage of plaques, we can discuss some research trials in which coronary artery blockages improved with diet, lifestyle, and drugs. Such trials give us an idea of what is required to achieve angiographic regression. The first report of coronary angiographic regression came from a study reported from the University of Southern California in Los Angeles. People who had undergone coronary bypass surgery at least 6 months previously were divided randomly into a group that received high dose drug treatment and a group that received modest drug treatment. The high dose drug treatment was **colestipol** 15 grams twice a day (about a tablespoon of a powder mixed with water, swallowed as a slurry, twice a day) and **niacin** also given in large doses. The films from coronary angiography were presented to “blinded” reviewers (“blinded” doesn’t mean that they couldn’t see the films, of course, but only that they did not know which film came before treatment and which came after treatment). After 2 years and again after 4 years, one out of six patients had an overall angiographic result that was better after treatment than before. Their arteries had opened up. Although it would seem disappointing that only one out of six improved, even this modest result was cheered, because it had never been seen before.

In 1987, a new drug came on the market. Lovastatin is, like penicillin, a complex chemical made by a mold or fungus. Merck pharmaceutical company developed lovastatin in the United States, following its discovery in Japan. **Lovastatin** (originally branded as **Mevacor**) continues to be an excellent drug in the statin class, along with **pravastatin (Pravachol)**, **simvastatin (Zocor)**, **fluvastatin (Lescol)**, **atorvastatin (Lipitor)**, and **rosuvastatin (Crestor)**. These drugs given as small, once-daily tablets (5 to 80 mg) reduce LDL cholesterol more than any other drug, and similar to the effect of combining huge doses of colestipol and niacin as mentioned above.

Hopes were high that lovastatin and other statins might reverse atherosclerosis. But it did not happen. Research trials testing statins against placebo (sugar pill) medication showed that the usual progression of blockages (becoming more and more narrow with time) could be slowed by about half in patients given statins, but reversal occurred only in rare patients. In 2002, atorvastatin (Lipitor) given at a dose of 80 milligrams daily did lead to angiographic regression in most of a group of patients with familial hypercholesterolemia. This is an inherited condition found in 1 out of 500 people around the world, who usually have cholesterol levels of 350 milligrams/deciliter (mg/dl) without treatment. The reason for such a good response (actual regression) in these patients might have been their very high initial cholesterol levels.

Combining a statin with certain other cholesterol-modifying drugs in high enough doses might lead to angiographic regression in most people with coronary atherosclerosis. Some of the promising results have been achieved by the same research team in Seattle mentioned earlier,

who showed probable removal of most of the cholesterol from atherosclerotic plaques in the carotid arteries. One treatment program included lovastatin 40 milligrams daily combined with high doses of colestipol. Another treatment program used niacin and colestipol in doses similar to those used in the Los Angeles study. The niacin-colestipol combination actually worked better in the Seattle trial than it did in the Los Angeles trial. Possible reasons are that more of the Seattle patients had high cholesterol levels or that the Los Angeles patients might have had less “active” atherosclerosis.

The Seattle research team also studied patients who had coronary atherosclerosis with low levels of HDL “good” cholesterol. LDL “bad” cholesterol levels in these patients were either normal or slightly high. Patients in the test group received simvastatin (Zocor) in a low to moderate dose and niacin in fairly high doses of 2,000 to 4,000 mg daily, usually divided into morning and evening doses. Patients who were randomly chosen to take placebo (that is, no real cholesterol-modifying medication) had the usual progression or worsening of coronary blockages. Patients in the test group showed slight angiographic regression.

Drug treatment is generally more powerful than dietary and lifestyle treatment for lowering LDL cholesterol and raising HDL cholesterol, but the research trial with the best result for angiographic regression used lifestyle and dietary training rather than drugs. An early, successful **study of lifestyle treatment** for anginal chest pain was actually planned and run by a medical student at Baylor College of Medicine in Houston, Texas in the early 1980s. With funding from a Houston developer, a group of people with anginal chest pain were housed together for a month in an elegant hotel, where they ate a vegetarian diet, exercised, and underwent relaxation and group therapy sessions. Their symptoms and their radionuclide stress tests improved. Later as a cardiologist in San Francisco, the same researcher refined his methods for measuring improvement in coronary blockages and ran a randomized research trial, in which 48 coronary patients were randomly chosen to enter a test group (with counseling and dietary change) or a control group (neither counseling nor dietary change). Exercise training in the test group was similar to that performed in cardiac rehabilitation programs all over the United States. Relaxation and visualization sessions had enthusiastic participation by the test patients; in fact, most patients did more than what the doctor asked. Many “vegetarian” diets include eggs and milk products, but this diet allowed only egg whites and nonfat dairy products. Thus the diet was more vegans than vegetarian. It was extremely low in fat. Both saturated and unsaturated fat added up to only 7% of total calories, compared to 35% in the usual American diet. The result of this lifestyle and dietary program after one year was angiographic regression of about 2% in the average coronary blockage. A follow-up study after 5 years showed further angiographic regression. This is as good as or better than the best drug trials.

Another research trial using dietary change to achieve angiographic regression was performed at St. Thomas Hospital in London. The key dietary changes were strong reductions in **dietary saturated fat and cholesterol** from animal food products (milkfat, meat, and eggs), calorie restriction to bring overweight patients down to normal body weight, and increases in polyunsaturated fatty acids (plant oils) and omega-3 fatty acids (fish and fish oils). In the group treated with this rigorous diet, angiographic regression was achieved. The coronary blockages opened up just a little, compared with the usual result of closing further in people not treated with diet.

In every one of the successful diet and drug trials performed so far, the measurement of angiographic regression improved by 0.2% to 2% per year. But the average measurement of total blockage, at the start of treatment, was in the range of 20% to 40%. Therefore, we have to ask how meaningful the results were. Diet, lifestyle, and drugs are not likely to replace balloon catheters, stents, and bypass grafts for the treatment of coronary or other arterial blockages anytime soon. Heart catheterization and/or bypass operations remain necessary to relieve anginal chest pain in most patients. Even so, the prevention of heart attacks and strokes needs diet, lifestyle, and drugs much more than heart catheterization and surgery, as we have already discussed.

Calcium, coronary calcium scans, and a dog that won't hunt

When doctors use the term “hardening of the arteries” to refer to atherosclerosis, people may think of bony hard arteries. In many cases, this is actually correct. “Bony hard” is an accurate description of many old, advanced plaques, in which a large amount of calcium is found. The calcium mineral in these plaques is very similar to bone calcium. Recently it has been discovered that cells of the arterial wall make many of the same proteins and enzymes made by bone cells.

Another fairly new discovery is that calcium deposits are found in early atherosclerosis as well as late, advanced atherosclerosis. Tiny microscopic nodules of calcium salts are found in the lipid-rich core of both early and mature atherosclerotic plaques. What is the calcium doing there? This question is being studied in the laboratory. Perhaps the best answer so far is that calcification is part of the inflammatory process. It results from inflammation in a lipid-rich tissue. Is the calcium good or bad? Again the answer is not known. We are quite sure, for example, that cholesterol is bad. Get rid of cholesterol, and the whole arterial wall can heal and improve. But at this time, we don't know how to get rid of the calcium, nor do we know how to prevent calcium deposits forming in the lipid-rich core. So it's not possible to tell whether calcium in the artery wall is good or bad. For now, calcium should be viewed as neutral, neither harmful nor beneficial – just there.

Calcium in the coronary atherosclerosis is good in one way. It allows us to see and measure atherosclerosis in the coronary arteries long before the atherosclerosis would usually cause a heart attack or anginal chest pain. The reason is that calcium shows up in an x-ray, while cholesterol does not. Ever since we have had x-rays, radiologists have noticed calcium deposits in large atherosclerotic arteries in the body. But the small size of the coronary arteries, the fact that they are surrounded by ribs and other bones, and most of all, the constant motion of the heart and its arteries all conspired to make the job of seeing calcium in the coronary arteries very difficult. In the early 1980s, a space-age computed tomographic (CT) scanner called an **electron beam CT or ultrafast CT** was invented. The electron beam CT scanner forms a 3-dimensional image of the heart in only 30 to 100 milliseconds, compared to one full second for a conventional CT scanner. The difference in timing is critical, making it possible for the electron beam CT to “freeze” the motion of the heart. Instead of seeing coronary calcium only in a few elderly people, the electron beam CT can visualize and measure very small amounts of coronary calcium in half of all men by ages 50-55 and in half of all women by ages 60-65. The new technology had 2 problems, however. First, it did not produce as sharp an image as conventional scanners, and thus the electron beam CT had few real uses beyond calcium detection in the coronary

arteries. Second, each electron beam CT scanner was pricey, more than \$2 million each with installation. The next breakthrough occurred in the late 1990s, when software programs were developed that allowed conventional CT scanners, found in every hospital in the United States, to visualize coronary calcium deposits almost as well as electron beam CT. The conventional CT used in this way can be called a “gated CT.”

Research studies today are asking how to use this new technology to help in the detection and treatment of coronary atherosclerosis. Every year we learn a little more, but it will likely be a decade or two before we really understand how useful it might be and how to use it best. Some facts are becoming clear, as follows: (1) The amount of calcium seen on an electron beam CT (and probably gated CT as well) gives a very good estimate of how much atherosclerosis is present in the coronary arteries. (2) Older people have more calcium than younger people. This is mostly due to older people having more atherosclerosis, but it appears that older people have more calcium than younger people for the same amount of atherosclerosis. (3) African Americans have less calcium than Caucasians and Asians for the same amount of atherosclerosis. (4) A younger person, particularly a man under age 50 or a woman under age 60, can have enough atherosclerosis to have a heart attack or heart-related death without any calcium at all seen on electron beam or gated CT. (5) Even so, the presence and amount of calcium in the coronary arteries is a very strong predictor of future heart attack or heart-related death, about as strong a predictor as LDL cholesterol, HDL cholesterol, smoking, diabetes, and high blood pressure all considered together. (6) For people who have chest pain suspected to be from coronary blockage, stress tests and heart catheterizations should be performed. They are still the best procedures to make a diagnosis of blockage. (7) Finally, whether treatment of cholesterol and other risk factors will prevent a rising amount of calcium in the coronary arteries and whether a rising amount of calcium means an even higher risk of heart attack are unanswered questions at this time.

A older friend once told me a saying that he used for any proposal that looked reasonable at first glance, but on close inspection or testing was very unlikely to work. His brief comment was “That dog won’t hunt!” It might have floppy ears, a big nose, and perhaps a loud bark, but it could not point out the birds or retrieve the ducks. **The dog that won’t hunt** here is **chelation therapy**. Let me point out that chelation therapy does work for lead poisoning and for certain types of iron overload in the body. It is a necessary medical procedure for those reasons. The theory of chelation therapy is that metals, including calcium, can be removed from tissue by adding fluid with a dissolved chemical that binds up the metal, makes the metal dissolve, and removes the metal as the chemical and its bound metal are washed away. In fact, you can remove calcium from atherosclerotic plaques by chelation in the laboratory. So this dog has floppy ears and a big nose. There are several problems: The strength of the chelating fluid used in laboratory experiments for removing calcium would kill any animal or person. It can only be used on dead tissue. Furthermore, as stated above, we don’t know whether removing calcium from plaques would help at all in atherosclerosis. Nobody has ever reported an experiment on chelation in animals with atherosclerosis. This may be because nobody who does animal atherosclerosis research has ever figured that chelation had enough of a chance to work to make it worthwhile performing the experiment. Then again, 85% of all the calcium in an animal or human is in the bones, not in the arteries. Chelation that really removes calcium from the arteries would likely also remove much of the calcium from bones. If you could prevent heart

attacks in this way, you might end up just a jelly blob lying in bed, toothless and unable to get up and walk.

The argument is made that by getting the conditions just right, you can remove calcium from the arteries, stop chest pain, and cure atherosclerosis. How can we tell if it works? One thing has been clearly learned. We cannot rely on testimonials, even if the clinic has a list of people who say, “My anginal chest pain disappeared!” Anginal chest pain can wax and wane, get better and worse, for no apparent reason. Chelation therapy, like any other medical treatment, must be tested in a clinical trial where people are randomly chosen to receive real treatment or sham (placebo) treatment. Such trials have been done. There was no hint of benefit for real chelation treatment over sham treatment. But there is one very evident result of chelation therapy. The patient feels tingling around the lips or in the fingers, as calcium levels in the blood are mildly reduced. So this dog has a kind of bark as well. But it won’t hunt.

Summary – a story of cells, cholesterol, and clotting

Arteries, which are blood vessels that carry blood from the heart out to all the tissues of the body, are formed by a remarkable interaction between lining cells – endothelial cells – and arterial wall cells – smooth muscle cells. The faster blood flows through an artery, the larger it will grow. We can take advantage of this fact by using exercise to create rapid blood flow through the coronary arteries. Exercise seems to strongly prevent coronary atherosclerosis, and a key mechanism may be the production of nitric oxide by endothelial cells exposed to rapidly flowing blood.

The intima – the innermost layer – of the artery is the place where atherosclerosis develops. Atherosclerosis is a disease in which cholesterol builds up in the intima and damages the inner lining of the artery. This disease is so dangerous to human life that it causes about one-third of all deaths in modern, industrialized countries of the world.

The arterial intima is a connective tissue. However, unlike almost all other connective tissues in the body, the intima has no lymphatic vessels to drain away excess proteins that leak across the endothelial lining. Because of the lack of lymphatics, the concentration of low density lipoproteins (LDL, sometimes called “bad cholesterol”) in the arterial intima is 10 times higher than it is in any other connective tissue in the body. This sets the stage for atherosclerosis. The concentration of LDL in the intima is about equal to LDL concentration in blood. The LDL concentration in blood is a strong risk factor for atherosclerosis. Other factors that raise the risk for atherosclerosis include tobacco use, high blood pressure, diabetes, male sex, and low levels of high density lipoproteins (HDL, or “good cholesterol”).

In addition to endothelial cells and smooth muscle cells, inflammatory cells enter the arterial wall from the blood in atherosclerosis. The high concentration of LDL in the intima seems to be a major reason for the entry of inflammatory cells. C-reactive protein in the blood is a marker for inflammatory stimulation in the body, and C-reactive protein is also a very strong risk factor for atherosclerosis.

The earliest lesions (abnormal spots) of atherosclerosis are fatty streaks, which contain inflammatory cells loaded with cholesterol, called foam cells. As time passes, some fatty streaks

develop another kind of cholesterol deposition outside of cells in the deep intimal layer. These cholesterol deposits become the lipid-rich core of the atherosclerotic plaque. The plaque grows by fibroproliferation to become a thickened area in the inner artery wall. The deep lipid-rich core also expands, and it may undermine and erode the living artery wall tissue all the way up to the inner surface of the artery wall. If this happens, the inner surface can break or rupture. Weakening of the arterial tissue seems to be caused by a combination of cholesterol and inflammation. When flowing blood comes into contact with the lipid-rich core of a ruptured plaque, the blood can clot within one to a few minutes.

LDL are lipoproteins that carry cholesterol into the artery wall. HDL are lipoproteins that can pick up cholesterol from the artery wall and direct the cholesterol back to the liver. This is consistent with the fact that people with high levels of HDL have fewer heart attacks and strokes.

The final event in the history of some atherosclerotic plaques is the blood clot that forms over the ruptured plaque. If the clot blocks enough blood flow to the heart or brain, then a fatal heart attack or stroke can happen. Certain medications, including aspirin, can help to prevent the formation of clots and thus help to prevent heart attacks and strokes.

Reversal or regression of atherosclerosis is a goal often sought by patients. Sometimes regression cannot be achieved. When it does occur, it is difficult and slow. Widening of the narrowed channel for blood flow, called angiographic regression, could happen in one of two ways. Either the plaque shrinks, or the whole arterial wall relaxes and expands. Most angiographic regression probably results from relaxation and expansion of the whole arterial wall. If only one cholesterol-modifying drug is used, angiographic regression is not achieved in most patients, but combinations of drugs can achieve regression. Two trials of diet and lifestyle have also achieved regression. Significant arterial narrowings are usually narrow the channel by 70% or more. When angiographic regression is seen, the average extent of the regression is one to a few percent. This is not enough to replace the need for coronary bypass operations and heart catheterization balloon and stent procedures.

While bypass operations and balloon-stent procedures effectively relieve anginal chest pain, they usually do not reduce the risk of heart attacks very much. One of the discoveries of the 1990s is that giving drugs such as statins to lower LDL can reduce the risk of heart attacks and strokes by 25% to 40%. Early evidence also suggests that risk can be reduced further by using drugs that raise HDL. These discoveries have led to a new idea that vulnerable atherosclerotic plaques – that is, plaques prone to rupture because of a large lipid-rich core – can be stabilized to prevent rupture. A recent research study using magnetic resonance imaging brings up the possibility that most of the cholesterol in an atherosclerotic plaque might be removed by using drugs that lower LDL and raise HDL. This could lead to stabilization of the vulnerable plaque.

Calcium is deposited in the lipid-rich core of early atherosclerotic plaques, and late atherosclerotic plaques sometimes become heavily calcified. This fact is useful in detecting coronary atherosclerosis at an early stage and in measuring the extent of coronary atherosclerosis. Special techniques of CT scanning (computed tomographic scanning) are needed to detect and measure coronary calcium deposits. Research studies today are asking how CT scanning might be used to help fight coronary atherosclerosis and prevent heart attacks. Like other ways of diagnosing heart disease, CT scanning is not foolproof. Many questions remain to

be answered. The presence of calcium in coronary atherosclerosis might seem to support the idea of chelation therapy in treatment. However, on closer inspection, the evidence says that chelation therapy won't work and doesn't work.

Thousands of diseases affect humankind. It is a remarkable fact that just one disease – atherosclerosis – causes about one-third of all human deaths in modern societies, as well as much suffering and early death. Just 30 to 40 years ago, coronary heart disease and stroke were mostly accepted as facts of life, and it was thought that little could be done about them. We now have coronary care units, bypass operations, and sophisticated heart catheterization procedures that are effective in people with chest pain, but don't work very well to prevent future heart attacks and strokes. As we learn more about atherosclerosis, and especially about the roles of cholesterol and clotting, it is becoming clear that most (and someday nearly all) heart attacks and strokes can be prevented.