

**Nutrition and Medicine, 2006**  
**Tufts University School of Medicine**  
**Lipids / Cardiovascular Disease I/II:**  
Learning Objectives

**Margo N. Woods, D.Sc.**

1. Identify each of the major lipoproteins and briefly describe their role with respect to atherosclerosis.
2. Describe the pathogenesis of atherosclerosis.
3. List the ATP III NCEP risk factors for cardiovascular disease that modify the therapeutic goal for LDL cholesterol levels. Indicate which are amenable to lifestyle modification and describe what is meant by the term “CHD Risk Equivalents”.
4. Describe what factors are used to calculate how the 10-year risk of CVD is determined and where to obtain a tool to calculate this number.
5. Define metabolic syndrome and discuss how it affects cardiovascular disease risk.
6. Enumerate the major classes of dietary fat. Indicate how each alters plasma lipoprotein levels and/or other cardiovascular disease risk factors.
7. Dietary *trans* fatty acids and omega-3 fatty acids alter cardiovascular disease risk in different ways. Discuss how and the biological basis for these effects.
8. Indicate whether quantity or quality of dietary fat alters cardiovascular disease risk and why.
9. Identify one dietary factor, other than dietary fat, that has been suggested to alter cardiovascular disease risk and discuss the current state of knowledge.

## **Nutrition and Cardiovascular Disease:** Answers to Learning Objectives

### **1. Identify each of the major lipoproteins and briefly describe their role with respect to atherosclerosis.**

Basic components of lipoproteins

- Fatty Acids

Fatty acids are hydrocarbon chains with a methyl and carboxyl end. Most fatty acids have an even number of carbon atoms that are arranged in a straight chain. The majority of dietary fatty acids vary in chain length from 4 to 22 carbons. Although by no means the most metabolically active, fatty acids with 16 and 18 carbons comprise the bulk of the fatty acids in both the diet and the human body. Individual fatty acids are distinguished from each other not only in chain length, but in degree of saturation, conformation, and location of double bonds. Fatty acids with no double bonds are referred to as saturated. Those with a single double bond are monounsaturated and fatty acids with multiple double bonds are polyunsaturated. The double bonds within unsaturated fatty acids can appear in the more common *cis* configuration, where hydrogen atoms are on the same side of the carbon chain or in the *trans* configuration, where hydrogen atoms on opposite sides of the carbon chain. The presence of double bonds, per se, and their number, position and conformation allows for fatty acids to occur as multiple isomers.

Geometric isomers of fatty acids result from differences in the conformation (spatial orientation) of the double bond(s). In practical terms, the presence of a *cis* relative to a *trans* double bond results in a greater bend or kink in the carbon atom chain. This kink impedes the fatty acids from aligning or packing together, thereby lowering the melting point of the fat. The presence of a *trans* double bond reduces the internal rotational mobility of carbon atoms and is less reactive to chemical change than a *cis* double bond.

Positional isomers of fatty acids are defined by differences in the location of double bonds within the acyl chain. These differences result in small alterations to the melting point of the fatty acid but large differences in the way the fatty acids are metabolized. The most common distinction made among potential isomers of fatty acids is the location of the first double bond from the methyl end of the acyl chain. Fatty acids in which the first double bond occurs 3 carbons from the methyl end are called omega-3 fatty acids, frequently denoted  $\omega$ -3 or n-3 fatty acids. Major dietary sources of  $\omega$ -3 fatty acids include soybean (7% of total fatty acids) and canola (10% of total fatty acids) oils which are rich in  $\gamma$ -linolenic acid (18:3  $\omega$ -3), and fish, especially fatty fish, which is high in eicosapentaenoic acid (EPA, 20:5  $\omega$ -3) and docosahexaenoic acid (DHA, 22:6  $\omega$ -3). The class of fatty acids in which the first double bond occurs 6 carbons from the methyl end is termed  $\omega$ -6 fatty acids. Major dietary sources of  $\omega$ -6 fatty acids include corn, safflower, soybean and sunflower oils, which are rich in linoleic acid (18:2  $\omega$ -6). Enzymes that metabolize fatty acids to bioactive compounds distinguish among positional isomers.

The metabolic products of the different positional isomers of fatty acids have different and at times opposite physiological effects.

Most double bonds occur in a non-conjugated sequence; that is, they are separated by a carbon atom not involved in a double bond. Some of these occur in the conjugated form; that is, without an intervening carbon atom separating the double bonds. Conjugated double bonds tend to be more reactive chemically, for example, may be more likely oxidized. Although there is considerable speculation regarding their role in disease progression, the current state of knowledge is insufficient to make any firm conclusion.

- Triglyceride

Triglyceride, commonly referred to as triglyceride, is composed of three fatty acids esterified to a glycerol molecule. Each position of the three carbons comprising the glycerol molecule allows for a stereochemically distinct fatty acid bond position; sn-1, sn-2 and sn-3. The fatty acid moieties of the triglyceride molecule account for ~ 90% of its weight, depending on the length of the constituent fatty acids. A simple triglyceride contains three identical fatty acids and is exceedingly rare in nature. A triglyceride with two or three different fatty acids is called a mixed triglyceride and comprises the bulk of the lipid in both the diet and body. The melting point of a triglyceride is determined by the specific fatty acids esterified to glycerol moiety: chain length; number, position and conformation of the double bonds; and the stereochemical position of the fatty acids. In vivo, triglyceride serves as a storage form of energy and as a reservoir for fatty acids that

Mono- and diacylglycerides have one and two fatty acids, respectively, esterified to glycerol. Rarely present in nature, they are intermediate products during triglyceride digestion and are frequently added to processed foods for their ability to act as emulsifiers. Recently, a cooking oil composed solely of diacylglycerides has been introduced into the market and some health benefits have been attributed to it. Limited experience and scientific literature makes it difficult to determine at this time whether there is an advantage of diacylglycerols compared to traditional cooking oils composed of predominantly triglycerides.

- Phospholipid

A phospholipid is composed of two fatty acids esterified to a glycerol molecule and one polar head group attached via a phosphate linkage. Phospholipid molecules are amphipathic. The fatty acids confer hydrophobic properties and the polar head group hydrophilic properties. Long chain fatty acids are preferentially esterified to the number 2 position of the glycerol backbone of the phospholipid molecule. The most predominant polar head group of phospholipid; choline, serine, inositol or ethanolamine, vary in size and charge.

In vivo, due to their amphipathic nature, phospholipids serve as the major structural components of cellular membranes and are critical constituents of lipoproteins particles necessary for the transport of lipids in the bloodstream. The fluidity of cell membranes is

determined, in part, by the fatty acid profile of the constituent phospholipids. Cell membrane associated phosphatidylinositol, although a minor constituent quantitatively, is the predominant source of arachidonic acid. Arachidonic acid is a substrate for cyclooxygenase and 5-lipoxygenase, resulting in the formation of prostaglandin. Other phosphatidylinositol derived compounds, inositol (1,4,5) triphosphate and diacylglycerol, play important roles in cell signal transduction pathways as components of second messenger cascades. Signaling through various phosphoinositidies have a role in mediating cell growth and differentiation, apoptosis, intracellular vesicle trafficking, ion channel activation, insulin action, cytoskeletal changes and motility.

- Cholesterol and cholesteryl ester

Cholesterol is an amphipathic molecule that is composed of a steroid nucleus and branched hydrocarbon tail. Its occurrence in the food supply is for the most part restricted to fats of animal origin. About 40% to 60% of dietary cholesterol is absorbed.

Cholesterol occurs naturally in two forms, either free or esterified to a fatty acid at carbon number 3. Free cholesterol is a critical component of cell membranes and along with the phospholipid fatty acid profile, influence fluidity. Intracellularly, free cholesterol is an important mediator of cholesterol homeostasis. It inhibits the activity of 3-hydroxy 3-methylglutaryl CoA (HMG CoA) reductase, the rate limiting enzyme in de novo cholesterol biosynthesis thereby minimizing intracellular cholesterol accumulation. It increases the activity of acyl CoA cholesterol acyltransferase (ACAT), the intracellular enzyme that esterifies free cholesterol thereby lowering intracellular concentrations. It decreases the synthesis of LDL cell surface receptors, thereby diminishing the uptake of additional cholesterol from plasma. It is critical for all these factors to work in concert to allow for adequate free cholesterol for optimal cellular functioning while limiting the build-up of free intracellular cholesterol due to its cytotoxic nature.

An ester of cholesterol is formed when a fatty acid is esterified to cholesterol. Cholesteryl esters are less polar than free cholesterol. As a consequent, whereas lipoprotein associated free cholesterol is localized to the surface of the particle, cholesteryl ester is sequestered in the core. The majority of cholesterol in plasma is carried on LDL. Cholesteryl esters are formed in plasma as a result of the activity of lecithin cholesterol acyltransferase (LCAT) and account for about two-thirds of the circulating cholesterol. Intracellularly, cholesteryl ester is stored in lipid droplets and accounts for a major portion of atherosclerotic plaque. In the arterial wall cholesteryl ester is either derived from lipoprotein particles or is synthesized endogenously as a result of the activity of ACAT.

## Lipoproteins

- Chylomicrons

Chylomicrons are intestinally derived lipoprotein particles formed and secreted after the ingestion of fat. Their main function is to provide a mechanism whereby dietary fat

(triglyceride), cholesterol, and other fat-soluble compounds are carried from the site of absorption (intestine) to other parts of the body for subsequent uptake and potential metabolism or storage. The first step in the formation of chylomicron particles is the resynthesis of triglyceride and phospholipids from fatty acids, and glycerol, sn-2 monoacylglycerides or lysophospholipids respectively. This process occurs on the smooth endoplasmic reticulum. The fatty acid composition of the chylomicron triglyceride, but not phospholipid, reflects the fatty acid composition of the diet. A large percent of the cholesterol that enters the enterocyte is re-esterified, a reaction catalyzed by ACAT, prior to incorporation into the chylomicron particle. The re-esterification processes are facilitated by fatty acid-binding protein.

Chylomicrons particles are the largest of all the lipoprotein subclasses. The structure is similar to that of all the other lipoprotein particles that will be discussed. The core of the spherical particle is composed primarily of apolar components; triglyceride and cholesteryl ester. The surface of the particle is composed of the more polar constituents; phospholipid monolayer, apolipoproteins and free cholesterol. Fat-soluble vitamins are sequestered in the core of the chylomicron particle.

In humans, the distinguishing apolipoprotein of intestinally derived lipoproteins is apolipoprotein (apo) B-48, whereas the distinguishing apolipoprotein of hepatically derived particles is apo B-100. Apo B-48 is a large hydrophobic protein synthesized on the rough endoplasmic reticulum. It results from mRNA editing and is approximately 48% of the molecular weight of apo B-100. Additional apolipoproteins on the surface of a chylomicron particle include apo A-I, apo A-IV, apo A-II, apo C and apo E. Recent work suggests that the release of apo A-IV is stimulated by lipid feeding and has a role in the regulation of upper gut function and satiety. It has further been suggested that apo A-IV may be involved in the long-term regulation of food intake and that chronic ingestion of a high fat diet blunts the intestinal apo A-IV response to lipid feeding, hence predisposes to obesity.

Chylomicrons are assembled from apo B-48 and triglyceride accumulated in the smooth endoplasmic reticulum. Microsomal triglyceride transfer protein (MTP) is responsible for transporting and inserting the triglyceride into the nascent chylomicron core as the particle is then transferred into the lumen of the endoplasmic reticulum. Some data suggest that small apo B-48 containing particles fuse with a large, independently formed triglyceride apo B-48 free particles prior to secretion. Carbohydrate is added to the nascent chylomicron particle just before release from the the Golgi apparatus by exocytosis from the cell.

Chylomicrons are released from enterocytes into the lymph before being channeled from the thoracic duct to the subclavian vein. Once in circulation, the triglyceride component of chylomicron particles is hydrolyzed by lipoprotein lipase and apolipoproteins are transferred to other lipoprotein particles. Lipoprotein lipase is synthesized in adipose tissue, heart and skeletal muscle, and migrates to the capillaries where it functions. Apo C-II is a critical cofactor for the reaction whereas apo C-I and apo C-III inhibit the reaction. The hydrolysis of triglyceride from chylomicrons in circulation accounts for the

delivery of ingested fat from the gastrointestinal system to peripheral tissue for oxidation, metabolism and storage. This process also results in the production of lipids and apolipoproteins that form HDL. Chylomicron particles depleted of the triglyceride component are termed chylomicron remnants and are taken up by the liver via either the LDL-receptor or LDL-receptor-like protein receptors. The components of chylomicron particles are either used by the liver directly or are incorporated into newly synthesized hepatically derived lipoprotein particles.

- Very low density lipoprotein (VLDL)

VLDL is hepatically derived particles that mediate the transport of fat from the liver to peripheral tissue. The triglyceride in VLDL is synthesized from fatty acids derived from de-novo lipogenesis, cytoplasmic triglyceride, lipoproteins taken up directly by the liver and exogenous free fatty acids. The major apolipoprotein in VLDL is apo B-100. Apo B-100 is synthesized on the rough endoplasmic reticulum and transferred to the Golgi apparatus where, with the involvement of MTP, it is incorporated into the nascent VLDL particle. Inadequate triglyceride or the absence of MTP results in internal degradation of apo B-100. This degradation is facilitated by the association of nascent apo B with a cytosolic chaperone protein, heat shock protein 70. In plasma, VLDL also contains apo E and apo C, which are either present at the time of secretion or acquired once in circulation.

The lipid components of VLDL particles are similar to those of chylomicrons, however, the relative proportion of triglyceride is less. This results in smaller denser particles. Once in circulation, the initial stages of VLDL metabolism are similar to that of chylomicron metabolism. Lipoprotein lipase hydrolyzes the core triglyceride. The resulting fatty acids are taken up by cells locally and are oxidized for energy, used for the synthesis of structural components (phospholipid) or bioactive compounds (leukotrienes, thromboxanes), or stored (triglyceride). Triglyceride depleted particles, VLDL remnants, can either be taken up directly by receptor mediated mechanisms in the liver or remain in circulation and be progressively depleted of triglyceride.

- Intermediate density lipoprotein (IDL)

The delipidation of VLDL results in the progressive formation shift in the composition of the lipoprotein particle from one defined as VLDL to intermediate density lipoprotein (IDL) and eventually LDL. This process is facilitated by not only lipoprotein lipase, but also hepatic lipase. This second lipase has the capacity to hydrolyze both triglyceride and phospholipid. The progressive depletion of triglyceride from the lipoprotein particle results in a marked increase in the relative proportion of cholesterol. As VLDL is depleted of triglyceride, apo C and apo E are transferred to other lipoproteins in circulation. The ultimate product is LDL, a cholesterol-rich particle containing only a single copy of apo B-100.

- Low density lipoprotein (LDL)

LDL can be taken up by an apolipoprotein mediated or scavenger receptor. There are a number of LDL receptors belonging to the LDL receptor gene family. They include the LDL receptor, LDL receptor-related protein (LRP), apo E receptor 2 protein, multiple epidermal growth factor-containing protein 7, VLDL receptor, LRP1B, megalin, LRP 5 and LRP 6. Whereas the LDL receptor mediates the uptake of apo B-100 or apo E containing lipoproteins, the other members of the LDL receptor gene family recognize multiple ligands and appear to play diverse biological roles. Once LDL is taken up by the cell it disassociates from the receptor and the receptor can be recycled. The LDL particle fuses with a lysosome and is subsequently degraded. This step is critical for whole body cholesterol homeostasis because the cholesterol taken up from circulation and released from the lysosome has three distinct effects. It inhibits the activity of HMG CoA reductase, down regulates the synthesis of LDL receptors, and increases the ACAT activity. The actions have the effect of decreasing the rate of de novo cholesterol biosynthesis, amount of LDL taken up by the cell, and level of free cholesterol in the cytosol, respectively. Long chain saturated fatty acids have been reported to further suppress LDL receptor activity. Alternatively, LDL can be taken up by a scavenger receptor on macrophages in various tissues. This system predominates after LDL particles are modified or oxidized as they circulate in plasma.

- High-density lipoprotein (HDL)

HDL particles are derived from the liver, periphery and intestine. They participate in “reverse cholesterol transport” by shuttling cholesterol from the peripheral tissues to the liver for excretion, metabolism or storage. An integral part of this process is scavenger receptor (SR)-B1. This hepatic HDL receptor selectively takes up the cholesteryl ester component of HDL, thus promoting the ability of HDL to pick up additional cholesterol from peripheral tissue.

HDL is a heterogeneous group of particles that differ in both the apolipoprotein composition and size. All HDL particles contain apo A-I. However, other apolipoproteins associated with HDL can include apo A-II, A-IV and C=III. HDL particles reportedly protect other lipoproteins from oxidative modification. This activity appears to be related to the presence of apo A-I, paraoxonase and platelet-activating factor acetylhydrolase. Plasma HDL levels are inversely related to triglyceride and risk of developing cardiovascular disease.

Tangier disease is an autosomal recessive disorder characterized by the virtual absence of HDL cholesterol. HDL-mediated cholesterol efflux, and intracellular lipid trafficking and turnover are abnormal in fibroblasts from Tangier patients. The genetic defect encoding for a member of the ATP-binding cassette (ABC) transporter family has been identified in these individuals. The ABC transporter is integral to the process of reverse cholesterol transport. Individuals with a mutation in the ABC transporter have very low levels of HDL cholesterol and develop premature atherosclerosis.

## **2. Describe the pathogenesis of atherosclerosis.**

Answer: The contemporary view of the pathogenesis of atherosclerosis is called the response to injury hypothesis. Formulated in 1973 and modified in subsequent decades, it states that the lesions of atherosclerosis are initiated as a response to some form of injury to the arterial endothelium. The injury increases permeability to plasma constituents, including lipids, and permits blood monocytes and eventually platelets to adhere to the endothelium.

Below are the proposed factors which contribute to the development of atherosclerotic plaques:

a. Endothelial injury

A variety of factors can cause endothelial damage, including hypertension or other hemodynamic forces, toxins such as those in cigarette smoke, hypoxemia, irradiation, or immune complex deposition. Three manifestations of this injury appear to be most important: increased endothelial permeability, increased monocyte adhesion, and increased endothelial cell replication. Blood monocyte adherence to endothelial cells is probably mediated by the induction of specific receptor molecules on the surface of injured or activated endothelial cells.

b. The inflammatory/immunologic response to injury

Following endothelial damage, monocytes and other inflammatory cells adhere to the damaged endothelium. Monocytes migrate into the intimal layer, differentiate into macrophages and release inflammatory cytokines. Macrophages ingest lipid and become foam cells. Although monocytes and macrophages express the LDL receptor, the rate at which they take up native LDL is too low to generate foam cells. They can, however, take up a modified (oxidized) form of LDL that is not recognized by the LDL receptor. The specific receptor on macrophages for oxidized LDL is called the scavenger receptor. Foam cells may therefore be considered to be specialized macrophages. They are present in variable numbers in all stages of atheromatous lesions. Atheromatous lesions begin early in life as fatty streaks and eventually develop into atheromatous plaques. Plaques can enlarge over time, producing progressive narrowing of the blood vessel lumen. Plaques can become calcified and can also rupture. Thrombosis can occur, causing acute occlusion of the lumen, leading to tissue hypoxia and death (such as may occur during a myocardial infarction).

c. Smooth muscle cell proliferation

Production of growth factors, cytokines, and other compounds by endothelial cells and inflammatory cells induces vascular smooth muscle cells to migrate from the media to the intima. There, smooth muscle cells proliferate, possibly in an attempt to repair the damaged endothelium. However, this proliferation promotes the progressive growth of atherosclerotic lesions, as does lipid accumulation within foam cells. Smooth muscle cell proliferation is also thought to be a critical factor involved in restenosis after angioplasty.

d. Hyperlipidemia

Increases in plasma levels of LDL (or other lipoprotein) may increase the rate of lipid penetration into the artery wall. Local modification (oxidation) of LDL may render it

more atherogenic. For example, oxidized LDL is more rapidly ingested by macrophages through the scavenger receptor, is chemotactic for circulating monocytes, increases monocyte adhesion, inhibits motility of macrophages already in lesions, stimulates release of growth factor and cytokines, is cytotoxic to endothelial cells and smooth muscle cells and is immunogenic. This suggests that antioxidants (such as vitamin E) might be effective in preventing atherosclerosis by reducing LDL oxidation.

e. Platelet activation and thrombosis

Plaque rupture leads to the exposure of procoagulants (such as tissue factor and von Willebrand factor) to circulating blood. Platelets aggregate and adhere to the plaque and the surrounding vessel wall, leading to thrombosis and vascular occlusion or embolism. Factors which reduce platelet aggregation (possibly omega-3 fatty acids) may prevent thrombosis and reduce ischemic events.

**3. List the ATP III NCEP risk factors for cardiovascular disease that modify the therapeutic goal for LDL cholesterol levels. Indicate which are amenable to lifestyle modification and describe that is meant by the term “CHD Risk Equivalents.”**

The Third Report of the Expert Panel on the Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III) from the National Cholesterol Education Program (NCEP) has defined elevated serum LDL cholesterol is a major cause of CVD. A large number of clinical trials in diverse populations have shown a reduction of CVD risk with LDL-lowering therapy and therefore, treatment of elevated serum LDL cholesterol is the primary target of therapy. The target level of LDL varies by patient, depending upon whether the patient a) has documented CVD, b) has two or more risk factors for CVD but does not have CVD or if CVD status is unknown and c) has a “CHD Risk Equivalents.”

Answer: The 5 major risk factors (besides elevated serum LDL cholesterol) for CVD are:

- Cigarette smoking
- Hypertension (BP  $\geq$  140/90 mmHg or on antihypertensive medication)
- Low HDL cholesterol ( $<$  40 mg/dL)\*
- Family history of premature CHD (myocardial infarction or sudden cardiac death in a male relative  $<$  55 years old or a female relative  $<$  65 years old)
- Age (men  $\geq$  45 years or women  $\geq$  55 years)

\*HDL cholesterol ( $>$  60 mg/dL) is a negative risk factor (i.e. it is protective)

Additional risk factors for CVD (which do not modify the serum LDL cholesterol goal but which are nonetheless important to address clinically) include obesity (particularly abdominal obesity), a sedentary lifestyle and/or an “atherogenic diet.” Emerging risk factors include hypertriglyceridemia, elevated lipoprotein (a), small dense LDL particles, elevated homocysteine, elevated inflammatory markers (such as C-reactive protein), prothrombotic factors, and impaired glucose tolerance. Individuals with the metabolic syndrome (see subsequent question) also have an elevated risk of CVD. These factors are considered as emerging because there is insufficient data on which to formulate treatment

guidelines. For example, recent data (2006) is not supportive of lowering homocysteine levels but providing supplemental folic acid.

The NCEP guidelines describe “CHD Risk Equivalents.” These are conditions in which the absolute risk of major CHD events (e.g. myocardial infarction or sudden cardiac death) is similar to the risk of events seen in individuals with known CHD. Individuals with “CHD Risk Equivalents” should therefore be treated like patients with established CHD, with similar therapeutic goals. “CHD Risk Equivalents” include:

- Diabetes
- Other forms of atherosclerotic disease, e.g., peripheral artery disease, carotid artery disease, abdominal aortic aneurysms
- Multiple risk factors which confer a 10-year risk of CVD  $\geq 20\%$

**4. Describe what factors are used to calculate the 10-year risk of CVD is determined and where to obtain a tool to calculate this number.**

|   |   |
|---|---|
| The risk assessment tool below uses information from the Framingham Heart Study to predict a person’s chance of having a heart attack in the next 10 years. This tool is designed for adults aged 20 and older who do not have heart disease or diabetes. To find a patients risk score, enter their information in the calculator below. The actual tool can be found on <a href="http://hp2010.nhlbihin.net/atpiii/calculator.asp?usertype=pub">http://hp2010.nhlbihin.net/atpiii/calculator.asp?usertype=pub</a> . |   |
| Age:  | <input type="text"/> years                                    |
| Gender:   | <input type="checkbox"/> Female <input type="checkbox"/> Male |
| Total Cholesterol:  | <input type="text"/> mg/dL                                    |
| HDL Cholesterol:  | <input type="text"/> mg/dL                                    |
| Smoker:   | <input type="checkbox"/> No <input type="checkbox"/> Yes      |
| Systolic Blood Pressure:  | <input type="text"/> mm/Hg                                    |
| Are you currently on any medication to treat high blood pressure.   | <input type="checkbox"/> No <input type="checkbox"/> Yes      |

Basic facts:

Total cholesterol –

- Less than 200 mg/dL 'Desirable' level that puts you at lower risk for heart disease. A cholesterol level of 200 mg/dL or greater increases your risk.
- 200 to 239 mg/dL 'Borderline-high.'

- 240 mg/dL and above 'High' blood cholesterol. A person with this level has more than twice the risk of heart disease compared to someone whose cholesterol is below 200 mg/dL.

#### HDL cholesterol

- Less than 40 mg/dL A major risk factor for heart disease
- 40 to 59 mg/dL The higher your HDL, the better
- 60 mg/dL and above An HDL of 60 mg/dL and above is considered protective against heart disease.

#### **5. Define metabolic syndrome and discuss how it affects cardiovascular disease risk.**

The “metabolic syndrome” (formerly called “Syndrome X”) is characterized by a constellation of interrelated metabolic risk factors in one individual. In the clinical setting, the metabolic syndrome should be diagnosed when an individual has three or more of the five following metabolic risk factors\*:

- Abdominal obesity (waist circumference > 40 inches for men or > 35 inches for women)
- Triglycerides > 150 mg/dL
- Low HDL cholesterol (< 40 mg/dL for men or < 50 mg/dL for women)
- Blood pressure > 130/85 mmHg
- Elevated fasting glucose (> 110 mg/dL; however, more recent guidelines released by the NHLBI, the AHA and the ADA suggest lowering this to > 100 mg/dL)

\*These guidelines are from the ATP III; the World Health Organization suggests slightly different diagnostic criteria for metabolic syndrome.

The metabolic syndrome is also associated with other metabolic risk factors that may be more difficult to assess during routine clinical assessment. These include insulin resistance, small dense LDL particles, prothrombotic states, and proinflammatory states.

In light of this constellation of CVD risk factors, individuals with the metabolic syndrome have increased CVD risk. With the increase in overweight and obesity in the U.S. and other developed nations, the metabolic syndrome is becoming increasingly common. According to the ATP III, “the increasing prevalence of the metabolic syndrome threatens to partially reverse the reduction in CHD risk that has resulted from a decline in serum LDL cholesterol levels in the U.S. population, which has occurred over the past three decades.” Therapy should include a healthy diet and physical activity, with the goal of achieving and maintaining a healthy weight and improving lipids, blood pressure, and glucose tolerance/insulin sensitivity. Pharmacologic therapy for hypertension, dyslipidemia, and glucose intolerance may also be recommended.

**6. Enumerate the major classes of dietary fat. Indicate how each alters plasma lipoprotein levels and/or other cardiovascular disease risk factors.**

Dietary fat is a major source of energy, providing 9 kcal per gram (versus 4 kcal per gram of protein and carbohydrate). Dietary fat also increases palatability of meals, delays gastric emptying, and aids in the absorption of the fat-soluble vitamins (A, D, E, and K) in the small intestine. The essential fatty acids linoleic acid (18:2, n-6) and alpha-linolenic acid (18:3, n-3) cannot be synthesized by the body and so must be consumed in the diet. Arachidonic acid is sometimes classified as an essential fatty acid, however, it can be synthesized from alpha-linolenic acid. Arachidonic acid can substitute for part of the alpha-linolenic acid requirement.

- Saturated fatty acids

Saturated fat is one of the principal dietary determinants of LDL cholesterol level. In general, saturated fatty acids increase plasma LDL cholesterol by decreasing LDL receptor-mediated catabolism. This is thought to be mediated by both decreased LDL receptor messenger RNA expression and decreased membrane fluidity. Saturated fats also increase HDL cholesterol by decreasing HDL clearance from the plasma. However, saturated fats have a greater effect on LDL than on HDL.

Not all saturated fatty acids affect plasma lipids in the same way. Stearic acid (18:0), does not raise total and LDL concentrations like other saturated fatty acids because a relatively higher proportion of it is converted in the liver to oleic acid (18:1), a monounsaturated fatty acid. Current data suggest that stearic acid may increase the risk of thrombosis, at least in the short term. Ultimately, though, the distinction between stearic acid and other saturated fats may not be important in dietary advice to patients because stearic acid and other saturated fatty acids are found in the same foods (beef and dairy products).

Major sources of saturated fatty acids are meat and full fat dairy products (butter, milk, cheese, eggs, ice cream, etc). Other sources include coconut, palm, and palm kernel oils. Today, 12-14% of an average American's daily calories come from saturated fat, down from 18-20% several decades ago. As a result, average LDL cholesterol levels have also declined during this time.

- Monounsaturated fatty acids

When substituted for dietary saturated fat, monounsaturated fat has a hypocholesterolemic effect, lowering plasma total and LDL cholesterol while having a minimal lowering effect on HDL cholesterol. When substituted for dietary carbohydrates, monounsaturated fat has a neutral effect on LDL and HDL concentration. In individuals with impaired glucose tolerance or diabetes, substitution of monounsaturated fat for carbohydrates may improve triglyceride and HDL cholesterol levels.

Major sources of monounsaturated fat include canola and olive oils. Other sources include avocado, sesame seeds, almonds, cashews, pecans, peanuts, peanut butter, and peanut oil.

- Polyunsaturated fatty acids

There are two types of polyunsaturated fatty acids: omega-6 (n-6) fatty acids and omega-3 (n-3) fatty acids, the later type will be discussed in the question below. The substitution of polyunsaturated fat in place of dietary saturated fat lowers LDL cholesterol more than substitution with monounsaturated fat, but it may also lower HDL more.

The primary omega-6 fatty acid in the diet is linoleic acid (18:2, n-6), which serves as a precursor for arachidonic acid (20:4, n-6). The major sources of polyunsaturated fatty acids are most vegetable oils, especially corn, soybean, sunflower and safflower oils.

**7. Dietary trans fatty acids and omega-3 fatty acids alter cardiovascular disease risk in different ways. Discuss what distinguishes them from other fatty acids and the biological basis for these effects on cardiovascular disease risk.**

- *Trans* fatty acids

Diets high in *trans* fatty acids increase plasma LDL cholesterol and decrease plasma HDL cholesterol levels. Other adverse effects of *trans* fatty acids have been reported but wait to be validated.

*Trans* double bond containing fatty acids occur naturally at low levels and dairy products and meat. They are produced during the hydrogenation of vegetable oils, during which the liquid oil is heating in the presence of metal catalysts and hydrogen. Hydrogenated oils are commonly used to make commercially prepared packaged foods, baked goods, and fried foods. Hydrogenated fat is favored by food processors because the resulting products are more resistant to oxidation than products made with liquid vegetable oil, hence have a longer shelf life. As of January 1, 2006, the US Food and Drug Administration requires that the *trans* fatty acid content of foods be listed on the Nutrition Facts label (for amounts > 0.5 g).

- Omega-3 fatty acids

The omega-3 fatty acids include the plant derived alpha-linolenic acid (ALA; 18:3, n-3), rich in canola and soybean oils and the long chain omega-3 fatty acids eicosapentaenoic acid (EPA; 20:5, n-3) and docosahexaenoic acid (DHA; 22:6, n-3), rich in marine products, especially cold water fish. The long chain omega-3 fatty acids, EPA and DHA, have cardiovascular benefits in addition to those provided by omega-6 fatty acids. In hypertriglyceridemic individuals they decrease triglyceride levels in a dose dependent manner by inhibiting hepatic triglyceride synthesis. For this purpose, doses in the range of 3 to 5 grams per day are recommended. In high doses (>5 g per day), omega-3 fatty acids can produce minor reductions in blood pressure. Additional anti-atherosclerotic

and cardioprotective effects have been observed at the level of 1 gram per day in individuals with established disease. These may include:

-modulation of the inflammatory response by altering the series of prostaglandins, leukotrienes and thromboxanes synthesized by the body. The omega-3 fatty acids increase the E3 series while the omega-6 fatty acids increase the E2 series, which have opposite metabolic effects. Omega-3 fatty acids may thereby reduce platelet aggregation and thrombosis.

-Anti-arrhythmic effects. In some clinical trials (GISSI-Prevenzione trial, n=11,323), consumption of omega-3 fatty acids reduced the risk of sudden cardiac death in subjects with (or at risk for) CVD. This is presumed to be due to a reduction in the risk of arrhythmias, possibly mediated through incorporation of omega-3 fatty acids into cardiac cell membranes, modification of eicosanoid pathways, modulation of ion channel function or signaling pathways, or direct effects on the myocardium. Recent clinical trials in the area (2006) have not support these findings and the area remains unresolved.

As indicated, major sources of the plant derived omega-3 fatty acid, ALA, are canola and soybean oils. Other sources include walnuts, flaxseed, flax oil, canola oil, dark green leafy vegetables, and soybeans. In humans, the conversion rate of ALA to EPA and DHA is very low, estimated to be less than 5% of total intake. The major sources EPA and DHA include cold water fish such as mackerel, swordfish, lake trout, herring, sardines, albacore tuna and salmon. There is some concern about the level of contamination of these fish with mercury, polychlorinated biphenols (PCBs), and dioxins. Mercury contamination is of greatest concern in young children or women of reproductive potential (can impair neurological development of the growing child or fetus). For all other individuals, the potential risk from contamination is thought to be considerably less than the risk of developing cardiovascular disease. Of the fish listed above (high in omega-3 fatty acids), swordfish, some types of mackerel (not Atlantic), and albacore (“white”) tuna have the highest levels of mercury contamination; the other fish generally have low levels of contamination. The American Heart Association recommends that healthy individuals eat fish twice a week for cardioprotective effects. Individuals with pre-existing CVD would need to eat fish daily or take omega-3 supplements to consume the amount shown to be beneficial in clinical trials.

- Dietary Cholesterol

The observation that dietary cholesterol increased blood cholesterol levels and was associated with the development of arteriosclerosis was originally made early in the 20th century in rabbits. In humans, a positive correlation has been repeatedly observed between dietary cholesterol and both blood cholesterol levels and CVD risk, although relative to SFA, the effect is modest. Whether the increase in plasma cholesterol levels induced by dietary cholesterol is linear or curvilinear, or whether there is a break point or threshold/ceiling relationship beyond which individuals are no longer responsive, remains to be determined. With few exceptions, dietary cholesterol is present in foods of animal origin. Therefore, restricting saturated fat intake is likely to result in a decrease in dietary cholesterol.

**8. Indicate whether quantity or quality of dietary fat is the most important determinant of cardiovascular disease risk and why.**

Dietary fat provides a major source of metabolic energy. One gram contributes 9 calories, a little more than twice that contributed by protein or carbohydrate, which is 4 calories per gram, and somewhat more than that contributed by alcohol, 7 calories per gram. In addition to energy, dietary fat is necessary for the absorption of fat soluble vitamins (vitamins A, D, E and K, and carotenoids) and in some cases serves as a carrier for these nutrients in the diet.

When considering the issue of dietary fat in terms of quantity in relation to cardiovascular disease risk and lipoprotein profiles, the emphasis is on triglyceride and HDL cholesterol levels. Relatively consistent evidence indicates that under isoweight conditions, that are when body weight is maintained at a constant level, diets low in total fat (<20% of energy) result in an increase in triglyceride levels and an decrease in HDL cholesterol levels, hence increases the atherogenic profile. Hypocaloric periods have a mitigating effect on this trend. However, it is unlikely that most individuals will remain in negative energy balance for long periods of time. Taking these existing data into consideration the NCEP, in revising their guidelines for ATP III, concluded that the total fat recommendation should be changed from less than 30 of calories from fat to 25% to 35% of calories from fat. This had the effect of ending the focus on the total fat content of the diet that had existed prior to 2000.

There has been some concern that taking the emphasis off the total fat content of the diet would promote increased body weight. However, the long term studies available on the relationship between percent of energy from fat and body weight have not, for the most part, supported a strong relationship. Two recent reviews have concluded that even major downward shifts in the fat content of the diet, 10% to 15% of energy, resulted in only modest weight loss, 1.0 kg, over a 12 month period in normal weight subjects and 3 kg in overweight or obese subjects. Noteworthy, the longer term studies where there was a drastic reduction in dietary fat indicate that in no case was weight gain reported. Since the most important approach to control obesity appears to be prevention, this area is still the subject of further long term investigation.

**9. Identify one dietary factor, other than dietary fat, that has been suggested to alter cardiovascular disease risk and discuss the current state of knowledge.**

- Fiber

Dietary soluble fiber, primarily  $\beta$ -glucan, has been reported to have a modest independent effect on decreasing blood total and LDL cholesterol levels. A recent meta-analysis concluded that 3 grams of soluble fiber (equivalent of three servings of oatmeal) reduced both total and LDL cholesterol levels approximately 5%. Most evidence suggests that soluble fiber exerts its hypocholesterolaemic effect by binding bile acids and cholesterol in the intestine, resulting in an increased fecal loss and altered colonic metabolism of bile acids. The fermentation of fiber polysaccharides in the colon yields short-chain fatty

acids. Insoluble fiber, that primarily from cereal grains, has little effect on blood lipid levels but has been consistently been associated with decreased risk of cardiovascular disease or progression of established lesions. This is in contrast to fiber derived from fruits and vegetables which has shown no such relationship.

- Soy Protein

The potential relationship between soy protein and the risk of developing CVD has a long history dating back to the 1940's. Despite this relatively protracted lead-time attempt at more precisely defining this relationship has been slow in coming and somewhat inconsistent. Renewed interest developed in the relationship between soy protein and blood lipid levels after a meta-analysis was published in the mid-1990's suggesting that soy protein resulted in significant reductions in total and LDL cholesterol levels, with the most pronounced effect in hypercholesterolemic individuals. Changes in HDL cholesterol levels were not significant. Whether the effect on total and LDL cholesterol levels was attributable to the soy protein, per se, or other soybean derived factor(s), the most likely of which the constitutive isoflavones, was yet to be determined. Since that time a number of well controlled studies have reexamined the effect of soy protein and/or isoflavones on blood lipid levels in humans. The results of more recent studies are variable. Declines in LDL cholesterol levels attributable to the substitution of 25 grams to 50 grams of soy protein for animal protein range from a null to small (3 to 6%) in normocholesterolemic and hypercholesterolemic individuals. Changes in HDL cholesterol levels were highly variable, ranging from -15% to +7%. Soy derived isoflavones do not appear to have an independent effect of blood lipid levels. On the basis of the most recent data it can be concluded that, although helpful when used to displace products containing animal (saturated) fat from the diet, despite the current claims, individuals should be cautioned against an over reliance on the casual use of soy protein containing foods or the use of isolated isoflavones to control serum lipid levels.

- Plant Sterols

Sterols are the designation for a group of compounds that are essential constituents of cell membranes in animals and plants. Cholesterol is the major sterol of mammalian cells. Phytosterols, such as beta-sitosterol, campesterol, and stigmasterol, are the major sterols of plant cells. In humans, plant sterols are not synthesized, are poorly absorbed and appear to interfere with cholesterol absorption. It is this later property that has been exploited in the use of these compounds as blood cholesterol lowering agents. Maximal LDL cholesterol lowering attributable to plant sterols occurs at a dose of about 2 g per day. Although a relatively wide range of responses has been reported, the majority of work suggests an expected LDL cholesterol lowering of about 10% in hypercholesterolemic subjects. A wide range of plant sterol enriched foods are currently available, as are tablets and soft gel capsules containing plant sterols. Few side effects of plant sterols have been reported with the exception of decreased levels of circulating carotenoids.

- Antioxidant nutrients

Considerable interest had been generated in the potential benefit of dietary supplementation with vitamin E and other antioxidant nutrients in reducing CVD risk. Support from this hypothesis came from two sources. First from the epidemiological observations suggesting that vitamin E supplement use was associated with decreased risk of CVD. Second from the *in vitro* work demonstrating that vitamin E in LDL was correlated with decreased susceptibility of the lipoprotein particle to oxidation and that in cell culture oxidized LDL resulted in foam cell formation. A number of well controlled and relatively larger recent intervention studies have failed to demonstrate a benefit of vitamin E or other antioxidant vitamins. At this time the data do not support a recommendation to use antioxidant vitamins for the prevention or management of CVD.

- Folic acid

Animal evidence had demonstrated a strong link between plasma homocysteine levels and cardiovascular disease, via damage to the vascular matrix that can result in proliferation of endothelial cells; facilitating oxidative injury of the vascular wall, disturbing endothelium-dependent vasomotor regulation and promoting arterial thrombosis. Further, epidemiological and clinical data suggested that elevated plasma homocysteine levels in humans were associated with increased risk for cardiovascular disease. For example, carotid artery medial-intima thickness was shown to be positively associated with elevated levels of homocysteine. In a review of epidemiological studies appearing in 1995 its authors concluded that “Higher folic acid intake by reducing homocysteine levels promises to prevent arteriosclerotic vascular disease”. However, it should be recognized that the relationship between diet and plasma homocysteine is complex, and does not solely rely on folate status. Plasma levels of homocysteine tend to be inversely associated not only with plasma folate, but also with plasma levels of vitamin B12 and vitamin B6, presumably through their role as catalysts for the enzymes involved in homocysteine metabolism, methylenetetrahydrofolate reductase, methionine synthase and cystathionine beta-synthase, respectively.

In 1991, a large scale population intervention trial with folate supplementation reported a significant decrease in the risk of children born with neural tube defects. Subsequently, the Food and Drug Administration mandated that all enriched flour, rice, pasta, cornmeal and other grain products contain 140 ug of folic acid per 100 g. This resulted in a secular decrease in plasma homocysteine and a rise in folate levels. Concomitant with these changes, anticipation for a potential beneficial role of the fortified folate in reducing cardiovascular disease risk was high – but now the enthusiasm has been somewhat tempered as a result of new studies. A number of folate or folate and other B vitamin intervention studies have successfully increased serum homocysteine levels but have not resulted in decreasing either death from cardiovascular disease or all cause mortality. Of greater concern, a number of these studies have suggested potential averse effects of folate supplementation.