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SERUM LIPIDS AND ATHEROSCLEROSIS: A REVIEW

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ARTERIOSCLEROSIS includes all forms of arterial disease except those that are frankly inflammatory in character. The several sub-divisions of arteriosclerosis include:[1]

1) Regenerative intimal thickening which is a rection to disuse. Examples include "involution" of the ductus arteriosus and hypogastric arteries after birth, and changes in the walls of the arteries of the uterine wall after delivery such that the lumen of each vessel is reduced to a size consistent with the needs of the resting uterus.

2) Elastic intimal thickening which occurs only in muscular arteries and occurs until young adulthood normally. Only in the renal arteries is it associated with medial atrophy when the vessels are exposed to prolonged hypertension such that the muscular vessels become more like elastic arteries.

3) Ectasia is a process of progressive dilatation with advancing age, and is found in the aorta, and in any large elastic artery in the late senile period. Elastic fibers of the vessel wall are replaced by collagen fibers, causing a loss of elasticity. It has been observed that where ectasia is pronounced there is relatively little atheromata.

4) Mockeberg's syndrome is one of calcification in the medial coat of muscular arteries, chiefly in the limbs, and especially in males. This does not result in luminal narrowing. The process, of unknown cause, begins in childhood and is found in almost all elderly people.

5) Intimal atherosclerosis is rarely absent in the adult and is by far the most common form of arterial disease although it is asymptomatic unless it becomes severe in some vital area. The disease begins in the intima and is largely restricted to it, but may also involve the media in advanced cases, especially in muscular arteries of coronary artery size. It begins with intimal plaques of loose fibrillar connective tissue and is followed by deposition of lipoid material, especially cholesterol, both in macrophages and in the intercellular fluid.[2] Calcium salts may be deposited later in necrotic tissue. As this process of connective tissue proliferation and lipid deposition continues, the lumen of the vessel is narrowed and may occlude.

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6) Arteriolosclerosis is an aging process, more marked in cases of prolonged hypertension, and consists of replacement of the arterioles muscular coat by subintimal deposition of hyaline material.

7) Medial fibrosis is also an aging process of unknown cause. In this process connective tissue deposition occurs in the media and replaces the muscle itself.

It is seen from this discussion that only atherosclerosis causes symptomatic luminal narrowing. Its very commonness has led many persons to confuse the terms "arteriosclerosis", and "atherosclerosis". The remainder of this discussion will be limited to atherosclerosis, and will emphasize current thoughts in its etiology.

Since lipids are found in atheromata which, in the past, have been found principally in elderly people with high serum cholesterol levels, lipids have been considered to be of major importance in the causation of atherosclerosis. The serum lipids are subject to both hereditary and environmental influences. Genetic disorders include familial hypercholesterolemia, and essential hyperlipemia (hypertriglyceridemia). Of the environmental factors, diet and stress have been given most attention. Among other factors are sex, dietary deficiency in "essential" fatty acids, fatty acid chain length and degree of unsaturation. Certain diseases also have a conditioning effect on serum lipids. These include nephrosis, hypothyroidism, biliary cirrhosis, Laennec's cirrhosis, pancreatitis, and diabetes mellitus.[2,3,4,5]

It would appear that some human chemical or anatomical peculiarity predisposes man to lipid deposition in the walls of large and medium sized arteries. Atherosclerosis has been produced in experimental animals but only by marked dietary alterations, or by surgical means (induced diabetes mellitus, hypothyroidism, etc.). Since the anatomy of man's circulatory system is essentially the same as that of other mammals, chemical differences are considered to be of greater significance.

The lipids of the plasma of man are largely bound to the alpha and beta globulins, and are thus called alpha or beta lipoproteins. The nonesterified, or free, fatty acids are bound to the plasma albumin fraction.[2,3,6]

The serum lipids include free and esterified cholesterol, phospholipids, glycerides, and nonesterified saturated and unsaturated fatty acids. It is easier to consider "abnormalities" of serum lipds than to define what is "normal" since the statistical mean of one population may differ from that of another and their incidence of clinically manifest atherosclerosis may also vary. The "normal" serum cholesterol level in humans varies with age, sex, diet, etc.[3] In American adults, the normal range for serum cholesterol[3] has been considered to be 125 - 260 mgm, per 100 ml. However, even in the upper part of this range, the cholesterol level and the extent of atherosclerosis were not well correlated.[5] In other populations, the adult serum cholesterol level is considerably lower. It has also been noted that cholesterol level and sites of atheromatous lesions — aortic, coronary artery, or cerebrovascular — are not well correlated.[2]

SERUM LIPIDS

Each of the serum lipid components and their interrelationships has been considered important in the genesis of atheromata at one time or another.

Ingested fats are either hydrolysed in the small intestine to glycerol and free fatty acids, and resynthesized to neutral fats in the intestinal wall, or emulsified and absorbed as such.[8,9] Most dietary fats then enter the systemic circulation through the intestinal lacteals and thoracic duct in the form of chylomicra. These may cause milkiness of the serum and probably consist largely of triglycerides.[2]

Triglycerides

The triglycerides are formed by esterification of glycerol with three fatty acids, either three molecules of one fatty acid or a mixture of fatty acids. (Figure 1). Triglycerides constitute the most widespread class of lipid in nature, and occur mainly in the fat storage deposits of plants and animals. Excess carbohydrate may be stored as triglyceride and serum triglyceride levels are highest on a high carbohydrate diet.[8,11,12] The triglycerides may also function as a major vehicle for transport of esterfied fatty acids to sites of utilization for energy.[2]

The fatty acids of triglycerides may be saturated or unsaturated. It has been claimed that an inadequate intake of unsaturated fatty acids may be the cause of the high serum lipid levels found in Western civilization. Since these high serum lipid levels are supposedly related to the incidence of atherosclerosis, this is a matter of importance.[12] The triglycerides synthesized by the mammalian body on a fat free diet are all saturated or monounsaturated.[13] These lipids, as well as cholesterol, are synthesized in the cytoplasm and nuclear mitochondria, especially in liver, intestine, skin, kidney, and adipose tissue. The principle precursor is acetic acid.[13] Whereas a deficiency of "essential" fatty acids, notably linoleic and arachidonic acids, has been found to cause a deficiency syndrome in rats, it has not been seen in humans.[8,12] In any case, adequate amounts of these lipids are available in ordinary diets in which fats provide 20 per cent or more of the total calories.[8] The ordinary American diet is 40 per cent fat.[11]

FORMULAS

$R-COOH$

$R-COOR'$

FATTY ACID AND ESTER

CH_2-COOH
$CH-COOH$
CH_2-COOH

GLYCEROL

CH_2-COOR
$CH-COOR'$
CH_2-COOR''

TRIGLYCERIDE

CH_2-COOR
$CH-COOR'$
$CH_2-PO_2HO-(CH_2)_2-N-(CH_3)_3-OH$

LECITHIN, A PHOSPHOLIPID

Figure 1

The saturation or unsaturation of lipids is measured by the iodine number of the molecule. This is defined as the number of grams of iodine absorbed by 100 grams of the lipid, and indicates its degree of unsaturation, not the nature of the unsaturated acids present.[10] For examples of unsaturated and saturated fatty acids see Table I. The serum cholesterol level appears inversely related to the mean iodine number of the dietary lipids, being higher as the mean is less than ninety, and remaining equally low for all mean values above 100.[12] In a study conducted in South Africa it was found that whites with evidence of previous myocardial infarctions had a mean serum lipid iodine number of 136 and 151 mg. cholesterol esters per 100 ml., whites without evidence of atherosclerosis had an iodine number of 160 and 141 mg. cholesterol esters per 100 ml.; and Bantus on a native diet (low racial incidence of clinical atherosclerosis) had an iodine number of 179 and 91 mg. cholesterol esters per 100 ml.[13]

It has also been found that the length of the chain in saturated fatty acids may be of importance. Butter and coconut oil cause an elevation of serum cholesterol greater than most other saturated lipids, including those with the same iodine number. These are also unusually good sources of short and intermediate length fatty acids.[11,12]

Nonesterified fatty acids

The nonesterified fatty acids are normally present in relatively low concentration but have a rapid turnover rate. They consist of the common dietary fatty acids, as palmitic, stearic, oleic, and linoleic acids. They represent one important form in which fatty acids are transported from storage sites to working cells. Since their level falls after a normal meal they may constitute an important energy source in the fasting state — they are released from adipose tissue and rapidly cleared from the circulation. Circulating nonesterified fatty acids contribute fifty per cent, or less, of the body's basal caloric needs in the fasting state; esterified fatty acids may also serve as a fasting energy source. It has also been noted that the non-esterified fatty acid levels are inversely related to serum sugar and amino acid levels, again confirming their metabolic role.[2]

Cholesterol

Cholesterol intake has frequently been mentioned as an etiologic factor in atherosclerosis. However, Keys was among the first to show that total dietary fat was more important than dietary cholesterol in determining serum cholesterol levels.[15,16] Yet hypercholesterolemia is also found in several disease states in which a precocious and severe degree of atherosclerosis is found. Among these diseases are diabetes mellitus, hypothyroidism, the nephrotic syndrome, and familial hyper-cholesterolemia.[5] However, most persons with clinically manifest atherosclerosis have "normal" cholesterol levels.

Phospholipids

The phospholipids are another group of serum lipids of possible atherogenic importance (Figure 1). The phosphoglycerides, as lecithin, are among the predominant

forms, but phosphoinositides and sphingolipids are also found both in the plasma and in atherosclerotic plaques.[24,25,26] In fact, their concentrations in the plaques are higher than in the plasma, implying *in situ* synthesis.[26] The plasma phospholipids are principally phosphoglycerides and serve an unknown function.

The lipid components and their concentrations in aortic atherosclerotic plaques have been studied by several investigators. Some report that plasma and plaque components and concentrations are essentially the same,[27] whereas others find them to be significantly different implying *in situ* synthesis.[7,24,26] It is known that lipids may be synthesized in the walls of arteries[26] but whether or not this fact has a bearing on the development of atherosclerosis is not known.[2]

Another means of measuring the significance of plasma phospholipids is by consideration of the ratio of serum phospholipid to either serum total cholesterol (P/C ratio) or to total serum lipids (P/L ratio). It has been noted that visible lipemia occurs when the P/C ratio is less than one, or P/L ratio less than 0.30. It has been noted also, that the P/C ratio is less than 1.0 in each of the hyperlipemic conditions that are found with early atherosclerosis. However, it is greater than 1.0 in biliary cirrhosis, where hyperlipemia is not associated with precocious atherosclerosis.[2,28]

It has also been found that the P/C ratio is greater than 1.0 in mammals resistant to the development of atherosclerosis, in new born humans, and decreases with maturation, especially in the male, being lowest in those who have experienced a myocardial infarction. However, giving males estrogens changes their P/C ratio toward that of young women, and giving people androgens tends to do the reverse.[3] The P/C ratio is also lower in atherosclerotic plaques than in the serum.[7]

An expression has appeared in the literature which is related to the P/C ratio. This is "colloidal stability." It refers to the fact that lipids are the only hydrophobic component of the serum and that mechanisms to prevent their precipitation must exist. One is to bind them to proteins, and most of the plasma lipid is found in lipoproteins, whose physical characteristics are those of their protein component.[2] However, the size of the lipid particle is also of significance and the active groups of the phospholipid molecules appear to partially form the surface of the lipid complex so that dissociation or precipitation in vascular tissue is less likely to occur.[2,11,23]

FATTY ACIDS		
PALMITIC	$C_{16}H_{32}O_2$	SATURATED
STEARIC	$C_{18}H_{36}O_2$	SATURATED
ARACHIDIC	$C_{20}H_{40}O_2$	SATURATED
OLEIC	$C_{18}H_{34}O_2$	ONE DOUBLE BOND
LINOLEIC	$C_{18}H_{32}O_2$	TWO DOUBLE BONDS
LINOLENIC	$C_{18}H_{30}O_2$	THREE DOUBLE BONDS
ARACHIDONIC	$C_{20}H_{32}O_2$	FOUR DOUBLE BONDS

Table I

A more objective measurement of the size of lipid particles is by means of the ultracentrifuge. Particles separated are measured in Svedberg flotation (S_f) units. Those of the S_f 10 - 20 size are found in circumstances that predispose to atherosclerosis and are absent in mammals resistant to atherosclerosis, and in the more resistant ages and sexes of man.[3,5] They tend to be inversely related to the P/C ratio.

Lipoproteins

The plasma cholesterol is normally transported in the beta lipoproteins in man. In those groups that are relatively resistant to atherosclerosis a large portion of the serum cholesterol is found in the alpha lipoproteins. The alpha lipoproteins contain forty per cent lipid, and are not influenced by dietary habits or increasing age. The beta lipoproteins are 75 per cent lipid and are more labile, being influenced by dietary composition, fasting, increasing age, and gonadal hormones.[2,3,17] It is also to be noted that the beta lipoproteins vary directly with serum cholesterol levels, and some observers feel that this is the lipid fraction of greatest significance in he genesis of atherosclerosis.[2]

It is known that the serum cholesterol and beta lipoprotein levels vary with the total dietary lipid, and that qualitative factors are also of importance. Among these are the origin of the lipid, that is from animal or vegetable sources, its proportion of polyunsaturated fatty acids, and its proportion of short and intermediate length saturated fatty acids.[2] Some investigators feel that the monounsaturated fatty acids may also play a role,[2] but Keys disagrees.[11]

However, several other dietary variables also may influence serum cholesterol and phospholipid levels when the caloric content of the diet is held constant. A drastic decrease in dietary protein is associated with decreased serum lipid levels — but this may be due to the fact that our proteins are principally of animal origin. The type of carbohydrate in the diet may also be of significance with starch and leguminous carbohydrates tending to lower serum cholesterol levels and sucrose tending to elevate them. A substantial decrease in calories from fat in the diet with a compensatory increase in carbohydrate calories will also reduce serum cholesterol levels but an associated marked elevation in serum triglyceride levels is sometimes seen.[2]

Treatment of hypercholesterolemia

Since the body is capable of synthesizing cholesterol, as well as other lipids, from simple precursors,[13,17,18,19] control of serum cholesterol levels would appear to be difficult, if not impossible. However, several means by which cholesterol levels might be reduced are known. One is by reducing the total dietary lipid. However, studies[11,12] have shown that the highest serum lipid levels and visible lipemia occur on a fat free diet and that a diet in which corn oil contributes about forty per cent of the total calories will optimally lower both serum cholesterol and phospholipid levels, where only dietary measures of controlling serum cholesterol levels are used.

Another means of lowering the serum cholesterol level is by decreasing cholesterol absorption from the gut.[20] This may be accomplished by feeding the

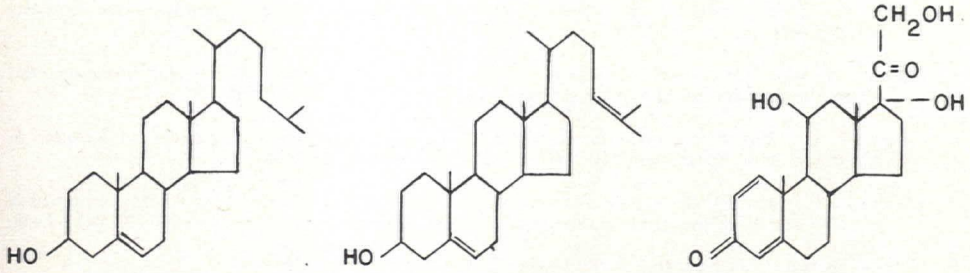
patient plant sterols (phytosteroles, B sitosterol).[15] In fact, it has been claimed,[11] and denied,[12] that the cholesterol lowering effect of corn oil is due to its constituent beta sitosterol. It is also to be noted that although cholesterol levels may be lowered with beta sitosterol, this has not been shown to reduce the rate of clinically manifest atherosclerosis.[15]

Triparanol (MER/29) is a biochemical inhibitor of cholesterol biosynthesis, blocking it at the very last stage; i.e., the conversion of desmosterol (24 dehydrocholesterol) to cholesterol. By use of this agent both serum and total body cholesterol have been reduced. However associated changes in the skin, hair and lens of the eye have been reported. It has also been stated that adrenocorticosteroid production is not influenced by reduced total body cholesterol,[20,21,22] however, suggestions to the contrary have recently been made.[23] This drug is no longer available for clinical use. Figure 2.

Most of the recent studies of dietary influences on serum cholesterol levels have stressed the importance of polyunsaturated fatty acids. It is generally accepted that a replacement of ad lib feeding by a diet in which corn oil, which is high in polyunsaturated fatty acid content, was the sole source of fat, will generally result in a significant decline in cholesterol levels until a new steady state is achieved.[2,11,12] However, the percentage of fat calories was important with 40 per cent calories from corn oil being superior to either zero per cent or 70 per cent fat calories.[11,12] Adding up to sixty grams carbohydrate[16] or sixty grams cholesterol daily[4] did not then influence serum lipid levels. However, an isocaloric change from vegetable to animal fats would rapidly return serum lipids to their previous levels.

The following lipid sources were approximately as beneficial as corn oil: safflower oil, cottonseed oil, peanut oil, and chicken fat.. The following caused elevation of serum cholesterol levels: coconut oil, butter, lard, palm oil, olive oil, and beef fat.[11]

The means by which polyunsaturated fatty acids lower serum lipid levels is unknown. However, during their utilization fecal cholesterol and its metabolites increase so cholesterol metabolism may be accelerated. More unsaturated fatty acid



CHOLESTEROL DESMOSTEROL PREDNISOLONE

Figure 2

is also found esterified to cholesterol which may decrease its tendency to separation from the serum.[7]

Conclusion

Evidence for the role of hyperlipemic states as a causative factor in the genesis of atherosclerosis is merely suggestive at this time, not conclusive. Similarly, evidence for the beneficial effects of polyunsaturated dietary fatty acids is not conclusive. For these reasons no general recommendations for changes in the average American diet have been accepted.[12] However, in hyperlipemic states the available information would suggest that measures be taken to inhibit persistence of high serum lipid levels.

Summary

The recent literature concerning lipids and atherosclerosis has been reviewed. Some of the means by which serum hypercholesterolemia may be reduced toward more normal levels are discussed.

Synopsis

Atherosclerosis is one of the major illnesses affecting members of Western civilization today. Due to its very commonness the term is frequently confused with "arteriosclerosis". The serum lipids are felt to be of importance in the genesis of atherosclerotic lesions, and each is discussed. Some of the means in current use to lower serum cholesterol levels are also briefly considered.

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