REVIEW OF LITERATURE
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The majority of people below the age of 55 years are afflicted with atherosclerosis and have one or more identifiable risk factors other than aging per se. The risk factor concept implies that a person with at least one risk factor is more likely to develop a clinical atherosclerotic event and to do so earlier than a person with no risk factors. The presence of multiple risk factors further accelerates atherosclerosis. They vary in terms of importance in the population. There is general agreement from an epidemiologic perspective that hypercholesterolemia, hypertension and cigarette smoking may be the most potent factors involved in the causation of atherosclerosis. Risk factors also vary in terms of their potential reversibility with current techniques of preventive management.

Thus, age, sex and genetic factors are currently considered to be irreversible risk factors, whereas continually emerging evidence suggests that elimination of cigarette smoking and treatment of hypertension reverses high risk of atherosclerosis attributable to these factors.

These factors are not mutually exclusive and they clearly interact. For example, obesity appears to be causally associated with hypertension, hyper-
glycemia, hypercholesterolemia and hypertriglyceridemia. Genetic factor may play a role by exerting direct affects on arterial wall structure and metabolism or they may act indirectly via such factors as hypertension, hyperlipidemia, diabetes and obesity. Aging appears to be one of the more complex factors associated with the development of atherosclerosis, since many of risk factors in themselves are related to aging, e.g. elevated blood pressure, hyperglycemia and hyperlipidemia.

HISTORICAL ASPECTS OF ATHEROSCLEROSIS

Atherosclerosis has been recognised in humans for thousand of years. Lesions of atherosclerosis were identified in Egyptian mummies as early as the fifteenth century B.C. Long has discussed the development of clinical pathological correlations that evolved during the era when autopsy examination permitted the development of an understanding between the degree of atherosclerosis and the incidence of myocardial infarction and stroke. In the mid nineteenth century, Virchow proposed the idea that some form of injury to the artery wall associated with an inflammatory response resulted in what was then considered to be a degenerative lesion of atherosclerosis. This idea was subsequently modified by Amschek and further included the role of platelets and thrombogenesis in atherosclerosis as expanded by Duguid in 1948. Many of
modern views of atherosclerosis stem from the work of
John French who noted that the structural integrity of
the endothelial lining of the artery represented a key
element in the maintenance of normal arterial function
and that alterations in endothelial lining of the artery
represented a key element in the maintenance of normal
arterial function and that alterations in endothelial
integrity might precede a sequence of events that leads
to the various forms of the lesion of atherosclerosis.
Thus over the years a number of theories concerning the
etiology and pathogenesis of atherosclerosis have been
developed. At least three of these deserve elaboration
and comment. These are response to injury hypothesis,
monoclonal hypothesis and the lipogenic hypothesis.

THE LESIONS OF ATHEROSCLEROSIS

Examination of atherosclerotic lesions with
modern techniques of cell and molecular biology has
revealed that each lesion contains significant element
of all the three cellular phenomenon. These are smooth
muscle proliferation, formation by the proliferated
cells of large amounts of connective tissue matrix
including collagen, elastic fibres and proteoglycans and
accumulation of intracellular and extra cellular lipid.
In each instance, the relative degree to which each of
the cells responds to different atherogenic stimuli
determines the unique combination that defines the type
and the extent of the resulting lesion (Rose and Glomset, 1976).

The lesions of atherosclerosis occur principally within the inner most layer of the artery wall, the intima. They include fatty streaks, the fibrous plaque and so called complicated lesions (Mc Gill, 1977). Secondary changes have been noted in the media of the artery underlying lesions, principally in association with the more advanced lesions of atherosclerosis.

**THE FATTY STREAK**

The process of atherosclerosis begins in the childhood with the development of flat, lipid rich lesions called fatty streaks. These lesions consist of a small increase in the number of smooth muscle cells together with some macrophages within the arterial intima. Both of these cell types contain deposits of cholesterol and cholesterol esters. The lesions are yellowish and sessile in appearance and cause little to no obstruction of the affected artery and no clinical sequelae.

**THE FIBROUS PLAQUES**

The fibrous plaque is grossly white in appearance and becomes elevated so that it may protrude into the lumen of artery. If this lesion progresses sufficiently it can occlude the lumen and compromise the vascular supply of the involved tissue. The principal change that occurs within the arterial intima during the
development of the fibrous plaque consists of proliferation of smooth muscle cells. These cells usually form a fibrous cap due to deposition by the cells of new connective tissue matrix and the accumulation of intracellular and extracellular lipids. This fibrous cap covers a deeper deposit of varying amounts of extracellular lipid and cell debris (Ceer and Haust, 1972).

**ADVANCED LESION**

The complicated lesions of atherosclerosis occur in increased frequency with increasing age. The fibrous plaque can become vascularised both from the luminal as well as medial aspects. In the complicated lesion, the necrotic "lipid rich core" increases in size and often becomes calcified. The lesions may become increasingly complex as a result of haemorrhage and calcification and the intimal surface may disintegrate and ulcerate and become involved with thrombotic episodes that may lead to occlusive disease. Such thrombi may then organise and further increase the thickness of the plaque while progressively reducing the size of the arterial lumen. It is not uncommon that as the intimal lesions progress, the number of smooth muscle cell in the underlying media decreases and media undergoes atrophy, which can sometimes results in aneurysmal changes rather than lead to thrombotic occlusion of artery.
<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Value</th>
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<tbody>
<tr>
<td>LDL</td>
<td>≤ 100 mg/dl*</td>
</tr>
<tr>
<td>HDL</td>
<td>≥ 50 mg/dl*</td>
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<tr>
<td>LDL/HDL (ratio)**</td>
<td>≤ 2.0</td>
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<tr>
<td>Triglycerides</td>
<td>≤ 150 mg/dl+</td>
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</tbody>
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* These numbers are based on values found in very low risk groups from population in several countries (The lipid research clinics Population studies. Data Book, volume. I, 1980; Conference on Health effects of blood lipids, 1979).

** The ratio of LDL/HDL is a very good index of risk (Gordon et al., 1977). Thus, with levels of HDL greater than 50, higher levels of LDL may be associated with low incidence of vascular disease.

+ The triglyceride value is arbitrary. It represents the value two standard deviations above the mean for an adult population (conference on Health effects of blood lipids, 1979). Higher values are thought to represent a group of metabolic abnormalities. This may have no relationship to vascular disease in presence of below average total plasma cholesterol (≤200 mg/dl) or "ideal" HDL (≥50 mg/dl).
In addition, HDLs serve as acceptors of lipid, especially free cholesterol from various tissues. HDLs are the substrate for Lecithin:cholesterol acyltransferase (L-CAT), which catalyzes the conversion of both free cholesterol to cholesterol ester and lecithin to lysolecithin. Cholesterol esters are transferred from HDLs to other lipoproteins nonspecifically as well as by a cholesterol-ester transfer protein. This process provides core constituents for triglyceride depleted particles such as chylomicron remnant. Apolipoproteins A-I and A-II are major proteins of HDLs. Hepatic lipase is involved in the metabolism of HDL phospholipids and triglycerides. Both the liver and the kidney appear to be major sites of HDL catabolism, and HDL receptor has been reported to exist in a variety of cell types.

EFFECTS OF DIETARY CHOLESTEROL IN LIPID METABOLISM

For 70 years the addition of dietary cholesterol has been known to increase plasma cholesterol levels and induce arteriosclerosis in experimental animals. In 1912 it was identified as the constituent of animal foods which would readily elevate the serum cholesterol level and produce atherosclerosis in experimental animals. Subsequently cholesterol rich diets have regularly caused hypercholesterolemia, atherosclerosis, and even at times myocardial infarction in a large number of species of
experimental animals, including primates (Taylor et al., 1950; Armstrong et al., 1967 and 1970). For a time, however, dietary cholesterol was considered of little importance in human lipid metabolism. By the early 1960s, a decisive effect of cholesterol in the diet of man upon the serum lipid levels was clearly demonstrated in series of metabolic ward experiments being carried out in normal volunteers (Conner et al., 1961; Conner et al., 1964 and Beveridge et al., 1960).

Dietary cholesterol is absorbed by the gut in amounts proportional to intake up to a dietary level of perhaps 1200 to 1500 mg/day. Only about 40 percent is absorbed (Conner and Lin, 1974; Grundy et al., 1969).

In man, absorbed cholesterol is transported initially in chylomicrons, largely as esterified cholesterol and reaches a peak concentration in plasma 48 hours after a given meal. After the action of lipoprotein lipase in peripheral tissues, it then circulates as cholesterol rich remnants before removed by liver. The cholesterol of remnants contributes its mass to the total body pools of cholesterol (Shatacharya et al., 1976).

There is good evidence that cholesterol of dietary origin is transferred ultimately into other lipoprotein classes, especially in low density lipoprotein (LDL) and contributes in this manner to elevations of total plasma cholesterol (Conner and Conner, 1977).

The increased plasma cholesterol was transported chiefly in LDL for the normal and hypercholester-
terolemic subjects. Slight increases occurred in HDL. In hypertriglyceridemic subjects however, both VLDL and LDL cholesterol increased, each accounting for about 50 percent of total increase.

Most of the dietary cholesterol is quickly delivered to the liver where several effects may be observed. The increased cholesterol uptake may:

1. Inhibit new cholesterol synthesis.
2. Increase sterol excretion in the bile as bile acids or as cholesterol itself.
3. Increase excretion of cholesterol from the liver as newly synthesized lipoproteins primary VLDL, or
4. Suppress specific receptors for LDL uptake and degradation.

This leads us to a consideration of the mechanism whereby dietary cholesterol increased the total plasma cholesterol concentrations and also LDL cholesterol concentrations. The concepts of sterol balance suggests that dietary cholesterol may simple overload the disposal system. The input of sterols into the plasma tissue pool has two sources, from dietary cholesterol and from cholesterol synthesized mainly by the liver and gut. The rate of cholesterol synthesis in man is not very labile, most studies have shown a mild depression of synthesis from the ingestion of dietary cholesterol (Lin and Conner, 1981). Therefore, the total amount of sterol entering the body either from diet or by synthesis will be much greater in
individuals consuming a high cholesterol diet than in individuals consuming low cholesterol diet. Most studies to date have indicated that the bile acid and neutral steroid excretion fails to increase very much after ingestion of dietary cholesterol. Thus the ingestion of large quantities of dietary cholesterol may have two consequences. The first is a rise in plasma cholesterol and LDL concentrations. The second, a direct result of the first, is the ultimate deposition of an increased amount of cholesterol in tissues, particularly in arteries, to initiate and sustain the atherosclerotic process.

**DIETARY LIPIDS AND HDL**

Much research has indicated the importance of specific dietary components in modulating levels of serum lipids, but there is yet little information regarding the effects of these components on HDL. In short term feeding studies, marked reduction in dietary fat and isocaloric increase in carbohydrate resulted in a decrease in HDL cholesterol in conjunction with elevation of serum triglyceride and VLDL. Studies of HDL composition have shown a decrease in ratio of apolipoprotein A-I to A-II and a decrease in HDL cholesterol to protein ratio (Schoenfeld et al, 1976) consistent with a selective decrease in HDL₂ species (Blum et al., 1977).

There is evidence that substitution of large quantities of polyunsaturated fat for saturated fat in diet can result in lower levels of HDL lipids and
proteins (Nichman et al., 1967). An increase in the P:S ratio from 0.25 : 1 to 4 : 1 in real food diets fed to four normal subjects for five weeks resulted in reductions of HDL cholesterol and apolipoproteins A-I concentration of 33 and 21 percent respectively, with an associated reduction in HDL₂ : HDL₃ ratio (Shepherd et al., 1978). Other studies have however, reported either no change (Lewis, 1978; Shore et al., 1981) or increases (Jackson and Glueck, 1980) in levels of HDL cholesterol with feeding of diets enriched in polyunsaturated fat.

High dietary intake of cholesterol, in the form of three to six egg yolks per day, has been reported to produce increases in apolipoprotein E-containing HDL subspecies in humans (Mahley et al., 1978). This effect was seen whether or not there was an increase in total plasma cholesterol. Despite the fact that HDL containing apolipoprotein E represented only a minor fraction of the total HDL, its presence was shown to account for an increase of 2.6 to four times the binding of HDL to LDL receptors of fibroblasts as compared to pretreatment HDL (Mahley et al., 1981). But this was not observed in another study (Applebaum et al., 1979). Recently it has been reported that levels of HDL cholesterol and serum apolipoprotein A-I, but not apolipoprotein E increased with the feeding of diets high in both cholesterol and saturated fat (Tsun et al., 1974). As described earlier an increase in ratio of cholesterol to triglyceride in
HDL has been observed during the feeding of 750 mg of added dietary cholesterol in normal subjects (Mistry et al., 1977).

HABITUAL DIET AND CORONARY ARTERY DISEASE

Although diet is not usually considered a primary risk factor of cardiovascular disease, it has been associated with several other risk factors of cardiovascular disease, including abnormal lipid lipoprotein levels, hypertension, diabetes mellitus and obesity. Although the effects of consuming excessive sodium, sugar and total calories on hypertension, diabetes and obesity are well documented, the influence of diet on lipid and lipoprotein levels, and eventually on cardiovascular disease is still controversial (Stambler, 1978).

The Oslo heart study, a randomized controlled trial of primary CHD prevention among middle-aged men at high CHD risk has reported favourable and significant results in regard to CHD incidence and mortality based on reduced intake of saturated fat and cholesterol (without recommendation of high polyunsaturated fat intake).

DIETARY HABITS AND BLOOD LIPID LIPROTEIN

In adolescents with initial cholesterol levels greater than 200 mg/dl, a 50 percent decrease in cholesterol intake led to an appreciable drop (15.6%) in cholesterol levels, but the effects were much more modest (8.3%) in those with lower intial levels (Mc Gandy et al., 1972). In a large survey of school children, there was
no positive correlation between the low (80 to 130 mg/dl) the intermediate (157 to 170 mg/dl) and the high (194 to 426 mg/dl) cholesterol levels, with the mean daily intake of energy, sugar, fat, saturated fat and cholesterol (Weidman et al, 1978). However, in 7 different studies summarized recently, significantly albeit weak, correlations were noted between serum lipids and dietary P/S ratio of fat, cholesterol, protein, carbohydrate and sucrose (Mallies and Glueck, 1983). In a survey of school age children examining the influence of nutrients on HDL and LDL cholesterol, it was concluded that the higher intake of cholesterol and lower ratio of P/S was associated with higher values of LDL cholesterol. Larger intake of carbohydrates led to decreased HDL cholesterol and increased triglycerides (Khoury et al, 1980; Laskarzewski et al, 1979). Despite the relatively low magnitude of the partial correlation coefficients between dietary factors and serum lipids in children, it is still possible to conclude that nutrient intake of calories and fat play a small but significant role relative to serum lipids and lipoproteins (Mallies and Glueck, 1983).

**GENETIC FACTORS OF CORONARY HEART DISEASE**

The genetic aspects of coronary heart disease (CHD) have been extensively evaluated. Familial clustering CHD strongly suggests that genetic factors play an important role in etiology (Deutscher et al, 1970);
Epstein, 1964; Rose, 1960). Some studies suggest that familial aggregation of CHD may be influenced both by genetic characteristics of various risk factors.

**Familial Hypercholesterolemia**

Of the diseases producing hypercholesterolemia in man, familial hypercholesterolemia is the best defined clinically genetically and biochemically. The disorder, results from one of several genetic defects in a cell surface receptor that normally controls the degradation of low density lipoprotein (LDL). Familial hypercholesterolemia is characterized by three cardinal features.

1. **Selective elevation in the plasma level of LDL.**
2. **Deposition of LDL derived cholesterol in abnormal sites in the body, especially in tendons (forming xanthomas) in arteries (forming atheromas) and**
3. **Inheritance as an autosomal dominant trait with a gene dosage effect, that is, individuals inheriting two mutant alleles (homozygotes) are more severely affected than those inheriting one mutant allele (heterozygotes).**

Familial hypercholesterolemia was first genetic disorder recognised to cause myocardial infarction. To this day, it remains the outstanding example of a single gene mutation that causes both hypercholesterolemia and coronary atherosclerosis.
Heterozygous with familial hypercholesterolemia occur at a frequency of about 1 in 500 persons, whereas homozygotes constitute 1 in one million persons population. Heterozygotes can be diagnosed at birth because blood plasma from the umbilical cord contains a two to three fold increase in the concentration of LDL cholesterol. The elevated levels of plasma LDL persist throughout life, but symptoms typically do not develop until the third or fourth decade. The most important clinical feature is premature and accelerated atherosclerosis. Myocardial infarction begins to occur in affected men in the third decade, showing a peak incidence in the fourth or fifth decades. By age 60, approximately 80 percent have experienced a myocardial infarction.

Homozygotes have marked elevation in the plasma level of LDL from birth. Coronary artery atherosclerosis frequently has its clinical onset in homozygotes before age 10, and myocardial infarction has been reported as early as 18 months of age.

ELEVATED LIPID LIPOPROTEIN LEVELS AND RISK OF ATHEROSCLEROSIS

Cholesterol

Elevated total cholesterol is a risk factor for coronary heart disease. As the level rises above 180 mg/dl the risk of developing CHD increases. A cholesterol value of 220 mg/dl represents a nearly two
fold elevation in the incidence when compared with level of 180 mg/dl (Kannel et al, 1971).

Likewise, patients with angiographically defined CHD have significantly higher cholesterol concentrations than patients without CHD, with increased levels associated with a greater number of diseased vessels (Cohn et al, 1977).

**LDL Cholesterol**

LDL-C which approximately 75 percent of total serum cholesterol may be more specifically associated with coronary artery disease than is total cholesterol.

It has been known for many years that the reduction of elevated LDL in other primate species is followed by regression of arteriosclerotic lesions in coronary arteries in larger vessels (St. Clair, 1983). We have now conclusive evidence in humans that reducing elevated LDL cholesterol will reduce the incidence of clinical events attributable to coronary arteriosclerosis (The lipid research clinics coronary primary prevention trial results, 1984).

**HDL Cholesterol**

HDL levels have an inverse relationship with coronary artery disease (Gordon et al, 1977). The ability of HDL cholesterol to predict the developing of coronary atherosclerosis has been estimated to be four times
greater than LDL cholesterol and eight times greater than total cholesterol (Gordon et al., 1977). Each 10 mg/dl change in HDL cholesterol concentration with 50 percent alteration in cardiovascular risk (Bransika, et al, 1984).

Sub classes of HDL can be fractioned by sonal ultracentrifugation and include HDL\textsubscript{2} and HDL\textsubscript{3}. Among these subgroups HDL\textsubscript{2} appears to have the strongest inverse relationship with coronary artery disease and accounts for different levels of HDL-c between men and women (Gofman et al., 1954). The possible mechanism by which HDL cholesterol decreases atherosclerosis include:

1. Reversal of cholesterol transport from the peripheral cells to the liver for removal from the body (Miller and Miller, 1975).

2. Inhibition of LDL cholesterol uptake by cells at the LDL receptor sites.

**Triglycerides**

The level of plasma triglycerides, reflecting increased levels of VLDL also predict increased cardiovascular risk (Carlson et al, 1979 and Kannel et al., 1979). However, there is currently great debate as to whether VLDL is direct operative factor in producing vascular disease, or whether it is the association of increased LDL or decreased HDL levels which are causative (Bilheimer et al, 1972 and Berman et al, 1973). Thus VLDL triglyceride may only be a marker for other lipoprotein abnormalities.
LIPOPROTEINS : PATHOGENIC ROLE

Low density lipoproteins and intermediate density lipoproteins enter the arterial intima from plasma in man at rates directly related to their plasma concentrations (Neihaus et al, 1977; Nicoll et al, 1981) and accumulate particularly in regions already atheromatous. Endothelial injury greatly enhances this process, and may itself be caused experimentally by raised concentration of plasma lipids (Ross and Harker, 1976). The cholesterol of atheromatous lesions is principally derived from plasma (Zilversmit, 1963). The interactions of LDL with cells of atheromatous plaques have been studied in some detail. Smooth muscle cells and fibroblasts have receptors that mediate uptake of LDL (Goldstein and Brown, 1974 and Bierman and Albers, 1975). Its cholesterol is released by lysosomal degradation. Macrophages lack these receptors but acquire lipoprotein cholesterol by other processes, including receptor mediated uptake of altered LDL. In contact with cultural endothelial cells, LDL is modified, permitting macrophages to degrade it (Henriksen et al, 1981).

At the higher tissue concentrations resulting from pronounced hyperlipidemia substantial amounts of LDL enter macrophages and other cells independently of receptors – by fluid phase endocytosis and other processes (Steinberg, 1981). The foam cells of atheromatous plaques and xanthomas result from accumulation of lipoprotein
lipids by these processes and their degeneration leads to extracellular lipid deposits of atherosclerosis (Small, 1977).

**OTHER MECHANISMS**

These, then, are some of the mechanisms underlying the association between high concentrations of LDL, coronary heart disease and atherosclerosis. LDL may also contribute to other aspects of atherosclerosis by further actions. LDL, particularly light LDL in man and in animals fed cholesterol, is selectively mitogenic to arterial smooth muscle (Fleisch et al, 1982). Studies on cultured cells suggest that LDL and other mitogens derived from platelets and endothelium might lead to the smooth muscle hyperplasia of atheromatous plaques.

Most cells meet their requirements for cholesterol (for membrane synthesis) synthesis chiefly by uptake of plasma LDL and other lipoproteins, local synthesis of cholesterol is a further source in some tissues. Except for cells that synthesize lipoproteins and those using cholesterol for steroid hormone synthesis cells in the steady state must possess other mechanisms for releasing cholesterol at rates equal to their uptake and synthesis. Cultured cells show a net efflux of cholesterol (Fielding and Fielding, 1982). As the liver is the only organ that excretes cholesterol, mechanisms must exist for reverse, centripetal transport of cholesterol from peripheral cells to the liver, it was
suggested in 1968 that HDL participates in centripetal transport. Soon after Miller and Miller (1975) advanced the concept that this function of HDL by favouring mobilization of cholesterol from arterial wall, might explain the inverse relation between HDL and risk of coronary heart disease.

**The Lipoproteins: Predictors of CHD**

LDL and HDL together carry more than 90 percent of the cholesterol in plasma. Independent but interacting antagonistic but closely associated, they are the "Odd couple" of plasma. The epidemiological aspects of their associations with coronary heart disease have been studied in depth; these associations are strong, predictive and independent of other risk factors as we expect of casual relations.

Concentrations of LDL cholesterol are directly related to and are predictive of the risk of coronary heart disease over a wide age range (Gordon et al., 1981). This underlies the association between coronary heart disease and serum cholesterol, for the latter reflects LDL concentrations (Kannel et al., 1979). Mortality rates from coronary heart disease in different communities are directly and linearly related with serum concentrations of cholesterol and LDL cholesterol (Lewis et al., 1978). HDL cholesterol concentrations are even more strongly predictive of the risk of coronary heart disease in most
(Gordon et al, 1981) and Goldboumt and Medalie, 1979) but not all (Wicklund et al, 1980), studies, the relation being inverse; but unlike LDL, HDL cholesterol concentrations do not correlate inversely with mortality rates from coronary heart disease in different countries.

Hyperlipidemia as well as other risk factors run in families and have a tendency to "track" or maintain their rank overtime. Screening for hypercholesterolemia at age of 12 years, is fairly predictive of adult hypercholesterolemia close to 50 percent of the top quintile (88%) for cholesterol, were similarly placed at follow-up nine years later. Of interest was the observation that those who dropped out the top quintile at follow-up had a lower incidence of obesity, smoked less and were more active (Orchard et al, 1983).

Predictions from measurements of total cholesterol can be improved upon by measuring HDL cholesterol, which is known to be protective against CHD and contributes proportionately more to the total cholesterol concentration at childhood. In a survey of 6775 school children, a substantial proportion of those with hypercholesterolemia were attributable to HDL cholesterol levels (Morrison et al, 1979). A recent study examining the influence of family history of CHD, hypertension, obesity and diabetes on total cholesterol, HDL cholesterol and LDL cholesterol, concluded that, in view of
their variation with age, screening during adolescence permitted a more accurate identification of individuals likely to become "high risk" adults (Durant et al., 1982).

More precise estimation of risk can be obtained from samples of LDL and HDL cholesterol. The ratio of total cholesterol (reflecting LDL) to HDL cholesterol is about as efficient as any other lipid profile (Kannel et al., 1979). A ratio of 5 indicates the average high risk in affluent western populations, and ratios exceeding this are a definite cause of concern within the range of serum cholesterol values that are commonly encountered. A more optimal ratio is in the vicinity of 3.5 corresponding to half the standard risk and resembling that found in low CHD in incidence countries (Gordon et al., 1982).