REVIEW OF LITERATURE

GENERAL

The massive study on serum lipid lipoprotein profiles, in various laboratories in the world, in response to diet pattern on healthy and diseased individuals, is to reveal the mysteries of the most important pathogenic entity i.e. atherosclerosis. It occurs mostly of major arteries (large and medium sized) especially in aorta, coronaries and cerebral.

For the process of atherosclerosis hypercholesterolemia is one of important risk factors. A risk factor may be defined as "any habit or trait that can be used to predict an individual probability of developing that disease (Dhew publication, 1981). A more specific definition may be that "a risk factor is causative agent or condition that can be used to predict an individual's probability of developing disease." The altered level of these serum lipoproteins, in particular, elevated low density lipoproteins (LDL, and diminished high density lipoprotein (HDL) appears to be strongest among other lipid levels. More over other factors viz. age, sex, smoking, obesity, hypertension, stress, dietary habits and sedentary life style exert their influence on lipoproteins levels and thus the development of atherosclerosis. Many are reversible but others like age, sex, genetic factors, family history of past IHD are irreversible ones. There are at least three independent
prediction of risk for individuals. They are plasma cholesterol concentrations (Ross, 1986; Inkeles and Eisenberg, 1981), cigarette smoking (Wissler, 1976) and elevated blood pressure (Oberman, Harlan et al, 1969).

The lesion of atherosclerosis starts from 1-10 years of age with a fatty streak and later with advancing age fibrous plaque develops eventually giving way to advanced lesions.

To understand in broader sense, the accumulation of fat in the arterial wall is typical sign of atherosclerosis (Weitz et al, 1956). Thus uptake depends upon plasma lipid levels as well as individual arterial wall factors and the uptake is largely of LDL cholesterol. Significant hyperlipoproteinemia is considered in those individuals who when below 20 years age have total serum cholesterol exceeding 200 mg/dl or plasma triglyceride levels exceeding 140 mg/dl while in those above 20 years of age, the values should exceeds 240 mg/dl for STC or plasma triglyceride more than 200 mg/dl. Usually individuals who are afflicted with atherosclerosis have more than one risk factors at a time.

An analysis of blood plasma shows major lipid classes as given below in table 1. All the lipid in plasma are bound to proteins (Fredrickson et al, 1967 and Scanu, 1969) and thus named lipoproteins.
TABLE 1: Range and mean values of various lipid fractions in human plasma (mg/dl).
(Mayer, 1981 and Harper)

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lipid</td>
<td>570</td>
<td>360-820</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>142</td>
<td>80-180</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>200</td>
<td>107-320</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>55</td>
<td>26-106</td>
</tr>
<tr>
<td>Free fatty acid</td>
<td>12</td>
<td>6-16</td>
</tr>
</tbody>
</table>

HISTORICAL ASPECT

Lesions of atherosclerosis were identified in Egyptian mummies as early as fifteenth century B.C. In mid nineteenth century Virchow made concept of injury to the arterial wall associated with inflammatory response resulting in lesion of atherosclerosis. This idea was subsequently modified by Anitschkow and included platelets and thrombogenesis role in atherosclerosis. Modern view started to stem from work of John French who noted that the structural integrity of endothelial lining of artery was key to maintenance of normal functions and any breach to it might precede a sequential events to lesions of atherosclerosis. Thereafter over many years, many theories concerning the etiology and pathogenesis of atherosclerosis has been putforth of which response to injury, monoclonal hypothesis and lipogenic hypothesis needs mention.
ATHEROSCLEROTIC LESIONS

The lesion occurs principally in the intimal layer of artery wall in form of fatty streak fibrous plaque and advanced lesions.

This process starts from age of 1-10 years in form of flat lipid rich yellowish lesions called fatty streak. It has small increase in smooth muscle cell along with few macrophages in intima. The cells have cholesterol and cholesteryl esters and cause little to no obstruction of the affected artery and thus no clinical sequelae.

As the lesions progress it attains white colour and becomes elevated so as to protrude into the lumen of artery thus compromising vascular supply to the involved organ. The changes occur in form of fibrous plaque involving proliferation of smooth muscle cells and formation of fibrous cap by deposition of new connective tissue matrix and also of intracellular and extracellular lipid.

As lesion advances with age, the fibrous plaque become vascularized. In complicated lesions, the necrotic lipid rich core increases in size and often calcifies. Due to repeated haemorrhages and calcification the intimal surface may disintegrate and ulcerate thus inducing thrombotic episode leading to occlusion. This thrombosis may organize and further increase the size of plaque and reduce the size of arterial lumen.
As these intimal lesions progress, the smooth muscle layer in underlying media may decrease and atrophy and may lead to aneurysms.

**LIPOPROTEINS: TYPE AND CHARACTERISTICS**

These are high molecule weight globular particles that transport non polar lipids (primarily triglyceride and cholesteryl esters) through plasma. Each lipoprotein particle has a core consisting of hydrophobic lipids in form of droplet accounting for most of the mass. These lipids are triglycerides and cholesteryl esters in various proportions. Surrounding this is a polar surface of phospholipids along with unesterified cholesterol to stabilize the particle. Each lipoprotein particle also contains specific apoproteins on surface which helps in binding to specific enzymes or transport proteins on cell membranes. Thus helping to reach the site of metabolism.

Contd.....
**TABLE 2: Major classes of lipoproteins**

<table>
<thead>
<tr>
<th>Lipoprotein classes</th>
<th>Density range</th>
<th>Electrophoretic mobility</th>
<th>Major lipid constituents</th>
<th>Apoprotein constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicrons</td>
<td>$&lt; 0.94$</td>
<td>Origin</td>
<td>Triglycerides (Exogenous)</td>
<td>A-I</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A-II</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A-IV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B-48</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.94-1.006</td>
<td>Prebeta</td>
<td>Triglyceride (Endogenous)</td>
<td>B-100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phospholipids</td>
<td>C-I</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C-II</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C-III</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>E</td>
</tr>
<tr>
<td>LDL₁ (IDL)</td>
<td>1.006-1.019</td>
<td>Beta</td>
<td>Esterified cholesterol</td>
<td>B-100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phospholipids</td>
<td>E</td>
</tr>
<tr>
<td>LDL₂</td>
<td>1.019-1.063</td>
<td>Beta</td>
<td>Triglyceride</td>
<td>B-100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Esterified cholesterol</td>
<td>E</td>
</tr>
<tr>
<td>HDL</td>
<td>1.063-1.210</td>
<td>Alpha</td>
<td>Phospholipid</td>
<td>A-I</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cholesterol</td>
<td>A-II</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C-II</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>E</td>
</tr>
</tbody>
</table>

* VLDL = Very low density lipoproteins.
* LDL = Low density lipoproteins.
* IDL = Intermediate density lipoproteins.
* HDL = High density lipoproteins.

Source: Beigel and Gotto - Lipoprotein in health and disease, Diagnosis and management. The Baylor College of Medicine, Cardiology Series, 9,6, No. 1, 1986.

The density of plasma lipoprotein is determined by their relative amount of protein and lipid and electrophoretic mobility divide it into four major classes as chylomicron, HDL, LDL and VLDL. LDL has been further divided into LDL₁ (Intermediate density lipoprotein) (1.006-1.019 gm/ml) and LDL₂ (1.019-1.063 gm/ml). LDL₂ is major plasma component of LDL.
HDL has been divided into HDL$_2$ (1.063-1.125 gm/ml) and HDL$_3$ (1.125-1.210 gm/ml). Changes in HDL level are usually due to alteration of HDL$_2$.

The characteristics of apoproteins are shown in table 3.

**TABLE 3: Characteristics and functions of apolipoproteins.**

<table>
<thead>
<tr>
<th>Apolipoprotein</th>
<th>Lipoprotein density class</th>
<th>Approx. plasma conc. (mg/dl)</th>
<th>Approx. molecular weight</th>
<th>Reported functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-I</td>
<td>Chylomicrons, HDL</td>
<td>120</td>
<td>28,300</td>
<td>-Cofactor with LACT, -Structural role in HDL</td>
</tr>
<tr>
<td>A-II</td>
<td>Chylomicrons</td>
<td>35</td>
<td>17,400</td>
<td>-Cofactor with HL structural role in HDL</td>
</tr>
<tr>
<td>A-IV</td>
<td>Chylomicrons</td>
<td>15</td>
<td>46,000</td>
<td>?</td>
</tr>
<tr>
<td>Apo(a)LP</td>
<td>LDL, HDL</td>
<td>10</td>
<td>9,00,000</td>
<td>?</td>
</tr>
<tr>
<td>Beta-100</td>
<td>VLDL, LDL</td>
<td>100</td>
<td>2,50,000</td>
<td>-Binding protein cell receptor structural role in VLDL and LDL</td>
</tr>
<tr>
<td>B-48</td>
<td>Chylomicrons</td>
<td>Traces</td>
<td>1,20,000</td>
<td>-Structural role in chylomicrons</td>
</tr>
<tr>
<td>C-I</td>
<td>Chylomicrons, VLDL, HDL</td>
<td>7</td>
<td>6,300</td>
<td>-Cofactor with LACT</td>
</tr>
<tr>
<td>C-II</td>
<td>Chylomicrons, VLDL, HDL</td>
<td>4</td>
<td>8,800</td>
<td>-Cofactor with LPL</td>
</tr>
<tr>
<td>C-III</td>
<td>Chylomicrons, VLDL, HDL</td>
<td>13</td>
<td>8,800</td>
<td>-Inhibitor with LPL -Chylomicrons remnant uptake</td>
</tr>
<tr>
<td>D</td>
<td>HDL</td>
<td>6</td>
<td>32,500</td>
<td>?</td>
</tr>
<tr>
<td>E-24</td>
<td>Chylomicrons, VLDL, HDL</td>
<td>5</td>
<td>37,000</td>
<td>-Binding proteins cell receptor</td>
</tr>
<tr>
<td>F</td>
<td>HDL</td>
<td>2</td>
<td>30,000</td>
<td>?</td>
</tr>
<tr>
<td>G</td>
<td>HDL</td>
<td>-</td>
<td>72,000</td>
<td>?</td>
</tr>
<tr>
<td>H</td>
<td>Chylomicrons, 1.21 B</td>
<td>10</td>
<td>43,000</td>
<td>-Cofactor with LPL</td>
</tr>
</tbody>
</table>
* LCAT = Lecithin cholesterol acyltransferase.
   HL = Hepatic lipase
   LPL = Lipoprotein lipase.
   1.21 B refers to 1.21 gm/ml density free fraction.


**LIPID METABOLISM**

**EXOGENOUS PATHWAY**

The chylomicrons, large triglycerides rich particles are produced in the intestine from dietary fat. Hence they are normally not present in plasma after fast of 12-14 hours. They are catabolized by lipoproteins lipase (LPL) and hepatic lipase (HL) to form chylomicrons remnants, triglycerides form free fatty acids (FFA). Apo E facilitates the uptake of these remnants while Apo C-III inhibits it.

**ENDOGENOUS PATHWAY**

VLDL synthesis occurs in liver and is increased in obese persons and is inhibited by the uptake of chylomicrons remnants. VLDL, triglycerides and phospholipids are hydrolyzed by lipoprotein lipase and hepatic lipases. During this apo E and apo C of VLDL is transferred to HDL while apo B-100 remains within. Thus the end product of VLDL catabolism are LDLs.

LDLs are major cholesterol carrying lipoproteins in normal plasma in humans and most of it comes from VLDL catabolism while some are synthesized directly (in subjects of homozygous familial hypercholesterolemia).
The major protein of LDL is apo B-100, LDL is catabolized in various cell types by receptor dependent as well as receptor independent mechanisms. LDL when degraded in cell results to form free cholesterol which in turn inhibits the enzyme (3 hydroxy, 3 methylglutaryl coenzyme A and reductase) producing it.

Direct HDL production occurs in liver and intestines and also derived from chylomicrons and VLDL catabolism. Moreover, HDL serves as acceptor of lipid especially free cholesterol. Apo I and II are major protein in HDL. Hepatic lipases metabolise HDL phospholipids and triglycerides and liver and kidney are major sites for catabolism.

TABLE 4: Lipoprotein values

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-c</td>
<td>&lt;100 mg%*</td>
</tr>
<tr>
<td>HDL-c</td>
<td>&gt;50 mg%*</td>
</tr>
<tr>
<td>LDL/HDL-c (Ratio)**</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>&lt;150 mg%***</td>
</tr>
</tbody>
</table>

* These are based on values found in very low risk groups (the Lipid Research Clinics, Population Studies, Data Book Vol 1, 1980, Conference on Health effects of blood lipids, 1979).

** The ratio of LDL/HDL-c is very good index of risk (Gordon et al, 1977). Thus with HDL >750 mg% higher levels of LDL-c may be associated with low incidence of vascular disease.

*** Triglyceride value is arbitrary. It represents the value two standard deviations above the mean for an adult population (Conference on health effects of lipids, 1979). Higher values suggests groups of metabolic abnormalities.
CHYLOMICRONS

It is the largest of the lipoproteins originating from the gut mainly composed of triglyceride and transport dietary triglyceride and cholesterol from gut to site of metabolism or storage. In post prandial state it is detected/"creaming in the cold".

Dietary fat is broken down to free fatty acids and monoglycerides in intestine which then enter intestinal villi in jejunum reconstituting into triglyceride. In the cells of jejunum cholesterol is esterified to cholesteryl ester (Oleate). The triglycerides are then apo AI & II complexed with Apo B-48, apo A-IV within intestinal wall. The chylomicrons enter systemic circulation via lymphatics, Apo E and apo C proteins are added in lymph or blood. Chylomicrons are rapidly cleaned from blood by lipoprotein lipases and results in formation of partially degraded chylomicrons particles called remnants which are taken up by liver.

Chylomicrons in fasting state is abnormal and has been postulated that prolonged clearance of dietary remnant particles could be damaging to vascular endothelium and may predispose to atherosclerosis.

VLDL

It is endogenously produced lipoprotein(in liver) and contains apo B-100. It's synthesis is increased in obesity, alcohol use and diabetes, nephrotic syndrome and
hypothyroidism. Its function is to transport cholesterol and endogenously produced triglyceride. Clefsky et al (1976) noted biphasic plasma triglyceride curve. An initial peak occurred 1-3 hours after feeding was accounted by increase in chylomicrons levels in more than 98% and second peak after 4-7 hours accounted for rise in VLDL level in 82%.

IDL

It is formed from metabolism of VLDL of which roughly half is metabolized in mass and remaineder half is converted to LDL. The elevation of IDL is also thought to predispose for atherosclerosis.

LDL

It is produced from VLDL in plasma, and LDL supplied cholesterol to extrahepatic parenchymal cells, as adrenal cortical cells, lymphocytes, muscle cells etc. Thus Goldstein (1977) hypothesized the concept of LDL receptors and has been confirmed by many laboratories. These receptors are over cell surfaces to which LDL binds and by endocytosis, it is digested by lysosomes literating cholesterol for membrane synthesis and precurosr for steroid harmone synthesis. Liver uses LDL for synthesis of bile acids and free cholesterol secreted in bile. Diet high in fat and cholesterol causes elevation of LDL but varies in man.
Age related difference in rise of LDL was demonstrated by Arora and Gupta G (1987). They found our that rise of STC after feeding high fat breakfast for one week was much more pronounced in young volunteers (20-30 years) with major portion of rise contributed by HDL. Contrary in other persons the rise of STC was less marked and LDL mainly contributes to it.

HDL

It is produced in gut, by liver and also by peripheral catabolism of chylomicrons and VLDL. They are reservoirs for apolipoproteins. Some investigators have proposed that HDL facilitates cholesterol removal from cells particularly of reticuloendothelial system (Schnitz and Robenek et al, 1985). It is thus termed reverse endocytosis.

HDL is subdivided into several fractions in which HDL$_2$ and HDL$_3$ are important and best studied. HDL$_2$ are large and more lipid rich than HDL$_3$.

Patsh et al (1980) studied that an inverse relationship exists between the degree of post prandial lipemia and the concentration of HDL, apo A-I and HDL$_2$.

Concentration of HDL$_2$ is higher in woman than in man and are increased by oestrogen or physical activity(Exercise). Alcohol also increases both HDL$_2$ and HDL$_3$. Factors in which HDL lowers are hypertriglycercidemia, cigarette smoking. Exogenous androgen
administration lowers HDL levels in man (Furman et al., 1967). A drop in HDL level is seen in males at around the time of puberty (Beagtehole et al., 1980) and has been related to degree of sexual maturation (Frerich et al., 1978 and Morrison et al., 1979). Transient increase of HDL₂ has been reported at time of ovulation (Barclay et al., 1965). No changes in HDL is found during pregnancy. Decreased HDL is found in individual's first degree relatives and prepubertal and pubertal children of patients with a history of acute myocardial infarction and is attributes to genetic influences.

HDL level changes with age. In males levels are stable uptill puberty after which there is decline followed by stable levels in adulthood until 55-60 years where there is increase and then a plateau in older age group. In females there is a small linear increase in levels from childhood to about 60 years.

Reduction in obesity by mild exercise programme resulted in no increase in HDL cholesterol while drop in HDL levels are found in those with caloric restriction in absence of exercise.

LP (a)

It is an unique minor lipoprotein found mainly in density range of HDL. Although, it resemble LDL since it contains apo B, it also has apo A. It has been
known to be linked for CAD in women of all ages and in men 55 years or over (Dehlen and Guyton et al, 1986).

**RISK GROUP**

To relate risk to level of LDL then high risk group includes individuals with LDL more than 170 mg%. Low risk group for values less than 100 mg% and intermediate risk group for values 100-170 mg%.

Recently ratio of LDL to HDL has been used as another indicator of risk. Individuals with ratio greater than 5 as high risk group, values 3-5 at significant risk and at value 3 at average risk, value 2-3 at modest risk.

Thus high risk individuals are those with total LDL value more than 170 mg% or who have LDL/HDL ratio greater than 5.

**EFFECT OF DIETARY CHOLESTEROL UPON LIPID METABOLISM**

Dietary cholesterol is contained only in foods of animal origin (Connor, 1968). Plants and the oils contain plant sterols (Beta sitosterol) but not cholesterol.

Dietary cholesterol is absorbed by the gut in amount proportional to intake into dietary level of perhaps 1200-1500 mg/day of which only 40% is absorbed.
This absorbed cholesterol reaches a peak concentration in plasma about 48 hours after meal and the dietary cholesterol is transformed into other classes as described above in metabolism. The increase in plasma cholesterol is expressed chiefly as LDL for normal and hypercholesterolemic subjects. Only slight increase occurs in HDL.

Most dietary cholesterol is quickly delivered to liver which may:

a. Inhibit new cholesterol synthesis.
b. Increase sterol excretion in bile as acids or as cholesterol itself.
c. Synthesize other lipoproteins primarily VLDL.
d. Suppress specific receptors for LDL uptake and degradation.

Thus cholesterol in plasma has two sources viz dietary and endogenous production. Cholesterol synthesis is not menlabile and has little effect on synthesis from ingestion of dietary cholesterol. This ingestion of large quantities of dietary cholesterol induces rise in plasma cholesterol and deposition of increased amount in tissues particularly arteries to possibly initiate and sustain atherosclerosis. Plasma cholesterol levels increase as much as 25% or more over baseline after changes in diet of high fat content (Mattson, Erickson and Kligman, 1972).
INFLUENCE OF EGGS ON PLASMA LIPOPROTEINS

Adding eggs to an otherwise balanced diet has not been found to alter total serum cholesterol in middle aged man (Flyan et al, 1979).

Three factors influence the effect of dietary cholesterol on serum cholesterol in serum are:

a. Composition of the diet with respect to other nutrients including polyunsaturated fatty acids.
b. Unknown anti or pro-hypercholesterolemic agents.
c. Baseline level of dietary cholesterol from which observations are made.
d. Individual variability.

Some people are hyper responders while other hyporesponders (Katan and Beynen et al, 1983; Robert and McMurray et al, 1981). This responsiveness relates the extent to which exogenous cholesterol interacts with the cholesterol homeostatic system in each individual and thus regulate the rate of cholesterol biosynthesis.

Beynen and Katan (1985) reported the result of study of egg yolk feeding in three males and three female volunteers, all of whom were scientific workers at University and age ranging from from 26-42 years, and weight from 56 to 81 kgs with cholesterol level ranging from 136-215 mg% (mean+S.D. 191±27 mg%). First trial was in 1981 and second in 1982.

The volunteers were asked to consume habitual diet, but to omit eggs, butter, shell fish and limit
meat and fish to 100 gms/day for 10 days. After this six eggs per day were then added to same diet for 10 more days. In this change of diet energy intake increased from 2260±524Kcal/day to 2689±96 Kcal/day. Dietary cholesterol increased from 207±26 mg% to 1803±155 mg/day and dietary fat increased from 39±5 to 46±6 mg% but P/S remained constant 0.47±0.06.

**Serum** After 10 days of low cholesterol diet average serum total cholesterol decreased significantly from 191±27 to 168±21 mg%. Eating eggs along with increased cholesterol by 13% in both groups (a significant increase). Moreover LDL increased 18% and 24% and HDL$_2$ increased 23% and 49% in both first and second trial respectively.

In a double blind crossover trial by Sacks et al (1984) made diet changes for 3 weeks and adding egg increased dietary cholesterol from 97-318 mg per day. VLDL declined by 17% and HDL values remains constant. 13% increase in LDL but no significant change occurred in serum total cholesterol 175±27 vs 182±35) as a result of ingesting the egg. Individual change in LDL in response to egg consumption ranged from -22% - 44% indicating marked variability.

Packard et al (1983) found that increasing dietary cholesterol from 180±110 to 1470±80 mg/day by adding six eggs/day increased LDL levels 40% and HDL level 18%.
These above studies demonstrate that individual response to dietary cholesterol are highly variable. Habitual cholesterol intake of 200-1020 mg per day has no effect on steady state STC or LDL levels. On the other hand, short term cholesterol consumption in the range of 750-1000 mg/day increases both LDL and HDL in about 50% of normal individuals. This increase may be attributed to failure of these individuals to inhibit endogenous biosynthesis of cholesterol.

**EFFECT OF CRYSTALLINE Vs EGG CHOLESTEROL ON LIPID LIPOPROTEINS**

Ordinary human diet has no significant effect on concentration of STC in blood.

In a study at Minnesota University it was concluded that variation in intake of cholesterol over whole range of natural diet do not influence the serum level of normal adult man so long other elements of diet are constant.

With intake of 30 gms of cholesterol (Crystalline) per day for 29 days, Messinger et al (1950) produced serum elevation in 4 out of 5 subjects and were very small but when high cholesterol doses were given in form of cream and egg yolk fat the serum cholesterol rose sharply (average 10-20%).

Aancel, Keys et al (1956) concluded as follows:
1. Comparison made between 23 man who before and after
had voluntarily doubled their cholesterol intake and of 41 men who halved their cholesterol intake failed to show any response in serum cholesterol levels in 4-12 months while rest of their diet was more or less constant.

2. 119 Minnesota businessmen failed to show any significant increase of serum cholesterol with increasing dietary cholesterol.

3. In last it was thus concluded that in adult men serum cholesterol level is essentially independent of cholesterol intake. It is probable that infant, children and woman are similar.

In a study Williams and Conner found that when subjects were given diet containing 1425 mg of cholesterol as egg yolk for 3 weeks the mean rise in serum cholesterol was 69 mg% and mean rise in serum triglyceride was 33 mg%. On other hand when similar subjects were given 3600 mg of crystalline cholesterol for 3 weeks, the mean rise of serum cholesterol was 19 mg%, and serum triglyceride was decreased by 4 mg%, and loss of fat in stools increased. The review of Cook and Karvinen and Lin suggests that human gastrointestinal tract has a limited capacity for cholesterol absorption and all cholesterol above a certain amount is unabsorbed and passed out in stools.

Crystalline cholesterol has a lesser effect upon the serum cholesterol and phospholipid concentration
than did egg yolk despite the greater amount of former was consumed. The increased lipid loss in stool suggested that this form might be poorly absorbed. Moreover Edwards Cook and Riddell found that intestinal absorption of egg yolk cholesterol was four times more complete than was crystalline cholesterol dissolved in olive oil.

EFFECT OF DIETARY FAT ON LIPID LEVELS

The quantity and quality of fat in diet has effect on plasma lipid with subsequent chylomicrons formation and its circulation in blood is directly proportional to the amount of fat consumed and thus imparts characteristic plasma appearance after 3-5 hours of meal (Creamy).

The quality of fat may be divided into three major classes.

Long chain saturated fatty acid (Class I) may be readily synthesized in the body. Dietary intake of these have hypercholesterolemic effect and increase LDL concentration (animal fats is saturated).

Monosaturated fatty acids (Class II) are present in all animal and vegetable fats. It has neutral effect plasma lipids. They are also readily synthesized in body.

Polyunsaturated fatty acids (Class III) are important consituents of cellular membrane and severe as prostaglandin precursor. Since they cannot be synthesized in body then can only be obtained from dietary
sources and thus called essential fatty acids. Common ones are linoleic and arachidonic acid. These in general depress plasma cholesterol, LDL, triglyceride and VLDL concentration.

Plyunsaturated to saturated fatty acid ratio in a given fat or oil is designated as P/S ratio. Fats with high P/S ratio value of 2 or above are recognized as hypercholesterolemic.

Shore, Krauss and Butlerfield (1981) studied the effects of dietary ratio of P/S on serum lipid lipoprotein profile in healthy normolipaemic man. In all IDL, LDL, HDL, Apo B, total cholesterol were lowest at P/S 3. The percentage changes was greatest in individual with higher initial values.

**EFFECT OF CALORIES ON LIPID LEVELS**

The consumption of extra calories in excess of these needed for metabolic maintenance and physical activity results in obesity. It is a state with increase in cholesterol synthesis (Bennion and Grunay, 1975) and elevated levels of VLDL, LDL and reduction in HDL (Carlson et al, 1975; and Garrison et al, 1980).

The immediate metabolic consequence of excess calories is an increased supply of substrates for triglyceride synthesis in liver and subsequent increased VLDL (Olefsky et al, 1974). Moreover reduced clearance of VLDL from blood may be responsible for hypertriglycer
ridaemia of obesity. Enlarged adipose tissue cells may have a reduced capacity to remove circulating triglycerides because of their decreased sensitivity to circulating insulin which activates lipoprotein lipase.

**EFFECT OF CARBOHYDRATES ON LIPID LEVELS**

Increasing carbohydrates to 70-80% of calories increases serum triglyceride level and is called "carbohydrate induction" (Ahrens et al, 1961). Although it is a transient effect of increased carbohydrates in diet long term studies have found little effect on blood lipid levels. Thus to emphasize that population consuming high carbohydrate diets habitually have a low incidence of atherosclerosis and do not have markedly elevated plasma triglyceride levels (Conner and Conner, 1977).

Earlier studies suggesting that sucrose in diet had a definite hyperlipidemic effect compared to glucose and starch has not been confirmed by subsequent investigation (Key et al, 1960).

**RELATION OF LIPID LIPOPROTEIN LEVELS IN PRE AND POST MENOPAUSAL WOMEN**

In premenopausal women there is 10-25% cyclic suppression of total plasma cholesterol, HDL and LDL apo B during leuteal phase and HDL increases slightly during the second half of menstrual cycle (Kin and Kalkhoff et al, 1981).
In post menopausal women the lipid lipoprotein levels changes as rise in LDL and fall in HDL and towards male values.

Young women of child bearing age has significantly low incidence of CAD than man of same age group. But this difference of incidence decreases with advancing age suggesting protective ovarian functions and comes equal to that of male after age of 55-60 years. This fact is supported by study of Bengtson et al (1973), Oliver et al (1959), Gordon et al, (1978) that female undergoing early menopause were observed to have higher rate of CAD than with those of late menopause of same age group.

The possible reason for above fact has been oestrogen, a safety factor causing increase HDL, lowering of LDL and total cholesterol. Exogenous progestrone has just opposite effect on lipid lipoprotein levels (Bradley, 1982 and Wingerd et al, 1982).

**EFFECT OF ESTROGENS IN FEMALES**

A study from Howard Medical School, Stampfer et al (1985) examined subjects in which approximately 50% has used estrogen at some time and 35% were current users (primarlin or conjugated estrogen) in dosage of 1.2 or 0.6 mg/day. The risk of myocardial infarction either fatal or nonfatal, was approximately half of that who had never used them. Of the current users the risk was about one
third of that who never used estrogen. However, these results were not corrected for age, cigarette smoking, past use of oral contraceptives, hypertension, diabetes mellitus, obesity and family history.

Another study by Wilson et al (1985) gives conflicting results. The effect of estrogen use on morbidity from CAD in post menopausal group gives a over 50% elevated risk for cardiovascular disease compared with women group who had not taken oestrogen. Those who smoked while taking estrogen were having a higher risk. Mortality from CAD and from all causes did not differ from either those who took estrogen or those who had not.

**ORAL CONTRACEPTIVES AND CAD**

Since oral contraceptive have both estrogen and progestrone in varying quantities, and opposite effect of both on lipid lipoprotein profile, the study of Mammet et al (1975) was first to demonstrate an increased risk of acute myocardial infarction with its use. The relative risk in users is at 4.5 compared with non users.


Engle et al (1983) showed role of oral contraceptives in developing myocardial infarction without atherosclerosis in more than 80% of their studied subjects. However, cigarette smoking was common in subjects.
OBESITY AND SERUM LIPID LIPOPROTEINS

Obesity has direct relationship with all major common risk factor except smoking, strongest correlation is with blood pressure, hypertriglyceridemia, hypeinsulinemia and inversely with HDL concentration.

Knuiman et al (1982) showed positive correlation of body mass index with total cholesterol and inversely related to HDL.

In addition, obese persons continue to gain weight and HDL level continue to decline. It is well documented that low HDL confers greater risk for CAD but it remains to be determined that if primarily elevating HDL will decrease risk in obese, and moreover obese person who lose weight do not show an increase in HDL (Rossner et al, 1980). Thus exact role that HDL plays in cardiac risks related to obesity is unknown.

PHYSICAL ACTIVITY AND SERUM LIPID LIPOPROTEIN AND CAD

Long term physical activity lower blood pressure serum triglycerides, raises HDL level, particularly HDL₂.

Siltanen et al (1982) showed that vigorous physical conditioning at least 4 hours/week appeared to decrease mortality after an initial myocardial infarction and possibly during subsequent 2 years.

Hartung et al (1983) studied the relationship between exercise and alcohol consumption on HDL in runners and inactive men respectively. It was found that
exercise was a more significant determinant of HDL levels than alcohol. Those running 12 miles or more a week had lower levels of lipids and high HDL than did sedentary ones. Women were also studied later in 1986 and Hartung found similar correlation between exercise and lipid lipoprotein levels as in males.

**INFLUENCE OF EARLY FAT INTAKE AND SUBSEQUENT SERUM LIPID LIPOPROTEIN LEVELS**

Breast milk has high cholesterol content (20 mg/dl) but has decreased P/S ratio. Serum cholesterol was higher in first 9-12 months of life in breast fed babies, but little difference subsequently (Freidman and Goldberg, 1976) Huttunen et al, 1983).

The feeding habits and serum lipids in infants and children showed direct correlation between serum lipid levels and the amount of saturated fat as well as its P/S ratio in 6-10 months but not later in 3-4 years children. Thus type and duration of early feeding practices had little influence on subsequent serum lipid levels (Andersons et al, 1979).

**EFFECTS OF GENETIC FACTORS ON SERUM LIPIDS AND LIPOPROTEINS**

The progeny of parents who sustained myocardial infarction before age of 55 years showed significant hypercholesterolemia and/or hypertriglyceridemia, among 18-29% of such group and while in control group it was only in
5% (Glueck, 1983). It has also been shown that sons of aged 14-25 years of fathers with IHD had lower HDL levels than controls (Nupuf and Sutherland, 1979). However, no difference was found in total serum cholesterol and triglyceride in this study of Nupuf.

In contrast, Pometta et al (1980) found that prepubertal sons of parents who reported myocardial infarction had lower levels of cholesterol than controls but neither of studies mentioned above measured ratio of STC/HDL.

The genetic aspects of CAD is suggested by familial clustering of this disease (Deutscher et al, 1970; Epstein, 1964; Rose, 1960).

Few studies indicated that familial aggregation of CAD may be influenced by the genetics of risk factors and common environmental conditions encountered among family members (Goldstein, 1973; Deutscher et al, 1970). Thus multifactorial genetic and environmental inter-relationship of risk factors can explain familial aggregation of CAD (Jhonson et al, 1965, Epstein, 1964).

Further evaluation of genetical influence over CAD has been studied in twins. Cederlof et al (1967) noted concordance rate of CAD to be 21.7% in monozygotic twins, compared to 6.17% in dizygotic twins. Similarly Verschver (1959) observed a concordance rate of 19% and 8.5% respectively in mono and dizygotic twins.
James Nora (1980) pointed towards a single high risk factor in CAD to be in children of first degree relatives with myocardial infarction before the age of 55 years.

REPRODUCIBILITY OF LIPID LIPOPROTEIN LEVELS

It is a mode to study and find out whether the response of serum lipid lipoprotein levels to changes in cholesterol intake in man is variable after a period of time, and if so then what pattern of variability is found. Moreover, it may help to study the association of various risk factors which may contribute to this variability and thus may have following outcome:

a. The cholesterolemic response to dietary cholesterol in man is stable.

b. The cholesterolemic response to dietary cholesterol in man is variable.

If b, then it may show changes in lipid-lipoprotein levels suggestive of risk of developing any complications due to accelerated atherosclerosis in future and thus of developing cardiovascular/cerebrovascular accident.

This pattern of study would help us in following ways.

1. Indicate pattern of changes of lipid profile in individuals who were earlier studied so that high risk subjects may be sought. Moreover, the previous
pointers towards high risk subjects would be confirmed.

2. These subjects could be given proper medical/dietary advice to modify reversible risk factors so as to change the course of the subclinical picture and thus possibly decrease morbidity and sudden mortality due to complications of atherosclerosis.

3. The association of various risk factors can be properly understood if the subject remains constant in multiple studies over a period of many years (4-6 years) on similar protocols and the pattern of changes in lipid lipoprotein profile would guide us to this understanding.

4. The relation of lipid profile to any disease which may develop during this period can be understood.

A study over reproducibility of the variations between humans, is, the response of serum cholesterol to cessation of egg consumption was done by Beynen and Katan in their study from 1976-82.

In order to find out whether the variable response of serum cholesterol to changes in cholesterol intake in man is due to constitutional differences in responsiveness, they have reinvestigated in 1982. Thirty four healthy men and women who habitually consumed at least 1 egg/day and had participated in a trial in 1976, serum cholesterol was measured on the habitual diet (about 800 mg cholesterol/day) and after 3 weeks during which no egg or egg
containing products were consumed (about 300 mg cholesterol/day) serum cholesterol decreased by 6±16 mg% in 1975 and by 12±14 mg% in 1982 (Mean±SD). Individual responses varied from -39 to +19 mg%. The correlation between the responses in 1976 and 1982 was r = 0.32 (p < 0.05). The decrease in serum cholesterol was most pronounced for subjects with a low body mass index and a high level of HDL. They concluded that part of cholesterolemic response to dietary cholesterol in man is individually determined and stable for at least 6 years.

LIPOPROTEINS AS PREDICTOR OF CAD

More than 90% of plasma cholesterol is carried by LDL and HDL. Concentration of LDL cholesterol are directly related to and predictive of CAD over a wide range (Gordon et al, 1981). This relation underlies the association between CAD and serum cholesterol, for later reflects LDL concentration (Kannel et al, 1979). Moreover, morbidity and mortality rates from CAD in different communities are directly and linearly related with serum concentration of total cholesterol (STC) and LDL (Lewis et al, 1978). HDL concentrations are even more strongly predictive of the risk of coronary heart disease in most (Gordon et al, 1981 and Goldbourt & Medalie, 1979) but not in all the persons (Wiklund et al, 1980).
Hyperlipidaemias as well as other risk factors probably run in families and may thus support the above concept for the development of CAD in individuals.

A recent study examining the influence of family history of CAD, hypertension, obesity and diabetes on total cholesterol (STC), HDL, LDL, 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).

The ratio of LDL/HDL is about as efficient as any other lipid profile (Kannel et al, 1979). A ratio of 5 indicates average high risk, and beyond this are a definite cause of concern.