REVIEW OF LITERATURE
Numerous studies have implicated altered levels of plasma lipoproteins in the pathogenesis of atherosclerosis. In particular, elevated low density lipoprotein (LDL) and diminished high density lipoprotein (HDL) cholesterol levels appear to be strong risk factors for the development of atherosclerosis.

Research of the last two decades has revealed a rather complex set of events that control plasma lipoprotein level. Specific proteins have been implicated in the regulation of lipoprotein synthesis. Besides this many more factors like age, sex, cigarette smoking, obesity, hypertension, dietary habits and sedentary life style exert their influence on lipoprotein levels and development of atherosclerosis in their own way. Many of the risk factors are reversible but influence of age, sex and genetic factors are irreversible.

Atherosclerosis is essentially a degenerative process associated with advancing years, mainly affecting larger arteries, particularly the coronary and cerebrals.
The lesion of atherosclerosis to start with is a fatty streak. This lesion can be found in majority of children of 10 years of age. Second stage in advancing atherosclerosis is a fibrous plaque and finally it gives way to advanced lesion.

CHANGES IN LIPID LIPOPROTEIN LEVELS AFTER HIGH CHOLESTEROL DIET

Effect of long term and short term feeding of diet rich in cholesterol evokes variable responses and is subject to individual variation. Dietary fat and cholesterol causes changes in specific lipoprotein in a variety of species (Mahley et al, Arora R.C. et al). Quantitatively, a change in specific lipoprotein may be dramatic in one species than in another. These changes have been associated with the development of atherosclerosis in experimental models (Mahley et al). Considering effect of diet on individual lipoprotein fractions.

TOTAL SERUM CHOLESTEROL (STC)

In 1956 Ancelkeys, J.T. Anderson et al concluded that serum cholesterol level is essentially independent of the cholesterol intake over the whole range of natural human diets. But later on it was
proved beyond doubt that feeding cholesterol rich
diet for 2-8 weeks raises total serum cholesterol
in blood (Arora R.C. et al., Messinger et al.,
Conner et al., Deborah Applebaum et al.).

In an earlier report, Bruhn in 1940 observed
a 20% rise in mean cholesterol level after a fat
load. Effect of high cholesterol fat load on post
prandial cholesterol levels has also been studied
in the past by several workers, but insignificant
difference has been found between post prandial
and 10 to 14 hrs. fasting value (Albrink and Man
All these workers observed plasma cholesterol values
up to 24 hrs. after a test meal. On the other hand
Nikkila and Kemtinem in 1962 demonstrated a significant
decrease in cholesterol level six hours after a fat
diet in healthy soldiers.

Hanno Krauss, Pieter Groot in Oct. 1987
reported insignificant changes in total serum
cholesterol after feeding 0.5 gm/m² of cholesterol
and taking readings at 2 hourly interval for 14 hrs.

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 effect was much more modest (8.3%) in those with lower initial levels (M.C. Gandey et al. 1972).

In another large survey of school children, there was no positive correlation between the low (80-130 mg/dl), the intermediate (157 to 180 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 weak, correlations were noted between serum lipids and dietary P/S ratio (Nollie and Glueck 1983).

In a survey of school age children examining the influence of nutrients on LDL cholesterol, it was concluded that the higher intake of cholesterol and lower ratio of P/S was associated with higher value of LDL cholesterol. Strict vegetarians have been reported having lower serum cholesterol than lactovegetarians and nonvegetarians (Sacks F.M. et al., 1975, Knuiman J.T. et al, 1982).
Textured vegetable proteins lowered total serum cholesterol in hypercholesterolemic subjects with no change or a slight elevation of in HDL cholesterol, no effect or only minor changes have been observed in normolipidemic subjects (Sirtori C.R. et al., 1985).

The replacement of animal protein with vegetable protein in the diet has been suggested to reduce the diet linked atherogenic risk (Carroll K.K. 1982).

However, Sacks F.M. et al. 1983 found no appreciable correlation between total intake of protein, when consumed above minimum requirement and serum cholesterol level.

Work in animals showing that sucrose and fructose are atherogenic, prompted human studies, which have not shown consistent changes. In one study, isocaloric replacement of starch with sucrose in mixed diet did not lead to changes in serum cholesterol (Mann and Truswell, 1972), changes that were documented in another study (Reiser et al., 1978).
Early fat intake does not influence subsequent serum lipids level. Serum cholesterol was higher during the first 9-12 months of life in breast feed babies, but there was little difference subsequently (Friedman and Coldberg 1976, Huttunen et al. 1983).

In addition to its high cholesterol content (20 mg/dl), breast milk has decreased P/S ratio of fatty acids when compared to formulas. In a report dealing with feeding habits and serum lipids in infants and children, there was a direct correlation between serum lipids and the amount of saturated fat as well its P/S ratio in infants aged 6 to 10 month, but no such correlation was found in 3-4 years old children. The type and duration of early feeding practices had little influence on subsequent serum lipid levels (Anderson et al. 1979). Results of human studies, therefore do not agree with animals work, which suggest that a low post natal dietary cholesterol homeostasis. In fact it was shown that children aged 7 to 12 years who were fed low cholesterol formulas had a lower mean serum cholesterol than those fed cow’s milk or breast milk (Hodgson et al, 1976). Another study could not document an effect of a low versus a moderate cholesterol intake during first six month of life in response to large cholesterol
intake during second 6 month of life (Glueck 1972).

**HIGH DENSITY LIPOPROTEIN (HDL)**

High density lipoprotein are lipid-protein complexes defined by flotation in the ultra centrifuge between density 1.063 and 1.21 gm per ml, by the presence of major protein constituents, apolipoprotein A-I and A-II, and by alpha migration on electrophoresis. Three classes of HDL are separated on the basis of flotation rates on ultracentrifugation. HDL₂ have flotation rates between 0-3.5, HDL₂ have rates in excess of 3.5. The third and minor HDL₃ is sometimes found at d<1.063 and overlaps with the low density lipoprotein distribution. Recently Mahley and colleagues have identified a distinct sub type of HDL designated HDLₓ or apo E-HDLₓ. This is found in the plasma of cholesterol fed animals, and to a much smaller extent in humans fed high cholesterol, high saturated fat diets. HDLₓ differs from other sub type by presence of apolipoprotein E. This property confers an affinity for the low density lipoprotein receptor (Mahley and R.W. Wiegand, 1978).

The lipid constituents of HDL exhibit variations. Cholesteryl ester content may range from 10-20 percent,
Triglycerides are normally less than 4 percent. The ratio of cholesterol to triglyceride in HDL may show wide fluctuations with increase being observed after dietary cholesterol supplementation (Mistry P. et al., 1977) and decrease being found in patients with hyper triglyceridemia (Weisweiler P. et al., 1977), uremia (Brunner J.D. et al., 1977), and Ischemic heart disease (Carlson L.A. et al., 1975).

The bulk of HDL mass appears to arise from the interaction of precursor particle nascent HDL secreted by the liver and intestines, with lipids and protein released during the catabolism of triglyceride rich lipoprotein. A portion of HDL also arises from transfer and uptake of lipids, particularly free cholesterol from cell membrane.

**FACTORS MODULATING HDL LEVELS IN HUMANS**

(a) **Constitutional Factors**

In most population it has been demonstrated that women have higher levels of HDL than men at all ages following puberty. Exogenous adrogen administration lowers HDL levels in men (Furman, R.H. et al., 1967). A drop in HDL level seen in males at around the time of puberty (Beagtehole et al., 1960) has been related to the degree of

Transient increase in HDL₂ have been reported at or near the time of ovulation. (Barclay M. et al., 1965). No changes in HDL cholesterol have been found during pregnancy (Kinnunen P.J. et al., 1980).

There also exist a strong genetic influence in disease states. Reduced levels of HDL cholesterol is found in adult first degree relatives and prepubertal and pubertal children of patients with a history of Acute myocardial infarction (Micheli H. et al., 1979, Pometta D. et al., 1979, Robertson F.W. et al., 1980). Recently, evidence for autosomal dominant inheritance of low HDL levels has been reported in large kindred with a high prevalence of coronary disease (Vergaie C. et al., 1981). High level of HDL has also been reported in black American population (Tyroler et al., 1975).

HDL level also change with age. In males the levels are stable until puberty and adolescence, during which there is a decline followed by relatively stable levels in adulthood until ages 55-60, where there is an increase; and then a plateau in older age group. In females there is a small linear increase in
HDL-c from childhood to about 60 years, after which no age effect is apparent (Heiss et al., 1980).

(b) OBESITY AND HDL

HDL levels are lower in obese individual than in non-obese controls (Wilson D.E. et al., 1972; Carlson L.A. et al., 1975 and Glueck C.J. et al.). During the course of weight loss, an increase in HDL cholesterol concentration has been reported to occur in association with reduction in VLDL and total triglyceride concentration (Wilson D.E. et al., 1972). But in other studies HDL cholesterol showed either no change or a reduction (Widholm, K. et al., 1978, Thompson P.D., 1979, Howard B.V., 1979).

(c) PHYSICAL ACTIVITY AND HDL CHOLESTEROL

High levels of HDL-c are reported to be related with high level of endurance type exercise, including long distance runners, cross country skiers, lumberjacks, tennis player, and soccer player (Wood P.D. et al., 1977, Lehtonen A. et al., 1978, Lehtonen A. and Viikari et al., 1978, Vedak P.A. et al., 1980).

Reduction in adiposity, in combination with mild exercise program, resulted in no increase in HDL cholesterol, whereas a drop in HDL cholesterol
was found with caloric restriction in the absence of exercise (Waltman et al., 1980).

(d) **ALCOHOL AND HDL**

Alcohol ingestion has been reported to raise levels of HDL (Johansson B.G. et al., 1974, Beilfage et al., 1977). But the results of Glusak C.J. et al., 1980 were contradictory to the above statement.

In a large epidemiological study levels of HDL cholesterol and amount of habitual alcohol intake in moderate range have been independently correlated (Castelli W.F. et al., 1977).

(e) **RELATIONSHIP OF DIET AND HDL CHOLESTROL**

Diet is an important modulator of the synthesis, secretion, and concentration of serum lipoprotein. Conflicting reports have appeared on effect of dietary cholesterol on HDL levels.

T.A. Borden et al. in 1964 reported enhanced levels of HDL-c in rats fed cholesterol while D.E. Haft et al., 1962 and D. Kritchevsky in 1965 reported no change in HDL levels in cholesterol fed rats.

R. Reiser et al., 1966 and A.N. Howard et al., 1968 reported decreased level of HDL cholesterol in rats fed with high cholesterol diet.
K.A. Narayan 1971 demonstrated that HDL₂ decreased drastically about 50% in rats fed with high cholesterol diet. These results confirmed the earlier observation of Reiser et al., 1966 that rat serum HDL level was decreased irrespective of whether a saturated or unsaturated fat was used in the diet supplemented with cholesterol. In short term feeding studies, marked reduced in dietary fat and isocaloric increase in carbohydrate resulted in 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 (Schonfeldt et al., 1976) consistent with a selective decrease in HDL₂ species (Blum et al., 1977).

There is evidence that substitution of large quantities of poly-unsaturated fat for saturated fat in diet can result in lower levels of HDL lipids and proteins (Nichaman et al., 1987). An increase in the P/S fat ratio from 0.25:1 to 4:1 in food diet fed to four normal subjects for five weeks resulted in reduction of HDL cholesterol and apolipoproteins A-I
concentration of 35 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 1973, Shore et al., 1981) or increase (Jackson and Glueck, 1980) in levels of HDL cholesterol with feeding of diets enriched in polyunsaturated fats. High dietary intake of cholesterol, in the form of three to six egg yolks per day, has been reported to produce increase in apolipoprotein E-containing HDL₃ sub species in human (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 to the total HDL, its presence was shown to account for an increase of 2.6 to 4 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 level 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 (Tan et al., 1974).
A final consideration in evaluating the effects of dietary variables on HDL is that, while levels of HDL cholesterol and plasma apolipoprotein A-I are similar after overnight fast and the nonfasting state, (Henderson L.O. et al., 1980), changes in levels and composition of HDL have been shown to occur actutely after meals containing fat. Cholesterol, Phospholipid, and C-apolipoprotein levels in HDL₂ increases, and cholesterol in HDL decreases (Havel R.J. 1973, Baggio G. et al., 1980) in conjunction with transfer of chylomicron lipids to HDL during the course of their catabolism. Recently it has been shown that HDL apolipoprotein A-I levels increased when fat was consumed in divided doses over a 10-hours period, but not when the same amount of fat was ingested as a single load (Kay R.M. et al., 1980).

**LOW DENSITY LIPOPROTEIN-CHOLESTROL (LDL-c)**

LDL-c is generated by the degradation and removal of triglyceride from very low density lipoprotein (VLDL) in the plasma, their density is in the range of 1.019-1.063 and they contain apoprotein B₁₀₀. More than 75 percent of the total cholesterol present in the plasma is in the form of LDL-c.
One function of LDL is to supply cholesterol to a variety of extrahepatic parenchymal cells, such as adrenal cortical cells, lymphocytes, muscle cell, and renal cells. In 1977 Goldstein hypothesized the concept of LDL receptor. The presence of these receptors have been confirmed by many laboratories. LDL receptors are present on the cell surface of liver, adrenal cortical cell, lymphocyte muscle cell and renal cells. LDL that binds to this receptor is taken up by receptor mediated endocytosis and digested by lysosome within the cells. The cholesterol esters of LDL are hydrolyzed by a lysosomal cholesteryl esterase, and the liberated cholesterol is used both for membrane synthesis and as a precursor for steroid hormone synthesis. Liver uses the LDL-c for synthesis of bile acids and for generation of free cholesterol which is secreted into the bile.

In humans around 80 percent of LDL is removed from the plasma each day by the LDL receptor pathway the remainder is degraded by scavenger cell system in phagocytic cells in reticulo endothelial system.
Diet Induced Changes in LDL-c

Diet high in fat and cholesterol cause an elevation in LDL in most animals (Mahley R.W. 1978). The response in man varies, but in those subjects who have an elevation in plasma cholesterol, there is an elevation in plasma LDL levels. In 1979 Deborah-applebaum et al. demonstrated significant rise of LDL level in human volunteers after feeding 5000 mg of egg yolk cholesterol per day for 30 days.

Age related difference in rise of LDL was demonstrated by Arora R.C. and Gupta Gulab et al. in 1987. They found out that rise of total serum cholesterol after feeding high fat, high cholesterol breakfast for one week was much more pronounced in young (20-30 gm) volunteers with major portion of rise being contributed by increased HDL. Contrary, in older age person the rise of total serum cholesterol was less marked with LDL-c contributing mainly in the increased levels.

M.F. Baudet et al. demonstrated that, there was significant fall in level of LDL in five volunteers 3 hours and 5 hours often taking butter diet. They attributed this fall due to defect in VLDL hydrolysis
by serum lipases and due to metabolic blocking in liver or adipose tissue.

In addition to this the diet induced LDL are larger than LDL from the same species on low fat – low cholesterol diet. In a study performed by Rudel and co-workers in 1979 on rhesus monkey showed that, high cholesterol diet induced LDL have molecular weight which are 1.5 fold larger than those of control LDL. Further more St. clair and Leight in 1978 have reported that the diet induced, large LDL are capable of stimulating cholesteryl esterification and accumulation in smooth muscle cells to a greater extent than are normal LDL.

An additional alteration in the LDL, induced by the high cholesterol diets involve the apoprotein constituents. In normal LDL, the B-apoprotein is the major detectable apoprotein moiety, however in several species the LDL contain a variable amount of the E apoprotein following cholesterol feeding (Mahley R.W. et al., 1977, Rudel L.L. et al., 1979).

CONTROL OF PLASMA CHOLESTEROL LEVEL BY LDL RECEPTORS

A decade of intense investigation has established a central role for lipoprotein receptors in regulating
plasma cholesterol traffic. Operationally, the LDL/LDL receptor system can be considered the primary transport mechanism for endogenous cholesterol. LDL are generated in the plasma by the degradation of intermediate density lipoprotein (IDL). Generated LDL is removed relatively slowly from plasma by binding to LDL receptors in the liver and extra hepatic tissues, (Kita T. et al., 1982). In rabbits, rats, and hamsters, more than half of the total LDL receptors are located in the liver. However the precise distribution of these receptors in man is unknown.

REGULATION OF HEPATIC LDL RECEPTOR

Hepatic LDL receptors are suppressed whenever the livers content of cholesterol increases or its demand for cholesterol is reduced. Thus receptor suppression occurs when a high cholesterol diet is consumed, (Hui, D.Y. et al., 1981) or when bile acids are infused (Angelin B. et al., 1983). Conversely, LDL receptors increases when hepatic cholesterol synthesis is blocked by drugs compactin or mevinolin (Goldstein et al., 1982, and Bilheimer D.W. et al., 1983), when bile acid binding resins are given (Shepered J. et al., 1980), or when an ileal by pass is created (Spengel F.A. et al., 1982). Fasting has also been
shown to suppress LDL receptor in rabbits (Goldstein J.L. 1982). LDL receptors can be stimulated by thyroxine (Thompson G.R. 1981) and by pharmacologic doses of estrogen (Winder, E.E.T. 1980). Hepatic LDL receptors decline when rabbits are fed a diet composed only of sucrose and casein (Chao Y.S. et al., 1982). In dogs, hepatic receptors fall with ageing (Mahley R.W. et al., 1981).

All of the changes in receptor activity alter the rate of uptake of LDL by the liver and cause reciprocal changes in plasma LDL levels. Whenever hepatic LDL receptors are suppressed, the plasma LDL level rises; conversely, whenever these receptors are induced, the plasma LDL level falls.

**FAMILIAL HYPERCHOLESTEROLEMIA AND LDL RECEPTOR**

Familial hypercholesterolemia is best defined clinically, genetically, and biochemically as an autosomal dominant trait with a gene dosage effect. It occurs at a frequency of about 1 in 500 person.
The basic defect is reduced number of LDL receptors. In normal person about 45 percent of the plasma LDL pool is removed from the plasma daily by the receptors, whereas in familial hypercholesterolemia heterozygotes this value is 25-30 percent and in homozygotes it is about 15 percent. This receptor deficiency results in accumulation of LDL into the plasma, leading to raised level and premature atherosclerosis.

TRIGLYCERIDES AND VERY LOW DENSITY LIPOPROTEIN (VLDL)

The level of serum triglyceride (STG) rises considerably after fat ingestion. Rise in the triglyceride level after fat ingestion has been reported after giving different amounts of the fat load and measuring the blood levels at different time interval (Nikkila and Konttinen 1962, Denborough, 1963).

Angervall in 1963 has reported a significant correlation between fasting, 3½ hours valve and 7½ hours valve of serum triglyceride postprandially.

Clefsky et al., in 1976 noted a biphasic plasma triglyceride curve with an initial peak occurring 1 to 3 hours after feeding and a secondary peak after 4 to 7 hours. The primary peak was accounted by increase in chylomicron levels in more
then 98% cases, whereas secondary peak represented rise in very low density lipoprotein (VLDL) level in 82%.

In 1957 Richard J. Havel concluded that increment in the concentration of triglycerides in the serum following ingestion of fat is entirely the result of an increase in their concentration in VLDL.

Excess production of VLDL and triglyceride is more often due to secondary abnormalities than to primary factors, perhaps the most common cause is high caloric intake associated with obesity, excess alcohol and excess carbohydrate. Increased levels are also found in diabetes mellitus, nephrotic syndrome and hypothyroidism with obesity. Delayed clearance of triglyceride from the serum is noted in cases of ischemic heart disease after high fat diet (Arora R.C. et al., 1967, David F., Brown et al., 1961).

**VLDL - REMNANTS**

Also known and Beta VLDL these particles are smaller than normal VLDL and contain more cholesterol. Both of these characteristics impart atherogenic potential to VLDL remnants.
BETA VLDL IN CHOLESTEROL FED MAN

In addition to a report by Mistry P. et al. in 1976 that Beta VLDL can be induced by cholesterol feeding in man, preliminary studies from the Gladstone foundation laboratories for cardiovascular disease indicate that certain individual respond to high fat, high cholesterol diet by producing lipoproteins which are capable of delivering cholesterol to macrophages. The Beta VLDL may occur transiently as minor components of the human plasma fractions after diets high in fat and cholesterol are consumed, and may cause repeated cholesterol deposition in cells in the arterial wall over the years. The Beta VLDL, either chylomicon remnants or hepatic lipoprotein may represent the atherogenic particle postulated several years ago by Zilverman. This alteration in the lipoprotein fraction may represent the most significant diet induced changes in lipoprotein predisposing to accelerated atherosclerosis.

SERUM TRIGLYCERIDE AND EXERCISE

Reduced level of triglycerides are found after exercise (Cohen & Goldberg, 1960). On the other hand, Billimoria et al., 1959 found that the alimentary lipemia occurred early in exercising than in resting
subjects. The explanation put forward for decreased levels of triglyceride after exercise is that working muscles directly utilise triglycerides for energy production.

**CHYLMICRONS**

Chylomicrons are large lipoprotein particle containing dietary triglyceride and cholesterol. They are vehicle of lipid transport in exogenous pathway. There chylomicrons are secreted into the intestinal lymph and pass into the general circulation for transport to the capillaries of adipose tissue and skeletal muscle where they are acted upon by lipoprotein lipase liberating free fatty acids and monoglyceride. The remaining particle deprived of triglyceride is termed as chylomicron remnant which is rich in cholesteryl ester. This remnant travels to the liver, where it is taken up by chylomicron remnant receptor and metabolised.

Different fats give rise to specific type of chylomicrons. Triolein gives large distinctive spherical chylomicrons while those seen after tristearin are creamy and vary in shape and are comparatively small.
Chylomicrons are rapidly cleared from the plasma and normally are not present after an overnight fast. The detection of these particles in fasting plasma is always abnormal and may indicate presence of other hyper lipemias.

The simplest method to detect chylomicron in post prandial state is "creaming in the cold". Increased levels of chylomicron in the plasma may be found in cases of genetic defect involving the enzyme, Lipoprotein lipase and in familial form of hypertriglyceridemia.

ATHEROSCLEROSIS : A POST PRANDIAL PHENOMENON

The possibility of atherosclerosis being a post prandial phenomenon was first proposed by Zilversmit in 1973. He hypothesised that chylomicron remnant or Beta VLDL may occur transiently as minor components of the human plasma fractions after diet high in fat and cholesterol is consumed. And this may cause repeated cholesterol deposition in cells in the arterial wall over the years, while the fasting cholesterol level may remain normal during the lifetime.
If atherogenesis is a post prandial phenomenon then premature CAD must be common in hyperchylomicronemic states. However in familial lipoprotein lipase deficiency enormous quantities of chylomicrons accumulate in plasma, but accelerated atherosclerosis has not been reported (Fredrickson D.S. et al., 1978).

**ATHEROSCLEROSIS AND LIPID LIPOPROTEIN LEVELS**

(1) **Total serum cholesterol (STC)**

Elevated STC is a risk factor for coronary heart disease. At the level of 220 mg/dl the incidence of coronary artery disease (CAD) is nearly two fold as compared to level of 180/dl (Kamnet et al., 1971). Similarly patient with proved coronary heart (CAD) disease have significantly higher cholesterol concentration then patient without CAD (Cohn et al., 1977).

**TRIGLYCERIDES**

Several studies have shown that an elevation of plasma triglycerides is common in patients with CAD (Albrink et al., 1959, Halley S.B. et al., 1980). Carlson and Bottiger in 1972 reported that rates of CAD rose linearly with increasing plasma triglycerides. However, there is currently great debate
as to whether VLDL is direct operative factor in producing CAD, or it is the association of increased LDL or decreased HDL level which are causative (Bilheimer 1972).

**LDL CHOLESTEROL**

LDL-c which constitutes about 75 percent of the total serum cholesterol is more specifically associated with CAD 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 atherosclerotic lesions in coronary arteries in large vessel (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 atherosclerosis (the lipid research clinics coronary primary prevention trial results, 1984).

**HDL CHOLESTEROL**

HDL level 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 eighth times
greater than total cholesterol (Cordon et al., 1977).
Each 10 ml/dl change in HDL cholesterol concentration is associated with 50% alteration in cardiovascular risk (Bresnik et al., 1984).

Subclasses of HDL can be fractioned by zonal ultra centrifugation and include HDL₂ and HDL₃. Among these subgroup HDL₂ appears to have the strongest inverse relationship with CAD 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.

**FAT TOLERANCE TEST AND ITS IMPLICATIONS**

The concept of fat/cholesterol tolerance test is not entirely new. In 1907 Neumann, after giving a fat load studied the quantitative lipid changes in form of chylomicron count after a fat load.
Introduction of isotopes, revolutionised the study of lipid metabolism. Brinkowitz in 1963 pointed out that radioactive fat tolerance is a better index for determining the functional state of lipid metabolism.

Zilversmit et al., 1979 brought forward the view that atherosclerosis may be a post prandial phenomenon with chylomicron and VLDL remnants of post prandial phase contributing to the development of atherosclerosis. This concept again aroused interest in determination of post prandial changes in lipid fraction often a meal rich in fat and cholesterol.

Subsequent work by Hanno Krauss et al., 1987 did not revealed any significant changes in serum total cholesterol often a heavy fat cholesterol lead, but found significant difference in triglyceride levels.

Arora R.C. et al., in 1987 put forward the concept of triglyceride tolerance test which showed significant difference in peak levels of STG in normal healthy, patient of IHD and that of diabetes.

Diet prior to the loading test meal, may be decisive. Under metabolic ward conditions, significant difference in fat tolerance has been reported in healthy subjects on an isocaloric diet, when the
daily fat intake per kg of body weight was varied from 0.1 to 2 gm. (Harvel, 1957). The lowest intake gives the highest fat tolerance. In contrast to the above, no change in the fat tolerance has been noted when the fat content of the diet was raised from 40 to 54% for three weeks (Horlick, 1957).

In an interpopulation study, no difference has been reported in the fat tolerance of three different communities who consume 17,45 and 60% respectively of their total calories as fat (Bouchier and Bronte-Steward 1961).

Composition of the test meal also plays an important role. In human beings, glucose one hour and half an hour before as well as one and a half hour after a fat meal reduced or even eliminated the serum triglyceride rise (Albrink and Man 1956). Glucose addition to 131 I-labelled triolein caused a flatter triglyceride curve as compared to ingestion of the latter only (Berkowitz et al., 1959). The depression of free fatty acid (FFA) and serum triglyceride levels following increased glucose utilisation is thought to result from a decrease in the mobilisation of fatty acids from the fat depot of the body (Gordon 1957).
There is also evidence that an increase in hepatic fat synthesis may be important in the reduction of the serum FFA levels (Shoemaker et al., 1960).

Long term studies of the effect of dietary protein on lipid level indicate that low protein intake is accompanied by a depression of serum lipids (Olson et al., 1957).

In 1962 James F. Sullivan demonstrated that increasing the relative content of protein in a meal results in higher levels of serum triglycerides in the post prandial period.

In 1957 Richard J. Havel demonstrated fall in cholesterol level 4 hours after taking high fat diet in two male subjects.

**FACTORS MODIFYING FAT TOLERANCE**

(a) *Age*

Fat tolerance and age have shown difference responses. Chylomicron count has been shown to rise more after a fat load in subjects more than 50 years as compared to the younger group (Becker et al., 1949, 50) similar results were found in turbidity measurements
(Marder et al., 1952, Schwartz et al., 1952). Using the same chylomicron counting principles, exactly opposite finding have been observed, and significantly lower chylomicron count in response to fat loading in older subjects, over 50 years as compared to younger subjects has been seen (Crummer and Hilden, 1953).

In a more illustrative work by Herzstein et al., 1953, it was observed that the total fats persisted longer in serum after fat loading in older subjects.

(b) **Body weight**

No significant correlation between body weight and the duration of lipemia in response to fat meal has been seen (Barritt, 1956). The fat tolerance rose appreciably after weight reduction was enforced.

(c) **Exercise**

It has been observed that at rest the lipid level of normal subjects increased by 42% after 3 hours of fat meal and the maximum was attained after 4 hours, while at work these figures were 34% at 3 hours (Nissen, 1931). Higher chylomicron counts
after fat loading in person at rest than in persons at work have been seen (Marder et al., 1952).

(d) Smoking

In habitual smoker, response to a fat meal indicated a lower post prandial rise in serum fat than to non smokers (Konttinon and Rajasalmi 1965). One cigarette per hour caused the chylomicron count to rise in a group of young subjects but not in two elderly subjects (Marder et al., 1952).

REPRODUCIBILITY OF FAT TOLERANCE

By large, fat tolerance curve is reproducible over a period of six month with very little variation (Norten, 1950, Osmon et al., 1957). However Bronte Stewart and Blackburn 1958 found considerable variability in response to the same fat load. Although those who exhibited a "high curve" continued to do so and vice versa.

HYPERLIPIDEMIA AND DIABETES

Hyperlipidemia is a relatively common problem in patients with poorly controlled diabetes Mellitus associated with abnormal lipid metabolism, diabetes tend to have higher incidence of hypertension, obesity and 2-3 fold increase in cardio vascular morbidity
and mortality when compared with nondiabetics (Kannel W.H. et al., 1979). Many factors appear to contribute to this enhanced, atheromatous process in diabetes, including alteration in platelet function, clothing factors, arterial smooth muscle cell metabolism and possibly blood pressure regulation (Ganda, O.P. et al., 1980). Nevertheless, changes in plasma lipoprotein levels in diabetes remain of the most important associated risk factors in term of accelerated atherosclerosis (Santen, R.J. et al., 1972). In addition, diabetics may have altered lipoprotein structure and metabolism independent of increase in plasma lipid levels (Eckel R.H. et al., 1981, Howard, B.V. et al., 1978, Schonfeld, G. et al., 1974) and these altered lipoprotein may be associated with accelerated atherosclerosis. It is generally appreciated that anatomic evidence of accelerated atherosclerosis frequently develops in insulin dependent diabetic patient, 10-15 years after onset of diabetes.

**Abnormal Lipoprotein Metabolism in Diabetic Patient**

Hypertriglyceridemia is the most common lipid abnormality observed in diabetic patients. This is usually caused by accumulation of very low density
lipoprotein (VLDL) and rarely, chylomicrons in plasma (Nikkila, E.A., 1973). Hypercholesterolemia may also develop secondary to increased VLDL levels, though changes in plasma low density lipoprotein (LDL) and high density lipoprotein (HDL) levels also occur with variable degrees of diabetic control. Various mechanisms have been reported to account for abnormal lipid metabolism, and this appears to depend on both the type of diabetes, and degree of insulin deficiency. Removal of triglyceride from plasma into adipose tissue requires two major processes.

(1) Hydrolysis of triglyceride to FFA catalysed by lipoprotein lipase.

(2) Esterification of the fatty acids in the adipocyte with alpha-glycerophosphate derived from glucose.

Activity of lipoprotein lipase depends on amount of insulin present in the circulation. Its activity is inhibited by catecholamines, adrenocorticotropic hormone, glucagon, and thyroid stimulating hormone (Robinson and Wing, 1970). Thus in insulin deficient diabetics activity of lipoprotein lipase is considerably decreased resulting into elevated
level of triglyceride.

GENETIC FACTORS OF CORONARY HEART DISEASE (CHD)

The genetic aspects of CHD have been extensively evaluated. Familial clustering of 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 and by common environmental conditions encountered by family members (Deutscher et al., 1970; Epstein, 1964; Rissanen and Nikkila, 1977; Thomas, 1959; Thomas, 1956; Goldstein, 1973).

Thus, Epstein estimated that almost two thirds of familial aggregation of CHD may be accounted for by familial trends in blood pressure and cholesterol levels. Similarly Rissanen and Nikkila observed that familial trends of CHD could in part be explained by familial elevated serum lipids, hypertension and diabetes Mellitus. Slocck and Evans, 1966 pointed out that members of the same family tended to share common environmental conditions such as diet, smoking and sedentary habits. Deutscher et al., suggested that numerous factors may play a role in familial CHD which are basically determined by genetic influences.
and then altered by environment. Thus, the concept of multifactorial genetic and environmental interrelationship of risk factors can explain familial aggregation of CHD (Johnson et al., 1965, Epstein, 1964). Further evaluation of genetic aspects of CHD has been derived from twin studies. Cederlof et al. (1967) noted a concordance rate of CHD of 21.7 percent in monozygotic twins, compared to 6.1 percent dyzygotic twins. Similarly Verschuer (1958) observed a concordance rate of 19% and 8.5% respectively in monozygotic and dyzygotic twins. However such finding as suggested by twin studies may be explained by genetic attributes of risk factors which may be influenced by common environmental experiences. Not only is heredity though to be the most important risk factor of cardiovascular disease but also it may be the easiest and least expensive way to detect in children. James Nora (1980) has reported that, making no additional assumptions about environmental and other genetic factors. The single highest risk factor of cardiovascular disease in children is first degree relative with myocardial infarction before age 55 years. This conclusion is supported by a study of a random sample of 320 pedigrees in Utah, demonstrating that 80% of the men with coronary occlusions under 55 years of age clustered in 16% of
families. Several other studies have reported that risk factors of cardiovascular disease show parent-child clustering (Boulton, 1979; Glush, 1978; Marrison et al., 1980). These studies suggest that one of the factors that may explain the relationship between premature heart disease in parents and offspring, may be elevated level of serum lipids.

LIPOPROTEINS: PATHOGENIC ROLE

Low density lipoproteins (LDL) and intermediate density lipoprotein (IDL) enter the arterial intima from plasma in man at rates directly related to their plasma concentration (Neithaus et al., 1977; Nicoll et al., 1981) and accumulate particularly in region already atheromatous. Endothelial injury greatly enhances this process. The cholesterol of atheromatous lesions is principally derived from plasma (Zilversmit, 1968). The interactions of LDL with cells of atheromatous plaques have been studied in some detail. Smooth muscle cells and fibroblast 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 those receptors but acquire lipoprotein cholesterol by other processes,
including receptor mediated uptake of altered LDL. In contact with cultured endothelial cell, LDL is modified, permitting macrophages to degrade it (Henriksen et al. 1981). Normally equilibrium is maintained in influx and efflux of cholesterol from the cell. Transport of cholesterol from peripheral cells to the liver may be mediated by HDL, soon after Miller and Miller 1975 advanced the concept that this function of HDL by favouring mobilisation of cholesterol from arterial wall, might explain the inverse relationship between HDL and risk of coronary heart disease.

**THE LIPOPROTEINS : PREDICTORS OF CHD**

The association of lipoproteins with coronary heart disease has been studied in depth in epidemiological studies. These associations are strong, predictive and independent of other risk factors.

Concentration 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). 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; Goldbourt and Medalie, 1979) but not in all studies (Wiklund et al. 1980). The relation being inverse, but unlike LDL, HDL cholesterol concentration do not correlate inversely with mortality rates from coronary heart disease in different countries.

Hyperlipidemia usually runs in family, screening for hypercholesterolemia at age of 12 years, is fairly predictive of adult hypercholesterolemia close to 50% of the top quintile (36%) for cholesterol, were similarly placed at followup, nine years later, of interest was the observation that those who dropped out of the top quintile at followup had a lower incidence of obesity, smoked less and were more active (Orchard et al. 1983).

In childhood HDL contributes proportionately more to the total cholesterol concentration. In a survey of 6775 school children a substantial proportion of those with hypercholesterolemia were attributable to high HDL cholesterol levels (Morrison et al. 1979). The ratio of total cholesterol 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 ratio exceeding this are 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 incidence countries (Gordon et al. 1982).