CHAPTER II

REVIEW OF LITERATURE - I

LIPID METABOLISM AND DIABETES MELLITUS

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Lipid metabolism is of fundamental importance for the clinicians practicing in various fields of medicine. Foremost are the diabetologists and cardiologists who have long recognized that hyperlipidaemia is a common finding in diabetes mellitus and cardiovascular disease. Biochemical studies on parameters of lipid metabolism have revealed strong relationship between diabetes and cardiovascular diseases. However, exact mechanism of the occurrence of these disorders still remains obscure.

Dietary derived triglycerides may be directly incorporated into adipose tissue or may also be acted upon by the liver prior to adipose tissue storage. In the liver, the lipid may undergo lipolysis and reesterification or it may merely undergo some modification of lipoprotein structures not involving extensive lipolysis.

Plasma free fatty acids (FFA) are rapidly taken up by the liver. In the fasting state, between 30 and 50 percent of the circulating FFA may be removed. In muscle, FFA are readily utilized for energy, whereas liver converts most of the FFA to triglycerides and phospholipids.

Phospholipids are added to liver tissue when chylomicrons and lipoproteins are removed from the blood by liver. Associated with lipoproteins are cholesterol esters and free cholesterol. Liver regulates plasma cholesterol levels and governs bile acid production from cholesterol.

Triglycerides are synthesized by liver. The precursors for triglyceride synthesis are fatty acids and glucose. FFA in blood mainly come from adipose tissue lipolysis of circulating triglycerides. Triglycerides of endogenous origin are circulated mainly as very low density (prebeta) lipoproteins (VLDL). These particles are smaller than chylomicrons, being 300-800 A^{o} in diameter and have a smaller proportion of their total composition as triglyceride. This varies from about 70 percent in the larger VLDL to 50 percent in the smaller particles. The remainder of the lipoproteins consist of phospholipids

(about 20 percent), cholesterol (about 15 percent) and protein (about 10 percent). Physiology of triglyceride metabolism is extensively reviewed by Bagdade and Bierman (1970) and Robert Stout (1975).

Cholesterol synthesized by the liver and the cholesterol in the chylomicron removed by the liver enter into a single ("anabolic") hepatic pool which supplies the free cholesterol for synthesis of plasma lipoproteins. The cholesterol derived from catabolism of plasma lipoproteins is transported back into another ("anabolic") pool in the liver from which biliary cholesterol and bile acids are derived. It is suggested that the cholesterol derived from the catabolism of plasma lipoproteins is not recycled for synthesis of new lipoprotein and that the dietary and other hepatic cholesterol derived from intestines is first quantitatively incorporated into plasma lipoprotein before it is seen in the biliary cholesterol or bile acids.

Control of cholesterol synthesis has been excellently reviewed by Bortz (1973). Arterial and body cholesterol has its origin in both exogenous and endogenous sources. Several studies have indicated however, that the bulk of arterial cholesterol in the natural diseased state

is of endogenous origin (Zilversmit, D.B. 1957 and Chobanian et al 1962). Accordingly then, knowledge of the mechanism of cholesterol formation is of profound importance. The biosynthesis of cholesterol from its precursor acetyl CoA involves 26 steps (Popjak and Cornforth 1960; Frantz and Shroepfer 1967).

$$18 \text{ Acetyl CoA} \longrightarrow \text{Cholesterol} + 9\ CO_2$$

The fourth step, the enzymatic reduction of beta-hydroxy-betamethyl-glutaryl CoA (HMG CoA) to mevalonic acid, is an irreversible reaction (Rudney 1963) that leads nearly exclusively to cholesterol. It is at this step that one seeks and finds the prime locus of metabolic control for the biosynthesis of cholesterol. Work of Nepokroeff et al (1974) indicates that the relative concentrations of insulin, glucagon and glucocorticoids are important in the regulation of the diurnal variation of rat liver B-hydroxy-B-methyl-glutaryl CoA reductase activity. Except adult brain all body tissues are capable of synthesizing cholesterol, though liver and small intestine have been considered as the prime loci of the process (Dietschy and Wilson 1968). Utilizing several chemical balance and isotopic kinetic techniques, the

denovo human cholesterol synthesis has been estimated to be around 1 gm/day (Wilson and Lindsey 1965; Grundy and Ahrens 1969 and Nestel et al 1969). The inhibitory effect of cholesterol on its own production is highly specific. Tomkins (1953) and Siperstein (1960) showed that cholesterol feeding had no effect on the conversion of acetate to CO_2 or to fatty acids. This is particularly so in the liver (Hotta and Chaikoff, 1955; Dietschy and Wilson 1970). Siperstein and Guest (1960) were the first to show that the effect of dietary cholesterol was mediated at the HMG CoA reductase step. The early pathways of acetyl CoA seemed to be unaffected and the conversion of mevalonate to cholesterol was unhampered. It is suggested that there is a prompt mobilization of tissue cholesterol when plasma concentration of cholesterol is reduced (Sodhi and Kudchodkar 1973).

There is lack of agreements concerning the effect of experimental diabetes on cholesterol formation (Haff and Miller 1958; Clarenburg and Chaikoff 1966). Nicotinic acid has been known to inhibit cholesterol formation. Hyperthyroidism, growth hormone, ACTH, adrenaline, fat feeding, stress, nephrosis and several other factors increase cholesterologenesis. Cholesterol is essential

for human body as it is an integral part of the cellular membrane and serves as a significant participant in structure of plasma lipoprotein. It also acts as a precursor for the bile acids.

Phospholipids :

Phospholipids are highly important structural components of the plasma lipoproteins although their precise contribution is undefined (Scanu et al 1970). Role of phospholipids in activation of leukocyte lysosomal enzymes (Hawiser et al 1972), biologic calcification (Wuthier 1973), neuronal excitation (Cook et al 1972), cell fusion (Ahkong et al 1973 and Lucy 1970) and the regulation of the synthesis of RNA through an effect on RNA polymerase (Menon 1972) has been defined in recent past. Role of phospholipids in transport of lipid in choline deficiency liver is worth noting (Lombardi and Cler 1967; Cler and Lombardi 1970). Jackson and Gotto (1974) have critically reviewed the role of phospholipids in biology and medicine.

Phospholipids and vascular disease :

Tissue and plasma phospholipids are suspected to be involved in thrombosis and atherosclerosis. In vitro, phospholipids activate the aggregation of platelets and may

thus be involved in the platelet phase of hemostasis (Marcus et al 1972). The concentration of platelet phospholipids may be increased in certain clinical conditions, but without recognized importance in the pathogenesis of vascular disease. Platelet phosphotidylserine is increased in juvenile diabetes (Nordoy and Rodset 1970) and platelet phosphotidylethanolamine is elevated in patients with occlusive peripheral arterial disease (Kunz and Stummvoll 1971). Atherosclerosis is associated with an accumulation of phospholipid, cholesterol and cholesterol ester within the arterial plaque. Arterial tissue is capable of synthesizing phospholipids insitu (St.Clair et al 1969; Zilversmit 1970). The phospholipids from atherosclerotic lesions contain a greater proportion of saturated fatty acids than phospholipids do from the plasma lipoprotein (Portman et al 1967; Homma et al 1972). Unlike the phospholipids, the cholesterol and sterol portions of cholesteryl ester that accumulates in arterial plaques are derived from the plasma, presumably from circulating lipoproteins (Portman and Alexander 1966). Unesterified cholesterol accumulates faster within the arterial wall than esterified cholesterol. During the development of atheromatos plaque, relative proportions of sphingomyelin

increases whereas relative content of phosphotydylo-choline and phosphotydylethanolamine decreases. The accumulation of arterial sphingomyelin may be accelerated in squirrel monkey if the animals are placed on an atherogenic diet. The content of sphingomyelin is particularly increased in microsomal membranes (Portman et al 1969) and in the cell membrane of smooth muscles. Jackson and Gotto (1974) have proposed a hypothesis that there is an initial net increase in the arterial concentration of phospholipids that are rich in saturated fatty acids. The accumulation of cholesterol would then occur as a secondary phenomenon.

It is no longer rational to diagnose and treat disorders of lipid transport by measurement of serum cholesterol and triglycerides alone. Lipoprotein measurement in serum is essential. Serum lipoprotein abnormality is a common finding in diabetes and atherosclerosis. Lipoproteins have been classified by Fredrickson, Levy and Lees (1967). This classification is based on electrophoresis together with chemical estimation of cholesterol and triglycerides and a recent WHO Memorandum (WHO, 1970) has further modified this. Stone et al (1971) have also classified hyperlipoproteinaemia

based on results with membrane filtration, nephelometry and cholesterol estimation. Typing of lipoprotein patterns is very useful in the diagnosis and comparative studies of different populations suffering from genetic lipid disorders.

Hyperlipoproteinaemia can be detected by general appearance of plasma level of triglycerides and cholesterol in serum and/or lipoprotein electrophoresis on paper, agarosegel, polyacrylamide gel or cellulose acetate. The lipoprotein type numbered according to the system of Fredrickson, Levy and Lees (1967) is shown below.

Lipoprotein Phenotyping

Present nomenclature	Fredrickson lipoprotein type	Plasma cholesterol	Concentration of Glycerides	L.D.L. protein	Presumptive kinetic effect
Familial induced lipemia	I	Normal or high	Very high	Low	Impaired Chylomicrone removal
Familial hypercholesterolaemia	II (IIa, b)	High	Normal or high	High	Impaired beta lipoprotein removal
Broadbeta disease	III	High	High	Normal	Faulty conversion of prebeta to beta lipoprotein
Combined hyperlipaemia	II, IIb or IV	Normal or high	Normal or high	Unknown	Unknown
Familial hyperglyceridaemia	IV	Normal	High	Normal	Impaired prebeta lipoprotein removal
Mixed hyperlipaemia	V	High	High	Normal	Impaired removal of chylomicrons and prebeta lipoprotein

Hyperlipidaemia :

Recent studies have shown that transport of lipoprotein from plasma to skin occurs frequently in various disorders of lipid transport. Eruptive xanthoma appear on elbows, buttocks and knees of patients with hyperchylomicronaemia, xanthelasma indicates abnormality in cholesterol rich β-lipoprotein which deposits in coronary vessels and tendons. In broad beta disease, planter xanthoma appears on palms and soles and tuberous xanthoma appears on elbows and knees. Very rarely, when lipids are markedly elevated lipemia retinitis may be observed.

Types of hyperlipoproteinaemia :

Type I : This is exogenous hyperlipaemia characterized by the presence of chylomicrons in plasma 14 to 16 hours after the last meal of a normal diet. Fasting plasma samples are creamy. Cholesterol is elevated and triglyceride concentration may be 10 gm percent or even higher. This is very rare type (Fredrickson and Lees 1966). In most cases, it has been thought to be familial and inherited. There is absence or deficiency in postheparin lipolytic activity (PHLA) (Fredrickson et al 1963). Eruptive xanthomata often develop. Hepatosplenomegaly, lipemia retinalis, abdominal pain and occasionally pancreatitis may also develop.

Type II : This type is relatively common and is often associated with advanced atherosclerosis. It is characterized by increased plasma concentrations of beta-lipoproteins. There is marked hypercholesterolaemia and clear plasma in type IIa whereas there is slight elevation of triglycerides apart from that of cholesterol in patients with type IIb hyperlipaemia. Clinical symptoms present tendon and tuberous xanthomata, corneal arcus and accelerated atherosclerosis. Presence of hypothyroidism, obstructive liver disease, and nephrotic syndrome is ruled out.

Type III : This type is relatively uncommon. Serum lipoprotein electrophoresis shows broad beta band (trailing from beta to prebeta region). Cholesterol and triglyceride levels are highly variable. Abnormal glucose tolerance, hyperuraemia and xanthomata are found. Presence of hepatic disease, dysglobulinaemia or uncontrolled diabetes is likely.

Type IV : This is most common pattern which reflects an imbalance in the synthesis and clearance of endogenous glycerides. Type IV is associated with elevated concentration of plasma triglycerides and normal or elevated concentration of plasma cholesterol. Clinical picture reveals accelerated vascular disease, and

abnormal glucose tolerance. The pattern may be secondary to nephrotic syndrome, diabetes mellitus, pancreatitis and glycogen storage.

Type V : Type V hyperlipidaemia is associated with the presence in plasma of both exogenous and endogenous glycerides associated with increased concentrations of cholesterol. Plasma is often creamy. Electrophoresis shows chylomicrons and prebeta band. Insulin dependent diabetics and alcohol edicts are likely to get this kind of hyperlipidaemia.

Diabetes mellitus :

As the knowledge regarding diabetes is advancing it has become increasingly difficult to define this disorder in its perfect sense. Alexander Marble (1971) defines it as "a chronic, hereditary disease characterized by an abnormally high level of glucose in the blood and excretion of that sugar in the urine. The basic defect is an absolute or relative lack of insulin which leads to abnormalities of metabolism, not only of carbohydrate but also of protein and fat".

The disease is characterized by polyuria, excessive thirst and progressive loss of weight. Poor control of diabetes may lead to ketosis, acidosis, coma and death.

History of diabetes can be traced back to about 1500 B.C., when Chinese, Indians and Egyptians described the disease in their medical literature. Sushruta Samhita and Charak Samhita - the two well known medical classics (between A.D. 100 to 500) have noted 'sweet urine' and named the disease as 'madhumeha'. The name diabetes was introduced by Arexalus in the first century of the Christian era. Diabetic gangrene was first reported by Avicenna around 980 to 1037 A.D.

Thomas Willis (1675) found that the urine of diabetic patient was sweet. Dobson in 1775 showed that this sweetness was due to sugar. Rollo (just prior to 1800) was the first one to prescribe low carbohydrate, high protein and high fat diet for a diabetic.

Period between 1796 to 1850 was the era during which treatment of diabetes mellitus was suggested by Prout and later by Arebius. Chevreul in 1815 showed that sugar present in the urine of diabetic patients was identical to glucose. In 1841 Trommer reported a qualitative test for sugar in urine.

In 1869, Langerhans discovered the islets of pancreas which were later given his name. In 1889, Vonmering and Minkovski produced diabetes in dogs by

pancreatectomy. In 1922, Banting and Best extracted insulin from the cells of pancreas which when injected to a diabetic patient gave remarkable relief. The introduction of oral hypoglycaemic drugs in 1955 was another landmark in the treatment of diabetes. In the last two decades, interest in diabetes and its complications in relation to drug therapy has been greatly aroused.

Diabetes mellitus can be either of genetic origin or it may occur due to some nonhereditary factors. The hereditary type of diabetes which is the most common one can be classified into (a) growth onset type and (b) maturity onset type. The growth onset or juvenile type appears characteristically in childhood. Patients having this type of diabetes are prone to ketoacidosis and they are dependent on insulin all the time. In contrast, maturity onset type of diabetes is quite stable and can be controlled by diet or oral hypoglycaemic agents. Onset gradually occurs after the age of 40 years. In general, these patients are not ketosis prone and appear to retain substantial capacity for the production of insulin. The nonhereditary type of diabetes occurs due to damage or removal of pancreatic tissue and disorders of endocrine glands other than the pancreas.

Earliest stage of diabetes is a prediabetes stage which starts from the time of conception. Prediabetics have a strong hereditary predisposition for diabetes. Their glucose tolerance curve is within normal limits but insulin secretion in response to glucose stimulation expressed as serum insulin/blood glucose relationship is slightly abnormal (Conn and Fajans 1961). In recent years, wide variety of suitably controlled studies have been carried out to trace the development of the diabetic state even earlier to the emergence of detectable diabetes. Preliminary studies on prediabetes in Elliot P. Joslin research laboratory showed increased density and a fibrillar structure of vascular tissue and local thickening of the basement membrane of glomerular capillaries in biopsy tissues obtained from two nondiabetic sisters whose parents were diabetic (Camerini Davalos et al 1963). Soeldner et al (1968) found diminished secretory response of insulin in prediabetes. Williams and Gleason (1968) carried out graded prolonged glucose infusions in five prediabetic and seven normal persons, all nonobese males of 15 to 40 years. Prediabetics showed significant hyperglycaemia with diminished insulin response during

glucose infusion as well as a delay in achieving peak insulin levels. Pyke and Taylor (1967) showed a low insulin response to oral glucose loading in twin siblings of diabetic patients. Colwell and Lein (1967) also found similar results with prediabetic subjects. Cerasi and Luft (1967) reported sluggish and delayed plasma insulin response to glucose infusion in five prediabetic adult monozygotic twins with normal glucose tolerance. These workers have shown over the last eight years that the development of diabetics may be regarded as two step process. Prediabetes may be considered as an inherited condition which forms the prerequisite for the development of diabetes and is characterized by normal glucose tolerance and decreased insulin secretion on glucose stimulation (Kipnis 1968 and Luft 1968). These same individuals may exhibit normal insulin secretory responses to other secretagogues such as tolbutamide (Boden et al 1968). In some prediabetics the regulation of the hepatic glucose output resembles that of diabetics. In some, the lipolytic response to physical exercise is increased almost to the same extent as is found in diabetes. It is suggested that the genetically determined defect in diabetes mellitus involves the glucose-receptor mechanism. It also involves the inability to

perceive a glucose signal which accounts for the hypoinsulinemic response observed in prediabetic subjects during the gradual deterioration of insulin output.

Diabetes mellitus is controlled by diet alone, oral hypoglycaemic drugs or insulin depending upon the type and severity of the disease.

Epidemiology of diabetes mellitus :

Indian population :

Prevalence of diabetes varies in different segments of Indian population. Patel et al (1966) from Bombay, Rao et al (1966) from Hyderabad and Ahuja et al (1966) from Delhi observed the prevalence to be 2.43%, 4.12% and 26.4% (based on glycosuria alone) respectively. Bajaj et al (1975) have reported a high prevalence rate amongst selected communities from northern India.

No exact figure is available on total number of diabetics in India though five year long term study is being conducted by Gupta and others in different parts of India. During the years 1967-68, in a door to door survey 40,000 persons belonging to 8,002 families were screened to test for glycosuria, 1½ to

2 hours after the main meal of the day and a total number of 25,273 postprandial urine samples were received and 870 persons were positive for the test. Of these, oral glucose tolerance test (OGTT) was performed on 586 diabetics. In 1976, the test was repeated in 141 cases. Among the retested, 30 out of 56 florid ones had improved and 26 had deteriorated in their OGTT curves (Permeshvera 1977). Regarding incidence of diabetes in young people no proper statistics is available in this country. Gupta et al (1964) has reported an incidence of 1% for juvenile diabetes amongst diabetics in Ahmedabad and Tulloch (1962) has reported an incidence of 2.7% for juvenile diabetes in whole of the tropics. At the J.J.Group of Hospitals, Raheja and Talwalkar (1966) found 191 cases of young diabetics out of 1,867 diabetics attending the clinic. Vaishnava et al (1964) reported that 67% of their South Indian diabetic patients under the age of 20 years were females. However, in India, incidence of diabetes is probably more in males than in females (Modi et al 1959; Vaishnava et al 1964 and Tripathy and Kar 1965).

Other countries :

Latest figures indicating prevalence rates of

diabetes in other countries have been quoted by Jarret (1977). It is difficult to compare the prevalence rates of diabetes between populations due to differing methodology and interpretations. Very few diabetes detection compaigns have examined truly representative samples of the population.

Great Britain :

Physicians' panel studies of recent years show an estimate of atleast 5 per 1000.

In a Bedford Survey (British study) of a random sample (546 people), full standard glucose tolerance test was used as the detection method. In the Birmingham Survey of 345 people was similarly tested. These surveys were conducted by the Royal College of General Practitioners in 1963. Both the surveys showed 1.3% prevalence of diabetes on basis of glycosuria testing and 6.8% on basis of blood sugar testing. National Survey of Health and Development in Britain estimated prevalence of diabetes at the age of 20, about 2:10:0, which is similar to that from Switzerland and East Germany (Wodsworth and Jarrett 1974).

America :

In a National Survey by the Department of Health Education and Welfare in 1960-62, 7710 people were studied. Blood sugar was examined one hour after 50 gms oral glucose load. The number of people with high blood glucose increased with age and women had higher glucose level than men at all ages. According to statistics, in July 1970, the estimated diabetic population in U.S.A. was 3,000,000.

Pima has the highest known prevalence of diabetes 50% over the age at 30. (Bannett et al 1971). By contrast, the Athabaskan Indians (Mouratoff et al 1969) and the racially dissimilar Eskimos (Mouratoff et al 1967) in Alaska - both have a very low prevalence of clinically evident diabetes. West and Kalbfleisch (1970) have provided the only data for the prevalence of diabetes in those aged 35 and over in Bangla Desh, Malaya, Uruguay, Venezuela and Central America. Two groups in U.S.A. - the cherokee Indians and the inhabitants of Bangor Pennsylvania were also compared. The comparative prevalence rates were 2.0 percent in Bangla Desh, 3.3 percent in Malaya, 4.1 percent in Central America, 6.9 percent in Uruguay, 7.0 percent

in Venezuela, 17 percent in Bangor, Pennsylvania and 25 percent in the cherokee Indians. This tenfold variation was related to obesity.

Canada :

A conservative estimate is that there are probably 100,000 known diabetics and only a slightly less number of persons with undetected diabetes (Marks et al 1971).

France :

A 1953 estimate shows roughly 10 diabetics per 1000 population.

Sweden :

Recent studies suggest a current rate of 10 per 1000.

Denmark :

Studies made a decade ago, have reported nearly 23,000 diabetics (4 to 5/1000).

Israel :

From the data presently available on diabetics in Israel probably number atleast 20,000 or 10 per 1000 of the population. Cohen et al (1970)

in Israel estimated the prevalence of known diabetes mellitus in the population aged 2-16 years at about 1:6000.

Diabetes can be suspected in the nondiabetic uniovular twin of a diabetic, a child both of whose parents are diabetic, a child one of whose parents is diabetic and the nondiabetic parent with family history of diabetes and the mother of a big baby (alive or still born or having hyperplasia of islet cells of the pancreas in the still born child excluding Rh incompatibility).

In the present state of our knowledge, the earliest recognisable abnormal characteristic of the diabetic state is the diminished ability to utilize the carbohydrate load. There are many laboratory tests useful in determining the presence of diabetes (a) presence of glycosuria (b) OGTT (c) Intravenous glucose tolerance test (IVGTT) (d) Intravenous tolbutamide test (IVTT) (e) Cortisone glucose tolerance test (CGTT) and other more sophisticated procedures. A normal person is able to restore the blood sugar to fasting level or less in 1½ hours, after the ingestion of the standard dose of glucose, the most valuable point being 2 hours when the value should be below 120 mg%.

Experimental diabetes :

Total or subtotal pancreatectomy has been long used as a routine procedure for the production of experimental diabetes. There are more convenient methods in which alloxan, dehydroascorbic acid, quinoline derivatives, streptozotocin and other substances which cause diabetes are used. Alloxan reacts strongly with sulfhydryl groups. Quinoline derivatives exert their diabetogenic action through irreversible interaction with zinc which is present in considerable amounts in the beta cells of many species. Streptozotocin induces permanent diabetes in rats, monkeys and dogs with very low general toxicity. Many drugs including diuretics and antihypertensives can cause temporary diabetes. Hormones such as growth hormone can also cause diabetes. Nath and Chakrabarti (1950) have shown that daily injection of acetoacetate in gradually increasing doses could bring about a condition of progressive glucose in-tolerance in rabbits kept on horse gram diet (Nath and Chakrabarti 1950).

Spontaneous diabetes in animals :

Spontaneous diabetes has long been recognised in a wide variety of domestic animals and the small

laboratory animals - specially rodents. The larger number of observations is available for cats and dogs. Rapid increase of incidence in dogs is after eight years. The pancreas of such animals shows small and scarce islets having signs of hydropic degeneration (Gepts and Toussaint 1967). In the Chinese hamsters, diabetes may occur at a young age or in adulthood. The appearance of the acidoketotic catabolic type of diabetic syndrome is often preceded by nonketotic hyperglycaemia as well as glycosuria. Moreover, vascular lesions possibly comparable to those occurring in man, have been observed to occur spontaneously (Brook Lodge Workshop 1967). The mode of inheritance of the syndrome is unclear. In yellow obese mice, moderate hyperglycaemia is associated with insulin resistance. Another type of diabetes mellitus has been described in desert rats. As long as these animals are kept on their natural diet, they remain normal. When kept on concentrated laboratory diets, some develop fatal ketotic diabetes mellitus (Hackel et al 1966). Similar syndrome occurs in spiny mice (Acomys Cahirinus). Diabetes is also found in certain laboratory bred animals. Many features of the diabetic

syndromes observed in animals resemble some characteristics of different types of diabetes in man. This explains the interest taken in the study of these animals in recent years.

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