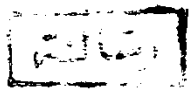
LIPOPROTEIN AND FREE FATTY ACIDS IN DIABETIC PATIENTS AFTER I.V CHALLENGE OF GLIBENCLAMIDE AND TOLBUTAMIDE

THESIS

Submitted for Partial Fulfilment for
the Degree of Master of Medicine

By
Fatma Sherif Mahmoud
M. B., B. Ch.



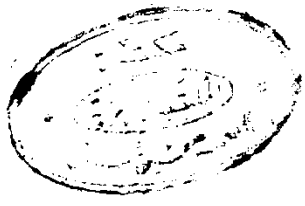
Supervised by

Prof. Dr. Ahmed Ghareeb
Chairman of General Medicine
and Endocrine Unit
Ain Shams University

Prof. Dr. M. Sabry Sallam
Prof of Clinical Pathology
Ain Shams University

Dr. Hussein El-Damassy
Ass. Prof. of Int. Medicine
Ain Shams University

Dr. Sayed Raafat
Ass. Prof. of Int. Med.
Ain Shams University



Faculty of Medicine
Ain Shams University

1982

ACKNOWLEDGEMENT

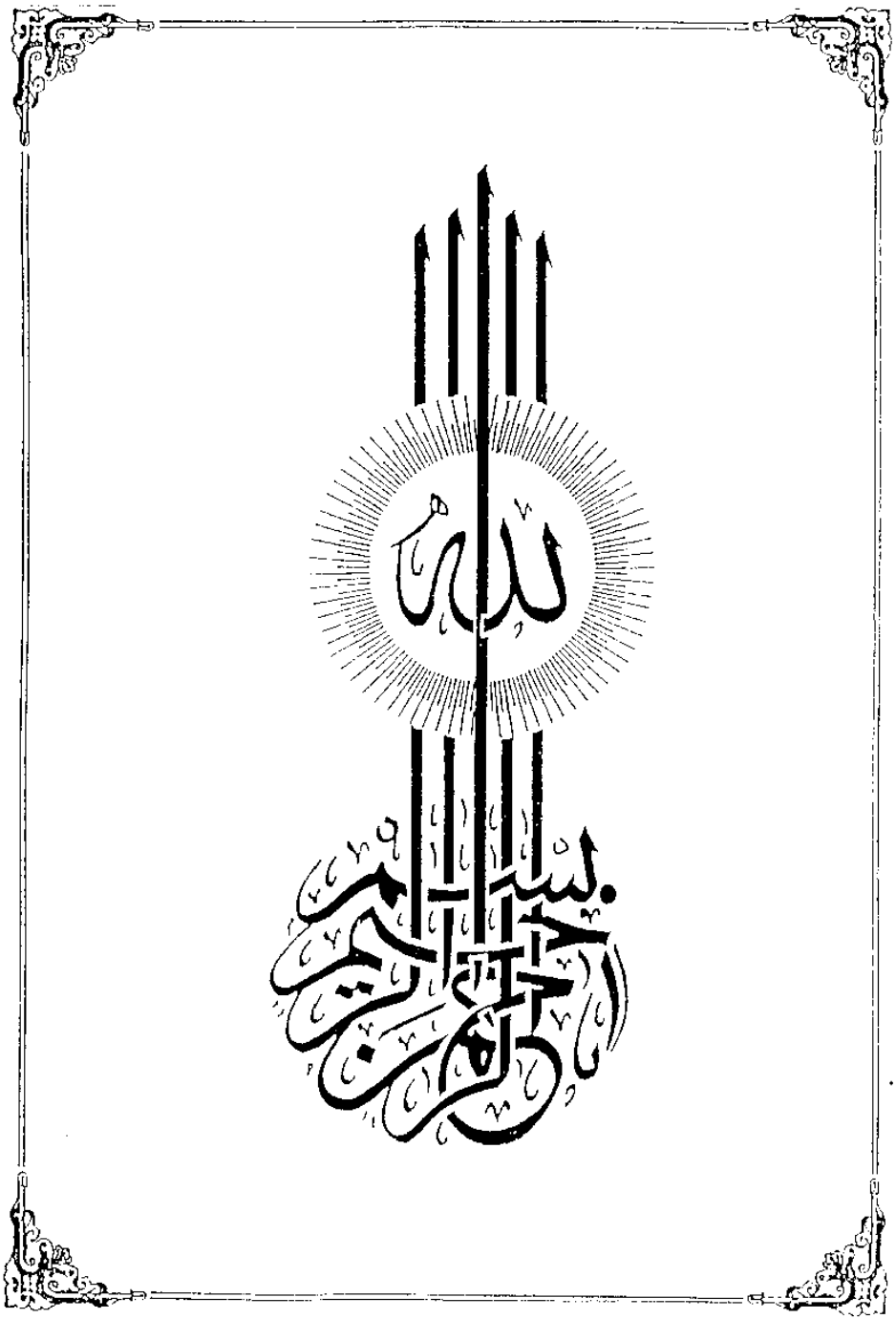
I wish to express my gratitude and appreciation to Pfofessor Dr. Ahmed Ghareeb, Professor of Medicine and Chairman of Endocrine Unit, for his consistent supervision, valuable suggestion as well as for reading and criticizing the manscript, and above all for his moral support and fatherly attitude, without his help the whole work would not have been possible.

I am also so grateful to Professor Dr. Sabry Sallam, Professor of Clinical Pathology for his trustful help.

I would like to offer a special gratitude to Dr. Hussein El-Damasy and Dr. Sayed Raafat for their keen interest and suprevision. I am so grateful to sincerity in effort and time afforded by them.

I would like to pay a special gratitude to Dr. Moghazi Ali Mahgoub for his valuable advice, guidance, kindness, and unfailing advice, and also Dr. Mohamed Amin Fikry for his generous help in accomplishing the statistical side of this work, and also Dr. Refky Fares.

I am also so grateful to sincerity in effort and time offered by the biochemists Azza and Mona Khedr, Lila Aziz and Magdy Abbas in excuting the practical part.



CONTENTS

AIM OF WORK

Atherosclerosis in the diabetic population tends to occur at an ealier age and with greater severity than in the non diabetic population. Hyperlipidemia and lipoprotein abnormalities play the major role in atherogenesis. In diabetes mellitus many authors report abnormalities of the lipid pattern.

Our aim is to study the lipoprotein pattern and free fatty acids in diabetic patients controlled either with insulin or oral sulfonylureas, and in uncontrolled diabetic patients, and compare both groups with healthy control. Then we will study the effect of insulin, tolbutamide and glibenclamide in a single intravenous bolus on blood glucose, serum cholesterol, serun triqly ceride, serum free fatty acids and lipoprotein pattern.

This is to clarify the most effective means of treating glucose intolerance and normalizing lipid dearangement to prevent atherosclerotic complications in diabetes.

REVIEW OF LITERATURE

INTRODUCTION

The relationship between diabetes mellitus and lipo proteins is of considerable interest, patients with diabetes have greater incidence of coronary artery disease than non diabetic population of similar age (Joseph & Guster.,1979). There are certain risk factors, such as hypertension, Cigarette smoking, and hyperlipidemia, that appear to play a role in the pathogenesis of atherosclerosis, these same factors are operative in diabetes (Kirk et al., 1980).

The major causes of morbidity and mortality in patients with diabetes mellitus are related to diseases of microcirculation and macrocirculation. Microcirculatory changes are most commonly found in the capillaries of the eyes, the Kidney, and in other areas of the body. The disease of the large sized and medium-sized arteries is athersclerosis , which appears to be indistinguishable from that observed in non diabetic patients (Strandness et al., 1964). However the disease appears at an earlier age in diabetic,is more extensive, and is associated with a higher morbidity and mortality (Santen et al., 1972).

Of the total lipids in human blood total

cholesterol forms about 33 percent and the phospholipids about 35 percent the remainder is neutral fat. In normal Egyptians the mean total lipids have been found to be 412.2 mg % ± 10.9 (Raafat, 1975).

Regarding the lipid and lipo-protein fractions in normal Egyptians, the mean total plasma cholesterol was 175.3 mg% ; and the lipoprotein pattern was alpha fraction 120.1 mg %, Beta fraction 212.5 mg % and the non mobile fraction 86.5 mg %. The Beta / alpha lipoprotein ratio was 1.8 .

In Diabetic Egyptians, there was marked derangement of the lipids and lipoprotein pattern.

The total serum lipids have been found to be 472.1 mg %, and total plasma cholesterol 218.6 mg % . Of the lipo-protein fractions, alpha forms 93.1 mg %, Beta. 275.9 mg %, and non mobile fraction 102.9 mg% The Beta / alpha ratio amount to 3.25 (Raafat, 1973).

Lipoprotein Metabolism in Diabetes Mellitus:

Diabetic patients who demonstrate premature athero sclerosis not infrequently have hyperlipidemia. Its prevelance is variable, depending on the type and severity of diabetes, glycemic control, nutritional status, age and other factors. (Ganda, 1980).

Epidemiologic data indicate that individual lipoproteins, or combination of lipids and lipo-proteins, are better predictors of risk for coronary artery disease than are the levels of total plasma lipids alone. Thus, low density lipoprotein cholesterol (LDL-chol) is a strongly positive risk factor, particulary at younger ages ($<$60yl); and high density lipoprotein cholesterol (HDL-chol) is an equally strong, though negative, risk factor at all ages (Kannel & Megee.,1979).

Cholesterol Metabolism :

In a statistical sense, the most frequent lipid abnormality in the diabetic is hypertriglyceridemia, with or without an associated increase in plasma total cholesterol (Simpson et al., 1979).

The work of Goldstein and Brown in cultured fibroblasts, lymphocytes, and other models established

the presence of a spesific LDL receptor pathway. Their work indicates that LDL - cell interaction includes binding of LDL at a spesific cell surface receptor site, followed by its entrance and lysosomal degradation . The free cholesteral thus released intracellularly ; (a) leads to suppression of a key enzyme, hydroxy methyl glutaryl (HMG) - COA-reductase, thus suppressing intracellular cholesterol synthesis, and (b) stimulates cholesterol erterification by activating the acyl COA : cholesterol acyl transferase (ACAT) system. These events lead to inhibition of LDL receptor when a critical concentration of intracellular cholesterol is available. If the LDL production exceeds its clearance via the receptor pathway, the consequent increased LDL concentrations in plasma facilitates its uptake in reticuloendo thelial system as well as arterial wall (Goldstein & Brown.,1977). In preliminary studies, no defects in LDL receptor binding have been found in diabetes perse (Chait et al., 1979). Insulin may also have important effects on HUG-COA reductase, the rate limiting enzyme in LDL metabolism. However, divergent results have been noted in the few studies done thus far. (Saudek & Ender.,1979).

High-Density Lipoprotein and Diabetes:

Accumulating evidence indicates a role for HDL in the prevention of cholesterol deposition in peripheral tissues . The mechanisms underlying the protective effect of HDL against atherosclerosis, and is the subject of intensive investigation, is presently unclear (Miller 1978) and (Tall & Small.,1978).

According to one attractive view the nacent HDL is secreted by the liver as a discoidal particle, containing mostly phospholipides. In the blood or in the periphery, the discoidal HDL picks up free cholesteral from the cell membranes and, perhaps, from remnant particles and IDL (generated by degradation of chylomicrous and VLDL). The lecithin cholesterol acyl transferase (LCAT) system, intimately active in this process, generates,cholesteral esters in the HDL from these lipids and converts them into a spherical shape. Each of these cholesterol ester- enriched particles, HDL and remnant, may then be recognized by hepatocyte receptors, leading to uptake, degradation and finally, billiary excretion of cholesteral (Tall & Small.,1978) and (Hamilton , 1978) . A preferential utilization of free cholesterol from high density lipo-proteins for

biliary cholesterol secretion has been demonstrated in msn (Schwartz et al., 1978).

The interaction of HDL and its subtypes (HDL_2. HDL_3) with the LDL receptor are poorly understood ((Daerr et al,, 1979). However, in cultured human fibroblasts, HDL some how reduces the binding of LDL, thereby reducing the entrance and degradation of LDL (Miller et al., 1977). In cultured human arterial cells, a competition of HDL with LDL - binding sites has also been shown. (Carew et al., 1976). Recent studies revealed that high cholesterol intake in man results in a qualitatively abnormal HDL particle (HDL_c or HDL_1), which is enriched with Apo E and like LDL, is atherogenic (Mahley et al., 1978).

Epidemiologic studies strongly suggested an inverse correlation of HDL- cholesterol level with the development of ischemic heart disease (Rhoads et al., 1976) and(Castelli et al ., 1977) .

Other investigators have shown a similar protective effect of HDL, as reflected by direct measurement of the plasma concentrations of apoprotein A-I and A-II

(Avogaro et al., 1979). Most of these studies revealed the inverse relation of HDL and atherosclerosis to be independent of other lipid abnormalities.

Several reports indicate an over all reduction of HDL-cholesterol levels in diabetic patients .(Gordon et al., 1977). One important finding is that diabetic Women, in general , have a greater lowering of HDL-cholesterol levels than do diabetic men when compared with non diabetic controls (Ganda et al., 1979 and Beach et al., 1979). This may, partly explain the relative increase in coronary heart disease in diabetic women relative to that of men as observed in Framingham and other studies (Bradley 1971 and Gordon et al,1977) . Another important observation is that the lowered HDL-cholesterol levels are particularly confined to non-insulin depend diabetics (Ganda et al., 1979) and (Stanton,1978). In some studies, insulin - dependent diabetics had significantly higher levels than the non diabetic controls of either sex (Nikkila,,1978). These observations, can be at least partly explained by the known inverse correlation of HDL cholesterol with adiposity and triglyaride levels (Glueck et al., 1976 and Schaeffer et al., 1978), and by a positive

correlation with lipoprotein lipase activity. (Nikkila , 1978) . The non-insulin dependent diabetic patients, whether on diet or on oral hypoylycemic agents, are more likely to be obese and hypertriglyceridemic . Yet the heterogenicity of the diabetic populations studies as well as differences in state of diabetic control have led to much confusion and controversy. However, several trends are emerging. One is that insulin - dificient diabetics or poorly controlled insulin - requiring diabetics have low HDL-cholesterol levels (Nikkila , 1978) , Nikkila 1973 and Bennion & Grundy ,1977), and in large part the HDL- chol returns toward normal with institution of insulin and improvement of diabetic control (Calvert et al., 1978).

In some reports the HDL- chol correlates with such indexes of diabetic control as glycosylated hemoglobin concentrations or plasma glucose levels. (Lopes & Virella1977 and Calvert et al., 1978) . Such patients have a highly significant correlation between HDL chol post heparin LPL, and adipose tissue LPL, and the initially subnormal levels of HDL- chol and adipose tissue LPL rise in response to insulin administration (Nikkila , 1978). In fact, several

different groups have reported recently that well controlled insulin - requiring diabetics have elevated HDL- chol levels. (Durrington., 1978) and Calvert et al., 1978). This may represent another argument for good diabetic control.

On the other hand, maturity - onset diabetes decreases HDL - cholesterol levels (lopes -virella et al., 197/ and Nikkila,1978), but only if plasma triglyceride levels and /or plasma glucose levels are elevated. In tnese subjects, there is no correlation between HDL-cholesterol and indexes of diabetic control (Nikkila., 1978 and Calvert et al., 1978). Of great concern are the reports tnat diabetics taking sulfonylureas have lower levels of HDL - chol than diabetics controlled by other means (Calvert et al., 1978).

Triglyceride Metabolism in Diabetes :

Insulin plays a critical role in the production and clearance of triglycerides in the diabetics (Brunzell & Bierman.,1978 and Nikkila et al., 1977). In severe