REGULATION AND ATTENUATION OF HYPERLIPIDEMIA IN RATS FED ON HIGH FAT DIET

THESIS

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By

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This thesis has not been submitted

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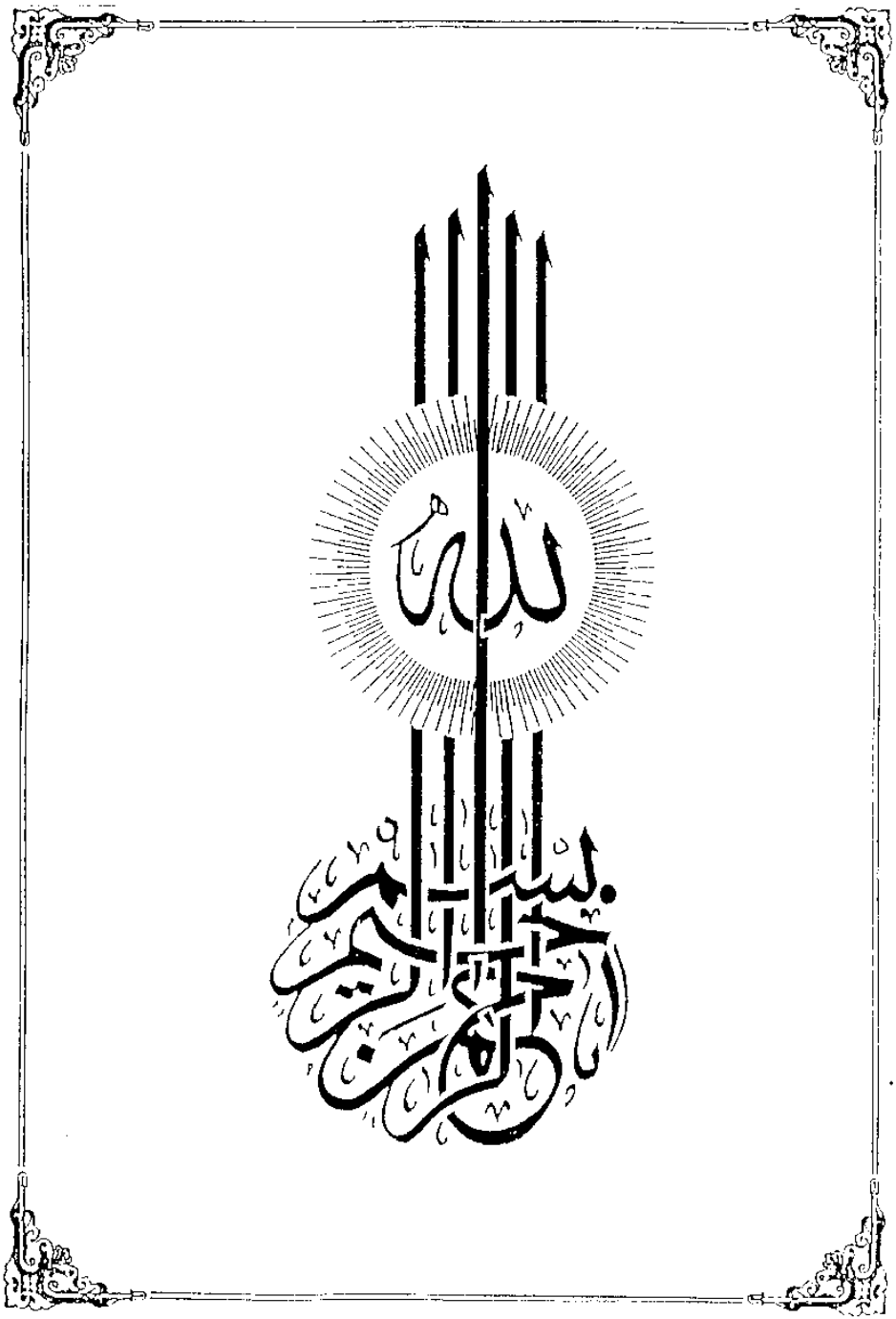

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ABBREVIATIONS USED

A	absorbanse
AR	analyitical grade
apo A	apo protein A
apo B	apo protein B
apo C	apo protein C
C	concentration
^{o}C	degree centigrade
Ca	calculated
ch	cholesterol
CHD	chronic heart desease
CPIB	α-chlorophenoxy isobytyryl ethyl ester
DG	diglycerides
FFA	free fatty acid
FH	Familial hyperlipoproteinemia
GABA	α-amino butyric acid
GAB	α-amino butyrate decarboxylase
GOT	glutamate-oxalsacetate transferase
GPT	glutamate-pyruvate transferase
hr	hour
HDL	high density lipoprotein
HDL-ch	high density lipoprotein cholesterol
HLP	hyperlipoproteinemia
HMG-CoA	3-hydroxy-3-methylglutryl-CoA reduction
i.m	intramuscular
IDL	intermediate density lipoprotein
LCAT	lecithine cholesterol acetyltransferase
LDL	low density lipoprotein
LDL-ch	low density lipoprotein-cholesterol
LDL-pL	low density lipoprotein-phospholipids
LPL	lipoprotein lipase
PL	pyridoxal
P.L	phospholipid
PLP	pyridoxal-5-phosphate
PM	pyridoxamine
PN	pyridoxine
S	serum
SE	standard error
TG	triglyceride
TL	total lipid
TLC	thin layer chromatograph
u RAC	uranylacetate
VLDL	very low density lipoprotein

CONTENTS

AIM OF WORK

There is a good correlation between atherosclerosis and the incidence of coronary heart disease (CHD) . Lowering the risk of CHD could be achieved by controlling the fat intake . However , in many cases treatment of hyperlipoproteinemic patient using suitable drugs is a must. Among drugs that in common use one could mentioned x-p-chlorophenoxy isoputyrate family such as clofibrate , finofibrate , fibric acid and gemofibrazil . All these drugs are now in use and they may exert undesirable side effects which sometimes are so sever that necessiate discontinuation of the theraby .

Some vitamins proved to exert potent effects on lowering the level of plasma lipids . The hypolipidemic property of nicotinic acid , ascorbic acid , and vitamin B12 have been throughly investigated by several workers.

The theme of this work is to regulate hyperlipidemia using non conventional approach . To achieve this goal rats were fed high fat-cholesterol diet containing massive dose of vitamin B6. Different doses of PLP were given to substantiate the effect of vitamin B6 already present in the diet .

Also , the effect of massive doses of vitamin B6 and PLP on the hypolipidemic property of clofibrate was studied aswell.The results are a break through in this area and are encouraging to pursue this finding .

I.A-1. The plasma lipids and lipoproteins:- Nature and distribution .

In the normal fasting human the major lipid fraction of plasma are cholesterol, cholesterolester, phospholipids, triglycerides, in addition to a small fraction of free fatty acids (which accounts for less than 5% of the total fatty acid present in the plasma). Since lipids are hydrophobic material (Hanahan, 1960), the problem presented is of transporting a large quantity in an aqueous environment (blood plasma) from the different tissues. Olson et al (1960) demonstrated that the more insoluble hydrophobic lipids were associated with more polar ones such as phospholipids, these complexes then combined with cholesterol and protein to form hydrophilic lipoprotein complexes.

Lipoprotein complexes differ in their densities, those differences have been exploited to achieve their separation by sequential ultracentrifugation into four main classes :- chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) (Lindgren et al, 1972), (Fig. 1)

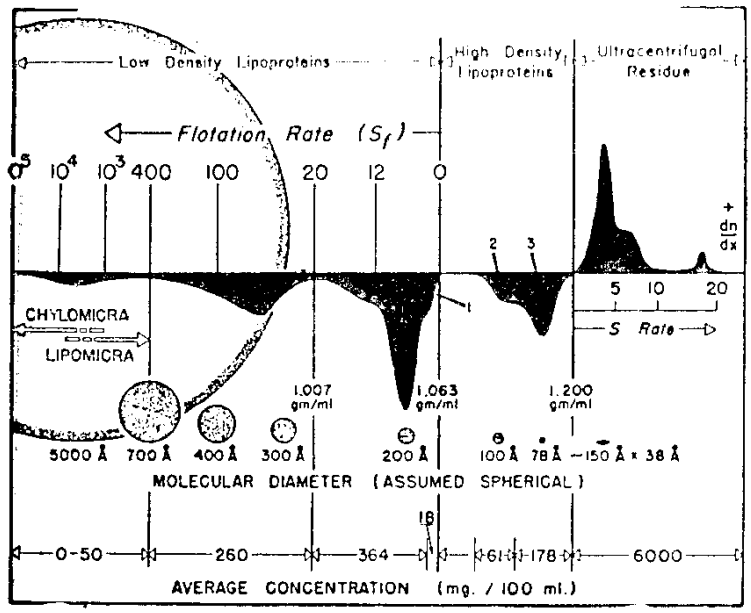

Fig. 1: ultracentrifugal composition of lipoprotein classes of human serum.

Lee et al (1976), and Brunzell et al (1978) reported that in human plasma, the LDL class separates in the density range (1.019-1.063 g/ml) and is characterized by its β-mobility on agarose gel electrophoresis and by the presence of apo B . In rats, however, when the lipoprotein fraction that separates in this same density range was further analyzed by rate zonal centrifugation, it was found to comprise besides apoprotein B- containing LDL at least one additional species that is enriched in a second apoprotein. Weisgraber et al (1977) designated this lipoprotein as HDL_1, because, like the other lipoproteins of the HDL group but unlike LDL, it had ∝ mobility by electrophoresis.

The composition of the different human lipoprotein classes is given in the following Table (Harper, 1979)

Table 1: Composition of lipoproteinsin human plasma

Fraction	Source	Protein (%)	Total Lipid(%)	Composition Percentages of total lipid Triacyl glycerol	Phospho lipid	Choles terol Ester	Choles terol (Free)	Free Fatty Acids
Chylomicrones	Intestine	1-2	93-99	88	8	3	1	
Very low density Lipoproteins (VLDL)	Liver and intestine	7-10	90-93	56	20	15	8	1
Low density Lipoprotein LDL 1 or IDL	VLDL chylo-micrones	11	89	29	26	34	9	1
LDL2		21	79	13	28	48	10	1
High density lipoproteins HDL1*	Liver ; ?intensine							
HDL2		33	67	16	43	31	10	
HDL3		57	43	13	64	29	6	6
Albumin-FFA	Adipose tissue	99	1	0	0	0	0	100

IDL,intermediate density lipoprotein, FFA, free fatty acid .
* This fraction is quantitatively insignificant.

Both human and animal plasma lipoproteins had been isolated and their densities were determined by the preperative ultracentrifugation.

Gherardi et al (1980) reported that human plasma lipoproteins were separated at the following densities, VLDL (d <1.006 g/ml), LDL (1.006-1.063 g/ml), HDL (1.063-1.210 g/ml). On the other hand, however, Calandra et al (1981),

and Tarugi et al (1982) reported that rat and rabbit lipoproteins were isolated at the following densities: VLDL (d< 1.006 g/ml), LDL (1.006-1.050 g/ml), HDL_1 (1.050-1.090 g/ml) and HDL_2 (1.090-1.210 g.ml).

Cohn et al (1984) by the use of preparative ultracentrifugation demonstrated that rat serum could be fractionated into the following lipoprotein density classes: VLDL (d < 1.006 g/ml), intermediate density lipoproteins (IDL; 1.006 < d < 1.030 g/ml), LDL (1.030 < d < 1.063 g/ml), and HDL (1.063 < d < 1.210 g/ml). Lipoproteins could be charactarized by the presence of one or more proteins or polypeptides which are known as an apolipoproteins or apoproteins. The two major lipoproteins of the HDL are designated A-I, and A-II. The main apoprotein of LDL is apoprotein B which is found also in VLDL, and chylomicrons. Apoproteins C-I, C-II, and C-III are smaller polypeptides found in VLDL, HDL, and chylomicrons, (Fredrickson, 1973) (Fig. 2).

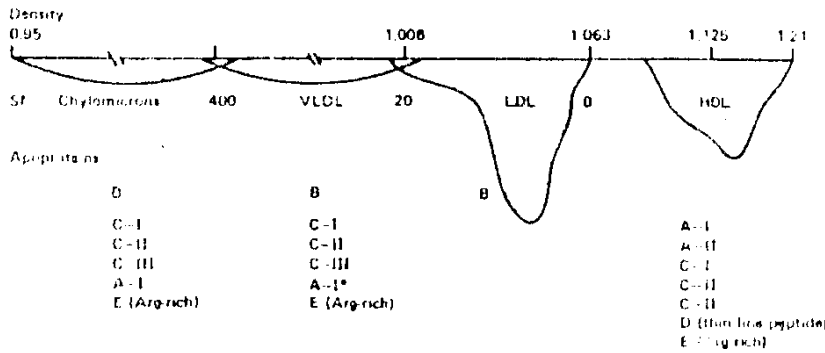

Fig.2:The 4 major plasma lipoprotein families depicted as Schlieren, patterns obtained in the analytical ultrcentrifuge. *Found only in intestinal VLDL (Modified from Fredrickson,1972-1973)

Apoprotein B generally recognized to be the characteristic apoprotein of the LDL, while apoprotein A-1, widely viewed as

a marker apoprotein for the HDL group of lipoproteins (Swany et al 1977).

Zannis (1980), Zannis et al (1982), and Calandra et al (1984) used the isoelectric focusing on SDS-PAGE technique to separate the apoprotein, and they concluded that rat apo A-1 consists of seven isoforms having the same molecular weight (27,000 daltons), rabbit apo A-1 contains five isoforms focusing in the 5.69-5.34 pH range, and human apo A-1 consists of five isoforms focusing in the pH range 5.91-5.0.

In addition to the use of technics depending on their density, lipoproteins may be separated according to their electrophoretic properties by paper or agarose electrophoresis at pH 8.6 (Fig. 3)[Scheig et al (1969)].

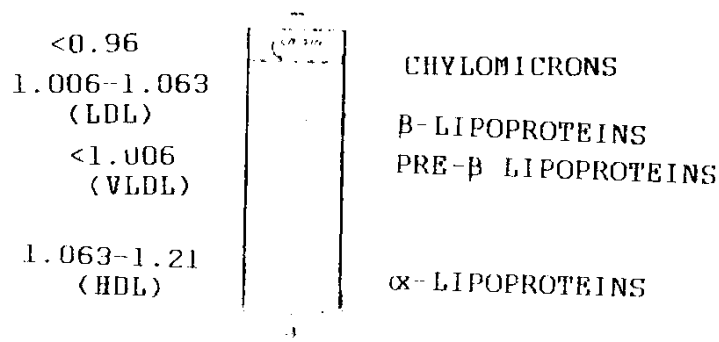

Fig.3: Separation of plasma lipoproteins by electrophoresis.

Considering the role of α and β lipoproteins, it has been reported that β-lipoproteins (LDL) differ from the α-lipoprotein (HDL) in transporting more of the total plasma

cholestrol, and in containing a higher concentration of both free and esterified cholesterol. It is probable that the abnormally high cholesterol : phospholipid ratio observed clinically in certain hyperlipidemic serum may be a reflection of elevated concentration of β-lipoproteins relative to that of the α-class which do not vary greatly in concentration . [Lipple et al, 1977; and Ahmed et al, 1979].

I.A-2. Hyperlipidemia and hyperlipoproteinemia (HLP)

In simplest terms, hyperlipidemia is defined as an elevation of plasma lipids. These lipids include cholesterol, cholesterol esters, phospholipids, and triglycerides. They are transported in plasma as a part of macromolecular complexes named lipoproteins. On the other hand, hyperlipoproteinemia means an elevation of one or more classes of plasma lipoprotein (Antonio et al, 1984).

Five pheno types of human hyperlipoproteinemia have been classified by Fredrickson (1967) each of which may be inherited or acquired (Table 2).

Table 2: Lipoprotein phenotype

Phenotype	Plasma Lipoprotein Present in excess
1	Chylomicrons
2a	LDL
2b	LDL + VLDL
3	*Beta VLDL
4	VLDL
5	Chylomicrons + VLDL

*Beta VLDL represents cholesterol rich VLDL remnants

In rats the problem is different,Mahley et al (1977) obeserved that the introduction of cholesterol in the commercial diet of the animals resulted in a marked shift in cholesterol from the HDL to the LDL. Nevertheless, Jaya et al (1981) studied alterations in the lipoproteins in cholesterol fed rats, and they reported that the increase in serum cholesterol in rats was mainly attributable to an elevation in VLDL cholesterol.

I.A-3- Hypolipoproteinemia

A number of conditions exist in which there is a deficiency in the amount of plasma lipoprotein and could be given in human after Fredrickson (1970) as follows:
(1) A-β lipoproteinemia, (2) Hypo-β lipoproteinemia, and (3) Hypolipoproteinemia (tinger disease).

I.A-4- Turnover of the Plasma lipoproteins

I.A-4-1 Chylomicrons and VLDL:

Chylomicrons are found in chyle which is transfered through the lymphatic system to the blood, whereas VLDL formation in the chyle is quantitatively less, and occurs even in the fasting state. The bulk of plasma VLDL in man and experimental animals is however of hepatic origin (Harper, 1983).

The clearance of labeled chylomicrons from the blood is rapid where the half-time of disappearance is of the order of minutes in small animals as rats (Havel et al 1970). On the other hand, chylomicrons and VLDL could be degraded by lipoprotein lipase enzyme which requires phospholipids, and apo C-II as cofactors for its activities that have been provided by chylomicrons and VLDL.Hydrolysis then takes place while the lipoproteins are attached to the enzyme on the endothelium. The triacylglycerol is hydrolyzed progressively through a diacylglycerol to a monoacylglycerol which is finally hydrolyzed by a separated monoglycerol hydrase. Reaction