Study of Total lipids, Trigly cerides and Cholesterol in normal Egytians

THESIS

BY

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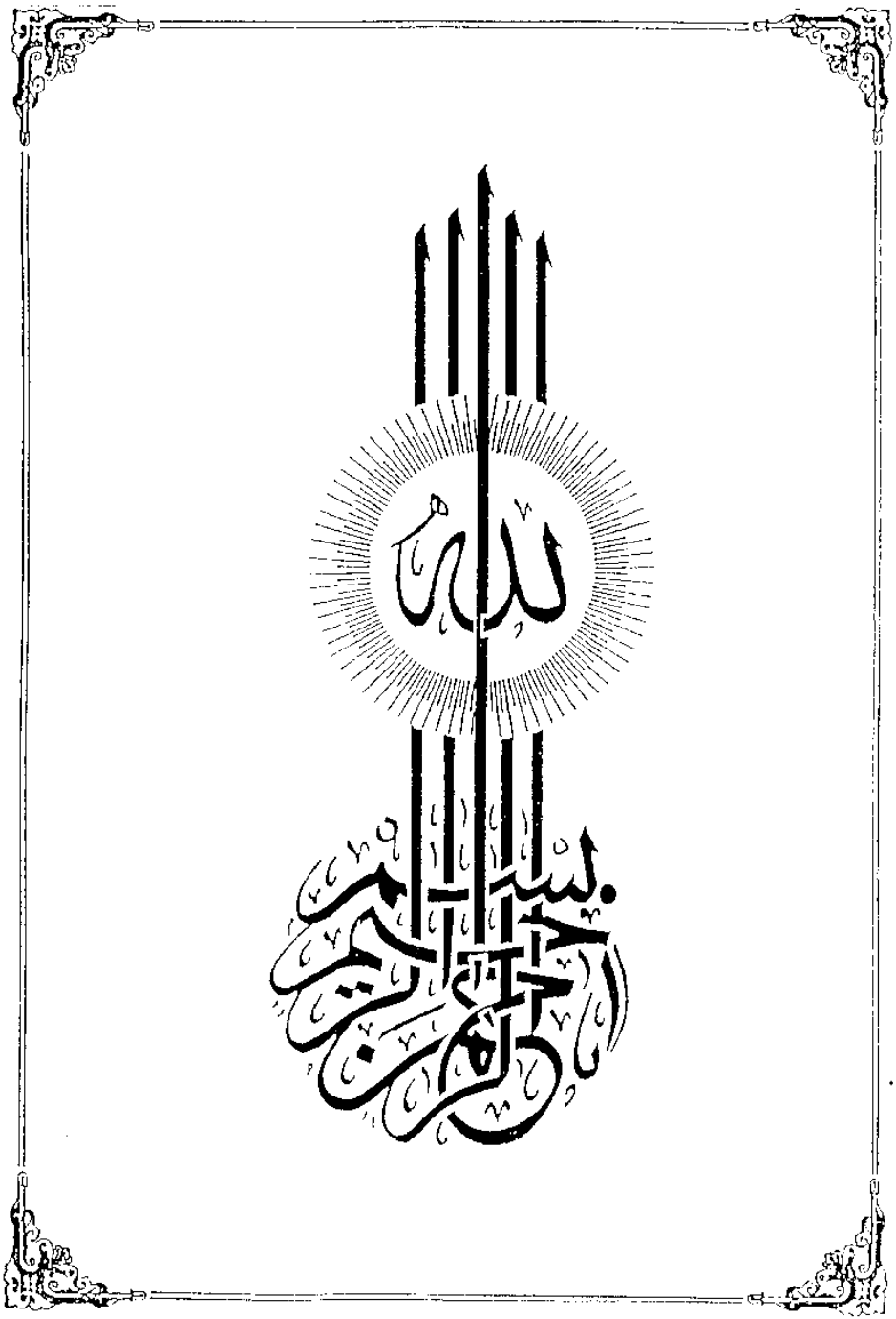

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Introduction

Lipids are a heterogenous group of compounds related either directly of potentially fo fatty acids. They are less dense than water, relatively insoluble in water and soluble in lipid solvents.

Bloor in 1937 classified lipids into:

1 - Simple lipids

- fats , esters of fatty acids with glycerol.
- waxes, esters of fatty acids with higher alcohol than glycerol.

11- Compound lipids

Esters of fatty acids containing groups in addition to an alcohol and fatty acid.

- phospholipids.
- glycolipids .
- lipoproteins.
- sulpholipids.
- aminolipids.

111- Derived lipids

Substances derived from the above groups by hydrolysis, but still possessing the general physical properties of lipids.

- saturated and unsaturated fatty acids.
- sterols and other steroids.

- glycerol.
- mono and diglycerides.
- Vitamins D and A.

In the human body, fat is in a continuous dynamic state serving as an efficient source of energy when stored in the adipose tissue (schoenheimer and Rittenberg, 1935). Fats has definite advantages as a source of energy over carbohydrates or proteins, its caloric value is higher and is associated with very little water in storage. Fats also serves as an insulating material in subcutaneous tissues and around vital organs (Henry and Winkelman, 1968) The clinical chemist is chiefly concerned with the lipids of serum or plasma.

Total serum lipids:

Lund and Godal 1961 made studies on serum lipids in healthy individuals and found that in men the total lipid content in serum averaged 567mg /100ml and in women 569mg / 100ml.

Total lipid content tended to increase with age in both sexes, reaching a maximum about the age of 60(Lund and Sivertssen, 1961).

Swahn, 1953 found an average serum total lipids of 805 mg/ 100ml for persons below the age of 40 and 943 mg/ 100 ml for those of more than 40 years with no sex difference.

Waris, 1958 found higher average values in men than in women (800mg/ 100ml and 690mg/ 100ml respectively) for individuals between the ages of 21 and 70 years.

The most widely used methods for the determination of total lipids in the blood serum have developed from the procedure of Bang (1918) who oxidized the lipids with acid chromate and measured the chromate consumed by iodometry. Under Bang's conditions, the oxidation was incomplete and an empirical factor had to be applied in calculating the results. Bloor, (1928) estimated total lipids by calculating the cholesterol concentration and theoretical factors for the oxidation of the cholesterol fatty acid mixture.

Bloor, (1947) described a method based on the measurement of the color change produced by the reducing action of fatty acid of cholesterol on a sulfuric acid-dichromate mixture. Recently; Bragdon, (1951), employing a modification of Bloor's method (1947) by applying the method to the unsaponified lipids of the serum.

Determinations of cholesterol and lipid phosphorus are also necessary for calculation of the results on the basis of oxidation factors.

The estimation of total lipids in this and other mod-
ications of the oxidation method is based on the
ssumption of molecular weights of the complex mixture
lipids which are present in the serum.

Kunkel, (1948) described the phenol turbidimetric
ethod for assay of total lipids.

Gravimetric procedures are the only direct quant-i-
ative techniques available for the determination of
otal lipids. Sperry, (1955) described a method
n which extraction is done by methanol-chloroform,
rification, evaporation to dryness, filtration,
ryness and weighing .

Colorimetric method based on the sulfo-phosphovanillin
eaction (Chabrol, 1961) is used in this work.

The lipid components of major interest are the trigl-
cerides and cholesterol.

Triglycerides:

Simple triglycerides are triesters of a fatty acid with glycerol, Mixed triglycerides contain more than one type of fatty acid per molecule.

Triglycerides circulate in the blood associated with proteins in the form of water soluble complexes termed lipoproteins (Searcy, 1962)

The appearance of a transient lipaemia after a high fat meal is due primarily to triglycerides in the form

of chylomicrons in the serum. (Henry, 1964). Recent attention has been focused on these triglyceride-rich fractions as possible indicators of atherosclerosis and other diseases resulting fom derangements in lipid metabolism. Hence determination of triglycerides is among the most frequently requested test in clinical chemistry.

As yet there is no general aggreement regarding the normal range for setum triglycerides. Fredrichson, (1967) had proposed a range of 10-190 mg/100ml be used depending on age, assuming the average american diet provides about 40% of its calories in the form of fat. Henry, (1968) suggested that each laboratory determine their own normal range. Attention should be given to the fact that certain measurement are influenced in clinically healthy individuals by diet, sex, age, diurual variation, physical activity, menstrual cycle, pregnancy and environmental factors (Searcy, 1969)

Carlson and Lindstedt, (1963) observed about 20% higher values for triglycerides in winter than in the end of summer.

The main approaches to the determination of triglycerides are : -

a - By difference

b - Determination of triflyceride glycerol

Determination of triglycerides by difference:

The total lipids, phospholipids, cholesterol, and cholesterol esters are determined gravimetrically then:

Triglycerides = Total lipids-phospholipids- cholesterol - cholesterol esters .

Determination of triglyceride glycerol :

The first step is extraction followed by removal of phospholipids, then hydrolysis of triglycerides to release glycerol which can be determined by chemical methods.

Cholesterol:

It is a steroid having the characteristic ring structure perhydro-cyclopentano phenanthrene carbon skeleton. It is the sterol which is almost exclusive to animals, including man, plants contain sterols, which are closely related to cholesterol but they cannot be converted to cholesterol by animals.

Cholesterol is present in all the cells, forming an integral part of the cell structure, the highest concentration being found in nervous tissue, liver and adrenal cortex.

There is ample evidence that tissue content of cholesterol can be influenced by many factors e.g nutrition, age and endocrine states (Lindholm, 1956) .

Age was found to be one of the important factors influencing cholesterol content of a given individual, increasing or decreasing according to the form, either total, ester or free and according to the tissue under consideration (Masoro , 1968).

The cholesterol in the human body is derived from two main sources, endogenous synthesis from acetyl Co - A and exogenous, with the diet from animal fat (Henry, 1964). Almost all the tissues are capable of synthesizing cholesterol, especially the liver, adrenal cortex, skin and intestine (Henry, 1964).

Cholesterol exists in various tissues and fluids of the human body in two main forms, either as free cholesterol e.g. large skeletal muscle mass, or in the esterified form e.g. liver, adrenal cortex, plasma and lymph.

The liver by possessing enzyme systems responsible for both hydrolysis and esterification of cholesterol esters, it plays an important role in the metabolism and regulation of plasma cholesterol ester level.

The role of free cholesterol as a precurser for the biosynthesis of steroid hormones is a well established fact.

In the plasma cholesterol is tranported as lipoprotein, the highest proportion being found in low density B lipoproteins.

Cholesterol assays:

Cholesterol, because of its medical importance has probably prompted the development of more methods for its measurement than other lipid fractions.

Extraction procedures (Kingsley, 1949) of serum with an organic solvent (e.g ethanol, ether, isopropanol, cholroform or acetone) with particular attention to temperature and proportion (solvents and serum) separates the lipid from proteins and denatures proteins (precipitation). A great deal of attention has been given to the selection of solvent systems that represent the initial step in many determinations; however several methods have been developed to by- pass this first step and substitute a direct reaction mixture.

The final step for spectrophotometric analysis most often requires a complete colorimetric reaction that is dependant on many variables, such as concentration of reactants (type and concentration of oxidizing agents) solvents, temperature, time and light (Henry, 1964).

The most popular, if not the most common is the Lieberman- Burchard color reaction (Lirberman, 1885- Burchard, 1890) in which a chloroform solution of cholesterol is admixed with concentrated sulfuric acid in acetic anhydride to produce a green colour with a maximum absorption peak at 620 um.

The method of Abell, (1958) has secured a firm position as a reference method and is practical as well as precise and accurate, it involves:

1 - Ethanolic alkali (KOH) to liberate the cholesterol from protein complexes (lipoproteins) and saponify the cholesterol esters.
2 - Extraction of the cholesterol into petroleum ether after an aqueous dilution of alcoholic solution.
3 - Measurment of the cholesterol in a portion of the petroleum ether layer after the Lieberman-Burchard reaction.

A direct colorimetric method using ferric chloride sulfuric acid reagent and glacial acetic acid solution of cholesterol has become increasingly popular in recent years (Zank, 1972) . It is a more sensitive reaction that yields a more intense and stable color than the Lie-

berman-Burchard color reaction, however it is also light sensitive.

Jung and Parekh (1971) have described a direct method employing ferric acetate-uranium acetate and sulfuric acid-ferrous sulfate reagents. The ferric acetate-uranium acetate is a unique precipitating agent that removes bilirubin with proteins, clears the serum of lipids and extracts cholesterol without need of solvents. When the acetate reagent extract is mixed with sulfuric acid-ferrous sulfate reagent, it produces a purple colour with a maximum absorbance at 560 nm, and maximum colour development within 15 minutes, it is stable for at least one hour.

Lind and Svirtssen, 1961 found average total cholesterol in men 240 mg/100 ml and in women 236 mg/100 ml, he found no significant sex differece in and age group.