TITLE PAGE

THE EFFECT OF PTEROCARPUS MILDBREADII SEED ON PLASMA HDL CHOLESTEROL OF ALBINO RAT

BY

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BC/2007/128

A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN BIOCHEMISTRY.

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CARITAS UNIVERSITY AMORJI-NIKE,
EMENE, ENUGU STATE.

AUGUST 2013

CERTIFICATION

This Research work has been approved as meeting the requirement for the award of Bachelor of Science (B.Sc) Degree in Biochemistry.

BY

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DEDICATION

This research work is dedicated to my brother Mr. Emmanuel Obi Onwuegbuke .C, Mr keneth onwuegbuke who supported me through the course of this work financially, morally and otherwise. I also want to thank my siblings for their love and care through out the course of this work.

ACKNOWLEDGEMENT

I want to thank God Almighty for his divine grace and strength during this project work. I also thank my brothers and siblings for their sincere support and encouragement during this research work.

I also express my profound gratitude to my Head of Department, Mr. Moses Ezenwali, my supervisor, Mr. P.O. Ugwudike and to all my lecturers and the entire Faculty of Natural Sciences.

To my friends Vin, Daniel, Ceasar, Chidimma, Ede Nnamdi Ede, Calister and my course mates that contributed in one way or the other for the success of this work also to my well-wishers.

I thank them immensely for the sacrifice they make in the course of this work.

Most importantly, my deepest and greatest thanks goes to Almighty God.

ABSRACT

This work was carried out to investigate the effects of pterocarpus mildbreadii seed on the HDL- level of albino rats.High density lipoprotein(HDL) is a fraction of cholesterol,which helps to transport cholesterol from peripheral of cell to the liver.High density lipoprotein is carried out to investigate cardio vascular diseases.Fresh oha seed (pterocarpus mildbreadii seed) was collected from Amokwe in udi local government Area Enugu state. They were dried at room temperature for about a month in an open laboratory space, groud into coarse form and weighed on an electrical weighing balance. 3% tween-80 was prepared by dissolving 3% tween -80 in 97ml of distilled water. The grounded pterocarpus mildbreadii was dissolve in the 3% tween-80.A total of sixteen wistar abino rats used for this analysis. The rats were divided into 4(four) groups. Group 1contain 4 rats and received 0.5 each of 3% tween-80. Group 2 contain 5 rats and were administered with 1400mg/kg b.w of pteridocarpus mildreadii solution Group 3 contain 3 rats and were administered with 2600mg/kg. b.w of pteridocarpus mildbreadii solution. Group 4 contain 4 rats and were administered with 500mg/kg. b.w. of Edmard MRT complex.

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CHAPTER ONE

INTRODUCTION

1.0 Pterocarpus mildbreadii are trees with edible parts successfully budded, pterocarpus species (P.Soyauxii and P. mildbeadii are reported to have different patterns of leaf flush, one spontanous and the other intermitted making the first suitable for commercial production and the second the home production (Okafor 1978). Pterocarpus mildbreadii being the major case study both the vegetable and the seeds.

The vegetable is majorly used in our various home for cooking. Pterocarpus mildbreadii (Oha seed) are not consumed by the people from the eastern part of Nigeria. So it cannot be majorly stated the content of this oha seed.

The demand for vegetable oils as a result of diminishing source of oils and fats creates the needs for new sources as well as exploiting sources that are currently unexploited in order to supplement the existing ones (Minzangi et al. 2011).

As industrialists continues to rely mostly on the popular vegetable oil like coconut oil, soyabeans oil, ground nut oil, palm oil etc. for the preparation and production of their various products.

Plant lipids also posses nutritive value and have an impact on human nutrition and the world economy.more than three quarters of the edible and industrial oils marketed annually are derived from seed and fruit triglycerol (Schimid & Ohlrogge, 2002). Seeds have nutritive and calorific value which makes them necessary in diets. They are good sources of edible oils and fats. The amount of energy provide by 1g of fat and oil when fully digested is more than twice as many joules of protein and carbohydrate do. Fats makes meals more satisfying, enrich its flavor and delay the unset of hunger (Odoemelan, 2005). Unfortunately, insufficient availability of animal protein and the costliness of available plant protein sources have also increased the need for research on revealing lesser known under-utilized legumes and oil seeds of nutritive value (Schimid & Ohlrogge, 2002).

CHAPTER TWO

LITERATURE REVIEW

2.0 The fats and oils used almost universally as stored form of energy in living organisms are derivatives of fatty acids (Nelson & Cox, 2005). The natural fat and oil are mixtures of glycerides and fatty acids belonging to a large group of water insoluble substances called lipids. Plants produced the majority of the world's lipids and most animals including humans depends on these lipids as a major source of calories and fatty acids plant lipid also have substantial impact on the world economy and human nutrition. More than three-quarters of the edible and industrial oils marketed annually are derived from seed and fruit triglycerols.

This figures are particularly impressive given that on a whole organism basis, plants store more carbon as carbohydrates than as lipids. Some plants are not mobile and since photosynthesis provide fixed carbon on regular basis, plant requirement for storage lipids as an efficient, light weight energy reserve are less acute than that of animals (Schmid & Ohlrogge, 2002). World supplies of fats and oil are reported to come from vegetable sources (68.1%) animal fats (28.2%) and marine fats

(3.8%). In Nigeria, there are abundant sources of lipids such as palm oil, coconut oil, cotton seed oil, soya bean oil, groundnut oil, etc. (Akpan et al. 2006).

2.1 LIPIDS

Lipids are important biological molecules. These compounds are sparingly soluble in water and highly soluble in organic solvents such as ether, chloroform, turpentine, Benzene. E.t.c. They are divers in both structures and functions therefore do not share a common molecular structure. Lipids may be broadly defined as hydrophobic or amphiphilic small molecules; the amphiphilic nature of some lipids allows them to form structures such as vesicles, liposomes, or membranes in an aqueous environment. Lipids function in energy storage, cell membrane structure, protective of living surfaces and chemical signals.

2.2 CLASSIFICATION OF LIPIDS

Lipids classification based on their chemical nature includes the simple lipids, compound lipids, derived lipids and lipids complexed to other compounds.

SIMPLE LIPIDS:- Are those containing fatty acids and glycerol or other higher alcohols. Compounds that exist under this class are the

A. Neutral fats – Triglycerides

B. Waxes

1. True waxes

2. Cholesterol esters

3. Vitamin A esters

4. Vitamin D esters

COMPOUNDS LIPIDS: These are fatty acids esterifies with an alcohol but in addition they contain other groups like the sulfate, nitrogen. Etc. common examples includes

A. phospholipids - Contain a phosphoric molecule and a fat molecules

B. Cerebroside – Contain a carbohydrates and a fat molecule.

C. Sulfolipids – Contain a sulfate radical.

DERIVED LIPIDS:- These compounds are derived from lipids or precursor of lipids. They include

A. fatty acids

B. fatty aldehydes

C. fatty alcohols

D. vitamins A,D,E,K

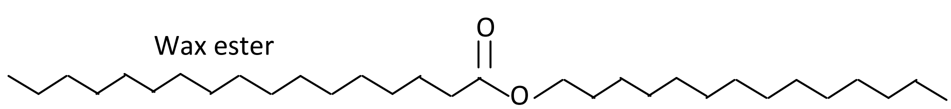
E. Hydrocarbons

2.3 TRIGLYCERIDES

Triglycerides results from the reaction between glycerol and fatty acid. They are the major energy reserve and the principal neutral derivative of glycerol found in animals. These molecules consist of glycerol esterifies with three fatty acids. If all the three fatty acids group are the same, the molecule is called a simple triglycerides. Eg tristerylgcerol and trioleoylglycerol. Mixed triglycerol contain two or more different fatty acids. Monoacylglycerol and Diacylglycerol also exist, but they are less common than the triglycerol. A significant number of fatty acids in plants and animals exist in the form triglycendes. These are the most abundant class of lipids. Most animal and plant fats are composed of mixtures of simple and mixed triglycerides. Triglycerol are stored as oil in seeds of many types of plants, providing energy and biosynthetic precursors during seed germination (Nelson &S Cox, 2005). Triglycerides are rich in high reduced carbons and thus yield large amount of energy in oxidative reaction of metabolism. Complete oxidation of 1 gram of triglyceride yield about 17 kg /g (Grisham & Garrett, 2005).

2.4 WAXES

Waxes are alcohol-based lipids that are extremely insoluble in water. If you have ever spilled your beverages on the wax paper wrapping of your sandwich, you have probably observed the way the liquid is repelled by the wax and forms beads. Because wax dose not dissolve in water, they form a thin layer over all the green tissue of plants that is both a chemical and physical barrier. This layer serve many purposes, for example to limit the diffusion of water and solutes, while permitting a controlled release of a volatiles that may deter pests or attract pollinating insects it provides protection from diseases and insects, and helps the plants resist drought. Waxes also have a water proofing and protective roles for insects.



Waxes can have a storage function, as in marine organisms and for example in the seeds of the jojoba plants. Bees use wax to produce the rigid structures of honey combs. The uropygial glands of birds secrets waxes, which they use to provide water-proofing for feathers (Christie, 2011).

2.5 PHOSPHOLIPIDS

These are the second most abundant class of lipids usually found in animal and plant cell membrane. Phospholipids contain a glycerol and fatty acids plus phosphoric acid and a low molecular weight alcohol. While fatty acids are typically composed of three fatty acids, phospholipids have two fatty acids. Phospholipids have water-hating “fails” and water-loving ‘heads “so that they form a double layer that helps protects our cellular machinery from the outside world. Examples of phospholipids found in the biological membrane are phosphatidylcholine (also known as PC, Gpcho or lecithin), phosphafidylethano lamine (Pt or Gpftn) and phosphatidylserine cps or Gpser).

2.6 FATTY ACIDS

In chemistry especially biochemistry, a fatty acid is a carboxylic acid with a long unbranched aliphatic tail either saturated or unsaturated. Their length varies between four or more than twenty carbons. Fatty acids form part of triglyceride and hence are the basic blocks and the main nutritional components of fats. When they are not attached to their molecules they are known as free fatty acids. The main fatty acids are important sources of fuel because their metabolism yields quantities of AIP. Many cell types can use either glucose or fatty acid for this purpose. In particular, heart and skeletal muscle prefer fatty acid as a source of fuel where as the brain relies on glucose or ketone bodies. Fatty acids make up the most commonest components of lipids in the body (Vasudevan & Sreekumari, 2007).

2.7 TYPES OF FATTY ACIDS

Saturated fatty acids do not contain any double bonds or functional groups along the acyl chain. They are long chain carboxylic acid with each carbon containing as many hydrogen as possible. In saturated fat, each carbon atom forms a single bond with hydrogen and other atoms in the molecule. This creates a fatty acid with a straight “tail” which allows many molecules to be parked tightly together in a relatively small space.

This fight packing is why saturated fats, like lard or butter, are solid at room temperature (Cholesterol database. com). They are non essential and can be made in adequate amounts to meet the body's physiologic and structural functions. The liver uses saturated fatty acid to make cholesterol therefore excessive dietary intake of saturated fat can significantly raise the blood cholesterol level especially the level of LDLs and increases the risk of developing cardiovascular diseases. Saturated fats are therefore classified as bad fats. Therefore it is recommended that daily intake of saturated fat be kept below 10% of total calorie. Saturated fatty acids are found primarily in animal products, including dairy items such as whole milk, cream, cheese and fatty meat like beef, pork e.t.c. Some vegetable products such as coconut oil, palm kernel oil are high saturates. Stearic acid (18:0) and palmitic acid (16:0) are the most common saturated fatty acid in nature (Grisham &S Garrett, 2005).

2.8 UNSATURATED FATTY ACID

Unsaturated fatty acid contains one or more double bonds between the carbon atoms. In nearly are naturally occurring fatty acids the double bond is in cis confirmation. These double binds create a link in the tail of the fatty acid, which means the molecule can not pack tightly together.

This is why an unsaturated fat, like olive oil is liquid at room temperature. Fatty acids in the trans-confirmation are not found in nature and are the result of human processing e.g. hydrogenation of fish or vegetable oil (Nelson & Cox, 2005). The difference in the geometry between the various types of unsaturated fatty acid as well as between saturated and unsaturated fatty acid, play an important role in biological process (cholesterol database.com). The incidence of cardiovascular diseases is conflated with diets high in saturated fatty acids. By contrast a diet that is relatively higher in unsaturated fatty acid especially poly unsaturated fatty acid reduce the risk of heart attacks and strokes.

2.9 ESSENTIAL FATTY ACIDS

Some fatty acids are deemed “essential” because the body can not make them on its own, but needs them to form important molecules and structures. Mono unsaturated and poly unsaturated are considered essential fats. The body does however produce all of the saturated fat that it requires. The body requires 20 fatty acids to function, but produces only 18 of theses. The remaining two are linoleic (Omega-3) and linolenic acid (Omega -6). These essential fatty acids are mostly found in plant and fish sources.

Arachidonic acid which is not found in plant . (Grisham & Garrett, 2005).

2.10 STEROIDS

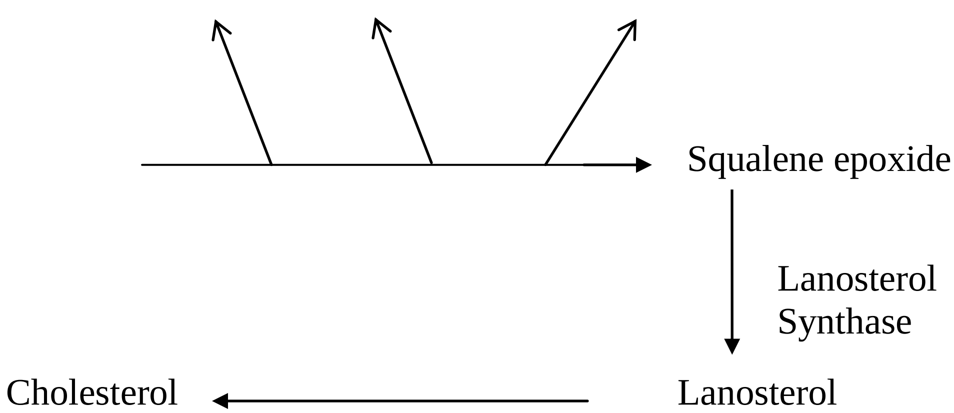
Steroids are oxidized derivatives of sterol lipids, such as cholesterol and its derivatives, are important component of membrane lipids. Steroids have a carbon backbone that consist of four (4) fused ring-like structures. They have different biological roles as hormones are chemical signals that regulate body function and development. The eighteen carbon (C18) steroids includes the estrogen family where as the (19 steroids comprise the androgens such as testosterone and androsterone. The C21 sub class includes the progestogens as well as the glycocorticoids . The secosteroids, comprising various forms of vitamin D, are characterized by cleavage of the B ring of the core structure (Delvin, 2006).

2.11 CHOLESTEROL

The name cholesterol originates from the Greek "Chole" (bile) and "Stereos" (solid), and the chemical suffix "OL" for an alcohol. Francois poulletier delasalle first indentified cholesterol in solid form in gall stones in 1967. However, it was only 1815 that chemist Eugene Cheverul named the compound “Cholesterol (Olson, 1998).

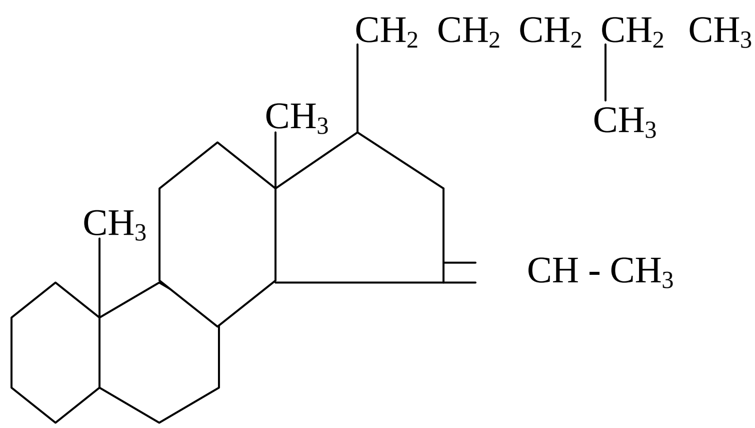
Cholesterol is the principal steroid of fat that is synthesized in eukaryotes such as fungi, and plants. It is almost completely absent among prokaryotes, including bacteria (pearson, et al. 2003). Cholesterol produces hormones and cell membrane and is transported in the blood plasma of all animals.iit is required to establish proper membrane permeability and fluidity. Biochemistry, cholesterol is of significant important because it is a precursor of a large number of important steroid which includes; bile acids, adrenocortical hormones, D-vitamins, cardiac glycosdies, sex hormones, sitosterols of the plants kingdom, and some alkaloids. It is the best known as steroid because of its association with atherosclerosis and heart diseases. Though, cholesterol is important an necessary for mammal, high levels of it in the blood can clog arteries and are potentially linked to diseases associated with the cardiovascular system. (national Health Service, 2010). Cholesterol is amphiphatic with a polar head group at c-3 (the hydroxyl group) and a non-polar hydrocarbon body (the steoid nucleus and the hydrocarbon side chain at c-17), about as long as 16 carbon fatty acids in its extended form. It has the molecular formular C 27 H460, with a molar mass of 286.65g/mole.

Its physical appearance is in the form of white crystalline power (safety MSDs data for Cholesterol, 2007), it has a density of $1.052 g/cm^3$, melting point of 148-150^oC (safety MSDs data for cholesterol, 2007). And boiling point of 360^oC. Its solubility in water is 0.095mg11(30^oC) and has a standard state at 25^oC,100pa. Sterols are structural lipids present in the membranes of most eukaryotic cells. The characteristic structure of this fifth group of membrane lipids in the steroid nucleus consisting of four fused rings, three with six carbons, and one with five carbons. The steroid nucleus is almost planar and is relatively rigid. The refused rings do not rotate about c-c bonds (Garrett, et al. 1999).



Formation of Cholesterol from Squalene.

Cholesterol is doubtless the most publicized lipid, notorious because of the strong correction between high level of cholesterol in the body (hypercholesterolemia), and the incidence of human cardio-vascular disease (Craford, 2003). Cholesterol is very essential in many animals, including humans, but it is not required in the mammalian diet. All cells ce;;s can synthesize it it from simple precursors.



Cholesterol |Structure (Voet and Voet, 2004)

A little more than half the cholesterol of the body arises by synthesis (about 700mg 11), and the remainder is provided by the average diet. The liver and intestine account for appropriately 10% each of total synthesis in humans. (Garette et al.S 1999). Virtually, all tissue containing nucleated cells are capable of cholesterol synthesis which occurs in the endoplasmic reticulum (ER) and the cytosol. The structure of this 27-carbon compound suggest a complex biosynthetic pathway, but all of its carbon atoms is the source of all carbon atoms in cholesterol which involved many biochemical reactions.

2.12 DIETARY SOURCE AND EFFECT OF DIET IN CHOLESTEROL LEVEL

Animal fats are complete mixture of triglycerides, with lesser amount of phospholipids and cholesterol. As a consequence all foods containing animal fat contain cholesterol to varying extents (Christie, and William, 2003).

Investigations indicate that a diet rich in animal facts tends to raise the level of cholesterol and the related fats and lipids in the blood (Encyclopedia, 2006) Major dietary sources of cholesterol include cheese, egg yolks, beef, pork, poultry, and shrimp (USDA National Nutrient Database, 2008). Human breast milk also contains significant qualities of cholesterol (Jensen et al. 1978). Evidence strongly indicates that people with such high levels are more likely to develop atherosclerosis and heart attacks than those with lower levels. The amount of cholesterol present in plant-based sources (USDA) National Nutrient Database 2008, Behrman &S Gopalan, 2005).

In addition plant product such as flax seed and peanuts contain cholesterol –like compounds called phytoserol, which are suggested to help lower serum cholesterol levels (Ostlund, et al., 2003). Total fat intake, and high intake of saturated fat, trans fat, (American \\\journal of Clinical Nutrients) and calories in excess of body requirement, pays a larger role in the elevation of the blood cholesterol than intake of cholesterol itself (Crowford, 2003). Saturated fat is present in full dairy products, animal fats, several types of oil and chocolate.

Saturated, Polyunsaturated and monounsaturated fats are thought to raise, lower and have no effect on serum cholesterol respectively. Trans fats are typically derived from the partial hydrogenation of unsaturated fats and do not occur in significant amounts in nature. Fat is most often encountered in margarine and hydrogenated vegetable fat, and consequently in many fast foods, snack foods and fried or baked foods.

2.13 FUNCTIONS OF CHOLESTEROL IN THE BODY

- Cholesterol is required to build and maintain membrane by modulating membrane fluidity over ranger of physiological temperature.
- Cholesterol reduces the permeability of the plasma membrane to protein and sodium ions.

- Within the cell membrane, cholesterol function in intracellular transport, cell signal and nerve conduction.
- Cholesterol assists in the formation of lipid raft in the plasma membrane.
- Cholesterol converts the bile in the liver and store in the gall bladder.

It is also important precursor molecule for the synthesis of vitamin D and steroids hormones including adrenal gland, hormone cortis and aldosterone and their derivatives. Cholesterol synthesis can be turned off or inhibited when cholesterol level are high. In addition to providing a soluble means for transporting cholesterol through the blood, lipoproteins have cell -targetting signals that directs the lipid they carry to contain tissues for this reason, there are several types of lipoprotein within blood called in order of increasing density. Chylomicrons, VLDL, IDL, LDL, and HDL.

The more cholesterol and less protein a lipoprotein has, the denser it is. The cholesterol within all the various lipoprotein is identical, although some cholesterol is carried as the free alcohol and some is carried as fatty acyl esters referred to as cholesterol esters, however, the different lipoprotein contain apolipoprotein which serves as ligand for specific receptor on cell membrane. (Olson, 1998). Cholesterol provides good number of vital functions in the body, the body makes its own cholesterol in the liver, because it is steroid and it is a necessary component of the cell membrane it is possible for more cholesterol to come from endogenous source than from the diet, the body packages this cholesterol for transport in the blood stream through several classes of lipoproteins like LDL, and VLOL, which serves as transport of lipids in the blood (Campbell & Farrell, 2008).

2.14 LIPOPROTEIN METABOLISM

It transports hydrophobic fats in plasma. The major lipoprotein circulating in the blood are chylomicrons, VLDL, LDLs, and HDLs. IDLs are derived from VLDLs in the formation of LDLs. Fatty acids are important cellular fuels and are stored as triacylglycerols in adipose tissue principally as triacyglycerol in chylomicrons and VLDLs. In adipose tissue, chylomicrons are rapidly degraded, and the remnant particles re-enter the circulation and are taken up by the liver. VLDLs are degraded in adipose tissue to LDLs which then circulates as the major transport lipoprotein for cholesterol. HDLs are lipoprotein that continuously circulate, the contain enzymes “cholesterol acyltransferase” that converts free cholesterol to cholestery esters.

2.15 **VERY-LOW DENSITY LIPOPROTEIN (VLDL)**

Very low densities lipoprotein are produced by the liver contain excess Triglycerides and Cholesterol that is not required by the liver for synthesis of bile acid (Javit; 1994). Very low density Lipoprotein are synthesized in the liver from glycerol and fatty acid and incorporated into VLDL along with hepatic cholesterol apoB-100 C-11 is the major Lipoprotein present in VLDL, when it is secreted, AOE and C-11 are obtained fro, HDL in plasma.

FUNCTIONS OF (VLDL)

Very low density lipoprotein (VLDL) carries triglycerides (endogenous triglycerides) from the liver to peripheral tissues for energy needs.

2.16 LOW DENSITY LIPOPROTEINS

Low density lipoprotein (LDL) molecules are the major carries of cholesterol in the blood and each one contains approximately 1,500 molecules ester. The shell of LDL particles are derived VLDL, but small part is directly released from the liver. The half of LDL in blood is about 2 days.

LDL receptor is a mosaic protein of 840 amino (after removal of signal peptide) that mediates the endocytosis of cholesterol rich LDL. It is a cell surface that recognizes the aPOProtein B-100 which is embedded in the phospholipids outer layer of LDL particles the receptor also recognizes the APOEProtein found in chylomicrons remnant and VLD remnant. (Campbell &S Farrell, 2008).

2,17 FUNCTIONS OF LDL

The main function of LDL is that they transport cholesterol from the liver to peripheral tissue. Also LDL concentration in blood has positive correlation with incidence of cardiovascular disease. About 75% of the plasma cholesterol is incorporated into the LDL particles. (Vasudevan & Sreekuman, 2007).

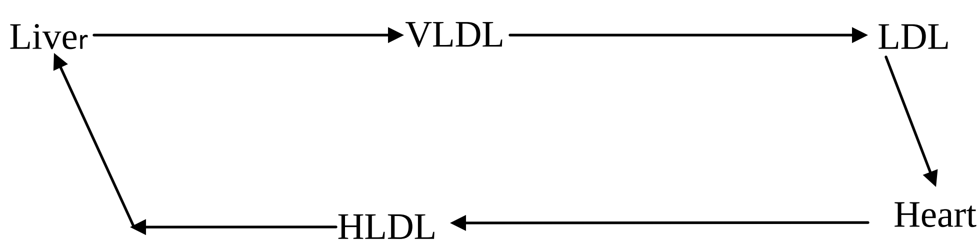
2.18 HIGH DENSITY LIPOPROTEIN

High density Lipoprotein are transporters of Cholesterol from the peripheral tissue to the Liver, which is later excreted through bile.

Cholesterol is used to synthesize hormone by some tissues in a process absorbs cholesterol from the blood stream and blood vessels wall and transport it to the liver for excretion through the bile, this is why they are referred to as cholesterol high density Lipoprotein and are the highest and densest because they contain the highest proportion of protein (Campbell & Farrell, 2008).

Cholesterol in general is susceptible to oxidation and easily forms oxygenated derivations known as osterol: they are oxidize mechanism from the ant oxidation. Secondary oxidation to lipid peroxidase and cholesterol metabolizing enzyme. Cholesterol is oxidized in the Liver varieties of the bile acid, the conjugated and non-conjugated along with cholesterol itself from the liver to bile.

Approximately 90% by the bile acid are reabsorbed from the intestine and the remainder are lost in feces.



Forward and reverse transport of cholesterol

(vascudevan & sreekunari, 2007).

One of the functions of HDL is excretion of cholesterol and acid prior esterifications with PUFA (Polyunsaturated Fatty Acids). This is an anti-atheorgenic. (Vasudevan & Sreekumar, 2007).

2.19 **CLINICAL SIGNIFICANCE**

The level of HDL is serum, is inversely repeated to the incidence of myocardial infractions. As it is anti-atherogenic or protective in nature, HDL is good cholesterol and it is highly desirable.

Hypercholesterolemia (high level of cholesterol in blood) and Lipid hypothesis abnormal cholesterol value, concentrationof functional HDL are strongly associated with cardiovascular disease because they promote atheroma development in arteries; atherosclerosis. This disease process leads to myocardial infraction (heart attack), stroke and peripheral vascular disease.

Hypocholesterolemia (Low level of cholesterol) low cholesterol level seem to be a consequence of an under lying illness rather than a cause of disease.

A change in diet in addition to other life style modifications can help to Reduced in take of animal product can help to lower cholesterol level.

2.20 **FAT SOLUBLE VITAMINS**

The fat-soluble vitamins are vitamin A, D, E and K. They differ from the water-soluble vitamins in several ways. They appear in fat and oily parts of food. They are digested only in bile because they are insoluble in water (food-infornet). They are absorbed from the small intestines, along with dietary fat, which is why fat malabsorption resulting from various diseases (e.g Crohn's disease, ulcerative colitis, cystic fibrosis) is associated with poor absorptiom of these vitamins. Fat soluble vitamins are primarily stored in the liver and adipose tissues. With the exception of vitamin K, fat soluble vitamins are generally excreted more slowly than water-soluble vitamins, and vitamin A and D can accumulate and cause toxic effects in the body(Kiran, 2011).

2.21 **VITAMIN A**

Vitamin A was the first fat-soluble vitamin identified (in 1913). Vegetables and fruit are riches vitamin A carriers. Most food that contain vitamin A are brightly colour (although not all brightly coloured food contains a lot of vitamin A). vitamin A-rich vegetables are carrots, sweet potatoes, spinach, winter squash and cantaloupe. Milk, cheese butter and eggs also contain vitamin A. the recommended amount of vitamin A is 1000 micrograms Re per day for men and 800 micrograms for women (food-info. Net, 2011).

Vitamin A is important for the maintenance of cornea and epithelial cells and thus, of vision. It also aids growth and bone reproduction and teeth. It plays a role in hormone synthesis and regulation and helps protect the body against cancer. There are some loss of vitamin A with cooking, but only after boiling for a comparatively long period. Retinoids are converted to retinol in the intestine and transported with dietary fat to the liver, where it is stored. A special transport protein; retinol binding protein (RBP), transport vitamin A from the liver to other tissues of all the carotenoids, beta-carotene has the highest potential vitamin-Activity. The active forms of vitamin A have three basic functions: vision, growth, immunity and tissue development. (Wardlaw et al. 2004).

2.22 **VITAMIN D**

Vitamin D has one characteristic that distinguishes it from all other vitamins: it can be produced by sunlight. This means that with regular exposure to sunlight, no additional in take of vitamin A is necessary. The RDA for vitamin D is 5 micrograms per day. Although the amount of synthesized vitamin D increases the longer the skin is exposed to sunlight, sunlight alone can never cause the vitamin D to reach toxic levels. Vitamin D from food is absorbed from the upper part of the small intestine, along with dietary fat and transported to the liver. In the liver, vitamin D is converted to calcidiol, an inactive form that circulates in the blood. Kidneys take up calcidiol and convert it to an active hormone form of vitamin D called calcitriol.

People with chronic kidney failure have very low levels of calcitriol and must be treated with this form of the vitamin. The most important function of vitamin D is to help regulate calcium and phosphorus blood levels. It also increase absorption of these minerals from the gastro intestinal tract (Kiran, 2011).

2.23 VITAMIN E

Vitamin E is abundant in vegetable and seed oils, which are found in margarine, salad dressing, soya beans oil and wheat germ oil has especially high concentration of vitamin E. Corn and sunflower oil rank second. Vitamin E comprises a family of eight naturally occurring compounds: four tocopherols and four tocotienols of which alpha-tocopherol is the only one to have vitamin-t activity in the human body. It is only the most common form of vitamin E in food. Vitamin T is highly susceptible to destruction by oxygen, metals, light, and deep-fat frying. As a result, prolonged food storage lowers the vitamin –E content of the food. The RDA for vitamin E is 10mg per day for men and 8mg per day for women (food-info.net).

2.24 VITAMIN K

Much of the body's supply for vitamin K is synthesized by bacteria in the digestive tract. Food sources of vitamin K are liver, green leafy vegetables, cabbage-type vegetables and milk. In 1992, the Danish researcher Henric Dam first noted that vitamin K played a critical role in blood clothing, and he named it vitamin "K" for "Koagulation". Vitamin K comprises a family of compounds known as quines. These include phylloquinone from plants and menaquinones from animal sources. The body needs to take in additional vitamin K in diet (food-info.net).

2.25 LIPID STORAGE IN PLANTS.

A plant stores reserved material in its seeds in order to allow seedling growth of the next generation until photosynthesis capacity can be established. The three major storage materials are oil, protein and carbohydrates and almost all seeds some of each. However, their proportions vary greatly. For example, the amount of oil in different species may range as little as 1-2% in grasses such as wheat, to as much as 60% of the total dry weight of oil seed. With the exception of the jojoba plant, which accumulates, wax esters in seeds, plant store oil as triglycerol (TAG).

2.26 LIPID BODY STRUCTURE AND BIOGENESIS

In the mature seed, TAG is stored in density packed lipid bodies, which are roughly spherical in shape with an average diameter of 1 um (Smith, 1994). This size does not change during seed development, and accumulation of oil is accompanied by an increase in the number of lipid bodies. The very large number of lipid bodies in an oilseed cell (often > 1000) contrast strikingly with animal adipose tissue where oil droplets produced in cytosol can coalesce into a few or only one droplet. The plant lipid bodies appear to be surrounded by a lipid mono-layer in which the polar head groups face the cytosol, while the non-polar acyl groups are associated with non-polar triaglycerol wthin.

When seed germinates, the TAG stored in the lipid bodies becomes the substrate of lipases. In at least some cases, peroxidation of polyunsaturated fatty acids by a lipid body lipoxygenase precedes the release of fatty acids from TAG(Fichteschere et al. 2002).

2.27 UNUSUAL FATTY ACIDS IN SEED TRIACYL GLYCEROL

The structural glycerolipids of all plant membrane contain predominantly 5 fatty acids (18:1, 18:2, 18:3, 16:0 and in some species, 16:3). However, the fatty acid composition of storage oils varies far more than in membrane glycerolipids. Altogether more than 300 different fatty acids are known to occur in seed TAG (Wifkowski et al. 1997). Chain length may range form less than 8 to over 22 carbons. The position and number of double bonds may also be unusual, and hydroxy, epoxy, or other functional groups can modify the acyl chain. Many of the different fatty acid structures, including hydroxyl, epoxy, acetylenic and conjugated varieties, are now known to originate from minor modifications in the amino acid sequence of the oceate desaturase.

The reason for the great diversity in plant storage oils is unknown. However, the special physical or chemical properties of the 'unusual' plant fatty acids have been exploited for years. In fact, appropriately one-third of all vegetable oil used for non-food purposes.

2.28 PROPERTIES OF LIPIDS

Lipids are formed structural units with a pronounced hydrophobicity. Lipids are soluble in organic solvents but not in water.

Water insolubility is the analytical property used as the basis for the facile separation from proteins and carbohydrates. Lipids in food exhibit unique physical and chemical properties. Their composition, crystalline structure. Meeting properties and ability to associated with water and other non-polar molecules are especially important to their functional properties in many food.

2.29 PHYSICAL PROPERTIES

Specific gravity:- Specific gravity is the heaviness of a substance compared to that of water at 4^{0}C (39^{o}F), and it is expressed without units. If something is 7.85 times as heavy as an equal volume of water (such as iron is) its specific gravity is 7.85.if the liquid you are comparing will float on this water, it has a specific gravity of less than one. If it sinks into the fresh water, the specific gravity is more than one. Specific gravity is measured with a hydrometer.

Melting point:- Thins is the temperature at which a compounds melts to liquid, often characteristic of particular oil and used as a means of identification. Particularly valuable as an index of purity, since impurities lowers the melting point.

Density:- Fats in liquid state have similar densities. The density of fat in solid state is higher. Density of fat is affected by an varies directly with degree of unsaturation and indirectly by average molecular weight.

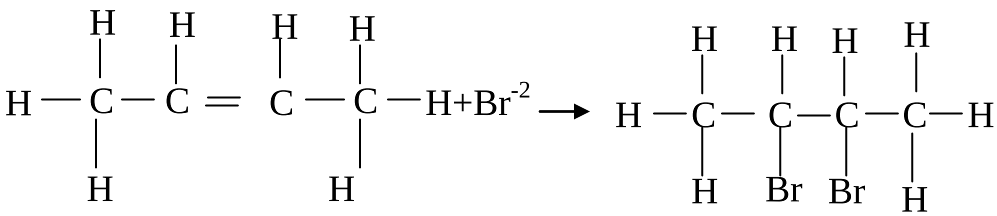
2.30 CHEMICAL PROPERTIES

Hydrogenation:- Unsaturated fatty acids may be converted to the corresponding saturated fatty acids by hydrogenation of double bonds.

$$\text{linolenic} \xrightarrow{+\,2H} \text{Linoloic} \xrightarrow{+\,2H} \text{oleic} \xrightarrow{+\,2H} \text{stearic}$$

Hydrogenation of oil can result to solidification and saturation (Vasudevan and Sreekumari, 2007). This process is known as hardening of oils. This process is commercially useful since many products are made from it e.g. soap, shortening, margarine etc.

Hydrolysis:- This is the commonest and oldest reaction of fats. fats and oil undergo hydrolysis on the presence of enzymes to yield fatty acid components. Also fats and oil undergo hydrolysis by action of strong base e.g. NaoH and KoH to yield salts of fatty acids and glycerol.



Polymerization:- Fatty acids undergo polymerization when oxidized at atmospheric temperature or in the absence of oxygen at elevated temperature. This process is commercially useful in “bodying” of oils in paint and vanish industries.

Etherification:- This is reverse of hydrolysis. Here fatty acids refers to the appearance taste or smell for fats and oils.

2.31 RANCIDITY

Fats and oils have a tendency to became rancid, thus the term rancidity refers to the appearance taste or smell for fats and oils.

Hydrolytic rancidity is due to partial hydrolysis of the triacyglycerol molecules due to traces of hydrolysis of the triacylglycerol molecules or due to traces of hydrolytic enzymes present in naturally occurring fats and oils.

Oxidative rancidity is the result of partial oxidation of unsaturated fatty with resultant formation of epoxides and peroxides of small molecular weight fatty acids by peroxides and free radicals. The same process, if it occurs in vivo will affect the integrity of biomembranes, leading to cell death.

2.32 ROLES OF LIPIDS

Lipids have many roles in the body, from serving a structural role in membranes and storing energy for later use to help the brain transmit nervous impulses and regulating function of the body.

2.33 STRUCTURE

Lipids make up the majority of cell membrane structure. Membrane consist of a bilayer of lipids, with their water-soluble (hydrophilic) head group effacing out and their hydrophobic (water-hating) fatty acid chain facing in proteins in the membrane have a variety of roles, but the lipids forms the physical barrier that separates the inside of the from the outside of the cell.

2.34 INSULATION

A layer of fat under the skin insulates animals from cold temperature. This is especially true for mammals that live in the arctic (polar bears) and water (whales, seals, dolphins); all these animals rely on thick layer of flat under their skin to keep their body warm.

2.35 **NERVE FUNCTION**

Nerves have long axons that conducts signals from one part of the brain or body to another cell in the brain or spinal cord. Some axons are very long-from the bottom of the foot to the spine, for instance. A thick layer of myelin, a special membrane lipid, helps nerve impulses travel more quickly axons.

2.36 **ENERGY SOURCE**

Lipids are stored in adipose tissue (fat cells) when more fat ingested than is needed immediately. In lean people 18% to 25% of the body mass is lipid (largely in cell membrane and adipose tissue). Muscles, fact cells and the liver metabolize lipids to form ATP during fasting, including over night. After eating, fat cells and liver cells can use fatty acids from the diet to make new liquids to store in adipose cells.

2.37 USES OF OHA (Pterocarpus mildbreadii seed)

i The leaves are edible e.g. use in cooking soup

ii. The bark is used for exudation

2.38 AIMS AND OBJECTIVES

Determination of the plasma HDL – cholesterol of an albino rats fed with oha seed extract.

CHAPTER THREE

3.0 MATERIALS

3.1 PLANT SEED

The fresh seed of pterocarpus mildbreadii were collected from Udi, Enugu State, Nigeria in the month of July,2013,and dried at room temperature.

3.2 ANIMALS

The experimental animal used for this research study were Wister albino rats of both sexes, of about 4 to 8 weeks old with average weight of 40 to 56g were obtained from the animal house of the college of medicine, University of Nigeria, Enugu campus. The animals were housed in the animal house at BRAIN PHOSEPHORYLAIONSHIP RESEARCH AND TRAINING CENTER, No 9 Ogui Road, 5th flour Right wing, former ACB Building Enugu and maintained standard pellets (Top feeds) MRT,5% tween-80 and grounded oha seed solution.

3.3 EQUIPMENT

The equipment used includes:

Centrifuge

Conical flask

Beaker

Measuring cylinder

Blender (gander)

Water bath

Refrigerator

Spectrophotometer

Syringe

Test tube

Weight balance

EDTA bottle,

Micro pipette

Capillary tube

cuvette

3.4 METHODOLOGY

Eighteen (18) albino rats of about four to eight weeks old were divide into four parts marked with picric acid as follows:

Group 1: Fed with control alone (3% tween - 80)

Group II: Fed with oha seed extract

Group III: Fed with oha seed extract

Group IV: Fed with MRT alone (control).

Determination of weight

The initial weights of the animals were taken before feeding commenced.

Preparation of Sample

The bark of dry pterocarpus mildbreadii seed were pealed off with hand and chipped into pieces and ground into powder using a blender (grinder). The resulting powder was mixed with 3% tween-80.

⇨ Weight of ground oha seed used 50.oog

⇨ Volume of 3% tween – 80 = 200m%

Concentration of oha seed

Powder

50 x 100 ml

= 250 mg/ml

The mixtures and the MRT were store in a refrigerator until the feeding time.

Sample

A standard = Absorbance of the standard.

PROCEDURE

This method is based on the principle that cupric ions, in an alkaline medium, interact with peptide colored complex.

High Density Lipoprotein (HDL)- Cholesterol Determination (using RANDOX commercial kit).

The determination of high density lipoprotein-cholesterol took two steps:

Procedure

A Precipitation step

The sample (500 µL) was pipette into labeled test tubes. Also, 1000 µL of HDL R_1 was pipette into a test tube labeled cholesterol standard, Spin for 10minutes at a minimum of 4000 rpm. The supernatant was carefully collected.

B. **Colorimetric step**

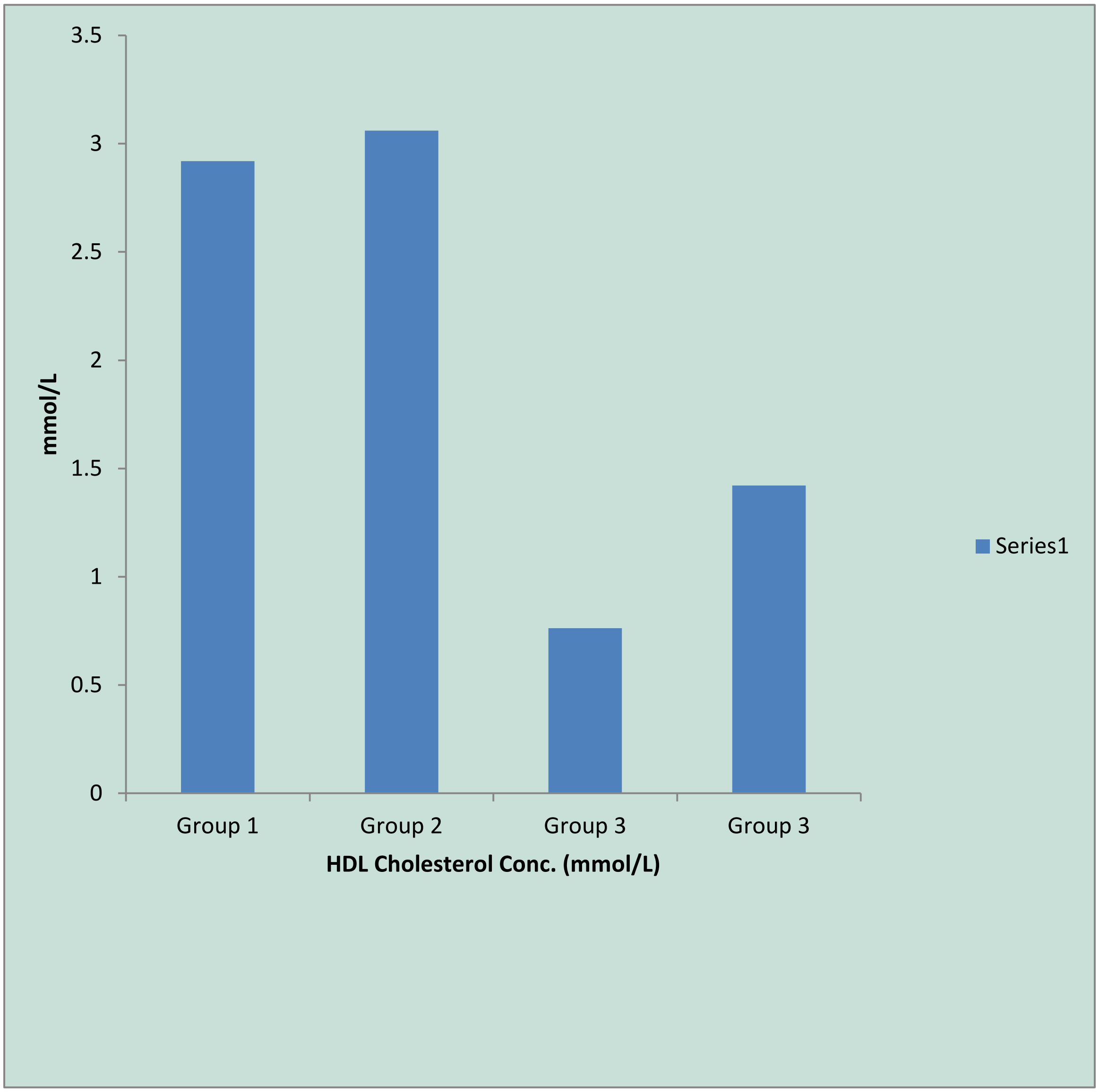
Distilled water (10 µL) was pipette in to the Blank test tubes (B.) Cholesterol standard was added into a test tube labeled (ST). 10µL of sample was added to a test tubes labeled (SA), 1000µL of Cholesterol R_1 was added to all the test tubes respectively and thoroughly mixed; the incubate for 10mins at 25^0C.

The absorbance (A) of the standard and sample at 500nm wavelength was measured against the Blank.

The HDL cholesterol concentration in the sample was calculated.

CHAPTER FOUR

4.1 CHOLESTEROL ACTIVITY



sFig. 1: Cholesterol Activit

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Oha seed has not been experimented.The blood lipoprotein are very vital in the causation of heart attack which when untreated causes CVDS(cardiovascular disorders).Any food or drugs that increases the deposition of lipoprotein,increases the chance of CVDS.

5.2 CONCLUSION

The analysis on the HDL cholesterol level of an albino rats shows that ,at a higher dose,the HDL concentration is lower,at a lower dose,the HDL cholesterol concentration increases,which shows that HDL is a good cholesterol and should be taken at higher dose .

APPENDIX

TABLES AND RESULTS

CONTROL GROUP 1

S/N	Mark	Weight (g)	Con’c of Dosage	
			Dosage	
1	Nose	45.78		0.5ml
2	Head	50.14		0.5ml
3	One ear	50.19		
4	Two ears	50.84		2.0

CONTROL GROUP 4 500mg/kg.b.w

S/N	Mark	Weight (g)	Con’c of Dosage	Volume
1	Two sides	45.25	22.625	0.48
2	2 rings @ tail	40.10	20.05	0.43
3	2 legs and 2 hands	56.00	28.30	0.60
4	Two ears	45.44	22.72	0.48
			93.695	2.0ml

GROUP 2 Dosage 1400 mg/kg/b.w

S/N	Mark	Weight (g)	Con’c of Dosage	Volume
1	One hand	42.24	59.136	0.237
2	One dot @ back	42.08	67.312	0.267
3	Two hands	44.41	62.174	0.248
4	One leg	43.12	60.368	0.241
5	One leg	47.34	66.276	0.265

GROUP THREE Dosage 2600 mg/kg/b.w

S/N	Mark	Weight (g)	Con'c of Dosage	Volume
1	Two legs	43.20	112.32	0.449
2	Base of tail	46.92	121.992	0.488
3	One H/one L	50.65	131.69	0.527
4	One leg/Nose	44.18	114.869	0.459
5	One side	47.56	123.656	0.495
			472.836	

GROUP ONE

S/N	Mark	A	B	c	Average	HDL-concentration in the sample
1	Nose	0.104	0.105	0.105	05.323	5.323
2	Head	0.094	0.093	0.093	2.241	2.241
3	One ear	0.034	0.046	0.046	1.565	1.565
4	Two ears	0.138	0.143	0.143	2.553	2.553
						2.920

Average of standard 0.616

GROUP TWO

S/N	Mark	A	B	C	Average	HDL-concentration in the sample
1	Base of tail	0.111	0.077	0.083	0.090	2.181
2	One dot at back	0.041	0.040	0.040	0.040	1.208
3	2 hands	0.078	0.078	0.077	0.077	3.11
4	2 dots at back	0.159	0.160	0.157	0.158	8.064
5	One leg	0.009	0.008	0.009	0.008	0.47
						3.006

GROUP THREE

S/N	Mark	A	B	C	Average	HDL-concentration in the sample
1	Base of tail	0.009	0.010	0.010	0.009	0.229
2	One hand/one leg	0.038	0.038	0.047	0.047	1.440
3	One side	0.003	0.001	0.001	0.016	0.62
						0.763

GROUP FOUR

S/N	Mark	A	B	C	Average	HDL-concentration in the sample
1	Two sides	0.012	0.011	0.011	0.011	0.318
2	2 rings at tail	0.066	0.070	0.068	0.068	1.158
3	2 legs/ 2 hand	0.033	0.052	0.052	0.045	2.298
4	2 hands/ 1 leg	0.038	0.039	0.039	0.038	1.918
						1.442

SAMPLE DILLUTIONN

S/N	Mark	Volume of plasma(ml)	Volume of water(mmol/L)	Dilution factor
1	Nose	0.03	1.6	6.33
2	Head	0.6	1.2	3.00
3	One ear	0.4	1.6	5.00
4	Two ears	0.8	1.6	2.25
5	One hand	0.6	1.2	3.00
6	One dot at back	0.5	1.4	3.80
7	Two hands	0.4	1.6	5.00
8	The dots at back	0.3	1.6	6.33
9	One leg	0.4	1.6	5.00
10	Base of tail	0.6	1.4	3.33
11	One hand / one leg	0.5	1.4	3.80
12	One side	0.4	1.6	5.00
13	Two sides	0.5	1.4	3.80
14	Two rings at tail	0.9	1.0	2.11
15	2 legs and 2 hands	0.3	1.6	6.33
16	2 hands / one leg	0.3	1.6	6.33

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