

**ASSESSMENT OF VARIOUS BIOCHEMICAL
CHANGES IN HYPERTENSIVE AND
HYPERTENSIVE-PRONE ADULT NIGERIANS IN
JOS, NIGERIA**

BY

SAMUEL ISEZUO, SALAMI

B.Sc (Hons) Biochem, (Unilorin) M.Sc Biochem, (Unijos)AIMLS

(Chem. Path.) (FSMLT, Jos)

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CERTIFICATION

This is to certify that the research work for this thesis and the subsequent preparation of this thesis by **SAMUEL ISEZUO SALAMI** - PGMS/UJ/9498/96 were carried out under my supervision.

G. E. ANEKWE (M.O.E)
PROFESSOR & SUPERVISOR

G. I. ADOGA
PROFESSOR & HEAD OF DEPARTMENT

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To God be the Glory for He has once more given me a reason to acknowledge that:

JESUS IS LORD INDEED and HE IS ABLE

SAMUEL ISEZUO SALAMI,
April, 2004.

DEDICATION

I dedicate this work to;
all having one form of cardiovascular disease
or the other and those that are prone to it.
And to Almighty God who is the ultimate healer.

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ABSTRACT

A total of 1,075 fasting Human Serum and early morning urine samples were assayed for various biochemical parameters. The parameters include: Blood lipids, Total Cholesterol, High Density lipoproteins, Low Density lipoproteins, Vitamin A, Sodium, Potassium, Chloride, Bicarbonate, Urinary glucose, Protein and Blood.

From the results obtained, the calculated phospholipids:cholesterol ratio was less than 1 amongst the hypertensives. This confirmed the work of Stroev and Makarova, 1986; Oforufuo and Nwanze, 1988. This also confirmed that these patients had pathological disorder – in this case, hypertension.

The mean systolic pressure of the hypertensives was 167mmHg with diastolic pressure of 110mmHg.

Results from the comparative urinalysis pattern of the hypertensives from two Governmental Hospital and two Missionary Hospitals, revealed some complications of hypertension which drug therapy could not control. However, such complications as proteinuria, glycosuria and haematuria were minimal where awareness and socio-economic stability were satisfactory.

The results of the lipid profile of the Burukutu consumers agreed with earlier studies where this local alcoholic beverage had a lowering effect

on serum total cholesterol of both sexes. Furthermore alcohol increased the HDL and TG concentration of the consumers.

The fact that individuals who were both alcoholics and smokers develop coronary heart disease faster earlier in life than alcoholics or smokers alone was well correlated in this study.

Vitamin A concentration – a lipid soluble vitamin, was estimated in children under one year of age by high performance liquid chromatography. The high point of the result was that exclusive breastfeeding still remained the best choice for babies under one year of age.

From the foregoing, it was suggested that if the lipid profile of women could be controlled within the normal reference values, incidences of high blood pressure due to high lipid profile molecules (cholesterol, lipoproteins etc) as age increases could be checked: thereby checking early on set of menopause.

Thus, a total of 200 adult Nigerian women, non-diabetic, non-hypertensive, non-obese and non-pregnant from three Hospitals (two Governmental and one Missionary) were assayed for lipid profile.

The results obtained showed that the lipid profiles were within the normal reference range and correlated very well with published fertility hormone assay results which had hitherto been used for monitoring on set of menopause.

Considering such factors as cost and ease of assay, affordability by the patients and reagent availability, the use of lipid profile measurement seemed a preferred alternative method, to the reported fertility hormone assays for measuring onset of menopause in Nigerian women.

CHAPTER ONE

INTRODUCTION AND REVIEW OF RELATED LITERATURE

The heart and its blood vessels make up the **CARDIOVASCULAR SYSTEM (CVS)** and together establish and maintain the circulation. The heart is a muscular organ which functions as a pump. The blood vessels are a successively dividing series of progressively smaller tubes beginning with the aorta, progressing to arteries, then to arterioles and finally reaching the capillaries. At this point, they gather successively into venules, veins and the vena cava to return to the heart.

The heart and its blood vessels are so arranged as to link the systemic circulation (peripheral blood vessels) and the pulmonary circulation (blood vessels of the lungs) in series, thus providing a continuous system for blood flow.

1.1 THE HEART

The human heart and the great arteries which arise from it form a u-shaped tube which lies obliquely across the thorax in the sagittal plane. The tube is divided longitudinally by a continuous septum into right and left channels, each channel being further divided by two transversely placed valves. Thus, the heart is divided into four chambers:

- (a) right atrium or auricle
- (b) left atrium or auricle
- (c) right ventricle
- (d) left ventricle

as shown in figure 1:

Figure 1: Anatomical Structure of the Heart

Arteries are supplied by the left and right coronary arteries, while venous drainage is by the coronary sinus, consisting of great, small and middle cardiac veins. The nerves are distributed to the heart from the cardiac plexus (which lie on the bifurcation of the trachea) and consist of sympathetic, parasympathetic and sensory fibres.

Microscopic structure of the heart consists of a layer of cardiac muscle called MYOCARDIUM.

Blood Pressure: This is the pressure (force) extended by the blood against any unit area of the vessel wall and is measured in millimeters of mercury (mmHg) by the sphygmomanometer. It can also be measured in centimeters of water (cm of water). It consists of systolic and diastolic pressures.

1mmHg = 1.36cm of water.

Systolic pressure (SP) is that pressure when the heart muscle is at the maximum contraction and is recorded first. Mean normal S.P. is 120mmHg. Diastolic pressure is that pressure when the left ventricle is in a state of relaxation mean normal diastolic pressure is 80mmHg.

Thus, mean blood pressure = 120/80mmHg.

Cardiac Output: This is the amount of blood pumped by the heart in a unit period of time. It is about 5000mls/minute in an adult person at rest.

Diseases of the CVS affect the heart and its components (heart valves, conducting system and myocardium) and the blood vessels;

primarily the arteries. Such diseases include ischaemic heart disease, hypertensive heart disease, congestive heart failure, atherosclerosis, angina pectoris, myocardial infarction to mention but a few.

1.2 HYPERTENSION

The word HYPERTENSION is used synonymously with essential hypertension as defined and classified by the World Health Organization (WHO) and recently modified by the Consensus Committee on hypertension. When a person is said to have hypertension, it is generally meant that his or her mean arterial pressure is greater than the upper range of accepted normality. Usually, a mean arterial pressure of greater than 110mmHg under resting conditions is considered to be hypertensive. This level normally occurs when the diastolic pressure is greater than 90mmHg and the systolic pressure is greater than 135 – 140mmHg.

In other words, hypertension could be defined as systolic pressure or Diastolic pressure greater than 160mmHg and 90mmHg respectively.

Before the turn of the 19th century, early workers often did not find cases of established hypertension in Africa. Donnison in 1929 did not find a single case of hypertension in over 1800 patients attending hospital over a period of two years in a Kenyan village. As late as 1935 some primitive African communities had been described to have very low prevalence of hypertension. In these population groups, blood pressure did not rise with age.

Following the independence of most African countries in the 1960s, reports of hospital and community – based prevalence studies of hypertension began to emerge, indicating increasing prevalence in most African countries (Wokoma 2002). Target organ complications of uncontrolled hypertension such as heart failure, stroke and nephrosclerosis constitute the commonest morbidity and mortality factors in most African urban communities. Thus, hypertension and its target organ complications have become a major public health priority in developing countries like Nigeria.

From epidemiological perspectives, therefore, hypertension is an emerging non communicable disease (NCD) which was hitherto either non-existent or extremely uncommon in Nigeria before 1900, but is now afflicting millions of Nigerians with increasing prevalence. This increase coincides with the transformation of Nigerian societies from a primordial agrarian and exercise – intensive society, to European type of industrialized and sedentary urban lifestyles.

Essentially, hypertension could be grouped into two broad classifications:

- (a) those with unknown cause(s) – ESSENTIAL OR PRIMARY HYPERTENSION
- (b) those with known causes – NON-ESSENTIAL OR SECONDARY HYPERTENSION.

ESSENTIAL HYPERTENSION: This is hypertension of unknown origin and 90% of hypertensive cases fall into this group. However, in most patients with essential hypertension, there is a very strong hereditary tendency (Canessa, 1984). The hypertension could be mild (D.P. of 90 – 104mmHg), moderate (D.P. of 105 – 119mmHg) or severe (D.P. of 120 – 130mmHg).

NON – ESSENTIAL HYPERTENSION: The cause of the hypertension is known. Examples are hypertension following renal artery constriction (renal hypertension), hypertension due to corticosteroids e.g. as in Conn's syndrome, hypertension due to neurological and psychological factors, hypertension due to coarctation of the aorta and in some cases, hypertension due to pregnancy.

AETIOLOGY: It occurs during the course of such maladies as toxic goitre, in certain forms of cardiac disease, atherosclerosis and toxæmia of pregnancy.

In the majority of cases, however, the origin cannot be found. Risk factors include height, weight, family history of congestive heart disease, smoking, therapeutics, diet, stress and sedentary life-styles.

Many patients with essential hypertension are identified in the course of routine examination and are asymptomatic. However, in those that are symptomatic, headache is the most consistent symptom related directly to the pressure elevation; most commonly, it is localized to the occipital region, present when the patient awakens in the morning and subsides spontaneously after several hours. Other complaints include

dizziness, fainting attacks, palpitation and easy fatigability. Epistaxis or haematuria, blurring of vision due to retinal changes, episodes of weakness or dizziness due to transient cerebral ischaemia, pain due to myocardial ischaemia and dyspnoea due to cardiac failure. Chest pain due to coarctation of the aorta or to a leaking aneurysm are less common presenting symptoms.

The important clinical features of hypertension include hypertensive encephalopathy, renal damage and cardiac failure. Essential hypertension has certain risk factors such as overweight, obesity resulting from the effects of a sedentary lifestyle, excessive consumption of refined carbohydrate and fatty foods, increased alcoholic consumption and cigarette smoking. (Wokoma, 2002).

Clinical investigation of the hypertensive patient is carried out by taking the history of the patient (clerking) and general examination of all the systems with particular attention to the CVS. Laboratory investigations include urinalysis, electrolyte, urea, lipid profiles analysis, uric acid, creatinine estimation and 24 – hour creatinine clearance where necessary. Other investigations include X-ray of the chest, electrocardiography and echocardiography.

1.3 LIPIDS

The word 'lipids' has long been used to denote a chemically heterogeneous group of substances, having in common, the property of insolubility in water but solubility in non-polar solvents such as chloroform, hydrocarbon, hot alcohols ether methanol, acetone or benzene. In addition, they are components of plants and animals. With the development of more efficient extraction procedures, especially chromatographic methods, the heterogeneity of lipid extracts has been established (Anekwe, 2002).

The dietary fats come from two major sources namely: vegetables and animals.

The animal sources include beef, liver (which contains adequate amount of phospholipids, cholesterol and fatty acids e.g. arachidonic acid) and intestines. Milk and ice cream are important sources of dietary triacylglycerol (triglyceride).

Similarly, the lettuce in salads or the cabbage provide a good supply of polyunsaturated fatty acid from the chloroplast's phospholipids and glycolipids. (Gurr and James, 1980). major plant sources of triacylglycerol include seed oils in the form of margarine, cooking oils, mayonnaises and salad creams.

The lipid content of these oils are almost entirely triacylglycerol (TAG). Milk contains different fatty acids depending on whether the

animal is a ruminant or non-ruminant e.g. human milk has a higher content of linolenic acid than cow's milk (Gurr and James, 1980).

Classification of Lipids

There are many types of classification of lipids, however, the most convenient and the one which has been most widely employed is the one originally suggested by Bloor (1925, 1926). This is as follows:

- (a) **Simple Lipids** e.g. neutral fats (esters of L- glycerol and fatty acids such as palmitic, stearic, oleic and linoleic acids) and waxes – true waxes; cholesterol esters, vitamin A esters or vitamin D esters.
- (b) **Compound or conjugated Lipids:** This group is distinguished by the presence in the molecule of products other than fatty acids and alcohol. In some cases, no alcohol is present and the fatty acids are combined in an amide – linkage rather than as esters. Examples are the phospholipids - lecithin, cephalin, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin and phosphatidic acids. (Folch 1941, Wolley 1943 and Klenk 1965). Cerebrosides (galactolipids and glycolipids) and sulpholipids.
- (c) **Derived Phospholipids:** This class includes derivatives of the first 2 classes of lipids obtained by hydrolysis and which still retain the lipid characteristics. They contain the important components of the non-saponifiable extract. Example include fatty acids, alcohols which could be straight – chain alcohol, sterols or alcohols

containing the β -ionone ring e.g. vitamin A, cryptoxanthin lutein and zexanthin. Hydrocarbons such as aliphatic hydrocarbon, carotenoids and squalene belong to this group. Others are vitamin B, vitamin E and vitamin K.

1.4 LIPOPROTEINS

Many proteins have a tendency to form complexes with other types of compounds, including lipids. There are two main types of such protein – lipid complexes or molecules called LIPOPROTEINS and PROTEOLIPIDS.

Lipoproteins in general, have the solubility properties of proteins i.e. they are soluble in water or weak salt solutions. On the other hand, proteolipids have the solubility properties of lipids. Furthermore, they are not present under normal conditions in blood plasma; however, practically all the plasma lipids are found in the lipoprotein (Folch et al 1963). Thus, lipoproteins are macromolecular complexes of lipids with specific proteins called apoprotein (Macheboeuf, 1929, Adair and Adair 1943).

During the last two decades, there has developed a steadily increasing interest in and literature on human serum lipoproteins and their relationship to health and disease. This followed the pioneering work of Macheboeuf (1929) who first isolated lipoproteins as an entity from horse serum by ammonium sulphate and sulphuric acid (0.2N) precipitation.

One of the principal differences between the lipoprotein classes is their relative lipid content i.e. their lipid to protein ratio. This ratio has been shown to vary from 99:1 (as in chylomicrons) to 1:99 (as in albumin – free fatty acid complex). In fact, this ratio classifies the densities of lipoproteins into different classes by ultracentrifugation. On the basis of density, therefore, lipoproteins can be divided into low and high density lipoproteins (Figure 2).

Figure 2: Composition of Human Serum Lipoprotein classes

Source: Gurr and James A.T., 1980. 185

Lipoproteins that float in a solution of 1.063g/ml density in the ultracentrifugal fields are considered to be low density lipoproteins (LDL) while those with a tendency to sediment are high density lipoproteins (HDL). LDL are of two classes: proper LDL in which density, $d = 1.006 - 1.063\text{g/ml}$ and very low density lipoproteins (VLDL) in which $d < 1.006\text{g/ml}$.

HDL are represented in plasma by two well defined and extensively studied classes: HDL₂ in which $d = 1.063 - 1.125\text{g/ml}$ and HDL₃ in which $d = 1.125 - 1.210\text{g/ml}$. However, separation of lipoproteins based on the charge they carry (electrophoretic technique) has been shown to give the following fractions:

- (i) ∇ -lipoprotein
- (ii) \exists -lipoprotein
- (iii) Pre- \exists -lipoprotein
- (iv) Chylomicrons or intestinal VLDL.

There is a classification of plasma lipoproteins based on the protein component which has been classified into three lipoprotein families:

- (a) Lipoprotein family A (LP – A)
- (b) Lipoprotein family B (LP – B)
- (c) Lipoprotein family C (LP – C)

These are characterized by the presence of apoproteins A, B and C respectively. Eight types of apoproteins have been isolated and characterized thus far: apo A – I, A – II, B, C – I, C – II, C – III, D and E.

Table 1: Apoproteins of the Lipoprotein Classes

Lipoprotein Class	Polypeptide Class	Importance	C-terminal amino acid
HDL	Apo – A1]	Major	glutamine
	Apo – A2]		glutamine
	Apo – C1]	Minor	serine
	Apo – C2]		glutamine
Apo – C3]	Alanine		
	Apo – C4]	Alanine	
LDL	Apo – B		
VLDL	Apo – C1]	Major	serine
	Apo – C2]		glutamic acid
	Apo – C3]		Alanine
	Apo – C4]		Alanine
	Apo – A]	Minor	Alanine
	Apo – B]		glutamine
Chylomicrons	Apo – C	Major	serine glutamine Alanine
	Apo – B] Apo – A]	Major	serine glutamine

Source: Gurr and James 1980. 188

DEFECTS IN LIPOPROTEIN METABOLISM:

Diseases of lipid metabolism generally seem to be due either to a defect leading to excess storage of the lipid, obesity could also be thought of in this way or to a defect in their transport. Since transport largely involves lipoprotein, the transport diseases fall into two main groups characterized either by an abnormally high or low level of a particular lipoprotein in the bloodstream. These are hyper- or hypo-lipoproteinaemias respectively.

HYPERLIPOPROTEINAEMIA:

This disorder may be secondary, that is the abnormal pattern is due to altered metabolism stemming from another recognizable disease which is treated, will lead to a normalization of the lipoprotein pattern. All remaining disorders are primary and in turn, may be heritable (*genetic in origin*) or induced by dietary imbalance.

Frederickson has provided us with a shorthand system to describe five types of hyperlipoproteinaemia, but it should be remembered that because of the gross heterogeneity of lipoprotein themselves, not all types of abnormality fall into these classification. The characteristics of these lipoprotein disorders are summarized in table 2.

Table 2: The Hyperlipoproteinaemias

Classification	Raised Lipids	
Cause		
Type I	Triacylglycerols free cholesterol	Lipoprotein lipase deficiency
Type II	Cholesterol	Unknown
Type III	Cholesterol Triacylglycerol	Unknown
Type IV	Triacylglycerol	Unknown
Type V	Triacylglycerol Cholesterol	Combination of causes of Type I and Type IV

Source: Gurr and James A. T. 1980. 196 – 97

HYPOLIPOPROTEINAEMIA:

These conditions in which lipoprotein levels are below normal or there is a complete absence of one lipoprotein class, are much rarer than those characterized by raised lipoprotein levels. They include:

- (a) Abeta – lipoproteinaemia (familial LDL deficiency).

NATURALLY-OCCURRING LIPOPROTEINS – ‘Membrane Types’:

Although in the past, there have been theories to explain the concentration difference inside and outside cells without invoking a physical barrier, and even in modern literature one can find such ideas, the idea of a membrane which preserves the integrity of the cell and regulates transport processes is generally accepted. The presence of lipids in cellular membranes was first proposed in the 19th century to account for the observed relationship between lipid solubility and the velocity of penetration of compounds into cells. Now that techniques for isolating membrane material free from soluble cytoplasmic components are readily available in many cases: the presence of lipid is in no doubt and the complete chemical composition of many membranes can be determined. This is an important preliminary in trying to determine the structure of the membrane - one of the greatest pre-occupation of modern biology.

1.5 Lipids in Nutrition, Health and Disease:

Lipids are structural materials, reserve fuels, barriers to the environment, vitamins, emulsifiers, flavours and aroma compounds, solvents. The animal body contains a variety of lipids serving a number of functions e.g. the polar lipids, phospholipids and glycolipids, form an important part of the structural material of cell membranes in all tissues. The only important neutral lipid here is cholesterol.

Furthermore, the TAG molecules constitute a pool of stored energy. Primitive man, without access to a deep freezer and uncertain of a constant food supply needed a portable reserve of fuel. Fat in the form of TAG was ideal because of its high energy value per gram and because it could be stored in compact anhydrous form. In today's affluent Western society, the need for fuel reserves has disappeared, but now that eating fulfils a social rather than a physiological role, and western man, moreover leads a more sedentary life, consumption of unwanted energy has led to a major social and medical problem, obesity. Skin waxes represent part of our barrier to the outside environment.

There are two ways in which the animal body can satisfy its needs for these lipids. It can either synthesize its own from the other raw materials – carbohydrate and protein available to it, or it can directly use dietary lipids with a minimum of retailoring.

It would be a mistake to think that the only role of dietary lipids is a structural or storage one. A completely fat-free diet would be extremely unpalatable, and it would probably, in view of the vitamin-like nature of linoleic acid, be lethal. Fats also affect the texture of food. Because of their detergent-like properties, polar lipids not only help in the emulsification of food in the gut but are used by manufacturers to aid the emulsification of foods before they are eaten, especially those whose texture is important to the palate e.g. spreads, creams and deserts.

Fats also play a solvent-like role, many vitamins are fat-soluble (vit. A, D, E, K) and are more efficiently absorbed if there is sufficient fat in the diet to 'carry' them. Vitamin E is a natural antioxidant and may exert a protective action against damages caused by peroxidation of membrane polyunsaturated fatty acid.

Finally, much of the aroma that stimulate our appetite for food comes from volatile breakdown products of lipids.

The Metabolism of Lipids in Relation to Disease

Toxic Effects of Lipids: Some dietary fats have harmful metabolic effects e.g. cyclopropene and long chain monoenoic fatty acids. The cyclopropene fatty acid inhibit desaturation of steric to oleic acid. The effect is to alter membrane permeability as demonstrated by 'pink-white' disease. If cyclopropene acids are present in the diet of the laying fowl, the permeability of the membrane is increased, allowing release of substrates including pigments into the yolk. By far the most important

edible oil containing cyclopropene acids is cottonseed oil in which the level ranges from 0.6 – 1.2%, although due to processing, the oil as actually eaten probably contains 0.1 – 0.5%.

Man has been eating cottonseed oil for years in such products as margarine, cooking oils and salad dressing but the intake of cyclopropene is very small. On this basis it is presumed that low levels have no adverse effects but whether prolonged ingestion of larger amounts by human would be deleterious is not known.

Fatty acids may play a role in regulating the body's immune defence system. Disturbances in lipid metabolism are involved in the aetiology of a number of major 'western' disease such as ischaemic heart disease, diabetes, obesity and perhaps cancer.

1.6 STERIODS

Steroids are nonsaponifiable lipids with specialized functions. Saponifiable lipids could be hydrolyzed by heating with alkali to yield soaps of their fatty acid components. However, cells also contain non-saponifiable lipids which contain no fatty acids and thus cannot form soaps and there are two major classes called steroids and terpenes.

Steroid denotes any substance which is a derivative of the condensed ring system cyclopentanoperhydro phenanthrene. Steroids are those members of the steroid class which contains a hydroxyl group capable of forming an ester. They are steroid alcohols. In a great many organisms and tissues, sterols exist as mixtures of the free alcohols and their

long chain fatty esters. Cholesterol is the major sterol of mammals, thus most of our knowledge is in cholesterol ester metabolism.

Cholesterol was discovered as a major component of gall stones in the 18th century by the French Chemist Chevreul and partly characterized it in 1816 and called it cholesterine. Later it was shown to be present in alcoholic extracts of blood and in 1859 Berthelot identified it as an alcohol and prepared cholesterol esters by heating the sterol with fatty acids at 200°C. In 1895, cholesterol palmitate and stearate were crystallized from extracts of the serum of dogs and other animals.

Figure 3: Structure of Cholesterol ester

Where: RCO = C16.0
 H C18.0
 ..O C18.1
 C18.2

1.7 CHOLESTEROL

It is widely distributed in all cells of the body but particularly in various tissues. It is the parent compound of all steroids synthesized in the body. It occurs in animal fats but not in plants, fungi or yeast. It is designated as 3-hydroxy-5,6-cholestene. Cholesterol as the most abundant sterol in animal products, occurs in meat, dairy products and egg yolk. It is found in all the lipoproteins of plasma, but mainly in the β -lipoprotein. However, much of the cholesterol in the body arises by synthesis, largely in the liver and only very little is absorbed from the intestine. The amount of cholesterol that is added daily to the body pool is balanced by an equivalent excretion in the bile, part is excreted as sterol and may be reabsorbed while part is degraded by the liver to bile acids and bile salts.

Cholesterol is always present in normal adult serum (3.5 – 6.5mmol/L). About 55% of total cholesterol of serum or plasma is composed of cholesterol itself and its esters and 15% consists of cholesterol derivatives such as 7-dehydrocholesterol. The total cholesterol of red blood cells is some 10 – 30% less than that of serum.

Control of Cholesterol Biosynthesis

Cholesterol biosynthesis is also controlled by the concentration of a specific protein-sterol carrier protein which binds the water insoluble intermediates of the sequence and thus makes them more readily available for the subsequent enzymatic steps.

The rate of cholesterol biosynthesis is altered not only by tissue levels of cholesterol and other steroids, but also by fasting, diurnal variations in food intake and in cancer-bearing animals. It is also inhibited when certain cholesterol – containing plasma lipoproteins bind to specific receptors on cell surfaces.

ATHEROSCLEROSIS

It is a disease of the intima of the arteries, especially of the large arteries, that leads to fatty lesions called atheromatous plaques on the inner surfaces of the arteries. The earliest stage in the development of these lesions is believed to be damage to the endothelial cells and underlying intima. The damage can be caused by physical abrasion of the endothelium by abnormal substances in the blood, or even by the effect of the pulsating arterial pressure on the vessel wall.

Once the damage has occurred, smooth muscle cells proliferate and migrate from the media of the arteries into the lesions. Soon thereafter, lipid substances, especially cholesterol begin to deposit from the blood in the proliferating muscle cells in the form of cholesterol esters, forming the atheromatous plaques. Because these plaques contain so

much cholesterol, they are frequently called simply cholesterol deposits. In the later stages of the lesions, fibroblasts infiltrate the degenerative areas and cause progressive sclerosis (fibrosis) of the arteries. In addition, calcium often precipitates with the lipids to develop calcified plaques. When these two processes occur, the arteries become extremely hard and the disease is then called arteriosclerosis or simply hardening of the arteries.

Obviously, arteriosclerotic arteries lose most of their distensibility and because of the degenerative areas, they are easily ruptured. Also, the atheromatous plaques often protrude through the intima into the flowing blood and the roughness of their surfaces causes blood clots to develop, with resultant thrombus or embolus formation.

Almost half of all human beings die of arteriosclerosis. Approximately two-third of these deaths are caused by thrombosis of one or more coronary arteries and the remaining one third by thrombosis or haemorrhage of vessels in other organs of the body – especially the brain, to cause strokes, but also in the kidneys, liver, GIT, limbs and so forth.

Despite the extreme prevalence of atherosclerosis, little is known about the cause. Therefore, it is necessary to outline the general trends of the causes and mechanism of formation.

CAUSES:

It is associated with abnormalities of lipid metabolism, but it is also exacerbated by almost any factor that injures the arterial wall. In particular, increase blood cholesterol is often related to atherosclerosis. But perhaps equally as important might be some undiscovered third factor that is inherited from generation to generation which causes increased arterial intimal degeneration or increased rate of cholesterol deposition in the arterial walls irrespective of the blood cholesterol concentration.

Mechanism: In the past, it had been believed that all that was necessary to cause atherosclerosis was to increase the amount of cholesterol circulating in the blood. However, the resulting deposits do not lead to subsequent arterial wall fibrosis and death of the animal. Furthermore, it has been difficult to achieve such deposits in carnivorous animals such as the dog, except by feeding extreme amounts of cholesterol and also removing the thyroid gland to prevent normal utilization of cholesterol.

Therefore, in recent years much more emphasis has been placed on the initial endothelial and intimal lesions as the primary cause of the ultimate atherosclerotic plaques.

Almost any factor that can cause damage to the endothelial cells will lead to the following sequence of events. First, platelets adhere to the endothelium, next, the platelets dissolve and some factor from the platelets causes proliferation of the sub-lying smooth muscle cells; these

then infiltrate the damaged region. Subsequently, cholesterol in ester forms and other lipids infiltrate the lesion until eventually lesions typical of human atherosclerotic plaques develop. The severity of these lesions is enhanced in the presence of hypercholesterolemia.

Atherosclerosis in the human being: Atherosclerosis is mainly a disease of old age, but small atheromatous plaques can almost always be found in the arteries of young adults. Therefore, the full-blown disease is a culmination of a lifetime of vascular damage and lipid deposition rather than deposition over a few years.

Far more men than women die of atherosclerotic heart disease. This is especially true of men younger than 50 years of age. For this reason, it is possible that the male sex hormone accelerates the development of atherosclerosis or that the female sex hormone protects a person from atherosclerosis. Indeed, administration of oestrogens to men who have already had coronary thrombosis has decreased the number of secondary coronary attacks in some clinical trials. Furthermore, administration of estrogen to chicken with atheromatous plaques in their coronaries has in some instances actually caused the disease to regress.

Atherosclerosis and atherosclerotic heart disease are highly hereditary in some families. In some instances this is related to an inherited familial hypercholesterolemia, the excess cholesterol occurring almost entirely in the low density lipoprotein. This probably results from lack of a lipoprotein receptor substance on the liver cell membranes that

recognizes the LDL and causes them to adhere to the cells. Normally, this adherence is required before the lipoproteins can deliver their load of cholesterol to the liver cells. In the absence of this, cholesterol can only leave the liver cells and the internal feedback mechanism of the liver cells causes prolific products of cholesterol, adding to that already in the LDL. Homozygous persons with this disease rarely live beyond the age of 20 years.

In other persons with hereditary atherosclerosis, the blood cholesterol level is completely normal. Inheritance of the tendency to atherosclerosis is sometimes caused by dominant genes, which means that once this dominant trait enters a family, a high incidence of the disease occurs among the offsprings.

Human beings with severe diabetes or severe hypothyroidism frequently develop premature and severe atherosclerosis. In both these conditions, the blood cholesterol is greatly elevated, which is at least part of the cause of the atherosclerosis.

Another disease associated with atherosclerosis in human beings as well as in experimental animals is hypertension; the incidence of atherosclerotic coronary heart disease is about twice as great in hypertensive people as in normal persons. Though the cause is unknown it possibly results from pressure damage to the arterial walls with subsequent deposition of cholesterol esters.

1.8 ELECTROLYTES

These are compounds which give rise to charged particles or ions in solution. When placed in an electrical field, positively charged ions or cations migrate to the cathode whereas negatively charged ions or anions migrate to the anode.

In clinical chemistry, the term electrolyte is taken to refer to inorganic ions only. Among this group, the physiological cations (H^+ , Na^+ , K^+ , Ca^{2+}) and the physiological anions (HCO_3^- , Cl^- , inorganic phosphate) are measured most often.

In practice, a request to the laboratory for electrolyte usually means Na , K^+ , Cl^- and HCO_3^- . Intracellular and extracellular concentrations of electrolyte may differ considerably thus the major extracellular electrolytes are Na^+ , Cl^- and HCO_3^- whereas the major intracellular electrolytes are K^+ , Mg^{2+} , PO_4^{3-} and SO_4^{3-} .

1.9 URINALYSIS

Urinalysis, with reference to proteinuria, glycosuria, haematuria and other relevant abnormal urinary constituent levels is often among the most commonly requested laboratory investigations in hypertensive. The usefulness of these parameters as diagnostic tools is still largely unclear. Harrington et al, 1973, reported that amongst other causes of non-essential hypertension includes the renal causes which can be attributed to kidney tumours, cyst or pyelonephritis. Symptoms of this renal cause range from mild to severe proteinuria, haematuria and renal failure. Early

detection and management of hypertension is of vital importance in order to reduce incidence of cardiovascular failure, stroke and possibly sudden death.

For proper management, such basic issues as cost of therapy, types of antihypertensives being used, patients co-operation among other factors must be considered.

The physical and chemical properties of urine have been recognized as an important indicator of health. In a routine urinalysis, it is required that urine colour, appearance, volume, odour, specific gravity, pH, protein, glucose, ketone bodies and occult blood be done as a screening test which should then be subjected to other quantitative measurements where some of these analytes are found positive.

The effect of antihypertensives on the renal physiology of hypertensive patients is better understood when the function of urine and maintenance of body homeostasis is fully examined.

THE KIDNEY

The kidneys are paired bean-shaped organ in the posterior part of the abdominal cavity on each side of the spine. The right kidney is located a little lower than the left, due to the space occupied by the liver. Each kidney is in a capsule of fibrous tissue, which is easily stripped off (Baker and Silverton, 1985). Underneath the capsule lies the cortex, followed by the medulla, which is made up of renal pyramids, then hilum where the renal arteries and nerves enter and the renal veins leave the kidney.

The basic unit of the kidney is the nephron. There are about one million nephrons in each kidney, each consist of a blind end tube. The nephron consists of a capillary network, called the glomerulus, and a long tubule which is divided into three parts, the proximal convoluted tubule (PCT), the loop of Henle (LH) and the distal convoluted tubule (DCT). Each nephron empties into a collecting tubule to which other nephrons are connected. The glomerulus and the convoluted tubules are located in the cortex of the kidney, while the loop of Henle extends down into the medulla.

The kidneys receive a large blood supplies from the renal arteries which arise from the abdominal aorta (Stewart and Dunlop, 1964). Approximately 20 – 25% of the blood that leaves the left ventricle of the heart enters the kidneys by way of the renal arteries. This means that in a normal adult, the blood passes through the kidneys at a rate of about 1200ml/min. After the renal artery enters the kidney it breaks up into smaller branches until thousands of tiny arterioles are formed. Each afferent arteriole then forms the capillary network of glomerulus. The glomerulus is surrounded by a structure called the Bowman's capsule, and the space that is formed between the capsule and the glomerulus is the Bowman's space.

The pressure of the blood within the glomerulus forces water and dissolved solutes with a molecular weight of less than 50,000 through the semi-permeable capillary membrane and into the Bowman's space

(Shaw and Benson, 1974). The remainder of the blood including blood cells, plasma proteins and large molecules leaves the glomerulus via the efferent arteriole and enters a second capillary network called the peritubular capillaries which surrounds the tubules.

FUNCTIONS OF THE KIDNEY

The basic functions of the kidney include:-

1. Maintenance of salt and water balance and control of osmolality and acidity of the blood.
2. The kidney enables the body to retain essential plasma constituents such as glucose, metabolites such as urea, uric acid, creatinine, sulphates and guanidine derivatives.
3. Excretion of drug metabolites and toxins.
4. Regulation of acid-base and electrolyte balance
5. Maintenance of body's internal environment (Homeostasis).
6. The kidney also undertakes endocrine functions such as the production of renin, by the juxtaglomerulus, prostaglandins and erythropoetin for production of blood. This accounts for presence of anaemia in renal failure. It also converts 25-hydroxycholecalciferol (25-(OH) D₃), to 1,25 dihydroxylcholecalciferol, which is the active form of vitamin D as well as the degradation of such hormones as insulin, glucagons and Aldosterone .
7. Formulation of urine and removal from the body:

Of these functions, the pronounced one is the formation and removal of urine and this involves the following processes:-

- (a) Glomerular filtration
- (b) Tubular reabsorption
- (c) Selective secretion

URINALYSIS: Principle and Procedure

The chemical investigation of urine constituents is a very simple and useful tool in assessing the overall function of the kidney in hypertensives. This is because most antihypertensives are a combination of diuretics with alpha or beta adrenergic blockers, angiotensin antagonists (Grimm et al, 1981).

For many years diuretic agents have constituted the standard initial drug therapy for hypertension. Numerous short and long acting diuretic agents became available to the physician and very few were associated with obvious clinical problems, except for hypokalemia and hyperuricaemia, all of which were considered either inconsequential or readily correctable (Culter 1983).

Because of the effectiveness of diuretics and the ease of treatment with these agents, many clinicians today still use them as first-step therapy for hypertensive patients (Cutler 1983). However, in view of recent findings with regard to metabolic effects, including newer hazards caused by hypokalaemia, and the adverse effects of these drugs on cholesterol ratio, the question arises as to whether the same results could not be

obtained from other drugs without incurring the risks posed by the diuretics (Lowenstein et al, 1982). Grimm et al 1981 recently showed that hydrochlorothiazide and chlorthalidone increase plasma triglycerides and total cholesterol in mildly hypertensive patients.

In view of the above side effects posed by the use of diuretics and their effect on the renal physiology, it is necessary to, regularly, in the course of diagnosis and prognosis of hypertensive patients, request for analysis of the urine sample of such patients as a guide towards changing both type and/or dosage of such diuretics or calcium channel blocker/diuretic combination.

PROTEINURIA AND HYPERTENSION

Proteinuria is probably one of the single most important indicator of renal disease. Proteinuria may at times reflect extra renal disease rather than intrinsic renal disorders (Ames, 1977).

There are two main mechanisms by which proteinuria can occur: Glomerular damage or a defect in the reabsorptive process of the tubules (Laurine, 1983). In glomerular damage there is increase permeability of the capillary walls, thereby allowing large molecules such as albumin to pass through and be seen in urine. This is seen in such conditions as glomerulonephritis, hypertension, pregnancy and diabetes mellitus (Laurine, 1983). Diminished tubular reabsorption presents with tubular proteinuria as seen in tubular acidosis, pyelonephritis, Wilson's disease and Fanconi syndrome (Laurine, 1983).

The concentration of protein in the urine is not necessarily indicative of the severity of renal disease. Proteins with large molecular weights are generally cleared at a lower rate than low molecular weight proteins and so it is possible to predict the type of renal disease by the amount and size of the proteins that are present (Wilson, 1975).

Severe, mild and minimal proteinuria all have different significance in evaluating renal diseases. Severe proteinuria ($> 3.5\text{g/day}$ (Kissner, 1979, Papper 1978) is characteristically seen in patients with glomerulonephritis, hypertension and amyloid disease.

In 1984, Mogensen described microalbuminuria as a predictor of mortality and morbidity in cardiovascular diseases. The reason for this remain speculative.

Jenczto et al in a study conducted in Poland analyzing cardiovascular fatalities over 10 years in over 5000 stress diabetic individuals aged between 18 and 67 years in 1991, demonstrated that hyperglycaemia, proteinuria and arterial hypertension among other diseases were mostly responsible. They also observed that proteinuria and hypertension carried greater risk in females than in males.

Proteinuria may be absent in some cases of renal disease. Examples include obstruction by kidney stones and tumours, congenital malformation of the kidney and during certain phases of acute and chronic pyelonephritis (Bradley et al, 1979; Kurtzman and Rogers, 1974).

Laurine (1983), also observed that the presence of protein in urine does not necessarily indicate the presence of a renal problem unless the existence of fever or emotional stress has been ruled out. This benign proteinuria also occurs during salicylate therapy, after exposure to cold and strenuous physical exercise.

GLYCOSURIA AND HYPERTENSION

Glycosuria is dependent upon the blood glucose level, the rate of glomerular filtration and the degree of tubular reabsorption among other factors. Generally, glycosuria occurs whenever the blood glucose level exceeds the reabsorptive capacity of the renal tubules. (Laurine, 1983). Renal glycosuria may occur in normal individuals thus permitting some glucose to spill into the urine. Diabetes mellitus – a pathological condition is the chief cause of glucosuria. This condition is characterized by a marked elevation of blood sugar level and an increase in urine volume.

The incidence of hyperglycaemia in hypertensives has been studied extensively. The presence of hyperglycaemia in stroke patients always creates a problem of determining a cause – effect relationship. This has led to the classification of some diabetic patients as “diabetes mellitus following stroke” (stress diabetes).

The hyperglycaemia in shock and acute stroke is also thought to be secondary to raised catecholamine levels (Jordan et al, 1972). Adrenaline causes hyperglycaemia by its action on the liver and also by depressing insulin release from the pancreas. (Mayer et al, 1973).

Hyperglycaemic responses are seen in hypertensive stroke patients with cerebral haemorrhage. Lokrou. et al, 1987 in a study observed that the prevalence of hypertension in diabetes and/or diabetes in hypertensives varies between 10 – 49% with a mortality rate in one series of 11.8% in Africa.

Furthermore, the use of diuretic and loop diuretics has been known to cause hyperglycaemia when used in high doses. The administration of thiazide diuretics or steroids can also result in the impairment of carbohydrate metabolism with resultant hyperglycaemia.

However, since diuretics are indispensable, there is need to use combination of several antihypertensive agents and to occasionally check the urine of hypertensive patients for the presence of glucose.

HAEMATURIA AND HYPERTENSION

The presence of blood in urine samples of hypertensive subjects has long been recognized. Harrington et al 1973 noted that symptoms of hypertension of renal origin include proteinuria, haematuria and renal failure. Laurine 1983 observed that a highly alkaline urine or urine sample having very low specific gravity (< 1.007) can cause red cells to lyse thus releasing their haemoglobin into the urine. There is some controversy regarding the number of red blood cells that can be present normally and the number that constitute haematuria (Freni et al, 1977).

Red cells may enter the urine anywhere from the glomerulus to the urethra. Thus, haematuria can occur in renal (kidney) diseases such as

glomerulonephritis (haemorrhagic nephritis) malignant hypertension, polycystic kidney disease, renal tumours, renal vein thrombosis amongst others (Laurine, 1983). Renal calculi may cause intermittent haematuria (Lytton, 1977). The finding of red cells in the microscopic examination and/or proteinuria helps to pinpoint the haematuria as originating in the kidney (Laurie, 1983). In hypertension, there is necrosis of arterioles and vessels so affected rupture to cause bleeding (Strasser et al, 1987).

In the male, urethroprostatitis can cause bleeding into urine (James, 1976). In females, haematuria is mostly due to contamination from vaginal discharge depending on age grade and whether catheterization method was used during collection (Laurine, 1983).

Vigorous exercise by normal individuals can result in haematuria. Fred and Natelson 1977 and Fred 1978, has concluded that this type of bleeding originates in the urinary bladder, but the mechanism for this occurrence is still only conjectural. Haematuria in hypertension is mostly due to renal vein thrombosis, renal arterial lesion or glomerulonephritis (Laurine, 1983). The presence of blood in the urine sample of hypertensives is therefore an indicator of a renal complication which should be investigated further.

THE MAIN OBJECTIVES OF URINALYSIS IN THIS RESEARCH WORK

The main objective of urinalysis in this research work is to:

1. Establish the informative role contained in simple analysis of urine samples of hypertensive individuals in monitoring treatment/complications that may arise in the course of treatment.
2. Establish a statistical difference if any in the management of hypertensives patients attending two different health centres based on such factors as level of awareness, economic stability and compliance to instructions on the part of the patients.
3. Make recommendations based on information from urinalysis of these subjects in the management of essential hypertension.

1.10 BURUKUTU

Burukutu is an alcoholic beverage prepared from grain which is produced mainly in the Savannah areas of Nigeria. Such grains include guinea corn, maize, millet, etc. A laboratory study on the alcoholic beverage (Burukutu) by Ogbonna et al (1983) showed that the final product was dark-brown in colour, biochemical analysis showed that it contained 5.2% sugar, 0.48% ash, 0.5% protein, 7.0mg/dl of vitamin C and 4% alcohol.

An alcoholic therefore, is an excessive drinker whose dependence on alcohol has attained such a degree that he shows a noticeable mental disturbance or an interference with his mental and bodily health (WHO 1982). According to Kato G. U. (unpublished lecture notes 1997), alcoholism represents a chronic disorder brought about by an excessive and prolonged use of alcohol that leads to physical damage and

seriously affects the individual health and social life and may lead to physical damage.

Most European countries consider anybody with blood alcohol concentration of 100mg/100ml and above to be under the influence of alcohol. Various organ disorders and biochemical alterations are induced by either ethanol itself or acetaldehyde (a product of ethanol metabolism). Alcohol has also been implicated as a cause of several haemolytic syndrome, some of which are associated with hyperlipidaemia and severe liver disease.

It has been reported that one beneficial form of a lipid fraction – HDL is that it increases with small amount of alcohol consumption (Nancy and Lee, 1984). An elevated cholesterol level usually reflects on an increase of β -lipoprotein (LDL) relative to the less variable alpha group (HDL). (Wells et al 1967). The serum neutral fat (TG) level is markedly elevated levels of 1000 to 10,000mg/dl are reported even when the patients are on a fat free diet (Wells et al 1967). Total serum cholesterol is increased but the increase depends on age, sex differences and dietary habits.

Fatty liver is the initial lesion in the liver produced by alcohol abuse which results in accumulation of fat. The alcoholic fatty liver itself exhibits little inflammation. The normal liver weighs 2.0 – 3.5kg and in rare cases, the liver may massively enlarge and weigh up to 4.0 – 5.0kg. This is characterized by the presence of fat in almost every hepatocytes and

the formation of numerous fatty cysts. The study of lipoproteins and lipid metabolism has been stimulated chiefly by a desire to relate their various changes to atherosclerosis and therefore, to coronary heart diseases and cerebral vascular disease.

Text explanation and related physiology of cholesterol

Cholesterol is the main lipid associated with atherosclerotic vascular disease. The liver metabolizes the cholesterol to its free form (Don Ladig and Robin 1992). Nearly 75% of the cholesterol is bound to LDL and 25% to HDL. Because cholesterol is the main lipid involved in atherosclerotic disease, high levels of free and bound LDL are associated with increased risk for atherosclerotic vascular disease (Kaslcn, Deska and Pagona 1992).

Since the liver is required to metabolize ingested cholesterol products, sub-normal cholesterol levels are indicative of severe liver disease (Moby's diagnostic reference 1990). The purpose of cholesterol testing is to identify the patients at risk for heart disease. Cholesterol testing is usually done as part of a lipid profile testing which also evaluates lipoproteins and TG.

Factors affecting cholesterol levels are:

- (a) Pregnancy is usually associated with increase cholesterol levels
- (b) Drugs that may cause increased levels include beta-adrenergic blocking agents, oral contraceptives anabolic steroids.

(c) Drugs that may cause decrease levels include androgens, bile salt binding agents, MAO inhibitors, Neomycin (oral), Niacin and nitrates.

1.11 SMOKING

Literatures concerning the effects of smoking revealed that smoking increases activities of lipoprotein lipase and hepatic TG lipase; thus increasing catabolic rate from VLDL to IDL and LDL; decreasing the levels of HDL, consequently accelerate the development of atherosclerosis (Goto et al 1988).

Smoking is the practice of drawing into the mouth or nose, the fumes of a burning vegetable substance with narcotic, sedative or stimulant property; the chief substance thus used are tobacco, opium, Indian hemp. The carbondioxide due to smoking has an effect on the capillary permeability leading to secretion of catecholamines with their effect on hormone sensitive lipase.

Epidemiological studies on relation between smoking and incidence of coronary and cerebral arterial disease shows that incidence of the disease is significantly higher among smokers and the risk is increasingly higher among those who started smoking early (Goto et al 1988). The daily exposure to cigarette smoke in the absence of cholesterol supplement diets can help accelerate the development of atherosclerotic lesion.

Results of separate survey conducted in children and adolescents suggested that the lipid and lipoprotein response to cigarette smoking increased cardiovascular risk. Higher LDL and VLDL levels have been observed for white boys and white and black girls who smoke (Webber et al 1991). These findings indicate that initiating even modest levels of cigarette smoking could have a long term atherogenic effects (Webber et al 1991).

Statistics presented by Buschello et al (1983) showed that plasma lipids and lipoprotein were related to the number of cigarette smoked per day. Heavier smokers (> 25 cigarette sticks per day) had significantly lower HDL and significantly higher LDL; total cholesterol and plasma TG levels than those who smoked < 25 sticks per day.

This study on alcoholics and smokers is undertaken to establish the effect of these lifestyles on serum lipids with a view to unravelling disease associated with disturbed lipid metabolism in smokers and alcoholics.

1.12 AGE:

The terms ageing and senescence are familiar but not precise. Usually senescent and senescence are used when talking about the changes which occur during the period of obvious functional decline in the later years of an animal's life span.

There have been many formal definitions of ageing processes and senescence. Medawar (1957) suggested that senescence could be defined as 'the change of the bodily faculties and sensibilities and

energies which accompanies ageing and which renders the individual progressively more likely to die from accidental causes of random incidence. Strehler (1962) defined senescence as the changes which occur generally in the post reproductive period and which result in a decreased survival capacity on the part of the individual organism.

Ageing processes have been defined by Maynard Smith (1962) as those which render individuals more susceptible as they grow older to the various factors such as intrinsic or extrinsic, which may cause death. Comfort (1960) said ageing is an, increased liability to die or an increasing loss of vigour with increasing chronological age or with the passage of the life cycle.

Shock (1961, 1962) and Weiss (1966) considered ageing as the sum total of changes during an individual's life span which are common to all members of his species or strain.

From the definitions above, the following facts are obvious:

1. That the changes which occur during ageing are deleterious. They increase the chances that an animal will die. Ageing therefore involves a decrease in the ability of an animal to cope with its environment.
2. That the deleterious age-related changes are cumulative.
3. That the processes involved are common to all members of a species and are inescapable consequences of getting older i.e.

ageing and senescence are fundamental, intrinsic properties of living organism.

However, for the purpose of this study, ageing will be considered as 'any time-dependent changes which occurs after maturity of size, form or function is reached and which is distinct from daily, seasonal or other biological rhythm'. This presumably includes all of the post maturation changes in an individual including senescence.

There have been a number of hypothesis attempting to explain the existence of senescence in evolutionary terms, and these include:

- (a) The somatic mutation hypothesis
- (b) The autoimmune theory
- (c) The free-radical hypothesis
- (d) The error catastrophe hypothesis

There is no general agreement about the fundamental cause or causes of ageing. Although there are many different ideas about how and why it may happen, no single theory is capable of explaining all the known facts. The structural and functional interrelations of the various components of cells, tissues and organs may make the end results of different types of damage very similar.

1.13 MENOPAUSE

The World Health Organization (WHO) defined menopause as the permanent cessation of menstruation resulting from the loss of ovarian follicular activity. Bradley (2002) defined menopause as the transition

period in a woman's life when the ovaries stop producing eggs, menstrual activity decreases and eventually ceases, and the body decreases the production of the female hormones estrogen and progesterone. Menopause is often used in place of a condition known as climacteric i.e. menopausal transition.

Menopause could be:

- (i) Physiological
 - (ii) Premature
- (a) Physiological menopause is that which occurs around 50 years of age. This is as a result of gradual loss of ovarian activity and eventual termination of menses.
- (b) Premature or early menopause is when it occurs before 40 years of age. This could be as a result of autoimmune disorder such as chromosomal irregularity, oophorectomy and family history.

Symptoms of menopause either physiological or premature include "hot flashes" by some women (about 75%) which can last up to five minutes each and are characterized by red, warm skin and perspiration.

Other possible symptoms are fatigue, irritability, insomnia, nervousness, night sweat, loss of bladder control, inflammation of the bladder or vagina, pain during sexual intercourse, occasional dizziness, tingling sensation, muscle and joint aches and pounding heart beat.

Menopausal transition is that period beginning with the first indication of the approach of menopause and ending with the final

menses (Burger, 1993). It is important therefore, to appreciate that menopause refers only to cessation of menstruation and occurs during the climacteric phase.

This period is characterized by hormonal changes associated with a rapid decline in ovarian follicle numbers; marked hormonal fluctuations are observable in individual subjects and there is a substantial increase in follicle-stimulating hormone (FSH) levels as menstrual irregularity occurs (Burger, 1993).

Menopause and cardiovascular system:

Cardiovascular diseases (CVD) are often thought of as a disease associated with men rather than women, although epidemiological data do not support this view (Beale *et al.*, 1996). Not only is death from CVD greater in women than men, but for women, it is currently the commonest cause of death. In USA, CVD is responsible for the death of more women than cancer; accidents and diabetes combined (Eaker, *et al.*, 1993).

Since the original observation that CVD rarely affects women before the menopause, estrogen deficiency has been strongly implicated in the aetiology of the disease. Analysis of data from a large prospective cohort study of 212,700 women strongly supports the role of estrogen in reducing the risk for CVD (Colditz *et al.*, 1987). After adjusting for age and cigarette smoking, women who had undergone bilateral oophorectomy and who had not taken estrogen after the menopause were found to have a significantly increase risk of CVD (relative risk 2.2) whilst the post-

menopausal use of estrogen replacement therapy (ERT) appeared to counter this effect and reduce the risk to 0.9 (Colditz et al, 1987).

Metabolic effect of menopause on lipids and lipoproteins

Whilst profound effects of exogenous sex steroids on lipids and lipoproteins have been demonstrated, there has been more controversy about the effects of endogenous female hormone (Stevenson, 1996). This is partly due to the fact that age and menopause are closely related and many cross-sectional studies have not included sufficient numbers or a wide range to permit the age adjustments necessary to determine any independent effects of the menopause.

In a study of 542 healthy non-obese Caucasian female aged between 18 and 70 years Stevenson et al, (1993) found that the standardized mean values for total cholesterol and triglyceride, LDL and HDL3-cholesterol were significantly higher in post-menopausal women, whilst those of HDL2 cholesterol were significantly lower. A previous analysis by Kuller et al, (1990) demonstrated the striking effect of the menopause on HDL2-cholesterol in accordance with Stevenson et al, (1993). These various changes in lipids and lipoproteins are considered as potentially risky in terms of Coronary Heart Disease (CHD).

Large scale studies attribute 30 – 50% of the cardioprotective effect of estrogen to a beneficial effect on lipid profile (Stampfer et al, 1996).

Women aged 20 – 50 years have a more favourable lipid profile than men of a similar age (Lower LDL and higher HDL).

However, at menopause, there is an associated detrimental change in lipid profile namely an increase in LDL and a concurrent decrease in HDL (Stevenson *et al*, 1993). This change is reversed with ORT.

At all ages and in all countries, diseases of the cardiovascular system are major causes of death and disability; though the pattern varies strikingly between different countries and age groups especially in developed countries and recently in developing countries particularly in Nigeria, where hypertension is common; affecting more than 1 in 10 of the adult population (Abengowe 1980). It affects both males and females with a high incidence between the ages of 25 and 40 years in Tropical Africa compared with 35 – 60 years in Temperate climates (Akinkugbe, 1976).

Hypertension was once regarded as a rare finding in Black Africans (Donnison, 1929); however epidemiological investigations into cardiovascular and cerebrovascular diseases in Nigeria and in other parts of the African continent now recognize that hypertension is extremely common and endemic in most communities in Africa (Fraser, 1959, Campbell *et al* 1962; Akinkugbe 1976, Ordman, 1984).

The predisposing factor has been blamed on rural to urban migration, urbanization, stress, obesity and excessive salt intake (Seedat *et al* 1976, Oviasu *et al*, 1980). The cause of the high incidence and

prevalence of hypertension may not wholly be blamed on socio-environmental factors. The pathogenesis of hypertension must stem from some imbalance or disturbance of some normal metabolic processes that could affect the vascular system.

Blood Lipids in Hypertension

Although there is still no definitive evidence to show that lipids or abnormal lipid metabolism, are causative agents in the development of coronary artery diseases, there is no question that lipid levels in tissues are affected. Animal experiments with a number of species have shown that increased levels of both saturated fats and cholesterol in the diet give rise to elevated plasma cholesterol levels and to atherosclerosis (Day et al 1971).

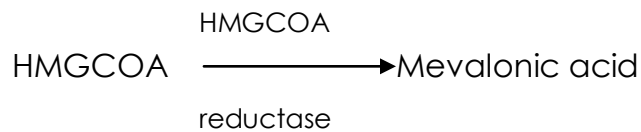
There is much argument as to whether dietary modification by increasing the level of linoleic acid and decreasing the level of saturated acids, is necessary or not. It is certainly true that plasma cholesterol levels can be lowered by such dietary changes and studies at clinics with a special interest in hyperlipidemia have shown that dietary modification can be quite effective and can be achieved very easily with readily available foodstuffs such as exchanging butter for "soft" margarines and using polyunsaturated cooking oil (Day et al 1971).

Cholesterol: This is a member of a large class of biological compounds called steroids. It is the most abundant sterol structure:

Other sterols found in mammalian tissues include dihydrocholesterol, 7-dehydrocholesterol and Bile acids. Mammals e.g. man can synthesize cholesterol in the body (liver mainly) using acetyl CoA molecules. It is unequivocally established that mevalonic acid, squalene and lanosterol are intermediates in the biosynthesis of cholesterol.

Regulation of Synthesis: Dietary cholesterol depresses liver synthesis of cholesterol but other tissues (all tissues except adult brain) which collectively synthesize more cholesterol than the liver, are not influenced by dietary cholesterol.

The enzymatic site of control (rate limiting step) is the reductase step at which the HMG CoA is converted to mevalonic acid (example of product inhibition of an enzyme sequence):-



HMGCOA reductase is an intrinsic membrane protein of the Endoplasmic Reticulum. The enzyme's active site extends into the cytosol. It uses 2 molecules of NADPH as the reducing agent and releases CoA, making the reaction irreversible.

This reductase reaction is irreversible and the inhibitory action of dietary cholesterol is directly on the enzyme activity and not due to repression of reductase synthesis.

Siperstein et al (1964) suggested that during the absorption – transport of dietary cholesterol, a specific cholesterol – containing lipoprotein is formed which functions as an inhibitor of the reductase. Bile acids have also been shown to inhibit hepatic cholesterol synthesis. Short periods of total fasting have been shown to affect hepatic cholesterol synthesis but not extra hepatic sites (Day et al 1971). However, some of Biochemical principles of the inhibitory mechanism which have been elucidated include:-

1. Feedback inhibition: Cholesterol is a feedback inhibitor of HMG CoA reductase, thus decreasing further cholesterol synthesis.
2. Hormonal regulation: HMG CoA reductase activity is controlled hormonally through a complex cascade of enzyme activations and inhibitions. The net effect is that glucagons favours formation of the inactive (phosphorylated) form of HMGCoA reductase and hence decreases the rate of cholesterol synthesis. In contrast, insulin favours formation of the active (unphosphorylated) form and results in an increase in the rate of cholesterol synthesis.
3. Sterol – mediated regulation of transcription:- The synthesis of cholesterol is also regulated by the amount of cholesterol taken up by the cells during lipoprotein metabolism. Chylomicron remnants internalized by liver cells and LDL internalized by cells of the liver and peripheral tissues provides cholesterol, which causes a

decrease in transcription of the HMGCoA reductase gene, leading to a decrease in de novo cholesterol synthesis.

4. Inhibition by drugs: Lovastatin and metastatin are reversible competitive inhibitors of HMGCoA reductase. They are used to decrease plasma cholesterol levels in patients with hypercholesterolaemia.

One important factor that influences the cholesterol content of a given individual is age. This distribution is typical of mammals and does not vary much from day-to-day or week – to – week. Plasma cholesterol is thus thought to be the causative agent in cardiovascular disease.

The total cholesterol (both free and esterified) in normal adult men ranges from 140 – 260mg/100ml of plasma i.e. 3.5 – 6.5mmol/L. Of the amount, most is in the esterified form. Although typical values are informative, it should be realized that plasma cholesterol varies widely between individuals; because of race as suggested by Siperstein and Fagan (1964).

In human infants, there is only about 35mg/100ml of plasma cholesterol, but this amount rapidly increases to about 150mg/100ml by the end of the first year of life. There is virtually no change until puberty is reached. Upon the onset of puberty, differences are noted between human male and female as ageing progress. In the male, it gradually increases till age 30 – 35 years, after which there is no clear change. In women, starting with puberty, plasma cholesterol varies with the menstrual

cycle; (increases during the follicular phase up till the point of ovulation but falls abruptly at ovulation; rises again slowly through luteal phase and falls abruptly just before menstruation) (Masoro, 1968).

In pregnant women, the level rises and reaches a maximum in the 8th month. At menopause and for the first decade thereafter, it rises to well above premenopausal values but then it begins to fall (Masoro, 1968).

Frederick Stare and collaborators (1966) reported the following complex relationship between dietary fat and plasma cholesterol:

1. Polyunsaturated fatty acids lower plasma cholesterol
2. Fats with monounsaturated fatty acids have no effect
3. Fats with certain saturated fatty acids e.g. myristic and palmitic acids increase plasma cholesterol.
4. Fats with longer-chain saturated acid e.g. stearic acid do not influence plasma cholesterol levels.
5. Plasma cholesterol levels rise markedly in hypothyroidism and is a diagnostic feature.

Total cholesterol is the standard lipid determination recommended in cardiovascular epidemiology for the following reasons:-

- (a) It correlates as well or even better than other lipid fractions with the prevalence of ischaemic heart disease.
- (b) It is known to be related to the risk of a future disease.
- (c) It is the fraction about which there is the most information

Lipids of Deranged Tissues

Considerable evidence has accumulated in recent years indicating that the biosynthesis of certain classes of lipids in deranged tissues is not under the same regulatory control as in normal tissues. Some evidence also exists to show that certain lipid classes are very useful in the regression of these derangements (Anekwe, 1982).

With regards to lipid metabolism and atherosclerotic tissues, much effort has concentrated on the effects of polyunsaturated fatty acids on plasma lipids. The work of Ahrens et al (1957) showed that it is not "animal" as compared to "vegetable" fats but saturated as compared to polyunsaturated fats which affect plasma cholesterol levels. Beveridge et al (1956) had earlier shown that corn oil, replacing part of the carbohydrate in a fat-free diet caused a plasma cholesterol lowering in excess of that achieved by the fat-free diet. Bronte-Steward et al (1956) demonstrated a positive effect of polyunsaturated oils if added to a diet containing animal fat and also showed that not only 18:2 fatty acids but also arachis oil and fats from marine animals could be used to lower plasma cholesterol levels. If the fats were hydrogenated the cholesterol lowering properties will be eliminated.

The mechanism of the action of polyunsaturated fatty acids on plasma cholesterol appears to be the increased excretion of acidic and neutral sterols in the presence of the polyunsaturated fatty acid (Anekwe, 1982). This had been shown earlier by Grandy (1975) and Nestle et al

(1973). Thus atherosclerotic tissues show regression of atherosclerosis in the presence of polyunsaturated fatty acids (Armstrong, et al 1970). Indeed, it is now accepted that diets in which part of saturated fats are replaced by PUFA appear to be useful and safe for long-term lowering of plasma cholesterol levels (Armstrong, et al 1970).

Studies on the lamellar structure of the abnormal Human serum lipoprotein by Hamilton et al (1971) showed that the electron microscopic image of whole serum from patients with cholestasis was strikingly different. This new structure appeared to be coin or disk – like. According to these workers, larger structures resembling myelin figures were also observed and often appeared with single disks and rouleaux; suggesting a structural transformation. Furthermore, they observed that VLDL and LDL were readily seen while HDL was not; suggesting that VLDL and LDL were larger in diameter (than normal size) and HDL was much smaller in size than normal size. This is in agreement with previous data by Eder, et al (1955, 1956) and Havel et al (1955).

The viscometric behaviour indicated that only moderately asymmetrical particles exist in the native serum of patients with cholestasis. The electron microscopic images are consistent with particles in the form of partially flattened vesicles, the walls of which are a continuous lipid bilayer of the width expected for an equimolar mixture of choline phosphatides and cholesterol. The unique disk like appearance

of the abnormal lipoprotein can be expected to facilitate investigation of its origin and metabolic fate.

Mineral Elements in Hypertension

Epidemiological studies by Adigun and Akinyanjuola (1989) suggest that hypertension is rare in populations whose habitual diet is low in sodium (Na) and high in potassium (K) while it is highly prevalent in societies that ingest diet with high Na and low K contents. Furthermore, this Na loading effect has been shown severally to cause no appreciable change in blood pressure in the salt-resistant rats but may raise blood pressure in the salt-sensitive rats (Adigun et al 1989a). This has also been shown in some individuals who are salt-sensitive (Sofola, 1991).

The precise mechanisms whereby dietary Na loading leads to the development and maintenance of hypertension in salt-sensitive animals and human subjects are yet to be established; though many have been proposed (Adigun et al 1989a; Poston 1987 and Akinkugbe, 1987). For instance, it has been hypothesized that Na loading may cause hypertension only in animals and human subjects with genetically determined susceptibility or defect (Akinkugbe), 1987 and Poston, 1987).

The genetic theory may also explain the observation that people with family history of hypertension are more susceptible in their blood pressure response to dietary Na (Strazzulo, et al 1983). Thus, in such individuals, a life-long abstinence from salt may prevent hypertension. On the other hand, in the absence of hereditary defect, the blood pressure

will remain normal inspite of high sodium chloride (NaCl) intake (Akinkugbe, 1987 and Strazzulo et al, 1983).

Studies by Poston (1987), Falase, (1987) and Dustan (1987) showed that Blacks respond differently to salt loading and showed differences in natriuresis when compared with the Caucasians, this observation provides an indirect evidence for the genetic susceptibility. However, the precise mechanism for the genetic defect is still unknown.

Other mechanisms proposed include:-

1. An abnormality of $\text{Na}^+/\text{H}_2\text{O}$ exchanges as the underlying kidney defect and is unmasked by high NaCl intake (Adigun et al 1989).
2. Abnormalities of transmembrane transport systems including membrane lipid content hypothesis (Bing et al 1986); Na/K Co-transport (Poston, 1987); Na^+/Na^+ or Na^+/H^+ exchange (Araoye, 1978 and Akinkugbe, 1987) and reduced Na^+ pump (Na^+ , K^+ ATPase) activity (Strazzulo et al 1983 and Skov, 1986).
3. Na^+ acting as a pressor agent through volume expansion (Chuwa, 1987 and Gray et al 1986) or through sympathoadrenal activation of renin – angiotensin system (Canessa, 1984 and Obel, 1989).
4. Activation of renin-angiotensin system (Canessa, 1984; Obel, 1989).
5. Prostaglandins (vasodilator natriuretic substances) and thromboxane (vasoconstrictor natriuretic substances), which show variable urinary level change with Na^+ loading (Chuwa, 1987 and Papanicolau et al 1985).

6. Deficiency of atrial natriuretic peptides or lack of sensitivity to the peptides (Papanicolau et al, 1985). This is an inherent abnormal pressure response originating from the CNS (Tobia, 1978) and resetting of baroreceptor activity in animals.

Of the many mechanisms proposed, the balance of experimental evidence appears to favour abnormalities of transmembrane transport systems, hypervolaemia and sympathetic activation.

In addition to the vital role trace metals play in enzymatic reactions, they (e.g. Zn^{2+} , Cu^{2+} , Fe^{2+}) have been critically examined and found to play some part in CVD (Osunkiyesi, et al 1986). Zn deficiency is known to cause among other things partial loss of taste sensation and thus a reduced threshold for salt taste has been found in the taste buds of some patients with essential hypertension (Russel et al 1983); causing excessive salt intake.

Iron deficiency can lead to blocking of haemoglobin (Hb) synthesis and other Iron – containing enzymes of respiration. With low levels of iron, the heart must sustain a greater cardiac output to provide tissues with adequate oxygen (Saltman, 1983). This resultant increased intravascular volume if sustained for long periods, may encourage the development of systemic hypertension (Haddy, 1983, Norman et al 1975).

Copper is important in the incorporation of iron into Hb; enhancing Hb synthesis and preventing the storage of iron in the liver (Saltman, 1983). Copper can thus indirectly affect blood volume.

Furthermore, Allen and Klevay (1978) showed that Cu deficiency causes hyperlipidaemia which in turn may cause impaired peripheral circulation. Also, this trace metal participates in tyrosinase and dopamine beta-hydroxylase – catalyzed reaction which are important in the synthesis of Catecholamines (Saltman, 1983). Excess of Cu may theoretically cause over-activity of the CNS with the ultimate development of systemic hypertension.

Indirect evidence for a deficiency of magnesium contributing to the development of hypertension in pregnancy has been suggested by the time-honoured specificity eclampsia (Saltman, 1983). Magnesium may therefore, like Ca^{2+} , be important in the development of systemic hypertension. However, magnesium levels do not appear to contribute significantly as a cause or effect of essential hypertension in the guinea savannah region of Northern Nigeria (Osunkiyesi, et al 1986).

Management of Hypertension

Among major risk factors for cardiovascular disease, hypertension is perhaps the most readily manageable. Management includes both medications and diet. Of recent, surgical management is another method through splanchnicectomy. However, side effects following splanchnicectomy may be quite disturbing to the patient (Cutler, 1983).

There has been a renewed focus on non-pharmacologic approaches to the control of hypertension. Disten (1984) urged that

therapies such as diet, exercise and behaviour modification be “pursued aggressively”.

Dietary Control of Hypertension

Nutritional therapy has proved to have a fundamental role in the care of hypertensive patients; thus avoiding where possible, some unwanted biochemical effects of drug treatment (Flamenbaum and Cohen, 1985). Thus, appropriate and prudent course of treatment is to implement dietary changes and to use drugs that do not have an adverse effect on lipid metabolism e.g. prazosin.

General energy nutrient balance: The ratio of protein carbohydrate and fat in the overall diet for a person with hypertension is an important consideration especially in the overall goal of weight management.

Suggested ratio by Adedeji and Onitiri (1990) include:-

1. that the patient should reduce his weight to normal or slightly below if obese or to 10% below his normal weight if not obese.
2. that carbohydrate should have the largest allowance (50 – 55%) of the total kilocalories with a large portion of complex carbohydrate.
3. that protein should make up about 15 – 20% (60 – 70g) and of high biological value.
4. that the diet should be moderately low in fat content, with a focus on unsaturated fat food forms.
5. that adequate vitamins and mineral elements must be included in the diet.

6. that sodium should be restricted, the degree of restriction to be determined by the physician.
7. that generous amounts of fruits and vegetables should be included in the diet.
8. that whole milk should be included in the diet.

In hypertensives patients vulnerable to K^+ deficiency, increased intake of K^+ - rich foods e.g. cooked meat (beef, liver); Fowl (Chicken, Goose); milk (whole milk, evaporated whole milk, powdered whole milk) and fruits (fresh grapes, mango, orange) should be encouraged (Adigun and Akinyanjuola, 1989).

The main source of dietary sodium is NaCl or common table salt. Others are baking powder and baking soda. In general, four levels of dietary sodium restriction have been used as basic food guides for deletion of higher salt or sodium foods by several workers (Adigun and Akinyanjuola, 1989; Sofola, 1991 and Araoye 1978):

- (i) Mild sodium restriction: 2 – 3g Na (70 – 130Meq)
 - Salt may be used lightly in cooking
 - No added salt is used
 - Foods in which salt is used as a preservative or a flavouring agent are removed e.g. pickles, olives, bacon, ham, chips and many other processed foods.
- (ii) Moderate sodium restriction: 1g Na (43.5Meq)
 - No salt is used in cooking

- No salt is added to the food
 - No salty foods are eaten
 - Vegetables with higher Na content are somewhat limited in use
 - Salt-free canned vegetables are substituted for regular canned ones.
 - Salt-free baked products are used
 - Meat and milk are used in moderate portions
- (iii) Strict sodium restriction: 0.5g Na (22Meq)

In addition to the above measures;

- Meat, milk and eggs are allowed only in small portions.
 - Vegetables with higher Na content are avoided.
- (iv) Severe sodium restriction: 0.25g Na (11Meq):

This level is rarely used because it is too highly limited to be practical and is not needed now with available drug therapy. Only low – sodium milk is used. Meat and eggs are allowed only occasionally.

1.14 AIMS AND OBJECTIVES OF THE RESEARCH WORK

Hypertension in the Nigerian predisposes to a high frequency of cerebrovascular disease other than through cerebral atherosclerosis (Osuntokun, 1977). The recent upsurge in hypertension in Nigeria is indeed an emerging epidemic of the so called Reaven Syndrome (metabolic syndrome or Syndrome X, (Reaven, 1988). This is as a result of the transformation of Nigerian community from a primordial agrarian and

exercise-intensive society to European type of industrialized and sedentary urban lifestyles.

Hence, the aims and objectives of this research work include the following:

1. To obtain certain biochemical parameters (urinalysis, lipid profile and electrolytes) which will enable one to explain the high prevalence and mortality rate of hypertension rather than the known atherosclerosis.
2. To be able to detect early enough based on objective (1) above, hypertensive – prone individuals, so as to prevent the devastating complications of the disease and the resultant casualty.
3. More recent comparative biochemical data on the Nigerian hypertensives are fragmentary. Adequately reliable and recent data should be of interest to the individual, medical personnel and the Government. This work is intended to be a contribution along this line.
4. To estimate the plasma lipid profile of adult Nigerian women within the age range 30 – 60 years.
5. To establish a pattern (true reflection) of lipid profile for the women of this age range.
6. Arising from objective 2 above, to estimate the age range at which menopause can set in among adult Nigerian women.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

1. Conc. sulphuric acid Diethyl ether, Glacial acetic acid, Petroleum ether (B.P. 40 – 60°C); phosphatidylcholine (lecithin) as lipid standard, Ammonium molybdate, conc. perchloric acid and Ethanol were obtained from the British Drug House (BDH) Limited, Poole, England.
2. Trichloroacetic acid and Ascorbic acid were obtained from Sigma Chemical Company, U.S.A.
3. Chloroform, methanol, acetic anhydride and copper sulphates were obtained from May and Baker Ltd., Dagenharm, England.
4. Phosphovanillin was obtained from Chemistry Department, University of Jos, Jos.
5. Reagents: Commercially prepared kits for lipid profile by BIOLABO were used.
6. Subjects: Adult women aged between 30 and 60 years, non-pregnant, non-hypertensive, non-diabetic and non-obese Nigerians from three hospitals were used.

All reagents used were of analytical grade.

Other materials used include test tubes and their stands, pipettes of different sizes, glass rods, water bath, sand bath, glass beads, filter paper (Whatman No. 1), Top bench centrifuge, Thin layer chromatography chamber, Development chamber, metallic iodine and grating spectrophotometer (CE 373 linear readout). Silica gel 60G Art 7731 No. 9628700 was used for coating the chromatographic plates (30g in 60ml of distilled water).

PREPARATION OF REAGENTS USED

1. Acetic acid: acetic anhydride reagent: 70mls of glacial acetic acid and 65mls of acetic anhydride were mixed together and stored in a brown bottle.
2. Ammonium molybdate reagent: 2.5g of ammonium molybdate were dissolved in 60ml of distilled water and then filtered through a filter paper (Whatman No. 1). The filtrate was transferred to a graduated flask of 100ml-capacity and labelled solution A.

25ml of distilled water was poured into a clean flask and 7.5ml of conc. H_2SO_4 added. This solution was poured into the solution labelled A and the mixture cooled to room temperature under running tap.

The mixture was finally made up to the 100ml-mark with distilled water. The reagent is usable within the period of a month.

3. Reducing agent: 1% solution of Ascorbic acid was prepared using 0.016% aqueous copper sulphate solution as solvent.
4. Vanillin – phosphate reagent: 4 parts (by volume) of conc. orthophosphoric acid was mixed with 1 part of 0.6% aqueous vanillin solution. The reagent was kept in a brown bottle at room temperature.
5. Phosphatidylcholine standard solution: Lecithin weighing 0.2026g was dissolved in 206ml of petroleum ether to make a concentration of 0.984mg/ml solution.
6. Lipid extraction reagent: The reagent was Ethanol: Diethyl ether (3:1 V/V).
7. Lipid purification reagent: This was 0.73% NaCl solution while chloroform: Methanol: 0.58% NaCl solution (3:48:47 V/V/V) was used in washing the purified lipid.
8. The trisolvent for the separation of the different phospholipids was chloroform: Methanol: Water (65:25:4 V/V/V). Tank time = 1 hour.
9. Trisolvent for the separation of the different Neutral lipids was petroleum ether: Diethyl ether: Acetate (80:20:1 V/V/V). Tank time = 35 minutes.

SOURCES OF SAMPLES

Two groups of samples (blood and urine) were obtained after an overnight fasting of 12 – 14 hours i.e. between 9.00am and 11.30am. The urine samples were collected into clean and sterile universal bottles.

One group of blood samples was obtained by venepuncture from patients attending the General Out-Patient Department (G.O.P.D.) of Jos University Teaching Hospital (JUTH), Jos; whose blood pressures were within the normal range (i.e. Normotensives) and do not present with any clinical signs and symptoms of hypertension. This is the control group.

The other group was obtained from patients attending the cardiology clinic of the Medical Out Patient Department (M.O.P.D.) of JUTH. They are the hypertensives (both newly diagnosed and those receiving treatment).

Elimination Criteria: In all the different groups any patient with clinical and Biochemical evidence of the following conditions was excluded from the study: Diabetes mellitus, past medical history suggestive of renal problems, or any other heart diseases, pregnancy, lactating or Nursing mothers and Hepatic diseases. Others include hepatic insufficiency hyperuricaemia, previous history of gout and evidence of secondary hypertension.

2.2 PROCEDURE

1. Total Cholesterol Determination: The method of Liebermann – Burchard reaction and enzymatic method were used.

(a) Libermann – Burchard Procedure:

Principle: The reaction of cholesterol with concentrated sulphuric acid (conc. H_2SO_4) to yield intensely coloured compounds – chiefly cholestapolyenes and cholestapolyene carbonium ions – has been widely used in the colorimetric assay of cholesterol. In the method employed, acetic acid and acetic anhydride are used as solvent and dehydrating reagents while conc. H_2SO_4 is used as a dehydrating and oxidizing reagent. A bright green colour appears which can be accurately measured spectrophotometrically at 570nm wavelength (Libermann, 1968).

The intensity of the coloured complex formed is proportional to the concentration of total cholesterol present in the serum.

Method: 3 test tubes were labelled Blank, Control and Test. To the tube labelled blank was added 0.2ml of distilled water and 5.0mls of acetic acid: acetic anhydride reagent. To the tube labelled control was added 0.2ml of cholesterol standard solution (200mg/dl) and 5.0mls of acetic acid: acetic anhydride reagent. To the tube labelled test was added 0.2ml of serum and 5.0mls of acetic acid: acetic anhydride reagent.

The test tubes were shaken vigorously and left for 5 minutes, then 1.0ml of conc. H_2SO_4 was added to each tube, shaken and left to stand for 10 minutes in a cold water bath.

The absorbance of each tube was read at 570nm wavelength using CE 373 spectrophotometer (linear read out).

Calculation: Calculation was done using the expression –

$$\frac{\text{absorbance of test}}{\text{absorbance of std.}} \times \text{conc. of standard}$$

and the result expressed in mg/dl cholesterol.

Conversion factor:

$$0.0259 \times \text{mg/dl cholesterol} = \text{mmol/L cholesterol}$$

METHODS

(a) CHOD – PAP kit method by Biolabo for serum total cholesterol estimation.

The kit has 3 vials:

- (i) Buffer solution (vial 1, 100mls): It contains phosphate buffer and chloro-4-phenol.
- (ii) Lyophilized enzymes (vial 2). It contains cholesterol esterase, cholesterol oxidase, peroxidase, cholic acid sodium salt and 4 amino-antipyrine.
- (iii) Cholesterol standard (vials 3, 5mls): Conc. = 5.17mmol/L

Preparations:

The entire content of vial 2 was transferred into vial 1 and stood for 10 minutes before mixing. This is the working reagent and is stable for at least 1 year at 2°C – 8°C.

(b) GPO kit method for serum Triglyceride estimation by Biolabo. This kit has 3 vials:-

- (i) Vial 1 – Buffer solution – 100mls: It contains 4-chlorophenol and mg. phenol.

(ii) Vial 2 - lyophilized enzymes: It contains lipase, peroxidase, glycerol kinase, Glycerol-3-P04 oxidase and 4-amino antipyrine.

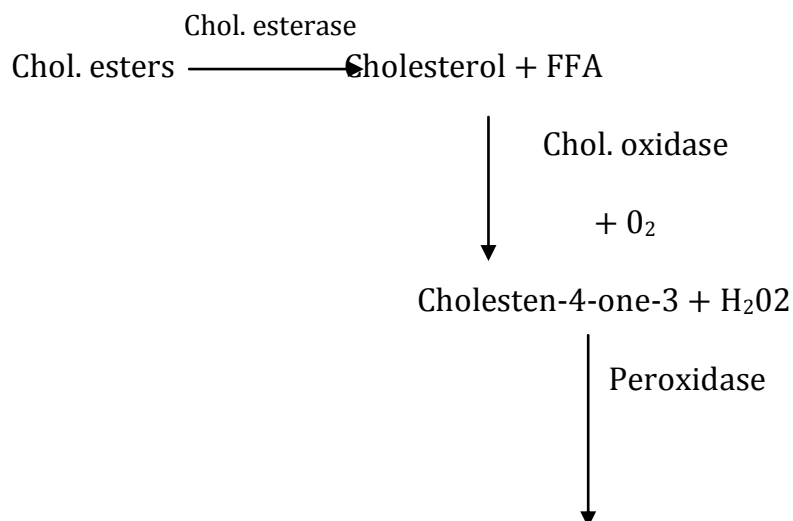
Preparation:

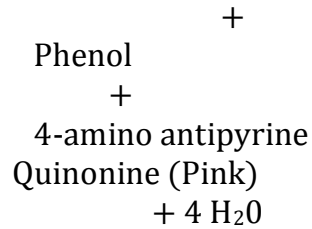
To reconstitute the working reagent, 15mls of the buffer solution was added to the lyophilized enzyme and mixed. This is stable for 45 days when stored at 4°C.

PROCEDURE

(i) Enzymatic estimation of total cholesterol by CHOD – PAP method using Biolabo kit.

Principle: Cholesterol esterase hydrolyses cholesterol esters to give cholesterol and free fatty acid. Addition of cholesterol oxidase in the presence of oxygen further oxidizes cholesterol to cholesten-4-one-3 and H₂O₂. Hydrogen peroxidase oxidizes H₂O₂ in the presence of phenol and 4-amino-antipyrine to give a pink coloured chromogen: Quinolone and H₂O. The intensity of the coloured complex formed is proportional to the amount of cholesterol present in the serum.





Protocol: Into each labelled test tube marked T (test), S (Std.) and B (Blank) was added 1ml of the working reagent, 10ul of serum sample was then dispensed into the tube marked T, 10ul of chol. standard into the tube labelled S and 10ul distilled water into the tube labeled B. The content of each tube was mixed and incubated at 37°C for 5 minutes. The absorbance of T and S were read using the spectrophotometer (Pye Unicam) at 500nm wavelength after zeroing the machine with the Blank.

Calculation: Chol. conc. = $\frac{\text{abs of T}}{\text{abs of S}} \times \text{Std. conc.}$

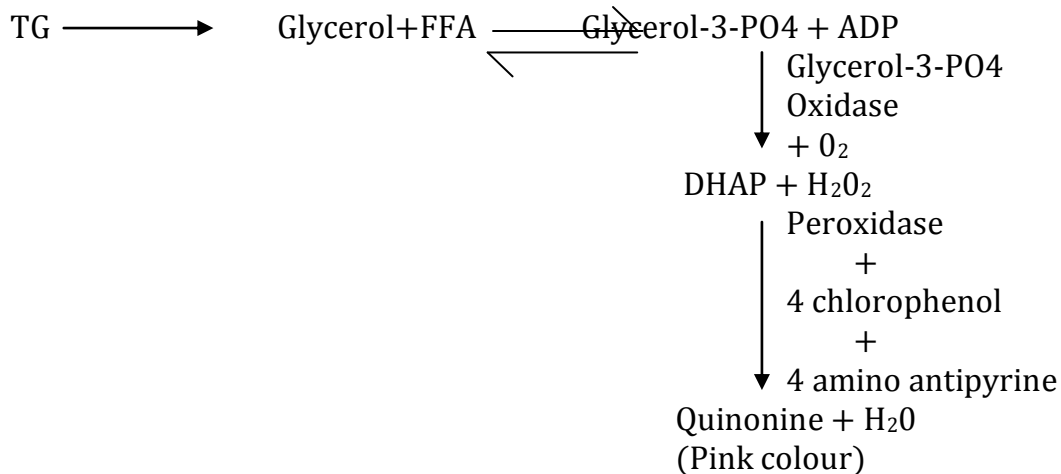
Ref. Range = 3.88 – 6.72mmol/L

(ii) Enzymatic estimation of serum Triglyceride using GPO – Kit method of Biolabo.

Principle: Lipase hydrolyses TG into Glycerol and FFA. In the presence of ATP, glycerol is converted to Glycerol-3-PO₄ and ADP by glycerol kinase. Glycerol-3-PO₄ is oxidized in the presence of oxygen to DHAP and H₂O₂ with 4 chlorophenol and 4-amino antipyrine to give a pink coloured complex: Quinonine and H₂O. The intensity of the coloured complex formed is proportional to the concentration of TG present in the serum.

Lipase

Glycerol kinase



Protocol: Into each labelled test tube marked T (test), S (Std.) and B (Blank), was added 1ml of the working reagent. 10ul were added into the tubes labelled T, S and B respectively. The contents of each tube were mixed and incubated at 37°C for 5 minutes. Using the spectrophotometer (Pye Unicam) the absorbance of the test and std. were read at 500nm wavelength after zeroing the machine with the Blank.

Calculation:

$$\text{Conc. of TG} = \frac{\text{abs of T}}{\text{abs of S}} \times \text{Std. conc.}$$

$$\text{Reference Range} = 0.5 - 1.75 \text{ mmol/L}$$

(iii) Enzymatic estimation of High Density Lipoprotein:

The method is the same for cholesterol estimation except that a precipitant is first used to precipitate, out of the serum, other forms of lipoprotein present in the serum, leaving only HDL. It is consequently estimated using the cholesterol kit.

2. Total Lipid Determination: The method is based on the ability of unsaturated lipid metabolite to produce by reaction with phosphovanillin reagent, a coloured compound (pink) whose colour intensity is proportional to the lipid concentration in the serum (Stroev and Makarova, 1986).

Procedure: 0.1ml of serum was transferred to a dry test tube labelled test and 2.9mls of conc. H_2SO_4 added. 0.2ml of distilled water and 5.8mls of conc. H_2SO_4 were added to another test tube labelled control. The contents of both test tubes were mixed thoroughly with a glass rod and placed in a boiling water bath for 10 minutes. After, the test tubes were promptly cooled under a stream of running tap water.

3mls and 6mls of phosphovanillin reagent were poured into two clean test tubes respectively. To the former test tube was added 0.2ml volume of the solution from the cooled sample test tube and to the latter, 0.4ml volume of the solution from the cooled control test tube. The contents were mixed thoroughly with a glass rod and placed in the dark for 45 minutes at room temperature to allow colouration to develop. The absorbance of both test solution and control was read using spectrophotometer CE 373 (linear readout) at 530nm wavelength.

- 3. Total Phospholipid Determination:** The method is based on a concentration measurement of organic phosphate released by acid hydrolysis (Stroev and Makarova, 1986).

Procedure

0.2ml of serum and 2.8ml of distilled water were added to sample test tube while 3ml of distilled water was added to the control test tube. 3ml of trichloroacetic acid was added to each test tube and mixed by shaking.

The sample solution was centrifuged for 15 minutes at 3000rpm. The supernatant liquid was decanted and the test tube placed upside down on a piece of filter paper to let the residual liquid drain off. 1ml of conc. perchloric acid was then added into both test tubes. Two glass beads were placed in each tube and mixed thoroughly. The test tubes were placed in the sand bath at 180°C for 20 – 30 minutes to hydrolyze the contents (until the solutions decolorize).

The test tubes were allowed to cool down in the air at room temperature and then 3ml of distilled water, 1ml of ammonium molybdate reagent and 1ml of freshly prepared reducing agent were added to each of the test tubes. The contents were mixed thoroughly and allowed to stand for 10 minutes at room temperature.

The absorbance of both sample solution and the control was read at 630nm using CE 373 spectrophotometer (linear readout).

- 4. Separation of blood serum lipids by thin layer**

Chromatography:

The method is based on different migration rates of serum lipid fractions (phospholipids, free fatty acid, cholesterol and Triglycerides) in a thin layer of absorbent as an organic solvent moves through it. The rate at which the fractions separated depends on their relative polarities; to identify the separated fractions, the chromatograms obtained were treated with iodine vapour (Anekwe 1983; Stroev and Makarova, 1986, Anekwe, 2002).

Procedure

1ml of serum was mixed with 10ml of ethanol: ether mixture in a standard flask of 25ml capacity. The flask was placed in a boiling water bath for 30 seconds and then cooled under a stream of running tap water and made up to the 25ml – mark with Ethanol: ether mixture. The lipid extract thus obtained was filtered through a defatted filter paper into a wide-mouth water bath. The dry residue was dissolved in 0.2ml volume of chloroform.

2 – 3ml of the trisolvent for phospholipid separation was poured into the chromatographic chamber. Using a Pasteur pipette, a drop of about 0.01 – 0.02ml of chloroformic extract was applied to a chromatographic plate so as to leave a space of 1cm between the spot applied and the plate edge. The

chromatographic plate was placed inside the chamber with the spotted end down, so as to immerse it 3 – 5cm deep in the solvent.

The chamber was covered with the lid to keep the vapour concentration at a constant level. The chromatography was allowed to proceed at room temperature until the ascending solvent front has reached a frontier line spaced 0.5cm from the upper edge of the chromatographic plate. The chromatographic tank time was 1 hour.

The plate was taken out of the chamber and dried in the air. Some metallic iodine crystals were placed on the bottom of the development chamber and the plate return into the chamber and covered. The sublimed iodine vapour was allowed to react with the chromatographically separated lipid fractions overnight.

The plate was taken out of the chamber and examined in ultraviolet light in the viewing cabinet. The procedure was repeated using a trisolvent for the separation of the different neutral lipids. The tank time was 35 minutes.

7. LDL - Cholesterol

This fraction of the lipoprotein is obtained by calculation after the total cholesterol, Triglycerides and HDL cholesterol have been determined. The expression below is used:

$$\text{LDL cholesterol (in mmol/L)} = \text{Total cholesterol} - \frac{\text{Triglycerides}}{2.3} - \text{HDL cholesterol (in mmol/L)}$$

This is FRIEDWALD FORMULA and it is applicable if plasma Triglyceride is less than 4.5mmol/L. This formula is based on the assumption that VLDL – cholesterol is present in a concentration equal to 1/5th of Triglyceride concentration. And this assumption is valid usually for Triglycerides concentration of less than 400mg/dl. At higher TG concentration inconsistencies in the VLDL TG/cholesterol ratio occur and the formula must not be used.

ELECTROLYTES

(A) MATERIALS

The following reagents of analytical grade were obtained and used:-

Sodium chloride, potassium chloride, mercuric nitrate, conc. nitric acid, Diphenylcarbazone, 95% ethanol, 0.5M sulphuric acid, 1M sodium hydroxide and Neutral red.

Distilled deionized water was used throughout. Pipettes of different capacities were used also. Flame photometer (Jenway type) was used for the sodium and potassium determinations.

(B) PREPARATIONS OF REAGENTS USED

- (i) Stock Sodium Standard (200mmol/L): 11.69gm of pure and dried NaCl was dissolved in 400mls of distilled deionized water and made up to 1 litre with distilled deionized water.
- (ii) Stock Potassium Standard (10mmol/L): 0.746gm of pure dried KCl was dissolved in few mls of deionized water and made up to 1 litre with deionized water.
- (iii) Working Na⁺/K⁺ Standard: Into 1 litre volumetric flask, 8mls of stock Na⁺ standard and 7mls of stock K⁺ standard were put in 1L flask and made up to 1 litre with deionized water. It was stored in polythene bottle.
- (iv) Mercuric Nitrate Solution: 2.9 – 3.0gm of HgNO₃ was dissolved in a few hundred mls of deionized water. 20 mls of conc. HNO₃ was

added and made up to 1 litre with deionized water. This amount of acid was strictly adhered to so that the end point could be sharp.

- (v) Diphenylcarbazone Indicator: 100mg of diphenylcarbazone was dissolved in 100mls of 95% ethanol and refrigerated at 4°C. The solution lasts for 1 month.
- (vi) Chloride Standard: 585mg of NaCl (pure and dried at 120°C) was dissolved in 400mls of deionized water and made up to 1 litre with deionized water.
- (vii) Working HCO₃⁻ Solution: Dilute 0.5M H₂SO₄ 1:100 and 1M NaOH 1:100 to obtain M/200 H₂SO₄ and M/100 NaOH respectively.

N/B: All solutions were prepared in CO₂ – free distilled, deionized water by boiling the deionized water, following it to cool before using it. Then, the 2 working solutions (M/200 H₂SO₄ and M/100 NaOH) were titrated against each other; adjustment was made accordingly until they neutralized each other with equal volumes.

- (viii) Neutral Red: 0.1g of neutral red was dissolved in 100mls of 95% ethanol.

(C) PROCEDURE

(i) Serum Na⁺ and K⁺ determination: By flame photometry method.

Principle:

Sodium and potassium solutions under carefully controlled conditions, when finely sprayed (aspirated) into a burner, the flame de-solvates the solution leaving solids (salts) which dissociates to give neutral ground state atom. Some of these atoms are excited in the flame thus moving into a higher energy state. When these excited atoms fall back to the ground state, light of characteristic wavelength is emitted (590nm for sodium and 770nm for potassium). This light then passes through a suitable filter unto a photosensitive element and the amount of current thus produced is measured. This is proportional to the amount of sodium or potassium present originally in the sample.

Technique

The serum sample was diluted 1:100 in a universal bottle with deionized water. For Sodium:

- (a) Turn the fuel adjust valve fully open
- (b) Turn on the fuel supply at source (i.e. cylinder)
- (c) Switch on electrical power
- (d) Depress ignition switch and hold down, until the 'FLM' indicator appears in the display window; release the ignition thereafter.
- (e) Insert the sodium light filter

- (f) Set the readout to zero with distilled deionized water by adjusting the "blank control".
- (g) Set the readout to 1.60 with working sodium standard solution.
- (h) Read the test.

For Potassium:

- (a) Insert potassium light filter
- (b) Set galvanometer with potassium working standard to 7.0 and continue as for sodium.

Serum chloride determination: By the mercuric nitrate titrimetric method of Schales and Schales (1971).

Principle

When mercuric nitrate solution is added to a solution containing chloride, unionized but soluble mercuric chloride is formed. At the end point, the first excess mercuric ions combine with the indicator (Diphenylcarbazone) to give a violet – blue coloured complex.

Technique

1 in 10 dilution of the serum was made in a universal bottle and 3 drops of the indicator added. The solution was mixed properly and then titrated from 2ml pipette (graduated in 0.01ml) with mercuric nitrate until a violet-blue colour was obtained. The same titration was carried out using 2ml of the chloride standard solution.

Calculation

$$\frac{\text{mls of HgNO}_3 \text{ required for the unknown}}{\text{mls of HgNO}_3 \text{ required for the standard}} \times \frac{100}{1}$$

Serum Hco₃ determination: By the method of Van Slyke et al (1932).

Principle

Acid (M/200 H₂S04) was added in excess of serum. Co₂ was liberated from Hco₃ an equivalent of H⁺ being removed by the formation of water. Excess of acid was then titrated with a standard alkali (M/100 NaOH). The end-point can be signalled by the indicator – neutral red.

Technique

Into a universal bottle was added the following: 5mls of deionized water, 0.2ml of serum sample, 2mls of M/200 H₂SO₄ and 2 drops of neutral red.

The resulting solution was titrated against M/100 NaOH and the titre noted.

Calculation: This was done using the expression:

$$\text{mmol/L HCO}_3^- = 2 - \text{volume of NaOH used} \times 50$$

Values obtained by this method were slightly higher in males than females.

URINALYSIS

(A) Study Subjects

A total of 100 subjects aged between 30 and 70 years both sexes were examined. The subjects were made up of 46 (16 males + 29 females) diagnosed hypertensives on treatment attending the MOPD of JUTH and 55 (24 males, 31 females) diagnosed hypertensives also on treatment attending consulting room (2) of UHS of UNIJOS. This latter subjects were mostly civil servants (staff of UNIJOS) and their relatives working in the University.

Elimination criteria applied for blood collection were also used for urine collection.

Patients were given clean, dry plastic universal bottles and instructed to pass, fasting, midstream early morning urine into the bottle to half the capacity (about 15mls) and submit same to the clinic in the early morning hours. The samples were then taken to the laboratory and analysed immediately after collection from the clinic with minimal delay.

(B) Analytical Procedures

Urinary Glucose

Test strip (Medi-Test Combi 10) was used for this test. The detection is based on the glucose oxidase – peroxidase – chromogen reaction. This reaction is glucose specific.

Principle

Glucose oxidase (3.2ul) which was incorporated into the test pad of the strip along with peroxidase (0.2ul) and ortho-toluidine (65ug), catalyses the oxidation of glucose in urine to glucuronic acid and hydrogen – peroxide. Peroxidase reduces the hydrogen peroxide to water and oxygen. The liberated oxygen combines with the chromogen; o-toluidine to give a colour ranging from green to bluish green depending on the concentration of glucose originally present in the urine sample.

Procedure

The test end of the strip was dipped into the specimen for a second and the strip placed against the edge of the specimen container to allow for drainage of excessive urine. Colour formed or change from the above reaction was compared with the colour chart and the result recorded accordingly.

Urinary Protein

Urine protein was measured with the use of test – strip (Medi – Test Combi 10). The detection is based on the “protein error” principle of indicators.

Principle

The reagent tetrabromophenol blue incorporated into the test-end of the strip at a concentration of 7.5ug activity/cm² and buffered to a constant pH value changes colour from yellow to greenish blue in the presence of albumin. Other proteins are indicated with less sensitivity. The

change in colour is due to a change in optical density or absorbance of the reagent.

Procedure

The test end of the strip was dipped into the urine sample for approximately a minute. The strip was then drawn across the rim of the container to remove excess urine. Colour formed from the above reaction was compared with the colour chart and the result recorded accordingly.

Blood in Urine

The presence of blood in urine was measured with the use of strip (Medi – Test Combi 10). The detection of blood in urine (haematuria) is similar to principle of occult blood and haemoglobinuria.

Principle

The detection is based on the Pseudoperoxidative activity of haemoglobin and myoglobin which catalyzes the oxidation of an organic indicator (tetramethylbenzidine 59ug) by an organic hydroperoxide (cumene hydroperoxide 253ug) producing a green colour.

Procedure

Test end of the strip was dipped into the urine for a second. It was then drawn across the edge of the container to allow for removal of excess urine. The resulting colour formed from the reaction was compared to a colour chat and recorded accordingly.

CHAPTER THREE**3.1 RESULTS**

A total of 1075 subjects have been studied so far. The distribution is as shown in Table 3.1

Table 3.1: Distribution of the total number of subjects studied

Analyte	No. of Hypertensives	No. of Normotensives	Total
1. Blood Lipids	100	50	150
2. Serum Electrolyte	-	120	120
3. Urinalysis Pattern (A)	100	-	100
4. Urinalysis Pattern (B)	92	-	92
5. Serum Lipids of alcoholics and smokers	116	84	200
6. Serum lipids of Burukutu consumers only	100	60	160
7. Vitamin A Conc. of children	-	53	53
8. Lipid profile of pre-post-menopausal women.	-	200	200
Total	508	567	1075

A total of two hundred (200) adult female Nigerians aged between 30 to 60 years non-diabetic, non-hypertensive, non-pregnant and non-obese from three hospitals were studied. The hospitals are:-

1. Jos University Teaching Hospital, Jos (JUTH)
2. Vom Christian Hospital, Vom (VCH)
3. Murtala Mohammed Specialist Hospital, Kano (MMSH)

The serum lipid profile of the subjects (total cholesterol, high density lipoprotein and Triglyceride) were analysed.

Table 3.2 showed the numerical distribution of the subjects from each of the hospitals.

TABLE 3.2: Numerical distribution of the subjects.

(AGE) IN YEARS	JUTH	MMSH	VCH
26 – 30	6	4	4
31 – 35	14	9	10
36 – 40	20	12	12
41 – 45	21	11	12
46 – 50	19	5	7
51 – 55	15	4	3
56 – 60	5	5	2
TOTAL	100	50	50

Table 3.3 Mean Blood Lipid Conc. of Hypertensive Subjects

Analyte	Normotensives n = 50	Hypertensives n = 100
Total Lipids (g/l)	5.50 ± 1.80	14.37 ± 2.31
Total Cholesterol (mg/dl)	207.19 ± 54.03	397.27 ± 113.08
Total Phospholipids (g/l)	2.37 ± 0.57	1.94 ± 0.71
PL: Chol. Ratio	1.14	0.49

The mean blood lipid concentration of 150 subjects studied is as shown in Table 3.3. This report showed that both total lipids and total cholesterol are higher in Hypertensives than the Normotensives while phospholipids is lower in Hypertensives than the Normotensives; though both values are within the reference range of 1.5 – 3.6g/L ($P>0.05$).

The high point of this study is that the calculated phospholipids; Cholesterol ratio is less than 1 amongst the Hypertensives. This confirms the work of Stroevev and Makarova (1986); Oforufuo and Nwanze (1988). This also confirms that these patients have pathological disorder – in this case, hypertension.

Furthermore, the patients studied had mean systolic pressure of 167 and Diastolic pressure of 110. This finding correlated well with the works of Mann et al (1988).

Table 3.4: Age – grouped sex – differentiated conc. of serum total cholesterol in mmol/L for both Normotensive and Hypertensive subjects.

Age (years)	Males		Females	
	Normo T.	Hyper T.	Normo T.	Hyper T.
11 – 20	-	12.1±2.3	4.8±0.3	11.7±1.1
21 – 30	5.7±1.0	12.5±2.1	5.8±0.1	12.1±1.6
31 – 40	3.2±0.5	14.3±1.8	5.8±0.1	16.7±0.5
41 – 50	5.0±1.5	10.8±1.5	3.8±0.2	10±0.7
51 – 60	6.59±0.3	10.0±1.6	4.7±0.5	9.9±0.3
61 – 70	-	8.7±1.0	-	8.4±0.2
71 – 80	-	7.5±1.0	-	8.0±1.0
MEAN, X	5.1±0.5	10.4±1.1	5.0±0.2	10.7±1.0

Table 3.4 is quite revealing. It was observed that the mean total cholesterol for both male and female hypertensives were high; higher than that of the normotensives of both sexes and even the reference range of 3.5 – 6.5mmol/L. This correlated well with previous studies ($P<0.05$).

When the age groups were considered, it was discovered that there was increasing total cholesterol concentration up to age group 31 – 40 years for male hypertensives and subsequently decrease even with increase in age. As for female hypertensives the pattern was similar; increase in serum total cholesterol concentration up to age group 31 – 40 years, thereafter, decrease in concentration even as age increases.

Table 3.5: Sex distribution of urinalysis pattern of Hypertensives from 2 Governmental Hospitals.

Hospital	Male	Female	Total
Jos University Teaching Hospital	16	29	45
University Health Services (UHS)	24	31	55
Total			100

From table 3.5, a total of 100 Hypertensives from 2 governmental hospitals were studied for urinalysis pattern. The distribution showed 45 patients (16 males and 29 females) from JUTH and 55 patients (24 males and 31 females) from UHS; both located in Jos North Local Government Area.

Table 3.6: Biochemical Parameters of the urine assayed.

Analyte	JUTH	UHS
Blood	30%	9%
Glucose	13%	9%
Protein	30%	36%

Table 3.6 shows significant proteinuria as a complication of the Hypertensives compared to other parameters ($P < 0.05$). About 30% haematuria is also observed amongst Hypertensives attending JUTH only.

Table 3.7: % sex differentiated of proteinuria amongst the hypertensives

Location	Male	Female
JUTH	12.5%	24.1%
UHS	8.3%	3.2%
Total	20.8%	27.3%

Table 3.7 shows that among the 45 patients from JUTH, more females had proteinuria (24.1%) than males (12.5%) whereas more males (8.3%) had proteinuria than females (3.2%) amongst the 55 patients from UHS. In all, more females (27.3%) showed proteinuria as a complication than males (20.8%). These percentages are however not significant ($P>0.05$).

Table 3.8: % Occupational Distribution of Proteinuria amongst the Hypertensives

Occupation	JUTH	UHS
Civil Servants	11.1%	70.9%
House Wives	51.1%	16.4%
Others	37.8%	12.7%

These results show that more Housewives attending JUTH have proteinuria as a complication than any other category of occupation (51.1%) ($P < 0.05$). However, more civil servants attending UHS have proteinuria as a complication than any other category of occupation (70.9%) ($P < 0.05$).

Table 3.9: Sex Distribution of Urinalysis pattern of Hypertensives from 2
Missionary Hospitals.

Hospital	Male	Female	Total
Vom Christian Hospital (VCH) in Jos South LGA	16	8	24
ECWA Evangel Hospital in Jos North LGA	26	42	68
Total			92

From Table 3.9, a total of 92 hypertensives were studied for urinalysis pattern. The distribution showed 24 patients (16 males and 8 females) from VCH located in Jos South LGA and 68 (26 males and 42 females) from ECWA Evangel Hospital located in Jos North LGA.

Table 3.10: % distribution of biochemical parameters of urine of hypertensives from the two Missionary Hospitals.

Analyte	VCH(n=24)	Evangel Hospital(n=68)
Blood	27%	26%
Glucose	5%	9%
Protein	68%	65%

From Table 3.10, proteinuria was a significant complication of the Hypertensives studied compared to other parameters. It is even higher than those patients from Government Hospital ($P < 0.05$).

Further analysis showed that more males than females had proteinuria from VCH but more females had more proteinuria than males from Evangel Hospital. This is shown in Table 3.11 ($P < 0.05$).

Table 3.11: % Sex Differentiated Proteinuria amongst the Hypertensives

Location	Male	Female
VCH	71%	29%
Evangel Hospital	41%	59%

Table 3.12: % Occupational Distribution of proteinuria amongst the Hypertensives.

Occupation	VCH	Evangel Hospital
Civil Servants	15%	15%
House wives	20%	10%
Others	65%	75%

Patients who engage in such occupations as farming, trading, driving and pastoral work which were grouped under others; showed significant proteinuria as a complication of their ailment – hypertension from the 2 hospitals ($P < 0.05$).

Table 3.13: Mean Concentration of total cholesterol of alcoholics and smokers.

Analyte	Male	Female
Total cholesterol (alcoholics)	3.0mMol/L \pm 0.1	3.3mMol/L \pm 3.2
Total cholesterol (smokers)	6.9mMol/L \pm 1.0	7.8mMol/L \pm 1.2

From Table 3.13, it was observed that both male and female alcoholics had lower total cholesterol compared to the reference range while both sexes for the smokers had higher concentration. This is a comparative study and it revealed that adults who indulge in both smoking and drinking stand a higher risk of developing heart disease ($P < 0.05$) than those who drink alcohol only.

Table 3.14 is also a comparative study of both smokers and alcoholics with respect to their mean High Density Lipoprotein (HDL) cholesterol concentration. Both male and female smokers had lower HDL levels than both sexes for alcoholics. This confirms the fact that the protective function of HDL for smokers is lacking or inadequate and therefore smokers are prone to heart disease more than the alcoholics only ($P < 0.05$).

Table 3.14 Mean Concentration of HDL concentration of both smokers and alcoholics.

Analyte	Male	Female
HDL (alcoholics only)	0.8±0.1	1.0±0.2
HDL (alcoholics and smokers)	0.5±0.1	0.6±0.1

Table 3.15: Mean concentration of lipid profile of Burukutu consumers (B.C.) (n = 100)

Analyte	Male		Female	
	B.C	Control	B.C	Control
Total cholesterol (mMol/L)	2.69±1.0	2.99±0.1	3.55±0.3	3.55±0.1
TG(mMol/L)	2.88±0.5	2.21±0.1	3.77±0.2	2.52±0.1

From table 3.15, the mean total cholesterol concentration of male Burukutu consumer is lower than the control and generally lower than the reference range of 3.5 – 6.5mmol/L for enzymatic method of total cholesterol estimation ($P>0.05$). This agreed with various studies done in which it was reported that alcohol has a lowering effect on the total cholesterol of the body. However, the mean Triglyceride concentration of both sexes for the Burukutu consumers were higher than those of the control and generally higher than the reference range($P<0.05$).

Table 3.16: Age-classified mean total cholesterol concentration for Burukutu consumers

Age (years)	Male	Female
21-30	3.69 \pm 0.1	3.48 \pm 0.2
31-40	2.46 \pm 0.1	2.83 \pm 0.3
41-50	2.45 \pm 0.2	2.35 \pm 0.3
51-60	2.02 \pm 0.2	2.02 \pm 0.2
61-70	1.17 \pm 0.1	2.00 \pm 0.1

Here, as age increases, the total cholesterol decreases for both sexes, confirming the lowering effect of alcohol on total cholesterol ($P < 0.05$).

Table 3.17: Age-classified mean Triglyceride concentration for Burukutu consumers

Age	Male	Female
21-30	2.87±0.1	3.64±0.5
31-40	3.62±0.1	3.52±0.5
41-50	3.31±0.3	3.52±1.1
51-60	2.02±0.4	3.94±0.3
61-70	2.76±0.1	2.85±0.2

From Table 3.17, as age increases, the Triglyceride concentration tended to increase for both sexes of Burukutu consumers, more so, up to the age of 50 years. This also agreed with previous report ($P>0.05$).

Table 3.18: Age-classified mean vitamin A concentration of group II Babies (formulated)

Age(Months)	Group II Babies
5-6	0.80(umol/dl) \pm 0.1
7-8	0.50(umol/dl) \pm 0.1
9-10	0.41(umol/dl) \pm 0.2
11-12	0.34(umol/dl) \pm 0.1

As shown in table 3.1 previously, a total of 53 babies aged between 5 months and 12 months were studied for serum vitamin A concentration. These babies were grouped into 2: group I were those exclusively breastfed (27 babies) while group II were those on formula (26 babies).

The serum total vitamin A concentration was estimated by High Performance Liquid Chromatography (HPLC) Group I babies had a mean vitamin A concentration of 0.91umol/dl which is within the reference range of 0.70 – 1.50umol/dl. However, group II babies had a mean vitamin A concentration of 0.44umol/dl and this is below the reference range.

Further analysis of group II babies based on their age is as reported in Table 3.18. A decreasing mean vitamin A concentration was observed even up to age 12 months (0.34umol/dl) ($P < 0.05$).

From this study, it was observed that group I babies had a normal vitamin A concentration; thus in the absence of any other predisposing factor, they are not likely to suffer any childhood disease that may be due to vitamin A deficiency. But for group II babies, it was observed that they have lower vitamin A concentration, thus, they are likely to suffer from childhood disease due to vitamin A deficiency.

In summary exclusive breastfeeding is still the best option for babies and should be encouraged among expectant and nursing mothers; the inconveniences notwithstanding.

Alternatively, addition of vitamin A supplement to the feeding formula should be encouraged right from source – the manufacturers.

TABLE 3.19: Mean and std. deviation of the biochemical parameters assayed.

Age (Years)	JUTH			MMSH			VCH		
	Chol. mmol/L	HDL mmol/L	TG mmol/L	Chol. mmol/L	HDL mmol/L	TG mmol/L	Chol. mmol/L	HDL mmol/L	TG mmol/L
26 – 30	4.3 ± 0.5	0.9 ± 0.2	1.7 ± 0.2	4.8 ± 0.4	1.2 ± 0.4	1.0 ± 0.3	5.2 ± 0.2	2.3 ± 0.3	1.5 ± 0.2
31 – 35	5.3 ± 0.5	1.4 ± 0.1	1.3 ± 0.1	5.4 ± 0.5	1.4 ± 0.4	0.9 ± 0.3	5.4 ± 0.3	2.5 ± 0.3	1.4 ± 0.3
36 – 40	5.7 ± 0.4	1.6 ± 0.2	1.1 ± 0.1	5.7 ± 0.1	1.7 ± 0.5	0.8 ± 0.3	5.6 ± 0.4	2.8 ± 0.2	1.3 ± 0.1
41 – 45	5.8 ± 0.5	1.6 ± 0.3	1.1 ± 0.1	6.0 ± 0.5	1.7 ± 0.6	0.8 ± 0.2	5.7 ± 0.3	2.8 ± 0.1	1.3 ± 0.2
46 – 50	5.6 ± 0.2	1.6 ± 0.4	1.1 ± 0.1	5.8 ± 0.4	1.7 ± 0.5	0.8 ± 0.2	5.7 ± 0.3	2.8 ± 0.1	1.3 ± 0.1
51 – 55	5.2 ± 0.3	1.2 ± 0.3	1.1 ± 0.1	5.4 ± 0.3	1.5 ± 0.5	0.8 ± 0.4	5.4 ± 0.3	2.8 ± 0.1	1.4 ± 0.2
56 – 60	4.4 ± 0.4	0.9 ± 0.1	1.3 ± 0.3	5.2 ± 0.6	1.0 ± 0.3	2.1 ± 0.3	5.2 ± 0.4	2.7 ± 0.2	1.8 ± 0.1
x ± S.D.	5.2 ± 0.3	1.3 ± 0.1	1.2 ± 0.3	5.5 ± 0.4	1.5 ± 0.2	1.0 ± 0.2	5.5 ± 0.4	2.6 ± 0.1	1.4 ± 0.4

OVERALL x ± S.D:- 1. Chol. = 5.4 ± 0.4

2. HDL = 1.8 ± 0.1

3. TG = 1.2 ± 0.3

Graph J.

Graph K

Graph V

CHAPTER FOUR

4.1 DISCUSSION

In this study, the blood lipids of the patients were determined and a ratio of phospholipids to cholesterol was estimated. For the hypertensives, it was found to be less than 1 (0.49) while that of the Normotensives was above 1 (1.14). This ratio agrees with the earlier works of Stroev and Makarova (1986); Oforufuo and Nwanze (1988).

The mean systolic pressure of the hypertensives was 167mmHg and Diastolic pressure of 110mmHg, as measured by the consultants. These figures agree with the study of Mann et al (1988) and Oforufuo and Nwanze (1988) among Nigerians with primary (essential) hypertension.

Apart from the use of blood pressure measurement to confirm the hypertensives, one could also use blood lipids specifically phospholipids and cholesterol concentration to ascertain that truly the patients have pathological disorder.

Comparative studies were carried out in 2 governmental (JUTH and UHS) and 2 Missionary (Vom Christian Hospital and ECWA Evangel Hospital) Hospitals; basically to establish the diagnostic relevance of simple urinalysis amongst hypertensives. The results were quite revealing as documented in Tables 3.5 and 3.6 previously. The results revealed some complications of hypertension which drug therapy

could not address. However, complications were minimal where awareness and socio-economic stability were very satisfactory, as observed among hypertensives attending Government Hospitals especially UHS. This might be attributed to the fact that most of the patients attending UHS were learned, secure financially and above all, obtained their drugs free on their clinic days. Thus, economic factors and level of awareness of the disease of by the populace appear to be key factors in the management of hypertensives. These factors should be well noted by all concerned.

Furthermore, efforts should be geared towards early detection in order to avoid further complication and resultant casualty. Also routine analysis should be encouraged and screening of individuals in communities to rule out high blood pressure should be done regularly. From this study (comparative urinalysis pattern), reliable and beneficial information can be derived. This will go a long way in reducing the cost of other complex tests usually requested by the physician for the hypertensives. It will also help the clinician in assessing the effect of diuretics, often included in the medication of the patients, on their renal physiology.

In Jos and its environ, Burukutu consumption is a common feature among the young and the old especially in the early hours of the day and late evenings. Both sexes are involved. Several reports have documented the lowering effect of alcohol consumption only on serum total cholesterol of both male and female adults.

Part of the result of this work correlated well with these previous studies and even went further to show that alcohol consumption increased the HDL and TG concentration of the consumers. This is an advantage to the heart of the individual. However, a major disadvantage was the rather high alcohol content of the local beverage.

In another closely related work carried out by this researcher, the effect of alcohol alone and alcohol and smoking on the lipid profile was studied. It was observed that those who only drink but did not smoke had lipid patterns resembling the Burukutu consumers i.e. low total cholesterol concentration, high HDL and high TG concentration. However, for those who both drank and smoke, the concentration of total cholesterol was high while the HDL and TG concentrations were low. This correlated well with the fact that alcoholics and smokers develop coronary heart disease faster in life than alcoholics or smokers alone.

Again, it is hereby recommended that a serious campaign against smoking and drinking among secondary school students and the youths generally be encouraged. They should be made to know the dangers of smoking and drinking early in life, and be persuaded not to engage in any of these.

It is an established fact that there is a correlation between vitamin A concentration and immunity. Breastfeeding is also known to be the best way of nursing a child. These facts are well corroborated in

this study. Thus, in the absence of any serious infection, children breastfed exclusively are not likely to suffer childhood diseases such as diarrhoea, vomiting, blindness, skin disease and measles. It is therefore strongly recommended that nursing mothers be encouraged to practise exclusive breastfeeding. Furthermore, the addition of vitamin A supplements to the baby formula should be made compulsory for infant formula producers.

Research reports from many parts of the developing countries indicate that the complication of uncontrolled hypertension are gradually displacing communicable disease as causes of adult medical admissions and death (Wokoma, 2002). These complications include hypertensive heart failure stroke and Ischaemic Heart Disease (Wokoma, 2002). For example, reports from dialysis centres in some African countries indicate that hypertensive nephrosclerosis is a primary cause of renal failure in most patients with end-stage renal disease (Gold, 1980). This is gradually superceding chronic glomerulonephropathics and chronic pyelonephritis as the leading cause of chronic renal failure in developing countries such as Nigeria (Seedat, Reddy, 1976).

Whereas communicable diseases exact a heavy toll on younger Nigerians, the emerging epidemic of hypertension takes its toll on adults, and on recent middle – aged Nigerian population on whom the young depend for survival. Thus, the future health and socio-economic implication for this scenario in Nigeria are grave; given the very poor

gross domestic product (GDP) of the nation, the very low per capital income and ever dwindling health budgets.

Currently, most Nigerians do not have social security benefits and virtually all antihypertensive drugs are imported, expensive and not readily accessible. Additionally, there are no special care units for the management of the complications of hypertension. From the foregoing, it is evident that Nigeria is ill-prepared and ill-equipped to cope with the challenges of the morbidities attributable to the emerging epidemic of hypertension in this millennium. There must be a change of focus from drug therapy to dietary, awareness on the part of the patients.

The reality of hypertension as an emerging medical problem of a non-communicable disease must be recognized and given proactive attention. This is so because it is not likely that in the near future, the Nigerian health systems will develop sufficiently to cope with the magnitude of health problems associated with the complications of poorly controlled hypertension.

Fortunately, the emergence of hypertensive complications is potentially preventable without the application of expensive technology. Thus, early and adequate blood pressure control will be beneficial in the prevention, delay and amelioration of complications (Multiple Risk Factor Intervention Trial Research Group 1982) (U.K. Prospective Diabetes Study Group, 1990, 1998).

From Table 3.19 and Graphs 5. 6 and 7, it was observed that as the age increased, both the cholesterol and HDL concentrations increased up to age range 36 – 40 years. Then they remained constant up to age range 46 – 50 years, thereafter, they decreased until age 60 years. A hyperbolic pattern of graph was obtained.

On the other hand, for TG, there was an initial decrease in concentration as age increased up to age range 36 – 40 years, it remained constant up till age 46 – 50 years and thereafter increased to age 60 years. A hypobolic pattern of graph was also obtained.

These results correlated with the results of Stevenson *et al* (1993). Based on the results obtained from this study, it was established that menopause was most likely to set in among adult Nigerian women from age 40 years (early menopause) up to age 50 years (physiological menopause) for the following reasons:

1. As age increases, the oestrogen concentration increased up to age 40 years. Oestrogen is known to have a cardio-protective function by enhancing corresponding increased in plasma cholesterol and HDL concentration up to this age, and a decrease in plasma TG concentration (Stevenson *et al*, 1993).
2. There is usually a decrease in oestrogen level in women after age 40 years, thus reversing the concentration of these lipids (Stevenson *et al*, 1993).
3. Decrease in oestrogen concentration is known to affect the development of ovaries (drastic reduction in maturation) and

consequently a cessation of menstruation resulting from the loss of ovarian follicular activity. This is termed menopause.

4. The cost of assaying fertility hormones (oestrogen inclusive) is so enormous and not so easily available, that an equally useful indicator of menopause which is cheaper and easily measured has become very necessary. Here, the lipid profile is most suitable considering the positive correlations noted earlier.

4.2 Summary of results:

The reality of essential hypertension as an emerging medical disorder with far reaching medical, demographic and socio-economic implications for Nigerians is no longer in doubt. The prevalence is increasing, afflicting the most productive segments of the populace; the aetiological risk factors stemmed out of a transition of the populace from rural and active lifestyle to urban and more sedentary populations. The complications arising from hypertension are fast becoming the commonest cause of morbidity, hospitalization and mortality. There is therefore the need for all concerned to give the attention needed for the prevention and control of hypertension.

From the human blood and urine samples analysed, the following facts represent the highlights of the results:-

1. The mean systolic pressure of the hypertensives was 167mmHg and diastolic pressure of 110mmHg.
2. It was found that there was an early onset of hypertension in Nigerians; for instance the mean age range was 30 – 35 years as against previous reports of 40 years and above for both sexes.

3. The calculated phospholipids to cholesterol ratio for the hypertensives was less than 1; confirming a pathological disorder; in this case, hypertension.
4. Urine analysis revealed that some complications of hypertension could not be controlled by the drug therapy.
5. Such complications as glycosuria, proteinuria and haematuria were minimal where awareness of the disease and socio-economic stability were satisfactory. For instance, this is true of patients from University Health Services – a Government Hospital where the level of education is higher and drugs were given to patients on their clinic days, free of charge.
6. Furthermore, complications are more common in female hypertensives especially housewives than in their male counterparts. This was true of patients attending Government Hospitals.
7. For patients attending Missionary Hospitals, the pattern was that those that engaged in other occupations such as farming, pastoral work and driving manifested more of the complications other than the other occupations, e.g. civil servants and housewives.
8. This work revealed that relevant and useful information to the patient and particularly to the physician could be obtained from simple urinalysis. Such information include: level of patients

education and awareness of the disease, socio-economic status, compliance with and affordability of drug therapy, lifestyle of the patients among other factors.

9. Burukutu – a locally brewed alcoholic beverage had a lowering effect on serum total cholesterol in both sexes (lower effects on male patients than female patients).
10. Furthermore, this alcoholic beverage increased the High density lipoprotein (HDL) and triglyceride (TG) concentrations in plasma of the consumers.
11. Estimation of vitamin A – a lipid soluble vitamin in children of one year and below was carried out by high performance liquid chromatography – a highly sophisticated separation technique. The result obtained showed that exclusive breastfeeding was better than the feeding of the commercial milk formula during the best first six months of life.
12. This research work was quite comprehensive in its approach to the assessment of both blood and urinary Biochemical parameters of hypertensives. It highlighted the fact that drug therapy alone would be grossly inadequate in the management of hypertension and perhaps other cardiovascular disease.
13. The lipid pattern obtained from the measurement of plasma lipids, in women aged 30 – 60 years showed that this could be used as an alternative method to estimate the menopausal age range of adult Nigerian women.

4.3 CONTRIBUTION TO KNOWLEDGE:

The data presented clearly impact strongly on the area of lipid Biochemistry as it affects adult Nigerians suffering from cardiovascular disease; viz hypertension and hypertensive – prone individuals. The study contributed to knowledge in the field along the following lines:

1. The work confirmed earlier reports, and also revealed the gross inadequacy of drug therapy alone in the management of hypertension.
2. The importance of simple urinalysis is once more brought into limelight as this would obviate or reduce the cost of elaborate tests which the patients could hardly afford.
3. Hypertension was found to have an early onset in life - earlier than previously known. There is the need therefore for all to be aware in order to avoid, or to reduce to the minimum, the medical and socio-economic consequences of this pathological state.
4. The need to appreciate such risk factors as smoking, drinking, dietary habits etc early in enough among the hypertensive – prone individuals was clearly demonstrated in the result of this study.
5. From the results too, the necessity to attend to such risk groups as youth, long distance drivers hoteliers, civil servants at the management level and other hypertensive-prone individuals

through counselling, workshop, public awareness campaign and the media was also well highlighted.

6. Dietary counselling as is being done for diabetic patients or sickle cell anaemia patients seems to be an open door for some breakthrough in the management of hypertensive patients and this work has clearly opened the door to interested researchers.
7. Lipid profile could be used to determine the menopausal age of women as an alternative method to the use of hormones. This method is cheaper, and readily available. Furthermore the results correlate very well with those from hormonal assays (fertility hormones).

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