PROXIMATE AND PHYTOCHEMICAL COMPOSITION OF AFRICAN YAM BEAN (*Sphenostylis stenocarpa*) AND ITS EFFECT ON THE LIPID PROFILE OF HYPERCHOLESTEROLEMIC RATS

A RESEARCH PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF HOME SCIENCE, NUTRITION AND DIETETICS, UNIVERSITY OF NIGERIA NSUKKA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.) DEGREE IN HUMAN NUTRITION

BY

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CERTIFICATION

Ikpa Sobechukwu Chinwendu, a post graduate student with registration number PG/MSc/11/58318, in the Department of Home Science, Nutrition and Dietetics, University of Nigeria, Nsukka has satisfactorily completed the research work for the award of the degree of MSc. in Human Nutrition. The work embodied in this research work is original and has not been submitted in part or full for any other diploma or degree of this or any other university.

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APPROVAL PAGE

This research work has been approved by the Department of Home Science, Nutrition and Dietetics for the award of Master of Science in Human Nutrition, University of Nigeria, Nsukka.

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DEDICATION

This project work is dedicated to the Almighty God, whose presence was felt throughout the period of this study.

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ABSTRACT

The study examined the proximate and phytochemical (saponins, tannins, phytate and flavonoid) composition of roasted cream coloured African yam bean (AYB) flour and the lipid profile of hypercholesterolemic rats fed diets supplemented with African yam bean. Different diets were formulated using the AYB flour. Group 1 consisted of the normal control rats (untreated rats), group 2 consisted of the hypercholesterolemic group of rats that were untreated. Groups 3-6 consisted of rats fed rat chow with 5%, 10%, 15% and 20% AYB flour supplementation, respectively. Proximate analysis was done using AOAC methods. Phytochemical and biochemical analyses were done in duplicates using standard methods. The proximate analysis showed that AYB flour contain 4.94% moisture, 27.66% protein, 4.29% fat, 3.66% ash, 9.57% crude fibre and 49.88% carbohydrate. The phytochemical analysis showed 0.77mg/100g saponins, 42.07mg/100g tannins, 9.08mg/100g phytate and 1.01mg/100g flavonoid. Total cholesterol level for the different supplementation groups showed significant decrease ($p<0.05$) with 20% supplementation showing the highest decrease (38.70%). Triglyceride levels decreased significantly ($p<0.05$) across all the groups. The 5% AYB supplementation showed the highest decrease (35.63%) and 20% supplementation the lowest decrease (28.01%). Low density lipoprotein (LDL) levels of the rats significantly ($p<0.05$) decreased across the groups. The control group also showed 50.34% decrease in LDL but 10% AYB supplementation showed the highest decrease (61.29%). Serum high density lipoprotein (HDL) concentration increased significantly ($p<0.05$) after supplementation with AYB. The 20% AYB supplementation showed the highest percentage increase (22.85%) relative to the other groups. There were significant decreases in serum total cholesterol, LDL cholesterol, triglyceride and increase in HDL cholesterol for the groups with AYB supplementation.

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CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Hypercholesterolemia is the presence of high levels of cholesterol in the blood, and is a form of "hyperlipidemia" (elevated levels of lipids in the blood) and "hyperlipoproteinemia" (elevated levels of lipoproteins in the blood) (Durrington, 2003).This is a major risk factor for cardiovascular disease especially coronary heart disease (Mudabmbi & Rajagopal, 2007). Cholesterol is the main sterol found in body tissues. Since cholesterol is insoluble in water, it is transported in the blood plasma bound to protein particles (lipoproteins). Lipoproteins are classified by their density; very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) (Biggerstaff & Wooten, 2004). All the lipoproteins carry cholesterol, but elevated levels of the lipoproteins other than HDL (termed non-HDL cholesterol), particularly LDL-cholesterol is associated with an increased risk of atherosclerosis and coronary heart disease (Carmena, Duriez & Fruchart, 2004). Dietary intervention trials have shown that the reduction of serum total and LDL cholesterol concentration is beneficial for the reduction of coronary atherosclerosis in people with or without coronary heart disease (Gould, 1992; Ornish, 1998).

Cardiovascular disease (CVD) is a class of diseases that involve the heart or blood vessels (arteries and veins) (Maton, 1993). It refers to any disease that affects the cardiovascular system, principally cardiac disease, vascular diseases of the brain and kidney, and peripheral arterial disease (Kelly & Fuster, 2010). According to the World Health Organization, chronic diseases are responsible for 63% of all deaths in the world, with cardiovascular disease as the leading cause of death (WHO, 2011). An estimated 17.3 million people died from CVDôs in 2008. Over 80% of CVD deaths take place in low- and middle-income countries. By 2030, almost 23.6 million people will die from CVDs (WHO, 2011). There are many causes of

cardiovascular disease but the most common are atherosclerosis and/or hypertension. The different types of cardiovascular disease include coronary heart disease (CHD), cardiomyopathy, hypertensive heart disease, heart failure, cor pulmonare, cardiac dysrhythmias, inflammatory heart disease (endocarditis, inflammatory cardiomegaly, and myocarditis), valvular heart disease, stroke or cerebrovascular accident, peripheral arterial disease. CVD typically involves the coronary arteries and thus is frequently termed coronary heart disease (CHD) or coronary artery disease (CAD). The risk factors of cardiovascular disease are in addition to high fat diet; age, gender, high blood pressure, high blood cholesterol levels, tobacco, smoking, excessive alcohol consumption, family history, obesity, lack of physical activity, psychosocial factors and diabetes mellitus (Kelly & Fuster, 2010).

Evidence shows that the Mediterranean diet improves cardiovascular outcomes (Walker & Reamy, 2009). The Mediterranean diet is made up of high olive oil consumption, high consumption of legumes, unrefined cereals, fruits, and vegetables, moderate consumption of dairy products, and moderate to high consumption of fish, low consumption of meat and meat products and moderate wine consumption. A 10-year study published in the Journal of American Medical Association (JAMA) found that adherence to a Mediterranean diet and healthful lifestyle was associated with more than a 50% lowering of early death rates (Knoops *et al*., 2004).

Legumes have benefits in terms of reduced risk of CHD. Legumes or dry beans have been shown to improve serum lipid profiles in patients with CHD (Anderson & Major, 2002; Bazzano, Thompson, Tees, Nguyen & Winham, 2009) and a growing body of evidence supports the positive effects dietary legume consumption confers on health, particularly in relation to risk of CHD. Epidemiological studies support the cardio protective effects of legumes as part of a healthy diet. In particular, one study examined the relationship between beans consumption and occurrence of CVD and reported that 1 serving per day of beans was

associated with a 38% lower risk of myocardial infarction (Kabagambe, Baylin, Ruiz-Narvarez, Siles & Campos, 2005). A second study by Bazzano, He & Ogden (2001) reported that individuals consuming legumes at least four times per week had a 22% lower risk of heart disease than individuals consuming legumes less than once per week. Majority of legume studies have examined the relationship between soybeans and heart disease. Some studies using navy beans and chickpeas have been conducted. In an early report, Anderson and Major (2002) demonstrated that consumption of navy beans in tomato sauce (baked beans) for 21 days decreased serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) concentrations in hypercholesterolemic men (Anderson, Gustafson, Spencer, Tetyen & Bryant, 1990). A research by Finley, Burrel, and Reeves (2007) reported that pinto beans consumption is also favourable. Cowpea commonly consumed in Nigeria has been linked to present significant reductions in plasma total cholesterol and non-HDL cholesterol (Frota, Mendonca, Saldiva, Cruz, & Areas, 2008). As Liu (2006) reported, diets with low glycemic index are associated with a reduced risk for the development of diabetes mellitus, obesity and cardiovascular disease.

All legumes contain phytochemicals which play metabolic roles in humans who frequently consume these foods. Phytochemicals, also referred to as phytonutrients, are found in fruits, vegetables, whole grains, legumes, beans, herbs, spices, nuts, and seeds and are classified according to their chemical structures and functional properties. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. There are many phytochemicals and each works differently. Presence of phytochemical components such as phytohemagglutinins, tannins, phytic acid, saponins, protease inhibitors, oligosaccharides and phytoestrogens in food legumes has both health benefits and adverse effects. These are some possible action; antioxidant, hormonal action, stimulation of enzymes, interference with DNA replication, anti-bacterial effect and physical actions. These

biologically active compounds in food legumes also have immense potential in biomedical application. On the other hand, phytochemicals have adverse effects as they limit the digestibility of proteins and carbohydrates or reduce the bioavailability of certain nutrients, interfere with normal growth, reproduction and flatulence production. Moreover, phytoestrogens have been linked with infertility problems. The synergistic or antagonistic effects of mixtures of these phytochemicals from food legumes, their interaction with other components of the diet and the mechanism of their action have remained a challenge with regard to understanding the role of phytochemicals in health and diseases. These have been associated with numerous health benefits, including reduced cardiovascular and renal disease risks, health care treatments including anti-aging, enhancement of brain function, and lower glycemic index for persons with diabetes, increased satiation and cancer prevention. Dietary intake of phytochemicals may provide health benefits, protecting against numerous diseases or disorders, such as coronary heart disease, diabetes, high blood pressure and inflammation (Bouchenak & Lamri-Senhadj, 2013).

Phytic acid (phytate), a type of phytochemical classified as anti-nutrient is found in many types of plant foods, such as grains, legumes (including peanuts and soybeans), nuts, and seeds. In legumes and seeds, phytic acid resides almost entirely in the endosperm (Schlemmer, Frolich, Prieto & Grases, 2009). Phytic acid has shown some capacity to reduce cholesterol and triglycerides, and positively impact the glycemic response of certain foods (Lee, Park and Chun, 2007). In some cases, phytic acid seems to have an ability to slow down a potential blood sugar spike following the ingestion of certain high-carbohydrate foods. Again, this may explain why high-fiber foods have been associated with improved blood sugar control. The potential benefits of phytic acid occur in instances with high dietary phytic acid intake. However, a high intake has also been associated with reduced mineral absorption**.** So, in order for us to get the best of both worlds, itôs important to discover some ways in

which we can minimize the negative effects while maximizing the beneficial effects. One way we can do this (specifically in regards to iron) is by incorporating more vitamin C (ascorbic acid) into our diet. These two work well together, with vitamin C placing iron in a chemical state that is more readily absorbed by the body (Hummers and Offeman, 1958).Preparation methods such as soaking, germinating, or fermenting can be very effective in reducing the amount phytic acid present in foods.

Tannin is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids. Tannins have traditionally been considered anti-nutritional but it is now known that their beneficial or anti-nutritional properties depend upon their chemical structure and dosage. Recent studies have demonstrated that products containing chestnut tannins included at low dosages (0.15ï 0.2%) in the diet of chickens may be beneficial (Schiavone, Guo and Tassone, 2008). Most legumes contain tannins. Red-coloured beans contain the most tannins, and white-coloured beans have the least (Reed, 1995). There is a growing consensus for the hypothesis that the specific intake of food and drink containing relatively high concentrations of polyphenols may play a meaningful role in reducing the risk of cardiovascular disease (Ashutosh, 2003).

Saponins are a class of chemical compounds found in abundance in various plant species. Saponins are attracting considerable interest as a result of their diverse properties, both deleterious and beneficial. Clinical studies have suggested that these health-promoting components, saponins, affect the immune system in ways that help to protect the human body against cancers, and also lower cholesterol levels. Saponins decrease blood lipids, lower cancer risks, and lower blood glucose response. The detergent qualities of saponins allow them to bind to bile and prevent its reabsorption. Once bound to saponins, cholesterol leaves the body in waste. A lower cholesterol level means less risk of heart attack or stroke.

Flavonoids (or bioflavonoids) (from the Latin word *flavus* meaning yellow, their color in nature) are a class of plant secondary metabolites. Flavonoids were referred to as Vitamin P (Benthsath, Rusznyak and Szent-Gyorgyi, 1937) probably because of the effect they had on the permeability of vascular capillaries. Flavonoids have been shown to have a wide range of biological and pharmacological activities in *in vitro* studies. Examples include anti-allergic, anti-inflammatory, antioxidant, anti-microbial (antibacterial, antifungal, and antiviral), anti-cancer, and anti-diarrheal activities (Yamamoto and Gaynor, 2001; Cushnie and Lamb, 2011; Friedman, 2007; Manner *et al.,* 2013). Inflammation has been implicated as a possible origin of numerous local and systemic diseases, such as cancer, cardiovascular disorders, diabetes mellitus, and celiac disease (Manach, Mazur and Scalbert, 2005; Babu, Liu and Gilbert, 2013; Ravishankar, Rajora, Greco and Osborn, 2013).

Preliminary studies indicate that flavonoids may affect anti-inflammatory mechanisms via their ability to inhibit reactive oxygen or nitrogen compounds (Izzi *et al.,* 2012). Flavonoids have also been proposed to inhibit the pro-inflammatory activity of enzymes involved in free radical production, such as cyclooxygenase, lipoxygenase or inducible nitric oxide synthase, and to modify intracellular signaling pathways in immune cells (Izzi *et al.,* 2012). Procyanidins, a class of flavonoids, have been shown in preliminary research to have anti-inflammatory mechanisms including modulation of the arachidonic acid pathway, inhibition of gene transcription, protein expression and activity of inflammatory enzymes, as well as secretion of anti-inflammatory mediators (Martinez-Micalo *et al.,* 2012). Among the most intensively studied of general human disorders possibly affected by dietary flavonoids, preliminary cardiovascular disease research has revealed the following mechanisms under investigation in patients or normal subjects (van Dam, Naidoo and Landberg, 2013; Tangney and Rasmussen, 2013):

- inhibit coagulation, thrombus formation or platelet aggregation
- reduce risk of atherosclerosis
- reduce arterial blood pressure and risk of hypertension
- reduce oxidative stress and related signaling pathways in blood vessel cells
- modify vascular inflammatory mechanisms
- improve endothelial and capillary function
- modify blood lipid levels
- regulate carbohydrate and glucose metabolism
- modify mechanisms of aging

Other components of the legume such as the protein, carbohydrate, fat, and dietary fibre have also been shown to improve CVD risk factors. The low content of saturated fatty acids and high content of fiber make pulses a good choice for a healthy diet. Dry beans are essentially fat-free and act to displace fat from the diet. Many of them have moderate amounts of oil that is predominantly unsaturated. Their complex carbohydrates together with dietary fiber result in low glycemic indexes of legumes and legume products, and play an important role in the prevention of diabetes. Dry beans contain a mixture of soluble fiber, which significantly lowers cholesterol and blood sugar concentrations, and insoluble fiber, which aids in gastrointestinal function. Dietary fiber has major protective effects against atherosclerotic cardiovascular disease (Anderson, Deakins, Floore, Smith & Whitis, 1990). Epidemiologic data suggest that intake of complex carbohydrates and dietary fiber is inversely related to coronary artery disease (Anderson *et al.,* 1990). Dietary fiber intake also slows development of atherosclerosis in animal models. Whereas soluble fiber clearly decreases serum cholesterol and LDL-cholesterol concentrations (Anderson *et al.,* 1990), the inverse relation between dietary fiber intake and coronary artery disease appears to be independent of serum cholesterol concentration. Findings from epidemiologic studies show a significant

relationship between increased protein intake and lower risk of hypertension and coronary heart disease. In experimental animal models feeding a variety of different plant proteins is associated with lower serum cholesterol concentrations than with feeding a variety of different animal proteins (Carroll, 1982). Legume proteins appear to have specific hypocholesterolemic effects. Less is known about the effects of other commonly consumed varieties, especially African yam beans (AYB) on heart diseases (Anderson & Major, 2002; Anderson, Smith, & Washnock, 1999).

African Yam bean (AYB) (Sphenostylis stenocarpa) is an herbaceous leguminous plant occurring throughout tropical Africa (Porter, 1992) and grows during the wet season (Allen & Allen, 1981). It is often cited among the lesser-known and underexploited species (Ene-Obong & Okoye, 1992; Bennett-Lartey, Ayensu, Monma, & Ito, 1993). It is cultivated both for the seeds and tubers because of its valuable and prominent source as plant protein (Nwodo & Nwinyi, 2012). Okeke, Ene-Obong, Uzoegbuna, Ozioko and Kuhnlein (2008) reported that AYB has medicinal value. According to Ngwu, Ndiokwelu, Ibaro and Nwachi (2012), AYB showed a significant reduction in the fasting blood sugar, blood pressure, appetite, urination, foot numbness and itching of diabetics in a rural community. Uguru and Madukaife (2001) who did a nutritional evaluation of forty-four genotypes of AYB reported that the crop is well balanced in essential amino acids and has higher amino acid content than pigeon pea, cowpea and bambara groundnut. The nutritional values of its whole and dehulled seeds have been reported (Masdac & Jerums, 2004). Nutritionally, AYB compares favourably with the cowpea bean which has been found to reduce blood cholesterol. Cowpea bean contains 53.3% carbohydrates, 25.9% proteins, and 2% fat (Frota *et al.,* 2008) while AYB has 62.6% carbohydrate, 19.5% protein and 2.5% fat (Okigbo, 1973). The aim of this study is to investigate the proximate and phytochemical constituents of AYB and its effect on hypercholesterolemia.

1.2 Statement of the Problem

This study is hinged on the problem of hypercholesterolemia and its adverse effects. Little effort has been made on the management of hypercholesterolemia through diet diversification programs especially using indigenous crops. A diet-based systemic approach to abolish these health disparities is desirable because it will assure the long-term solutions against the existing menace (Butt, Shahzadi, Sharif & Nasir, 2007). Recent studies suggest plant-based diets may be more effective for weight loss, blood sugar control, and reduction of cardiovascular risk factors, particularly blood cholesterol. Legumes have benefits in terms of reduced risk of coronary heart disease due to their many components such as phytochemicals. There are many phytochemicals and each work differently. The synergistic or antagonistic effects of mixtures of these phytochemicals from food legumes, their interaction with other components of the diet and the mechanism of their action have remained a challenge with regard to understanding the role of phytochemicals in health and diseases. Dietary intake of phytochemicals may provide health benefits, protecting against numerous diseases or disorders, such as coronary heart disease, diabetes, high blood pressure and inflammation (Bouchenak & Lamri-Senhadj, 2013).

High levels of lipids in the form of cholesterol (LDL) cause heart disease, stroke, poor circulation and kidney disease. High levels of lipids in the blood are due to the consumption of foods high in fat, or due to an inherited disorder. It could also be caused by medical conditions such as diabetes, obesity, hyperthyroidism, alcoholism, kidney disease, liver disease and stress. The best way to reduce lipid levels in the blood is to eat less fat, exercise regularly and loose weight if necessary (American Academy of Family Physicians, 1998). AYB is highly nutritious with low fat, high protein, mineral and dietary fiber (Ene-Obong & Carnovale, 1992). The potential role of AYB consumption on the prevention and treatment of chronic diet-related non communicable diseases such as obesity, diabetes, hypertension and

cardiovascular diseases has been suggested (Oshodi, Ipinmoroti & Adeyeye, 1997; Onyechi & Nwachi, 2008; Alozie, Udofia, Lawal & Ani, 2009).

This study seeks to determine the proximate and phytochemical composition of AYB and its effect on hypercholesterolemic rats.

1.3 Objective of the study

1.3.1 General Objective: The broad objective of the study is to determine the proximate and phytochemical composition of African Yam Bean and its effect on the lipid profile of hypercholesterolemic rats.

1.3.2 Specific Objectives: The specific objectives of the study were to:

1. assess the proximate composition of the flour;
2. assess some phytochemical constituents of the flour (saponins, phytate, tannins and flavonoid);
3. determine the serum total cholesterol, triglyceride, low density lipoprotein, and high density lipoprotein of adult Wister rats and
4. determine the effect of roasted AYB flour on the lipid profile of the adult Wister rats.

1.4 Significance of the study

The result of this study will generate scientific evidence that will be used by dietitians and other health care professionals in dietary counseling and for prevention and management of non communicable diseases (NCDs). This will be a means of diversification and upgrading AYB. It will provide basic data and information for sustainable commercial exploitation and increased use of the legume. The findings would increase the food use of AYB which will consequently increase the production of AYB by farmers and strengthen the food security of the people. It will create more areas of research based on AYB. It will also revitalize the place of AYB in the food system.

CHAPTER TWO

LITERATURE REVIEW

2.1 Hypercholesterolemia

Hypercholesterolemia is a condition characterized by very high levels of cholesterol in the blood. Cholesterol is a waxy, fat-like substance that is produced in the body and obtained from foods that come from animals (particularly egg yolks, meat, poultry, fish, and dairy products). The body needs this substance to build cell membranes, make certain hormones, and produce compounds that aid in fat digestion. Too much cholesterol, however, increases a person's risk of developing heart disease. Since cholesterol is insoluble in water, it is transported in the blood plasma bound with protein (lipoproteins). Lipoproteins are classified by their density (very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) (Biggerstaff & Wooten, 2004). All the lipoproteins carry cholesterol, but elevated levels of the lipoproteins other than HDL (termed non-HDL cholesterol), particularly LDL-cholesterol are associated with an increased risk of atherosclerosis and coronary heart disease (Carmena *et al.,* 2004). This is because while HDL helps remove cholesterol from cells and in turn, its excretion from the body, LDL carries cholesterol made from the liver and other sources to cells. In contrast higher levels of HDL cholesterol are protective (Kontush & Chapman, 2006). Elevated levels of non-HDL cholesterol and LDL in the blood may be a consequence of diet, obesity, inherited (genetic) diseases (such as LDL receptor mutations in familial hypercholesterolemia), or the presence of other diseases such as diabetes and an underactive thyroid (Durrington, 2003). Reducing dietary fat is recommended to reduce total blood cholesterol and LDL in adults (Hooper *et al*., 2012). In people with very high cholesterol (e.g. familial hypercholesterolemia) diet is often insufficient to achieve the desired lowering of

LDL and medications which reduce cholesterol production or absorption are usually required (Ito, McGowan, & Moriarty, 2011). If necessary other treatments, including LDL apheresis or even surgery (for particularly severe subtypes of familial hypercholesterolemia) are performed. More than 34 million American adults have elevated blood cholesterol levels (higher than 240 mg/dl).

Inherited forms of hypercholesterolemia, which cause even higher levels of cholesterol, occur less frequently. The most common inherited form of high cholesterol is called familial hypercholesterolemia. This condition affects about 1 in 500 people in most countries. Familial hypercholesterolemia occurs more frequently in certain populations, including Afrikaners in South Africa, French Canadians, Lebanese, and Finns.

2.2 Signs and Symptoms of hypercholesterolemia

Although hypercholesterolemia itself is asymptomatic, longstanding elevation of serum cholesterol can lead to atherosclerosis (Bhatnagar, Soran, & Durrington, 2008). Over a period of decades, chronically elevated serum cholesterol contributes to formation of atheromatous plaques in the arteries. This can lead to progressive narrowing or even complete blockage of the involved arteries. Alternatively smaller plaques may rupture and cause a clot to form and obstruct blood flow (Finn, Nakano, Narula, Kolodgie, & Virmani, 2010). A sudden blockage of a coronary artery results in a myocardial infarction or heart attack. A blockage of an artery supplying the brain can cause a stroke. If the development of the narrowing or blockage is gradual blood supply to the tissues and organs slowly diminishes until organ function becomes impaired. At this point that tissue ischemia (restriction in blood supply) may manifest as specific symptoms. For example, temporary ischemia of the brain (commonly referred to as a transient ischemic attack) may manifest as temporary loss of vision, dizziness and impairment of balance, aphasia (difficulty speaking), paresis (weakness) and paresthesia (numbness or tingling), usually on one side of the body. Insufficient blood supply to the heart

may manifest as chest pain, and ischemia of the eye may manifest as transient visual loss in one eye. Insufficient blood supply to the legs may manifest as calf pain when walking, while in the intestines it may present as abdominal pain after eating a meal (Durrington, 2003). Some types of hypercholesterolemia lead to specific physical findings. For example, familial hypercholesterolemia (Type IIa hyperlipoproteinemia) may be associated with xanthelasma palpebrarum (yellowish patches underneath the skin around the eyelids) (Shields C & Shields J, 2008), arcus senilis (white or gray discoloration of the peripheral cornea) (Zech & Berger, 2006), and xanthomata (deposition of yellowish cholesterol-rich material) of the tendons, especially of the fingers (James & Berger, 2006; Rapini, Bolognia, & Jorizzo, 2007). Type III hyperlipidemia may be associated with xanthomata of the palms, knees and elbows (James & Berger, 2006).

2.3 Causes of hypercholesterolemia

Hypercholesterolemia is typically due to a combination of environmental and genetic factors (Bhatnagar *et al*., 2008). Environmental factors include: obesity and dietary choices. Genetic contributions are usually due to the additive effects of multiple genes. However, occasionally it may be due to a single gene defect such as in the case of familial hypercholesterolemia. A number of secondary causes exist including: diabetes mellitus type 2, obesity, alcohol, monoclonal gammopathy, dialysis, nephrotic syndrome, obstructive jaundice, hypothyroidism, Cushingôs syndrome, anorexia nervosa, medications (thiazide diuretics, ciclosporin, glucocorticoids, beta blockers, and retinoic acid) (Bhatnagar *et al*., 2008).

2.3.1 Diet

Diet has an important effect on blood cholesterol but the size of this effect varies substantially between individuals (Howell, McNamara, Tosca, Smith, & Gaines, 1997). Approximately 50% of non-esterified cholesterol is absorbed in the intestine, but inter-individual variations in the efficiency of uptake and the effect of other dietary components

such as plant sterols and fiber content affect absorption (Lichtenstein, 1990). Moreover, when dietary cholesterol intake goes down, production (principally by the liver) typically increases, though not always with complete compensation, so that reductions in blood cholesterol can be modest (Stryer, Berg, & Tymoczko, 2007). Reductions in fat intake, particularly saturated fats, also reduce blood cholesterol (Sacks & Katan, 2002). Recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemia in 2011 were that people with familial hypercholesterolemia should follow The National Cholesterol Education Program (NCEP) Third Adult Treatment Panel (ATP III), Therapeutic Lifestyle Changes (TLC) diet and restrict intakes of total fat to 25 - 35% of energy intake; that saturated fatty acids should make up less than 7% of energy intake, and that cholesterol intake should be less than 200 mg per day. There is also evidence that inclusion of 2 g per day of plant sterol or sterol esters and 10 to 20 g per day of soluble fiber decrease dietary cholesterol absorption. Dietary changes can typically achieve reductions of 10 to 15% in blood cholesterol. Maintaining a healthy body weight through increased physical activity and appropriate caloric intake is also important. Overweight or obese individuals can lower blood cholesterol by losing weight - on average a kilogram of weight loss can reduce LDL cholesterol by 0.8 mg/dl (Ito *et al.*, 2011).

2.3.2 Genetics

Genetic abnormalities are in some cases completely responsible for hypercholesterolemia, such as in familial hypercholesterolemia where there is one or more genetic mutations in, for example, the LDL receptor. Even when there is no single responsible mutation to explain hypercholesterolemia, genetic predisposition still plays a major role, potentially adding to lifestyle factors and multiplying the risk of late complications. Multiple genes are involved, and hypercholesterolemia where there is a genetic predisposition is called polygenic hypercholesterolemia (Citkowitz & Isley, 2010).

2.4 Risk factors of hypercholesterolemia

There are several factors that contribute to high cholesterol. Some are controllable while others are not.

Uncontrollable Risk Factors

- Gender: After menopause, a woman's LDL-cholesterol level ("bad" cholesterol) goes up, as does her risk for heart disease.
- Age: Your risk increases as you get older. Men aged 45 years or older and women aged 55 years or older are at increased risk of high cholesterol.
- Family history: Your risk increases if a father or brother was affected by early heart disease (before age 55) or a mother or sister was affected by early heart disease (before age 65).

Controllable Risk Factors

- Diet: The saturated fat and cholesterol in the food you eat raise total and LDL-cholesterol levels.
- Weight: Being overweight can make your LDL-cholesterol level to go up and your HDL level go down.
- Physical activity/exercise: Increased physical activity helps to lower LDL- cholesterol and raise HDL-cholesterol (the "good" cholesterol) levels. It also helps you lose weight.

2.5 Diagnosis

Cholesterol is bound to protein molecules (called lipoproteins) as it travels through the blood stream. LDL and VLDL deliver cholesterol to the body. HDL removes cholesterol from the blood stream. This is why HDL is often termed ñgoodò cholesterol. LDL and VLDL cholesterol are more likely to damage the blood vessels if the levels are high. Total cholesterol is a combination of HDL, LDL and VLDL cholesterol. A blood test will show whether these blood cholesterol are high.

Cholesterol levels for adults

Total cholesterol levels:

<200mg/dl = normal

200 ï 239mg/dl = borderline high

Ó240mg/dl = high risk for artery damage, heart disease and stroke

LDL cholesterol levels:

<130mg/dl = normal

130 ï 159mg/dl = borderline high

Ó160 = increased risk for heart disease

HDL cholesterol levels:

Ó60mg/dl = reduced risk of heart disease

<35mg/dl = increased risk for heart disease.

Source: Albers (1996).

2.6 Diseases and conditions associated with hypercholesterolemia

2.6.1 Cardiovascular Diseases (CVD)

Cardiovascular disease is a class of diseases that involve the heart or blood vessels (arteries and veins) (Maton, 1993). Cardiovascular disease refers to any disease that affects the cardiovascular system, principally cardiac disease, vascular diseases of the brain and kidney, and peripheral arterial disease (Kelly & Fuster, 2010). The causes of cardiovascular disease are diverse but atherosclerosis and/or hypertension are the most common. Cardiovascular diseases remain the biggest cause of deaths worldwide, though over the last two decades, cardiovascular mortality rates have declined in many high-income countries. At the same time cardiovascular deaths and disease have increased at an astonishingly fast rate in low- and middle-income countries (Mendis, Puska, & Norrving, 2011). Although cardiovascular disease usually affects older adults, the antecedents of cardiovascular disease, notably atherosclerosis begin in early life, making primary prevention efforts necessary from childhood (McGall, McMahan, & Gidding, 2008). There is therefore increased emphasis on preventing atherosclerosis by modifying risk factors, such as healthy eating, exercise, and avoidance of smoking. Evidence shows that the Mediterranean diet improves cardiovascular outcomes (Walker & Reamy, 2009). In clinical trials the Dietary Approaches to Stop Hypertension (DASH) shows diet to reduce blood pressure (Sacks, Svetkey, & Vollmer, 2001), lower total and low density lipoprotein cholesterol (Obarzanek, Sacks, & Vollmer, 2001) and improve metabolic syndrome (Azadbakht, Mirmiran, Esmaillzadeh, Azizi T, & Azizi F, 2005); but the long term benefits outside the context of a clinical trial have been questioned (Logan, 2007).

Some of the CVDs related to high blood cholesterol are

- **Atherosclerosis** (also known as arteriosclerotic vascular disease or ASVD) is a condition in which an artery wall thickens as a result of the accumulation of fatty materials such as cholesterol. It is a syndrome affecting arterial blood vessels, a chronic inflammatory response in the walls of arteries, caused largely by the accumulation of macrophage white blood cells and promoted by low-density lipoproteins (plasma proteins that carry cholesterol and triglycerides) without adequate removal of fats and cholesterol from the macrophages by functional high density lipoproteins (HDL). It is commonly referred to as a hardening or furring of the arteries. It is caused by the formation of multiple plaques within the arteries (Maton *et al*., 1993). Atherosclerosis does not occur in children, but can begin forming as early as the teen years. Until progressing to an advanced stage, it is usually asymptomatic. Atheroma in arm, or more often in leg arteries, which produces decreased blood flow, is called peripheral artery occlusive disease (PAOD). Typically, atherosclerosis begins as a thin layer of white streaks on the artery wall (usually due to white blood cells) and progresses from there. According to United States data for the year 2004, for about 66% of men and 47% of women, the first symptom of atherosclerotic cardiovascular disease is heart attack or sudden cardiac death (death within one hour of onset of the symptom). The main cause of atherosclerosis is yet unknown, but is hypothesized to be fundamentally initiated by inflammatory processes in the vessel wall in response to retained low-density lipoprotein (LDL) molecules (Williams & Jabas, 1995). Once inside the vessel wall, LDL molecules become susceptible to oxidation by free radicals (Sparrow & Olszewski, 1993) and become toxic to the cells. The damage caused by the oxidized LDL molecules triggers a cascade of immune responses which over time can produce an atheroma. The LDL molecule is globular shaped with a hollow core to carry cholesterol throughout the body. The body's

immune system responds to the damage to the artery wall caused by oxidized LDL by sending specialized white blood cells (macrophages and T-lymphocytes) to absorb the oxidized-LDL forming specialized foam cells. These white blood cells are not able to process the oxidized-LDL, and ultimately grow then rupture, depositing a greater amount of oxidized cholesterol into the artery wall. This triggers more white blood cells, continuing the cycle. Eventually, the artery becomes inflamed. The cholesterol plaque causes the muscle cells to enlarge and form a hard cover over the affected area. This hard cover is what causes a narrowing of the artery and reduces blood flow (Mitchell, Kumar, Abbas, & Fausto, 2007).

- **Coronary heart disease** (CHD) is the narrowing or blockage of the coronary arteries, usually caused by atherosclerosis. Atherosclerosis (sometimes called ñhardeningò or ñcloggingò of the arteries) is the build up of cholesterol and fatty deposits (called plaques) on the inner walls of the arteries. These plaques can restrict blood flow to the heart muscle by physically clogging the artery or by causing abnormal artery tone and function. Without an adequate blood supply, the heart becomes starved of oxygen and the vital nutrients it needs to work properly. This can cause chest pain called angina. If blood supply to a portion of the heart muscle is cut off entirely, or if the energy demands of the heart become much greater than its blood supply, a heart attack (injury to the heart muscle) may occur.
- **Stroke**, or **cerebrovascular accident** (**CVA**), is the rapid loss of brain function(s) due to disturbance in the blood supply to the brain. This can be due to ischemia (lack of blood flow) caused by blockage (thrombosis, arterial embolism), or a haemorrhage (leakage of blood) (Sims & Muyderman, 2009) As a result, the affected area of the brain cannot function, which might result in an inability to move one or more limbs on one side of the body, inability to understand or formulate speech, or an inability to

see one side of the visual field (Donnan, Fisher, Macleod, & Davis, 2008). Risk factors for stroke include old age, hypertension (high blood pressure), previous stroke or transient ischemic attack (TIA), diabetes, high cholesterol, cigarette smoking and atrial fibrillation. High blood pressure is the most important modifiable risk factor of stroke (Donnan et al., 2008). High cholesterol levels have been inconsistently associated with ischemic stroke (Iso, Jacobs, Wentworth, Neaton, & Cohen, 1989). Patients with diabetes mellitus are 2 to 3 times more likely to develop stroke, and they commonly have hypertension and hyperlipidemia. Nutrition, specifically the Mediterranean-style diet, has the potential for decreasing the risk of having a stroke by more than half (Spence & Lees, 2006).

2.6.2 Obesity

Hypercholesterolemia is frequently found in patients with obesity, so that the average serum cholesterol level is significantly higher in overweight subjects than in lean ones, and usually a significant correlation exists between serum cholesterol and obesity (Tanner, 1951; Montoye, Epstein, & Kjelsberg, 1966). It should be borne in mind, however, that a great many obese patients have normal serum lipid concentrations. Hypercholesterolemia and obesity are known to be associated with the development of ischemic heart disease, although the association is relatively weak in the case of obesity (Keys, 1970). The Third National Cholesterol Education Program specifically recommends weight management for preventing hypercholesterolemia. Whereas randomized controlled clinical trials show that weight loss causes cholesterol lowering (Hutton & Fergusson, 2004). Evidence linking weight gain to increased cholesterol is derived primarily from cross-sectional associations, longitudinal studies of concurrent weight and cholesterol increases (Noppa, 1980; Grinker, Tucker, Vokonas, & Rush, 2000; Norman, Bild, Lewis, Liu, & West, 2003), and prospective epidemiological studies (Ishikawa-Takata, Ohta, Moritaki, Gotou & Inoue, 2002). Several

longitudinal studies report that changes in weight have greater effect on cholesterol levels in men than in women (Norman *et al*., 2003), while others report a greater effect in women, or no difference. The effects of changing body weight on plasma cholesterol concentrations may also vary with level of adiposity, as some studies suggest greater increases with weight gain among nonobese than obese men and women (Norman *et al.,* 2003).

2.6.3 Diabetes

Researchers are still figuring out exactly how diabetes changes cholesterol levels at the microscopic cellular level. They do know that high levels of insulin in the blood tend to adversely affect the number of cholesterol particles in the blood. High insulin levels act to raise the amount of LDL cholesterol (the "bad cholesterol") that tends to form plaques in arteries, and lower the number of HDL cholesterol particles ("good cholesterol") that help to clear out dangerous plaques before they break off to cause a heart attack or stroke. Diabetes also tends to cause higher levels of triglycerides, another type of fat circulating in the blood (Ross, 2011). Similarly, high cholesterol can also be a predictor of diabetes; elevated cholesterol levels are often seen in people with insulin resistance, even before they have developed full-blown diabetes. High or borderline high total cholesterol is common in diabetes and is present in 70% of adults with diagnosed diabetes and 77% with undiagnosed diabetes in the U.S. population. Although elevated LDL cholesterol is uncommon in people with diabetes who have total cholesterol of <200 mg/dl, other risk factors for coronary heart disease are very frequent (100% of men, 73% of women), and low total and LDL cholesterol may mask low high-density lipoprotein cholesterol (Harris, 1991). People with Type 2 diabetes, regardless of blood sugar control, tend to have increased triglycerides, decreased HDL, and sometimes increased LDL. This cholesterol profile tends to persist even if blood sugar levels are under control--pointing to an even higher likelihood of developing plaques. In fact, plaques formed in the arteries of people with Type 2 diabetes tend to be fattier and

less fibrous than in people with Type 1 diabetes, leading to an even higher risk of a plaque dislodging to cause a heart attack or stroke (Ross, 2011). Therefore, investigation of blood lipid levels and coronary heart disease risk factors should be routine in all patients with diabetes, and treatment strategies should include management of lipid disorders and the multiple other risk factors for coronary heart disease that are highly prevalent in these patients (Harris, 1991).

2.6.4 High Blood Pressure

High blood pressure can result from narrowed arteries such as those that occur with hyperlipidemia. Systolic blood pressure, or the top number, measures the force of blood leaving the heart and traveling through the body's arterial system. When the arteries are narrowed from plaque build up, the pressure rises to force the blood through smaller passageways.

2.7 Management of Hypercholesterolemia

Hypercholesterolemia can be managed by diet, exercise and medications. The specific treatment plan will depend on the cholesterol level (including LDL cholesterol) and on the history of coronary artery disease or risk factors for coronary artery disease.

2.7.1 Diet

The Adult Treatment Panel III (ATP III) of the National Cholesterol Education Program has for the past decade recommended non pharmacologic treatment as initial therapy in most patients with hyperlipidemia. The Therapeutic Lifestyle Changes (TLC) approach was based on the panel's review of the available evidence in 1999 that concluded that diet and exercise can have a beneficial effect on serum levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides. The TLC diet recommendations according to ATP 111 Guideline include

- Saturated fat less than 7% of calories
- Monounsaturated fat about 20% of calories
- Polyunsaturated fat about 10% of calories
- Protein about 15% of calories
- Carbohydrates about 50% of calories
- Fiber about 25 grams per day
- Cholesterol less than 200 milligrams per day

2.7.2 Dietary Factors

A large number of dietary factors may influence lipid levels. These include modification of nutritional components, consumption of specific foods, use of food additives and supplements, and major dietary approaches.

2.7.3 Nutritional Components

Comprehensive reviews of the evidence for dietary influences on levels of serum lipids and cardiovascular disease have been published (Katcher, Hill, Lanford, Yoo, & Kris-Etherton, 2009; Van Horn, McCoin, Kris-Etherton, 2008). Decreasing total fat intake and replacing saturated and trans fats with polyunsaturated and monounsaturated fats, along with limiting dietary cholesterol, lower total cholesterol, LDL cholesterol, and triglyceride levels. Compared with a baseline or Western diet, reducing saturated fat intake to 7 percent of total calories and limiting cholesterol to 200 mg per day reduce LDL cholesterol levels by 9 to 12 percent (Van Horn *et al*., 2008). A meta-analysis of 224 studies of dietary interventions showed that changes in total cholesterol levels were affected primarily by changes in intake of saturated and polyunsaturated fats, and dietary cholesterol (Howell *et al.,* 1997). Another meta-analysis of 60 controlled trials showed that replacing trans fats with polyunsaturated fats from unhydrogenated oils is the most effective measure for improving blood lipid

profiles (Mensink, Zock, Kester, & Katan, 2003). A meta-analysis of 67 controlled trials of dietary soluble fiber as a single intervention showed that the effects on total cholesterol and LDL cholesterol levels were modest. For example, the addition of three 28-g servings of oats per day decreases LDL cholesterol levels by 5 mg/dl (Brown, Rosner, Willett, & Sacks, 1999). Some persons had little change in lipid levels despite significant changes in fat and cholesterol intake (Cox, Mann, Sutherland, & Ball, 1995). This observed variation may be explained by genetic factors (Wallace, Mann, & Sutherland, 2000) or insulin resistance (Lefevre, Champagne, Tulley, Rood, & Most, 2005).

2.7.4 Specific Foods

Tree nuts are high in unsaturated fats and low in saturated fats. Recent reviews of published data conclude that consumption of tree nuts can reduce LDL cholesterol levels by 2 to 19 percent compared with lower-fat and Western diets (Griel & Kris-Etherton, 2006; Mukuddem-Peterson, Oosthuizen, & Jerling, 2005). Nuts are calorie-dense and therefore should be isocalorically substituted for other foods. Recommended amounts range from 1 to 3 oz (28-85g) per day, at least five days per week (Van Horn, *et al.,* 2008; Mukuddem-Peterson *et al.,* 2005). Soy protein can also be used to replace foods high in saturated fats and trans fats. A meta-analysis of 41 randomized controlled trials concluded that soy protein supplementation leads to small reductions in total cholesterol and LDL cholesterol levels (about 5 and 4 mg/dl respectively), as well as small increases in HDL cholesterol levels (about 0.8 mg/dl) (Reynolds *et al.,* 2006). The typical amount of soy protein used in studies has been 1.0 to 1.5 oz (28-43g) per day.

2.7.5 Additives and Supplements

A comprehensive review of plant sterols showed that these substances lower LDL cholesterol levels in persons at risk of coronary heart disease (Katan *et al.,* 2003). This review included a

meta-analysis of 41 trials showing that 2 g per day of either stanols or sterols reduces LDL cholesterol levels by 10 percent. These effects are additive with other diet or drug interventions. Fortified foods typically have 0.5 to 1 g of sterol or stanol per serving (Van Horn *et al.,* 2008).

2.7.6 Dietary fibre

Dietary fibre may act on each phase of metabolism (ingestion, digestion, absorption and excretion) to affect cholesterol metabolism (Brown *et al.,* 1999) such as the following:

1. Caloric energy of foods through a bulking effect
2. Slowing of gastric emptying time
3. A glycemic index type of action on absorption
4. A slowing of bile acid absorption in the ileum so bile acids escape through to the cecum
5. Altered or increased bile acid metabolism in the cecum
6. Indirectly by absorbed short-chain fatty acids, especially propionic acid, resulting from fiber fermentation affecting the cholesterol metabolism in the liver.
7. Binding of bile acids to fiber or bacteria in the cecum with increased fecal loss from the entero-hepatic circulation.

An important action of some fibers is to reduce the reabsorption of bile acids in the ileum and hence the amount and type of bile acid and fats reaching the colon. A reduction in the reabsorption of bile acid from the ileum has several direct effects.

1. Bile acids may be trapped within the lumen of the ileum either because of a high luminal viscosity or because of binding to a dietary fiber (Eastwood & Hamilton, 1968)

2. Lignin in fiber absorbs bile acids, but the unconjugated form of the bile acids are absorbed more than the conjugated form. In the ileum where bile acids are primarily absorbed the bile acids are predominantly conjugated.
3. The enterohepatic circulation of bile acids may be altered and there is an increased flow of bile acids to the cecum, where they are deconjugated and 7alpha-dehydroxylated.
4. These water-soluble form, bile acids e.g., deoxycholic and lithocholic are adsorbed to dietary fiber and an increased fecal loss of sterols, dependent in part on the amount and type of fiber.
5. A further factor is an increase in the bacterial mass and activity of the ileum as some fibers e.g., pectin are digested by bacteria. The bacterial mass increases and cecal bacterial activity increases.
6. The enteric loss of bile acids results in increased synthesis of bile acids from cholesterol which in turn reduces body cholesterol.

The fibers that are most effective in influencing sterol metabolism (e.g. pectin) are fermented in the colon. It is therefore unlikely that the reduction in body cholesterol is due to absorption to this fermented fiber in the colon.

1. There might be alterations in the end-products of bile acid bacterial metabolism or the release of short chain fatty acids which are absorbed from the colon, return to the liver in the portal vein and modulate either the synthesis of cholesterol or its catabolism to bile acids.
2. The prime mechanism whereby fiber influences cholesterol metabolism is through bacteria binding bile acids in the colon after the initial deconjugation and dehydroxylation (Gillissen & Eastwood, 1995).

3. Fermentable fibers e.g., pectin will by virtue of their providing a medium for bacterial growth increase the bacterial mass in the colon. The sequestrated bile acids are then excreted in feces.
4. Other fibers, e.g., gum arabic, are associated with a significant decrease in serum cholesterol without increasing fecal bile acid excretion

2.7.7 Dietary Approaches

Both low-fat and low-carbohydrate diets affect lipid levels. In a meta-analysis of randomized controlled trials (RCT) comparing these approaches, low-fat diets had the most favorable effects on total cholesterol and LDL cholesterol levels, whereas low-carbohydrate diets had the most favorable effects on triglyceride and HDL cholesterol levels(J. Nordmann, A. Nordmann, & Briel, 2006). An RCT showed that the total-to-HDL cholesterol ratio was reduced by 20 percent in participants following a low-carbohydrate diet, compared with a 12 percent reduction in those following a low-fat diet; this was a statistically significant difference (Shai, Schwarzfuchs, & Henkin, 2008). The reduction in the total-to-HDL cholesterol ratio is similar to the reduction in LDL cholesterol levels, and the total cholesterol level is reduced primarily by changes in the LDL cholesterol levels.

The Mediterranean diet is characterized by a high consumption of monounsaturated fats (primarily from olive oil) and low consumption of saturated fats (Katcher *et al.,* 2009). Other characteristics include limited consumption of red meat, dairy products, eggs, and poultry; increased consumption of fish, tree nuts, vegetables, and whole grains; and moderate consumption of wine. When two versions of a Mediterranean diet were compared with a low-fat diet, the Mediterranean diets lowered the total-to-HDL cholesterol ratio more than the low-fat diet (Estruch, Martinez-Gonzalez, & Corella, 2006).

The Portfolio Diet is a plant-based TLC diet that is a composite of four additional LDL cholesterolï lowering components: soluble fiber, soy and other vegetable proteins, plant sterols, and almonds (Kendall & Jenkins, 2004). In controlled settings, the Portfolio Diet has been shown to reduce LDL cholesterol levels by 29 to 35 percent, comparable to a combination of a diet low in saturated fats and cholesterol plus 20 mg of lovastatin (Mevacor) daily (Katcher *et al*., 2009). Among persons eating self-selected Portfolio Diet foods, 32 percent achieved LDL cholesterol reductions of more than 20 percent after one year (Jenkins, Kendall, & Faulkner, 2006).

The use of fibers and legumes for the management of hypercholesterolemia has been reported by many researchers. Among the mechanisms that have been proposed to explain the cholesterol-lowering effect of these legumes are the physiological action of legume components such as phytic acid, dietary fiber, saponins, phytosterols, proteins, and their amino acid profiles (Reynolds *et al.,* 2006). Cholesterol lowering effects are most often associated with gelling, mucilaginous, viscous fibers such as guar gum and pectin as well as other legumes. The mechanism responsible for the cholesterol lowering effect of dietary fiber is partly related to enhanced excretion of bile acids into faeces, and the hypotriacylglycerolemic effects associated with a decreased absorption of dietary lipids and a decreased activity of fatty acid synthase in the liver (Yamamoto, 2001). A study by Oboh and Omofoma (2008) showed a significant reduction in the amount of serum lipids in rats fed heat treated lima beans. The consumption of lima beans has been recommended to also lower cholesterol and promote cardiovascular health due to the presence of saponin in the legume. Guar gum, an annual legume crop a great source of dietary fiber almost in the soluble form helps to lower cholesterol and glucose levels (Pszczola, 2003). Another study of whole cowpea seed and cowpea protein isolate showed a significant reduction in plasma cholesterol and non HDL cholesterol (Frota *et al.,* 2008). Hypocholesterolemic effect have been

observed in studies with faba beans in rats (Macarulla *et al*., 2001), soy protein isolate in hamsters (Song, Lee, Murphy, & Hendrich, 2003), peas and blue lupine in pigs (Martins *et al.,* 2004, 2005). Some legumes like AYB are yet to be worked with, hence this study on the effect of AYB on plasma lipid of hypercholesterolemic rats.

2.8 African Yam Bean (AYB)

African Yam Bean (*Sphenostylis stenocarpa*) is a grain legume that belongs to the family fabacea. It is a vigorous herbaceous, climbing vine, reaching 1.5-2cm in height, with trifoliate leaves, the leaflets being up to 14cm and 5cm broad. The conspicuous flowers are mauvish pink, purple or greenish white in colour, about 2.5cm in length and borne on stout auxiliary pedicles. It has a glabrous seed pods which are linear, flat with both margins raised, 25-30cm long and 1-1.5cm broad, containing 20-30 seeds which may be ellipsoid, rounded or truncated and show considerable variation in colour and size, the largest are usually 1cm long and 0.7cm wide. Seed colour may vary from creamy-white or brownish-yellow to dark brown, sometimes with black marbling. There appear to be a number of varieties according to seed colour (Root Crops, 1987). The plant produces a small spindle-shaped, starchy tubers about 5-7.5cm long, smaller in size than those of sweet potatoes. These take between 5 and 8 months to mature and serve as organs of perennation as the above-ground parts usually wither and die in dry weather (Porter, 1992).

AYB has different names in different countries; Ijiriji, Ukpodudu and Asama in Igbo, Nsama in Ibibio, Ewa in Itsekiri, Sese in Yoruba, Girigiri in Hausa, Yam bean in English, Raya and Norouko in Sudan, Diegemtenguere in Mali, Haricotigname and Pomme detere du mossi in France (Onyechi & Nwachi, 2009; Ene-Obong, 2008).

2.8.1 Origin and Distribution of African Yam Bean

African yam bean is said to have originated from Ethiopia. Both the wild and the cultivated types now occur in tropical Africa as far South as Zimbabwe, throughout West Africa from Guinea to Southern Nigeria, being especially common in the latter and in Togo and Cote Dõlvore, and East Africa from Northern Ethiopia to Mozambique including Tanzania (Porter, 1992). AYB was first introduced into Nigeria in a grain legume shop held in IITA, Ibadan.

The distributional range for African Yam Bean is listed below;

North East tropical Africa ï Chad, Ethiopia

East tropical Africa ï Kenya, Tanzania and Uganda

West Central Tropical Africa ï Burundi, Central African Republic, Zaire

West Tropical Africa ï Cote dõivore, Ghana, Guinea, Mali, Niger, Nigeria, Togo

South Tropical Africa ï Angola, Malawi, Zambia, Zimbabwe

2.8.2 Nutritional Composition of African Yam Bean

Proximate analysis of AYB seed (Ene-Obong & Carnovale, 1992) showed that AYB contains about 54.03% carbohydrate, 2.1% fat, 21.6% protein, 19.12% dietary fiber and 3.1% ash. According to them, the seeds also contain calcium 46mg, iron 4.7mg, zinc 2.6mg, phosphorus 288mg, sugar 5.87% and starch 48.2%. The essential amino acid (EAA) content of AYB compares favourably with soybean, cowpea and pigeon pea. It has higher values in all EAA except for phenylalanine where its value was equal to that of cowpea but significantly lower than that of pigeon pea. It compares well with soybean in EAA except for methionine and tryptophan. Despite this limitation, AYB still has more sulphur-containing amino acids(SAA) than pigeon pea, cowpea and soybeans (Ene-Obong *et al.,* 1992). Nwodo *et al.,* (2012) reported that AYB contains the following: 1.96% moisture, 37.21% protein, 9.49% fat, 5.35% ash, 3.55% crude fiber and 44.4% carbohydrate.

2.8.3 AYB in diet-related non communicable diseases management

AYB has been implicated in the prevention, treatment and management of diet-related non communicable diseases such as obesity, diabetes and cardiovascular diseases (Oshodi, Ipinmoroti & Adeyeye, 1997; Onyechi & Nwachi, 2008; Alozie, Udofia, Lawal & Ani, 2009). A study by Ngwu, Ndiokwelu, Ibaro and Nwachi (2012), showed AYB to be effective in improving glycemic control and blood pressure in diabetics. Onyechi and Nwachi (2010) also reported that AYB reduced blood sugar in normoglycemic adults.

2.8.4 Constrains to the use of AYB

The most common constraints to the use of AYB include; low production and non-availability, hard-to-cook (HTC) phenomenon or long cooking time, low status of the crop (women being the sole producers) (Ene-Obong, 1992). The HTC phenomenon has been shown to be genetic and can be taken care of by genetic selective breeding. The HTC constraint can also be handled by simple domestic processing techniques like soaking over night (6-12 hours) can reduce the cooking time by 50%. Soaking was also able to reduce the levels of tannins and phytate (Ene-Obong & Obizoba, 1996). However, prolonged soaking was found to reduce calcium and iron values by 19% and 35% respectively (Ene-Obong, 2008). This is because substantial amount of these minerals are located in the seed coat of the legume.

2.9 Phytochemical and Anti-nutrient

Phytochemicals are a large group of plant-derived compounds hypothesized to be responsible for much of the disease protection conferred from diets high in fruits, vegetables, beans, cereals, and plant-based beverages such as tea and wine (Arts and Hollman, 2005). Based on their chemical structure, phytochemicals can be broken into the following groups.

Types of Phytochemicals

1. Phenolic Acids
2. Flavonoids (anthocyanins, flavonols and flavanols (Catechins &Epicatechins and proanthocyanidins (procyanidins and prodelphinidins))).
3. Stilbenes/ Lignans

Epidemiological studies suggest that consumption of a diet high in fruits and vegetables is associated with a reduced risk of chronic disease (Hung, 2004). Unfortunately, there is not yet enough evidence to support the concept that phytochemicals are responsible for these effects. Fruits and vegetables are important sources of a variety of beneficial agents including vitamins, minerals, fiber, and phytochemicals. More research is needed to fully explain the actions of phytochemical compounds in the human body (Halliwell, 2007). Hundreds of phytochemical compounds, with several different biological functions, have been identified in plant-based foods. Therefore, consuming a variety of plant-based foods helps to ensure that individuals receive the optimum benefits from the fruits and vegetables consumed (Manach, 2004). Due to the lack of food composition data and a true understanding of the absorption and metabolism of phytochemical compounds, the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and Its Panel on Dietary Antioxidants and Related Compounds of the Food and Nutrition Board at the Institute of Medicine chose not to create a Dietary Reference Intake (DRI) for these compounds. Therefore, a recommended intake for phytochemicals does not currently exist. Today, many health authorities such as the American Cancer Society and the American Heart Association recommend consuming a diet high in fruits and vegetables to ensure that an individual ingests an adequate amount of phytochemical compounds (American Cancer Society).

Anti-nutrients are natural or synthetic compounds that interfere with the absorption of nutrients. Nutrition studies focus on those anti-nutrients commonly found in food sources and beverages. Many traditional methods of food preparation such as fermentation, cooking, and malting increase the nutritive quality of plant foods through reducing certain anti-nutrients such as phytic acid, polyphenols, and oxalic acid (Hotz and Gibson, 2007). Such processing methods are widely used in societies where cereals and legumes form a major part of the diet (Phillips, 1993). An important example of such processing is the fermentation of cassava to produce cassava flour: this fermentation reduces the levels of both toxins and anti-nutrients in the tuber (Oboh and Oladunmoye, 2007). The major anti-nutrients mostly found in plant protein sources are toxic amino acids, saponins, cyanogenic glycosides, tannins, phytic acid, gossypol, oxalates, goitrogens, lectins (phytohaemagglutinins), protease inhibitors, chlorogenic acid and amylase inhibitors. Some of them determined in this study include;

2.9.1 Phytate

Phytic acid (phytate) is a substance found in many types of plant foods, such as grains, legumes (including peanuts and soybeans), nuts, and seeds. Itôs the storage form of phosphorus, an important mineral used in the production of energy as well as the formation of structural elements like cell membranes. Phytic acid is found predominantly (about 80% of it) in the bran, or outermost shell, of whole grains. In legumes and seeds, phytic acid resides almost entirely in the endosperm (Schlemmer, Frolich, Prieto & Grases, 2009). Phytate has been termed anti-nutrient due to its ability to bind to essential minerals such as iron, zinc, calcium, and magnesium in the digestive tract and inhibit their absorption by the body (Schlemmer *et al.,* 2009). Despite these, phytic acid actually has some potentially beneficial properties. Phytic acid can act as an antioxidant, particularly in regards to iron (Phillipy and

Graf, 1997). It is known that iron can behave as a free radical, contributing to oxidative stress in the body. In this context, phytic acidôs ability to sequester and trap iron is beneficial. In fact, it does such a good job of binding to iron that it can effectively neutralize any free radical. In addition to acting as an antioxidant, phytic acid also has been shown to exhibit some anti-cancer properties (Vucenik and Shamsuddin, 2006). Though research in humans is a bit scarce, there have been several studies demonstrating the potential positive effects of phytic acid in fighting cancerous tumor cells. This may partially explain why high-fiber diets tend to be associated with reducing colon cancer risk. Finally, phytic acid has shown some capacity to reduce cholesterol and triglycerides, and positively impact the glycemic response of certain foods (Lee, Park and Chun, 2007). In some cases, phytic acid seems to have an ability to slow down a potential blood sugar spike following the ingestion of certain high-carbohydrate foods. Again, this may explain why high-fiber foods have been associated with improved blood sugar control. The potential benefits of phytic acid occur in instances with high dietary phytic acid intake. However, a high intake has also been associated with reduced mineral absorption**.** So, in order for us to get the best of both worlds (if such a thing is possible) itôs important to discover some ways in which we can minimize the negative effects while maximizing the beneficial effects. One way we can do this (specifically in regards to iron) is by incorporating more vitamin C (ascorbic acid) into our diet. These two work well together, with vitamin C placing iron in a chemical state that is more readily absorbed by the body (Hummers and Offeman, 1958).Preparation methods such as soaking, germinating, or fermenting can be very effective in reducing the amount phytic acid present in foods. Some methods are better for different foods. In the case of nuts and legumes, soaking and germinating are most successful, but for grains and cereals, all three are effective. Another way to maximize benefit of phytic acid is to simply increase the consumption of foods rich in

iron, zinc, magnesium, and calcium that are naturally low in phytic acid. For example, consider eating more animal-based proteins.

2.9.2 Tannins

Tannin is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids. The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation (Ferrell and Thorington, 2006). Tannins have traditionally been considered anti-nutritional but it is now known that their beneficial or anti-nutritional properties depend upon their chemical structure and dosage. Recent studies have demonstrated that products containing chestnut tannins included at low dosages (0.15ï 0.2%) in the diet of chickens may be beneficial (Schiavone, Guo and Tassone, 2008). Most legumes contain tannins. Red-coloured beans contain the most tannins, and white-coloured beans have the least. Peanuts without shells have very low tannin content. Chickpeas (garbanzo beans) have a smaller amount of tannins (Reed, 1995). A review published in 2012 found growing consensus for the hypothesis that the specific intake of food and drink containing relatively high concentrations of flavonoids may play a meaningful role in reducing the risk of cardiovascular disease (CVD). Some polyphenols, particularly from the flavan-3-ol (catechin-type), have both anticarcinogenic-proapoptotic and mutagenic effects (Ashutosh, 2003). Some natural polyphenols share the properties of some anticancer drugs such as etoposide and doxorubicin.

2.9.3 Saponins

Saponins are a class of chemical compounds found in particular abundance in various plant species. More specifically, they are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produce when shaken in aqueous solutions, and structurally by

having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative (Hostettmann and Marston, 1995). Saponins are attracting considerable interest as a result of their diverse properties, both deleterious and beneficial. Clinical studies have suggested that these health-promoting components, saponins, affect the immune system in ways that help to protect the human body against cancers, and also lower cholesterol levels. Saponins decrease blood lipids, lower cancer risks, and lower blood glucose response. A high saponin diet can be used in the inhibition of dental caries and platelet aggregation, in the treatment of hypercalciuria in humans, and as an antidote against acute lead poisoning. In epidemiological studies, saponins have been shown to have an inverse relationship with the incidence of renal stones (Shi *et al.,* 2004). The detergent qualities of saponins allow them to bind to bile and prevent its reabsorption. Once bound to saponins, cholesterol leaves your body in waste. Peter R. Cheeke, Ph.D., from the Linus Pauling Institute, notes that many cholesterol-lowering medications perform the same role, and over time excretion of bile may help lower your cholesterol. A lower cholesterol level means less risk of heart attack or stroke. Eating more saponins may boost your immune function and fight off fungal infections, according to an article published in "ACS Chemical Biology" in March 2010. The study noted that saponins cause death of fungal cells, such as Candida albicans, which is responsible for yeast infections, thrush and many hospital-acquired infections. Saponins appear to enhance the immune system's ability to fight off viruses and parasites. Pharmaceutical manufacturers often include saponins in vaccines to increase their effectiveness. According to an article published in the "Journal of Nutrition" in 1995, saponins found in soybeans slow the growth of human cancer cells. These plant compounds may also cause the death of tumor cells, according to an article published in the journal "Phytochemistry Reviews" in June 2010. The exact mechanism of these cell deaths varies depending on the source and dose of saponins. Few studies on saponins used human subjects.

Animals and isolated cells in test tubes are the most common subjects. More research would provide a better picture of the potential role saponins play in cancer treatment and prevention.

2.9.4 Flavonoids

Flavonoids (or bioflavonoids) (from the Latin word *flavus* meaning yellow, their color in nature) are a class of plant secondary metabolites. Flavonoids were referred to as Vitamin P (Benthsath, Rusznyak and Szent-Gyorgyi, 1937) probably because of the effect they had on the permeability of vascular capillaries. Flavonoids have been shown to have a wide range of biological and pharmacological activities in *in vitro* studies. Examples include anti-allergic, anti-inflammatory, antioxidant, anti-microbial (antibacterial, antifungal, and antiviral), anti-cancer, and anti-diarrheal activities (Yamamoto and Gaynor, 2001; Cushnie and Lamb, 2011; Friedman, 2007; Manner *et al.,* 2013). Inflammation has been implicated as a possible origin of numerous local and systemic diseases, such as cancer, cardiovascular disorders, diabetes mellitus, and celiac disease (Manach, Mazur and Scalbert, 2005; Babu, Liu and Gilbert, 2013; Ravishankar, Rajora, Greco and Osborn, 2013).

Preliminary studies indicate that flavonoids may affect anti-inflammatory mechanisms via their ability to inhibit reactive oxygen or nitrogen compounds (Izzi *et al.,* 2012). Flavonoids have also been proposed to inhibit the pro-inflammatory activity of enzymes involved in free radical production, such as cyclooxygenase, lipoxygenase or inducible nitric oxide synthase, and to modify intracellular signaling pathways in immune cells (Izzi *et al.,* 2012).

Procyanidins, a class of flavonoids, have been shown in preliminary research to have anti-inflammatory mechanisms including modulation of the arachidonic acid pathway, inhibition of gene transcription, protein expression and activity of inflammatory enzymes, as well as secretion of anti-inflammatory mediators (Martinez-Micalo *et al.,* 2012). Clinical studies investigating the relationship between flavonoid consumption and cancer

prevention/development are conflicting for most types of cancer, probably because most studies are retrospective in design and use a small sample size (Romagnolo and Selmin, 2012). Two apparent exceptions are gastric carcinoma and smoking-related cancers. Dietary flavonoid intake is associated with reduced gastric carcinoma risk in women, and reduced aerodigestive tract cancer risk in smokers (Gonzalez, Sala and Rokkas, 2013; Woo and Kim, 2013). Among the most intensively studied of general human disorders possibly affected by dietary flavonoids, preliminary cardiovascular disease research has revealed the following mechanisms under investigation in patients or normal subjects (van Dam, Naidoo and Landberg, 2013; Tangney and Rasmussen, 2013):

- inhibit coagulation, thrombus formation or platelet aggregation
- reduce risk of atherosclerosis
- reduce arterial blood pressure and risk of hypertension
- reduce oxidative stress and related signaling pathways in blood vessel cells
- modify vascular inflammatory mechanisms
- improve endothelial and capillary function
- modify blood lipid levels
- regulate carbohydrate and glucose metabolism
- modify mechanisms of aging

Flavonoids have been shown to have (a) direct antibacterial activity, (b) synergistic activity with antibiotics, and (c) the ability to suppress bacterial virulence factors in numerous *in vitro* and a limited number of *in vivo* studies (Taylor, Hamilton-Miller and Stapleton, 2005). Noteworthy among the *in vivo* studies is the finding that oral quercetin protects guinea pigs against the Group 1 carcinogen *Helicobacter pylori* (Oh, Kim and Kim, 2010). Researchers from the European Prospective Investigation into Cancer and Nutrition have speculated this

may be one reason why dietary flavonoid intake is associated with reduced gastric carcinoma risk in European women (Zamora *et al.,* 2012). Additional *in vivo* and clinical research is needed to determine if flavonoids could be used as pharmaceutical drugs for the treatment of bacterial infection, or whether dietary flavonoid intake offers any protection against infection.

The presence of some nutrient inhibitors has been recorded in AYB. They include enzyme inhibitors (e.g. trypsin, chemotrypsin, etc), phytate, polyphenols (e.g. tannins), and other anti-nutrients. The level of these in AYB was found to be lower compared to the other legumes studied (Ene-Obong, 1992). A study by Akuirene (2007, unpublished) shows some of the antinutrient levels in mg/100g as phytate 4.58, tannins 10.11, saponins 0.05, oxalate 3.12 and trypsin 3.12. Trypsin inhibitor which had the greatest effect on the in vitro protein digestibility of the AYB is known to be heat labile. Thus the various heat treatments applied during preparatory procedures are effective in inactivating these inhibitors (Ene-Obong, 1992). The study conducted by Aburime (2011, unpublished) shows that processing methods lower the level of these antinutrients to a health beneficial level. In this study, roasting compared favourably with fermentation. Studies have shown that saponin protects from certain chronic diseases and cancer (Savage, 1993). Tannins have high antioxidant potentials that lower serum cholesterol and reduce the risk of coronary heart disease as well as cancer in the blood (Haslam, 1989; Hollman, 1985). Legume flavonoids play a fundamental role, being excreted by the plant in response to nodulation factors produced by the bacteria. Isoflavones, which are molecules mainly found in legumes, may have beneficial effects on human health, and their use can be of help in the fight against many diseases, including several types of cancer and cardiovascular disorders. Phytates also may help prevent cardiovascular disease and lower a food's glycemic load.

2.10 Proximate analysis

Proximate is used in the analysis of biological materials as a decomposition of a human-consumable good into its major constituents. They are a good approximation of the contents of packaged comestible goods and serve as a cheap and easy verification of nutritional panels. Proximate Analysis is a partitioning of compounds in a food into its different categories based on the chemical properties of the compounds.

From an industry standard proximates include five constituents:

- Ash
- Moisture
- Proteins
- Fat
- Carbohydrates (Calculation)

Analytically, four of the five constituents are obtained via chemical reactions and experiments. The fifth constituent, carbohydrates, is a calculation based on the determination of the four others. Proximates should nearly always add up to 100%, any deviation from 100% displays the resolution of the chemical test i.e. small variations in the way each test is performed chemist to chemist will accumulate or overlap the composition make-up.

There are additional ingredients that may fall under the category of one of the five constituents. Carbohydrates for example include but are not limited to:

- Dietary Fibers
- Sugars
- Sugar Alcohol

Whereas Ash includes but is not limited to:

- Dietary Minerals (Sodium, Potassium, Iron, Calcium)
- Vitamins (ϐ-Carotene, Retinol, Vitamin D_3 Vitamin D_2, B Vitamins)

Although proximates do not give the entire nutritional assay, they are an inexpensive way to track deviations from the quality of foods.

This analysis was an attempt to duplicate animal digestion. After extracting the fat, the sample is subjected to an acid digestion, simulating the acid present in the stomach, followed by an alkaline digestion, simulating the alkaline environment in the small intestine. The crude fiber remaining after digestion is the portion of the sample assumed not digestible by monogastric animals. In the proximate analysis of feedstuffs, Kjeldahl nitrogen, ether extract, crude fiber and ash are determined chemically. The determination of nitrogen allows the calculation of the protein content of the sample.

2.10.1 PROTEIN

Proteins are essential nutrients for the human body. They are one of the building blocks of body tissue, and can also serve as a fuel source. As a fuel, proteins contain 4 kcal (17 kJ) per gram. The most important aspect and defining characteristic of protein from a nutritional standpoint is its amino acid composition. Proteins are polymer chains made of amino acids linked together by peptide bonds. During human digestion, proteins are broken down in the stomach to smaller polypeptide chains via hydrochloric acid and protease actions. This is crucial for the synthesis of the essential amino acids that cannot be biosynthesized by the body (Genton, Melzer and Pichard, 2010).

Widespread popularity of high-protein diets has drawn controversy as well as scientific interest. By reducing intake of carbohydrates and increasing consumption of fats and proteins, such diets are thought to increase satiety, facilitate weight loss, and improve cardiovascular risk factors. In recent years, many randomized controlled studies have compared the effects of higher-protein diets on weight loss and cardiovascular risk factors with those of lower-protein diets. Emerging evidence from clinical trials indicates that higher-protein diets increase short-term weight loss and improve blood lipids, but long-term data are lacking. Findings from epidemiologic studies show a significant relationship between increased protein intake and lower risk of hypertension and coronary heart disease. However, different sources of protein appear to have different effects on cardiovascular disease. Although optimal amounts and sources of protein cannot be determined at this time, evidence suggests a potential benefit of partially replace refined carbohydrates with protein sources low in saturated fats.

Legumes are inexpensive sources of plant protein with potential to be used worldwide as substitutes for animal-protein sources. The protein content of most beans (uncooked) averages 20ï 25% by weight, whereas the protein content of soybeans is å36% by weight. Although dry beans are rich in essential amino acids, they fall short in methionine and tryptophan content, requiring the consumption of larger amounts of protein to meet the nutritional requirements for these essential amino acids (Geil and Anderson, 1994). Soybeans and dry beans may exert a major cholesterol-lowering effect through their vegetable protein content. In experimental animal models feeding a variety of different plant proteins is associated with lower serum cholesterol concentrations than with feeding a variety of different animal proteins (Carroll, 1982). Legume proteins appear to have specific hypocholesterolemic effects. Clinical studies using soy protein for cholesterol reduction date back to 1967, when Hodges *et al.* (1967) showed the cholesterol-lowering properties of soy in

male prison inmates. More than 40 studies have found soy to be effective at reducing cholesterol when added to or replacing animal protein in the diet (Anderson, Johnstone and Cook-Newell, 1995).

2.10.2 FATS

Fats, also known as triglycerides, are esters of three fatty acid chains and the alcohol glycerol.

The terms "oil", "fat", and "lipid" are often confused. "Oil" normally refers to a fat with short or unsaturated fatty acid chains that is liquid at room temperature, while "fat" may specifically refer to fats that are solids at room temperature. "Lipid" is the general term, as a lipid is not necessarily a triglyceride. Fats, like other lipids, are generally hydrophobic, and are soluble in organic solvents and insoluble in water.

Fat is an important foodstuff for many forms of life, and fats serve both structural and metabolic functions. They are necessary part of the diet of most heterotrophs (including humans). Some fatty acids that are set free by the digestion of fats are called essential because they cannot be synthesized in the body from simpler constituents. There are two essential fatty acids (EFAs) in human nutrition: alpha-linolenic acid (an omega-3 fatty acid) and linoleic acid (an omega-6 fatty acid). Other lipids needed by the body can be synthesized from these and other fats. Fats and other lipids are broken down in the body by enzymes called lipases produced in the pancreas

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample collection

African Yam Bean (AYB) seeds (cream coloured) was used for the study. The AYB was purchased from Orba International Market, Udenu L.G.A, Enugu State, Nigeria

3.2 Sample preparation

Seven kilogram (7kg) of cream coloured AYB seeds were handpicked and sorted to remove stones, dirt, damaged seeds and any other extraneous material. The seeds were then washed with deionised water and poured in a sieve to drain. The seeds were toasted in a local frying pan (agbada) over fire with continuous stirring using a wooden spoon until cracking (approximately 15 minutes). After toasting, the seeds were allowed to cool and milled into fine flour. A portion of the flour was analyzed immediately and the remainder stored in an air-tight container and kept in the refrigerator below -4^0C until used for dietary formulation for the rats.

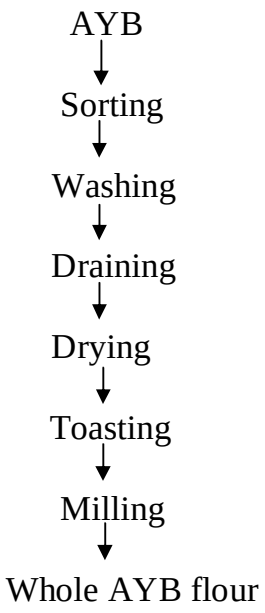

Fig 1: Production of whole AYB flour

3.3 Analytical Procedures

3.3.1 Proximate Analysis

The proximate determination of the food sample was carried out according to the procedure of AOAC (1995) for moisture, fat, protein, ash, carbohydrate and crude fiber. Protein was determined by micro kjeldahl method and carbohydrate was calculated by difference.

Moisture

It was determined by the drying method using oven with aluminum dishes at 78^0C for 3 hours. The dry samples were allowed to cool in desiccators and re-weighed. The samples were put back in the oven and re-weighed at one-hour interval until consistent weight is obtained after three weighing.

$$\%\text{Moisture} = \frac{W_2 - W_1}{\text{Wt of sample}} \times \frac{100}{1}$$

Where W_2 = initial weight of dish and sample

W_1 = final weight of dish and sample

Fat

Sohxlet extraction method was used to determine the fat content of the samples. Two grams of the sample was put in a fat extraction thimble and inserted into the Sohxlet extraction apparatus containing petroleum ether (60% BP). Heat was introduced to boil the solvent. The hot solvent was allowed to flow into the thimble containing the sample to extract fat. At the end of the extraction, the thimble was removed. The extracted fat and the container were kept under fume hood to dry. The dry container and fat were kept in desiccators to cool. The cooled container was reweighed and the difference in weight was the weight of the fat. The value obtained was expressed as a percentage of the original weight of the sample.

Ash

This was determined by combusting 20g of the sample in a muffle furnace at 500^0C for 5 hours. The ashed sample was cooled and re-weighed with the crucible. The percentage ash was calculated using the following steps:

Weight of crucible = A

Weight of crucible + ash = B

Weight of crucible + ash ï weight of crucible = weight of ash (C)

$$\% \text{ Ash} = \frac{\text{weight of ash}}{\text{Weight of sample}} \times \frac{100}{1}$$

Crude protein

Total protein was determined by Micro Kjeldahl method. The analysis of a compound of its protein content by Kjeldahl method is based upon the determination of the amount of reduced nitrogen present. Two hundred milligram (200mg) of the sample was weighed into a micro-kjedahl digestion flask. About 2.5g of anhydrous Na_2SO_4 and 0.5mg of $CuSO_4$ was added as catalysts to the sample and subjected to heat until a colourless solution (ammonium sulphate) is obtained. The solution was then made up to 100ml in a volumetric flask. The micro-kjeldahl distillation apparatus was positioned by allowing the tip to be about 2cm inside the digested sample. About 5ml of 60% NaOH solution was added through the funnel stop cork. On the introduction of steam into the distillation apparatus, NH_3 was liberated, condensed, collected with receiver flask containing 4% boric acid and mixtures of methyl red and blue in a ratio of 2:1 as end point indicator. As soon as NH_3 dropped in the beaker containing a mixture of boric acid, the purple colouration of the mixture changed to yellowish-green; a characteristic of alkaline gas (NH_3). Distillation continued until a reasonable volume is collected for titration.

There was a qualitative analysis of the distillate by titration of a navy blue colour with 0.01N HCl.

$$N\ (mg) = \frac{\text{Titer} \times 14.001}{\text{Weight of sample}}$$

The percentage of protein was determined by the multiplication of %N with the conversion factor 6.25

Crude fibre

About 2g of the sample was weighed into a 500ml beaker. The content was boiled for 30 minutes. It was filtered through a fluted funnel and washed with boiling water until the washing is no longer acidic. The sample was boiled for 30 minutes with 200ml NaOH, solar filtered with hot water using calico cloth, rinsed with 1% HCl and finally with methylated spirit. The residue obtained was collected into crucible and dried in an oven for 30 minutes. The content was cooled in a desiccator and weighed. This was taken to the furnace for ashing at 550^0C for 30 minutes. The ashed sample was removed from the furnace after the temperature has cooled to 200^0C and put into the desiccators and later weighed. The loss in weight between the incinerated residue before and after incineration was taken as the crude fiber content.

$$\%\ \text{Crude fiber} = \frac{\text{total weight of fiber} \times 100}{\text{Weight of the sample}}$$

Carbohydrate

The carbohydrate content of the samples was calculated by summing up the percentage values for protein, ash, fat and moisture. The total % value obtained was then subtracted from 100% to get the percentage of carbohydrate for the sample.

3.3.2 Phytochemical Analysis

The phytochemicals compositions were determined quantitatively.

Saponin Determination

About 20g of the sample was put into a conical flask and 100cm^3 of 20% aqueous ethanol added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55^{0}C. The mixture was then filtered. The residue was mixed with another 200ml of 20% ethanol. The combined extract was put in a water bath at 90^{0}C until the volume is 40ml. The concentrate was transferred to a 250ml separator funnel and 20ml of diethyl ether added. The mixture was vigorously shaken. The aqueous layer was recovered and the ether layer discarded. The purification process was repeated, 60ml of normal butanol added. The combined normal butanol extracts was washed twice with 10ml of 5% aqueous sodium chloride (recover the upper layer and discard the lower layer). The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight. The saponin content was calculated as percentage (Obadons & Ochuko, 2001).

Calculation:

W1 = Weight of empty beaker

W2 = Weight of empty beaker + dried sample

W2 ï W1 = Weight of residue (saponin)

$$\%\text{Saponin} = \frac{W2 - W1}{\text{Weight of sample}} \times \frac{100}{1}$$

Tannin Determination

Tannins was be determined by Van-Buren and Robinson (1981) method. About 500 mg of the sample was weighed into a 50 ml plastic bottle. About 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered mixture was pipetted out into a test tube

and mixed with 2ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 720nm within 10mins.

Flavonoid Determination

The flavonoid content was determined using the method described by Bohn and Kocipai-Abyzan (1994). Ten gram of each sample was extracted with 100ml of 80% aqueous methanol at room temperature and filtered with Whatman filter paper number 42 (125mm). The filtrate was transferred into a crucible and evaporated into dryness over a bath and weighed to a constant weight.

Phytate Determination

Onwuka (2005) method was used. The sample was first extracted using 0.2N HCl, then 1ml of the extract was pipetted into a test tube. Two milliliter of solution 2 was added and the test tube corked. The tube was placed in a water bath for 30mins the allowed to cool at room temperature. Four milliliter of solution 3 was added and mixed well. The absorbance was read at 519nm wave length.

Solution 2: This consists of 0.2g ammonium iron (111) phosphate in 100ml 2N HCl made up to 100ml with distilled water.

Solution 3: This consists of 10g biphyridine and 10ml thioglycollic acid in distilled water made up to 100ml.

$$\text{Concentration of phytate} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of standard}}{\text{Weight of sample}}$$

3.4 Biological Analysis

3.4.1 Animal and housing

Thirty-six male albino rats weighing between 78 - 100g were purchased from the animal house of the Department of Zoology, University of Nigeria, Nsukka. They were marked and randomly assigned to two groups; control group containing six rats and experimental group

containing thirty rats. This was done such that the difference in average weight per group will not exceed 5g. The rats were housed in wire cages kept in the animal house of the Department of Home Science, Nutrition and Dietetics, University of Nigeria, Nsukka. The rats were fed rat chow to acclimatize them for 5 days, prior to the study. Food and water were given *ad libitum*.

3.4.2 Experimental design

Blood samples were collected from all the rats for baseline analysis of lipid profile assay (serum total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL)). Group 1 (control group) containing six rats continued on rat chow for the period of the study. Hypercholesterolemia was induced in the remaining thirty rats by giving them rat chow supplemented with 25% coconut oil and 1% cholesterol for 30 days (Oboh & Omofoma, 2008). After 30 days, blood samples were collected from both control and experimental rats through ocular puncture and biochemical assay was carried out for serum lipid assayed for total cholesterol, triglyceride, LDL cholesterol and HDL cholesterol to ascertain if hypercholesterolemia has been induced. The hypercholesterolemic rats were then sub divided into five groups of six rats each. The first group received only rat chow. The second and third group received the diet containing 5% and 10% AYB flour supplementation respectively, the fourth group received diet containing 15% AYB flour, while the fifth group received 20% AYB flour supplementation. Animal management and experimental procedures were performed strictly in accordance with the requirements of the National Research Councilôs (NRC) Guide for the use of Laboratory Animal (NRC, 1985).

After 30 days of the feeding trial, the animals fasted for 12 hours and anesthetized with pentobarbital (60mg/kg body weight). Insertion was made into the heart region for collection of blood samples with the use of a needle and heparinized syringe. The blood samples were

collected into labeled bijou bottles containing heparin as anticoagulant and centrifuged immediately to obtain the serum. The serum samples were assayed for total cholesterol (TC), triglyceride (TG), low density lipoproteins (LDL), high density lipoproteins (HDL) using Quimica Clinica Applicada (QCA) cholesterol kit.

Table 3.1: Composition of experimental diets (g/day)

	Groups					
Dietary components (g)	1	2	3	4	5	6
Rat chow (commercial pellet)	100	74	69	64	59	54
Coconut oil	-	25	25	25	25	25
Cholesterol	-	1	1	1	1	1
AYB	-	-	5	10	15	20
Total composition	100	100	100	100	100	100

1 = normal control (untreated rats); 2 = hypercholesterolemic control rats; 3 = hypercholesterolemic rats fed 5% AYB; 4 = hypercholesterolemic rats fed 10% AYB; 5 = hypercholesterolemic rats fed 15% AYB; 6 = hypercholesterolemic rats fed 20% AYB.

3.4.3 Plasma Lipid and Lipoprotein Analyses

Total Cholesterol

Cholesterol measurements are used in the diagnosis and treatments of lipid lipoprotein measurement disorders. Lipids play an important role in the body; they serve as hormones or hormone precursors, aid in digestion, provide energy, storage and metabolic fuels, act as functional and structural components in biomembranes and form insulation to allow nerve conduction and prevent heat loss.

In clinical chemistry, over the last decade however, lipids have become associated with lipoprotein metabolism and atherosclerosis.

Assay Principle

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxidase and 4-aminoantipyrine in the presence of phenol and peroxidase

Test Procedure

Three (3) test tubes were set up in a test tube rack and labeled blank, standard and sample respectively. 10µl distilled H_2O was added to the blank test tube, 10µl standard specimen to the standard test tube and 10µl sample (that is, serum) to the sample test tube. To each of these test tubes was added 1000µl of the cholesterol reagent. It was thoroughly mixed and incubated for 10minutes at room temperature (20-25^oC) or 5 minutes at 37^oC in water bath. Measure of the absorbance of the sample (A_{sample}) against the blank was taken within 60 minutes at 505nm (Zoppi, Fellini, 1976).

Calculation: cholesterol concentration was calculated as below

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 200$$

Low Density Lipoprotein Cholesterol (LDL-C)

Principle:

LDL-C can be determined as the difference between total cholesterol and the cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethyleneglycol monomethyl ether.

Procedure:

About 0.1ml of the precipitant solution was added to 0.2ml of the serum sample and mixed thoroughly and allowed to stand for 15 min. It was centrifuged at 2,000 x g for 15 min. Determination of the concentration of the serum total cholesterol was as described by Assmann, Jabs, Kohnert, Nolte and Schriewer (1984).

Calculation:

LDL-C (mg/dl) = Total Cholesterol (mg/dl) ï 1.5 x Supernatant Cholesterol (mg/dl).

High Density Lipoprotein Cholesterol (HDL-C)

Principle:

LDL and VLDL (low and very low density lipoproteins) are precipitated from serum by the action of a polysaccharide in the presence of divalent cations. Then, high density lipoproteins cholesterol (HDL-C) present in the supernatant is determined.

Procedure:

HDL-C was determined using Quimica Clinica Aplicada cholesterol kit. 0.1ml of the precipitant solution was added to 0.3ml of the serum sample and mixed thoroughly and allowed to stand for 15 min at room temperature. Centrifugation was done at 2,000 x g for 15 min. The cholesterol concentration in the supernatant was determined (Albers, Warmick & Cheng, 1978).

Calculation: The concentration of HDL-C will be calculated as

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 200$$

The result will be expressed in mg HDL-cholesterol/dl.

Triglycerides

Clinical significance:

Triglycerols measurements are used in the diagnosis and treatment of diseases involving lipid metabolism and various endorcrine disorders e.g. diabetes mellitus, nephrosis and liver obstruction.

Principle:

The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

Procedure:

About 0.1 ml of the sample was pipetted into a clean labeled tube and 1.0 ml of tricloroacetic acid (TCA) was added to it, mixed and then centrifuged at 250 rpm for 10 minutes. The supernatant was decanted and reserved for use. The assay procedure was carried out as shown below:

Table 3.2: Procedure for Triglyceride Analysis

S/N	Blank	Standard	Sample
1. Distilled water	0.5	-	-
2. Standard solution (ml)	-	0.5	-
3. TCA (ml)	0.5	0.5	-
4. Supernatant (ml)	-	-	1.0
5. Reagent mixture (ml)	1.0	1.0	1.0

The mixtures were allowed to stand for 20 minutes at 25 ^{0}C and the absorbance of the sample and standards read against the blank at 540 nm.

Calculation: The concentration of triglyceride in serum was calculated as follows:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Standard concentration (mg/dl)}$$

3.5 Statistical Analysis

The statistical analysis was done with the Statistical Package for Social Sciences (SPSS) software, version 16. Data were expressed as mean ± standard error of mean (SEM) for each group of rats. Comparison between the control and experimental set of data was analyzed by ANOVA and p values <0.05 were indicative of significance. The Duncanôs New Multiple Range Test was also used to compare means.

CHAPTER FOUR

RESULTS

Table 4.1 presents the proximate composition (%) of roasted African yam bean (AYB). The moisture content was 4.94% and that of protein was 27.66%. On the other hand, fat, ash, crude fibre and carbohydrate were 4.29%, 3.66%, 9.57% and 49.88%, respectively.

Table 4.1: Proximate Composition of Roasted African Yam Bean (dry weight)

Nutrient	Composition (%)
Moisture	4.94±0.92
Protein	27.66±1.70
Fat	4.29±0.15
Ash	3.66±0.15
Crude fibre	9.57±0.42
Carbohydrate	49.88±0.48

Means ± Standard deviations of duplicate determinations

Table 4.2 presents the phytochemical and anti nutrient composition of roasted AYB flour (mg/100g). The AYB flour contained 0.77mg, 1.01mg, 42.07mg, and 9.08mg for saponins, flavonoid, tannins, and phytate respectively.

Table 4.2: Phytochemical and Anti-nutrient Composition of Roasted African Yam Bean Flour (mg/100g sample)

Phytochemicals	(mg)
Saponins	0.77±0.40
Flavonoid	1.01±1.32
Tannins	42.07±0.44
Phytate	9.08±2.14

Means ± Standard deviations of duplicate determinations

Food intake of rats during inducement of hypercholesterolemia and during experimental feeding with AYB supplementation is shown in Table 4.3. The negative control group fed rat chow only had a significant ($p < 0.05$) increase in food intake during the experimental period as against during inducement (81.10g vs. 72.11g). The food intake of the second group of rats was 70.71g during inducement and 68.31g during the treatment period. This was not significant ($p > 0.05$). The addition of 5% AYB to rat chow significantly ($p < 0.05$) increased the food intake of the rats (group 3) from 54.60g to 74.17g. On the other hand when 10% AYB was added to rat chow (group 4), it non significantly ($p > 0.05$) decreased food intake of the animals from 71.60g to 68.76g. However, as percent AYB was increased to 15% and 20% (group 5 & 6), there was a tremendous significant ($p < 0.05$) increase in food intake, particularly those of the group of rats fed 20% AYB (90.12g vs. 55.79g).

Table 4.3: Mean Food Intake of Rats Fed Various Diets

Experimental groups of rats	Food intake (g) After Inducement	After Treatment	% difference
1	72.11±2.82[a]	81.10±3.39[b]	↑12.47
2	70.71±3.42[a]	68.31±4.89[a]	↓3.39
3	54.60±2.10[a]	74.17±2.78[b]	↑35.84
4	71.60±3.06[a]	68.76±4.70[a]	↓3.97
5	68.88±2.50[a]	85.91±3.66[b]	↑24.72
6	55.79±2.66[a]	90.12±3.51[b]	↑61.53

Values are expressed as mean ± SEM, n = 6. Values on the same row with different superscripts are significantly ($p < 0.05$) different.1 = normal control (untreated rats); 2 = hypercholesterolemic control rats; 3 = hypercholesterolemic rats fed 5% AYB; 4 = hypercholesterolemic rats fed 10% AYB; 5 = hypercholesterolemic rats fed 15% AYB;6 = hypercholesterolemic rats fed 20% AYB.

Table 4.4 presents the body weight of groups of rats at baseline, inducement and experimental periods. The body weights of group 1 were 79.78g, 101.45g and 152.43g, respectively. The group 2 rats that were induced without treatment with AYB had higher body weight during experimental period relative to the baseline as well as inducement periods (201.40g vs. 100.12g and 127.82g). When 5% AYB was added to 95% rat chow, the weight gain significantly ($p < 0.05$) increased from 78.08g to 482.67g. There was also a significant ($p < 0.05$) increase in body weight when rat chow was supplemented with 10% AYB. However, the increase was lower than that of 5% (482.67g vs. 158.12g). When the diet was supplemented with 15% AYB, it caused increase that was significant ($p < 0.05$) from 85.62g to 182.87g.There was also a significant ($p < 0.05$) increase in body weight when the rat chow was supplemented with 20% AYB from 90.42g to 159.20g. However, the increase was not as much as that of rats fed 15% AYB and rat chow (182.87g).

Table 4.4: Mean Body Weight (g) of Rats after Inducement of Hypercholesterolemia

Group of rats	Baseline	After Inducement	After Treatment	% difference
1	79.78 ± 2.73^{a}	107.45 ± 0.64^{b}	152.43 ± 2.62^{c}	↑41.86
2	100.12 ± 6.30^{a}	127.82 ± 1.01^{a}	201.40 ± 8.16^{b}	↑57.57
3	78.08 ± 2.53^{a}	109.57 ± 5.89^{b}	482.67 ± 3.40^{c}	↑304.51
4	80.25 ± 3.21^{a}	119.57 ± 0.82^{b}	158.12 ± 1.38^{c}	↑32.24
5	85.62 ± 3.90^{a}	122.02 ± 6.84^{b}	182.87 ± 7.26^{c}	↑49.87
6	90.42 ± 3.14^{a}	107.83 ± 7.29^{a}	159.20 ± 7.10^{b}	↑47.64

Values are expressed as mean ± SEM, n = 6. Values on the same row with different superscripts are significantly ($p<0.05$) different.1 = normal control (untreated rats); 2 = hypercholesterolemic control rats; 3 = hypercholesterolemic rats fed 5% AYB; 4 = hypercholesterolemic rats fed 10% AYB; 5 = hypercholesterolemic rats fed 15% AYB; 6 = hypercholesterolemic rats fed 20% AYB.

Table 4.5 presents total cholesterol levels of rats fed different experimental diets (baseline, after induction and after test diet). The cholesterol levels of rats fed control diet (group1) decreased from 135. 80mg/dl to 132.52mg/dl though they did not receive the test diet. This was not significantly ($p<0.05$) different. The group 2 hypercholesterolemic rats that were fed rat chow without AYB supplementation after inducement had a significant ($p <0.05$) increase in cholesterol relative to the baseline and after inducement (267.01mg/dl vs. 141.55mg/dl and 216.03mg/dl, respectively). Group 3 had decreased blood cholesterol with the test diet relative to inducement (141.28mg/dl vs.189.78mg/dl), which was significantly ($p<0.05$) different. There were further decreases in cholesterol levels of rats in group 4 (211.72mg/dl vs. 145.14mg/dl) and group 5 from 224.40mg/dl to 162.10mg/dl, both were significantly ($p<0.05$) different. When 20% of the rat chow was supplemented with AYB, it caused a tremendous significant ($p <0.05$) decrease from 201.87mg/dl to 123.75mg/dl showing the efficacy of AYB in reducing total cholesterol.

Table 4.5: Mean Serum Total Cholesterol (mg/dl) Level of Rats Fed Different Experimental Diets

	Total cholesterol (mg/dl)			
Groups	Baseline	After inducement	After test diet	% difference
1	134.45 ± 1.83^{a}	135.80 ± 3.00^{a}	132.52 ± 5.34^{a}	↓ 2.42
2	141.55 ± 1.04^{a}	216.03 ± 2.97^{b}	267.01 ± 2.06^{c}	↑ 23.60
3	140.90 ± 2.59^{a}	189.78 ± 2.54^{b}	141.28 ± 2.90^{a}	↓ 25.56
4	132.57 ± 0.95^{a}	211.72 ± 6.04^{b}	145.14 ± 0.51^{a}	↓ 31.45
5	138.35 ± 0.48^{a}	224.40 ± 7.25^{c}	162.10 ± 1.22^{b}	↓ 27.76
6	139.60 ± 1.16^{b}	201.87 ± 1.75^{c}	123.75 ± 0.23^{a}	↓ 38.70

Values are expressed as mean ± SEM, n = 6. Values on the same row with different superscripts are significantly ($p<0.05$) different. 1 = normal control (untreated rats); 2 = hypercholesterolemic control rats; 3 = hypercholesterolemic rats fed 5% AYB; 4 = hypercholesterolemic rats fed 10% AYB; 5 = hypercholesterolemic rats fed 15% AYB; 6 = hypercholesterolemic rats fed 20% AYB.

Table 4.6 presents the triglyceride (TG) levels of rats fed different diets containing 0 to 20% AYB. The negative control group (group 1) that were on rat chow throughout the period of the study had slight increase in TG level from 128.33 to 132.45mg/dl which was not significantly ($p > 0.05$) different. On the other hand, group 2 that had 165.67mg/dl after inducement had a lower value (140.36mg/dl) at the end of the experiment. This was significant ($p < 0.05$). When 5% AYB was added to rat chow there was a significant ($p < 0.05$) decrease in TG level of the rats from 192.78mg/dl after inducement to 124.10mg/dl after the test diet. Group 4 had a significant ($p < 0.05$) difference from 187.92mg/dl after inducement to 128.50mg/dl after the test diet. Group 5 had a significant ($p < 0.05$) decrease from 201.38mg/dl after inducement to 140.70mg/dl after inducement. The 20% supplementation with AYB in group 6 caused a significant ($p < 0.05$) decrease from 182.75mg/dl to 131.57mg/dl. The diet containing 5% AYB had the highest percentage decrease (35.63%).

Table 4.6: Mean Serum Triglyceride (mg/dl) Levels of Rats Fed Different Experimental Diets

	Triglyceride (mg/dl)			
Groups	Baseline	After inducement	After test diet	% difference
1	128.33±6.36[a]	143.10±5.27[a]	132.45±5.48[a]	↓ 7.44
2	123.92±7.57[a]	165.67±7.41[b]	140.36±7.31[a]	↓ 15.28
3	144.58±1.58[a]	192.78±6.15[b]	124.10±2.73[a]	↓ 35.63
4	116.55±7.01[a]	187.92±1.79[b]	128.50±4.17[a]	↓ 31.62
5	115.07±3.23[a]	201.38±6.63[c]	140.70±4.01[b]	↓ 30.13
6	115.07±4.56[a]	182.75±2.30[c]	131.57±2.04[b]	↓ 28.01

Values are expressed as mean ± SEM, n = 6. Values on the same row with different superscripts are significantly ($p < 0.05$) different. 1 = normal control (untreated rats); 2 = hypercholesterolemic control rats; 3 = hypercholesterolemic rats fed 5% AYB; 4 = hypercholesterolemic rats fed 10% AYB; 5 = hypercholesterolemic rats fed 15% AYB; 6 = hypercholesterolemic rats fed 20% AYB.

Table 4.7 shows the effects of AYB on low density lipoprotein (LDL) cholesterol levels of the rats. Group 1 had a significant ($p<0.05$) decrease in LDL at the end of the experiment from 76.50 to 54.37mg/dl. The groups of rats that were induced without consumption of diet containing AYB also had decreased LDL level that was significantly ($p<0.05$) different (141.93 to 70.48mg/dl). Group 3 had the LDL level significantly ($p <0.05$) decrease from 106.33 to 56.02mg/dl. Group 4 had a significant ($p<0.05$) decrease of 61.29% (129.63mg/dl vs. 50.18mg/dl). Groups 5 and 6 equally showed significant ($p<0.05$) decreases from 134.58mg/dl vs. 61.68mg/dl and 71.10mg/dl vs. 58mg/dl respectively.

Table 4.7: Mean Serum Low Density Lipoprotein (LDL) (mg/dl) Level of Rats Fed Different Experimental Diets

	Low density lipoprotein (mg/dl)			
Groups	Baseline	After inducement	After test diet	% difference
1	51.47 ± 3.10^{a}	76.50 ± 3.26^{b}	54.37 ± 2.62^{a}	↓28.93
2	48.27 ± 6.04^{a}	141.93 ± 3.26^{c}	70.48 ± 0.70^{b}	↓50.34
3	49.55 ± 7.63^{a}	106.33 ± 9.86^{b}	56.02 ± 2.42^{a}	↓47.31
4	41.82 ± 2.53^{a}	129.63 ± 4.06^{b}	50.18 ± 3.16^{a}	↓61.29
5	39.88 ± 8.31^{a}	134.58 ± 9.83^{b}	61.68 ± 2.89^{a}	↓54.17
6	53.60 ± 7.71^{a}	71.10 ± 5.94^{b}	58.12 ± 4.69^{a}	↓18.26

Values are expressed as mean ± SEM, n = 6. Values on the same row with different superscripts are significantly ($p<0.05$) different. 1 = normal control (untreated rats); 2 = hypercholesterolemic control rats; 3 = hypercholesterolemic rats fed 5% AYB; 4 = hypercholesterolemic rats fed 10% AYB; 5 = hypercholesterolemic rats fed 15% AYB; 6 = hypercholesterolemic rats fed 20% AYB.

Table 4.8 presents the effects of AYB supplementation on high density lipoprotein (HDL) cholesterol levels of rats induced hypercholesterolemia. The group 1 rats had a slight increase (5.95%) after the test diet from 47.55mg/dl to 50.38mg/dl but this was not significantly ($p>0.05$) different. The group 2 rats continued to have decreased HDL cholesterol level (57.75mg/dl compared with 51.96mg/dl), but this was not significantly ($p>0.05$) different. The increase (19.83%) in HDL of group 3 from 55.23 to 66.18mg/dl was not significant ($p >0.05$). Groups 4 and 5 also registered significant ($p <0.05$) increases of 58.28mg/dl vs. 66.54mg/dl and 58.86mg/dl vs. 69.37mg/dl respectively. Group 6 had the highest significant ($p <0.05$) increase from 56.23mg/dl to 69.08mg/dl with a percentage difference of 22.85%.

Table 4.8: Mean Serum High Density Lipoprotein (HDL) (mg/dl) Level of Rats Fed Different Experimental Diets

	High density lipoprotein (mg/dl)			
Groups	Baseline	After inducement	After Test diet	%difference
1	50.68±5.28[a]	47.55±1.35[a]	50.38±1.19[a]	↑5.95
2	68.22±1.63[a]	57.75±1.70[b]	51.96±3.14[b]	↓10.03
3	59.85±4.09[a]	55.23±2.05[a]	66.18±5.49[a]	↑19.83
4	65.00±2.32[a]	58.28±1.55[b]	66.54±3.23[a]	↑14.17
5	74.02±2.71[a]	58.86±2.65[b]	69.37±4.90[a]	↑17.86
6	66.30±3.91[a]	56.23±3.75[b]	69.08±3.10[a]	↑22.85

Values are expressed as mean ± SEM, n = 6. Values on the same row with different superscripts are significantly ($p<0.05$) different. 1 = normal control (untreated rats); 2 = hypercholesterolemic control rats; 3 = hypercholesterolemic rats fed 5% AYB; 4 = hypercholesterolemic rats fed 10% AYB; 5 = hypercholesterolemic rats fed 15% AYB; 6 = hypercholesterolemic rats fed 20% AYB.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The proximate composition of AYB is presented in Table 1. This showed that AYB is a good source of protein, crude fibre, carbohydrate and minerals. The protein, carbohydrate, fat, and dietary fibre content of legumes have also been shown to improve CVD risk factors. The AYB flours had low moisture content. The moisture content was greater than what was observed by Nwodo *et al*. (2012) but lower than was observed by Nwosu (2013). The low moisture content of AYB flour showed that it has a high storage potential. Thus, the rate of deterioration of the seeds will be low in view of the little moisture level. This also suggests that the level of other nutrients might be high. The protein content were lower than what was reported by Ngwu *et al.* (2013) and Nwodo *et al.* (2012) but higher than that of Eneche (2003) and Ene-obong (1993) as reported by Alozie *et al.* (2009) and Nwosu (2013). The protein content surpassed those of other underutilized legumes that ranged from 12.5%-14% (Arinathan, Muhan & De Britte, 2003). It is also greater than the protein content of some commonly consumed legumes such as cowpea 25.9% (Frota *et al.,* 2008), 22.66 to 24.10% (Bala *et al.,* 2012). Findings from epidemiologic studies show a significant relationship between increased protein intake and lower risk of hypertension and coronary heart disease. In experimental animal models feeding a variety of different plant proteins is associated with lower serum cholesterol concentrations than with feeding a variety of different animal proteins (Carroll, 1982).The low fat content of the flour was not a surprise. It is known that pulses are high in carbohydrate and low in fat. The fat content is lower than that observed by Ngwu *et al.* (2013), and Nwodo *et al*. (2012) but higher than Nwosu (2013). The low content of saturated fatty acids and high content of fiber make pulses a good choice for a healthy diet. Dry beans are essentially fat-free and act to displace fat from the diet. Many of them have

moderate amounts of oil that is predominantly unsaturated. The ash content suggest that the flour will be a very good source of minerals. This is because it is known that the higher the ash content of a given food, the higher are the mineral content. The ash content observed is lower than what was observed by Nwodo *et al*. (2012) and Ngwu *et al*. (2013) but higher than was observed by Nwosu (2013). The fibre content of the flour has some nutrition implications. It is higher than what was observed by Nwosu (2013), Ngwu *et al.* (2013) and Nwodo *et al.* (2012).The presence of fiber shows that AYB can be used in the control of non-communicable diseases in humans. High fiber diet is known to reduce cholesterol in the blood and prevent atherosclerosis (Anderson *et al.,* 1995; Salvin *et al.,* 1997).The relatively high carbohydrate level of the flour is meant to suggest that AYB is a fair source of carbohydrate. The proximate composition indicated higher content of carbohydrate than the report of Ngwu *et al.* (2013), Nwodo *et al*. (2012) but lower than the report of Nwosu (2013).

Their complex carbohydrates together with dietary fiber result in low glycemic indexes of legumes and legume products, and play an important role in the prevention of diabetes. Dry beans contain a mixture of soluble fiber, which significantly lowers cholesterol and blood sugar concentrations, and insoluble fiber, which aids in gastrointestinal function. Dietary fiber has major protective effects against atherosclerotic cardiovascular disease (Anderson, Deakins, Floore, Smith & Whitis, 1990). Epidemiologic data suggest that intake of complex carbohydrates and dietary fiber is inversely related to coronary artery disease (Anderson *et al.,* 1990). Dietary fiber intake also slows development of atherosclerosis in animal models. Whereas soluble fiber clearly decreases serum cholesterol and LDL-cholesterol concentrations (Anderson *et al.,* 1990), the inverse relation between dietary fiber intake and coronary artery disease appears to be independent of serum cholesterol concentration.

The saponins content was high compared to what was reported by Ngwu *et al.* (2013) and Nwosu (2013). It has been reported that saponins due to their detergent qualities form insoluble complexes with cholesterol and bile making them unavailable for absorption (Oakenfull, 1996).

The tannins content was very high compared to what was observed by Ngwu *et al.* (2013) and Nwosu (2013). Tannins are known to have high potency antioxidant property that lowers serum cholesterol and reduces risk of coronary heart disease and cancer (Haslam, 1998). However, at high levels they form complexes with proteins, carbohydrates and certain metal ions (Nnam & Onyeke, 2003). These complexes inhibit enzymatic hydrolysis, digestibility and absorption of nutrients in the body. Tannins have traditionally been considered anti-nutritional but it is now known that their beneficial or anti-nutritional properties depend upon their chemical structure and dosage.

The phytate content was higher than which was observed by Ngwu *et al.* (2013) and Nwosu (2013). The phytate content of the sample would also help to reduce serum cholesterol. The potential benefits of phytate occur in instances with high dietary phytate intake. However, a high intake has also been associated with reduced mineral absorption**.** The presence of flavonoids is desirable. They have antioxidant properties to protect the body against cardiovascular disease and some forms of cancer (Nnam, 2011). The discrepancies between the phytochemical levels in this work and other works might be due to processing method, climatic condition and differences in soil conditions.

The increase in food intake of rats fed 5% AYB and decrease in those fed 10% AYB has some nutrition implications. It showed that increased AYB supplementation affects food intake. It is known that rats are very sensitive to food palatability. The observation might be that at higher (10%) AYB supplementation, the palatability of the diet decreased, which

consequently decreased the food intake. On the other hand, there were increases in food intake of rats fed 15% and 20% AYB combination with rat chow.

The increases in body weight of the animals fed rat chow with graded levels of AYB supplementation has great nutrition implications. It shows that the low level of AYB supplementation caused the animals to eat more food to maintain various body organs. On the other hand the decreases in body weight gain of rats fed diet supplemented with 10%, 15%, and 20% AYB suggest that the higher the AYB supplementation, the lower the body weight of the rats. The observation might imply that at higher supplementation, less of the nutrients were utilized for body weight and the rest might have excreted through both feces and urine.

The observed hypocholesterolemic effects of AYB are in agreement with those for other legume seeds reported by Macarulla *et al.* (2001), who studied the effects of faba bean in rats. Other studies that were effective include, study on cowpea seed and its protein isolate on hamsters (Frota *et al.,* 2008); soy protein isolate in hamsters (Song *et al.,* 2003); lima beans on rats (Oboh & Omofoma, 2008). Also the study conducted by Rotimi (2013) showed that AYB reversed diabetes-associated dyslipidemia.

The decreases in the total cholesterol levels of rats fed different experimental diet were a function of supplementation. This suggests that AYB may have affected cholesterol biosynthesis which resulted to reduction in the level of cholesterol in the blood. The values for the test groups after feeding with AYB were significantly ($p<0.05$) different and lower from the hypercholesterolemic control group. These results are in agreement with work on other legumes (Oboh & Omofoma, 2008; Frota *et al.,* 2008; Pande *et al.,* 2012). It also agrees with the work of Rotimi (2013) on diabetes-associated dyslipidemia using AYB. The possible mechanism of action on cholesterol biosynthesis could be by the combined effects of dietary fiber, saponins, phytosterols and other bioactive components present in beans (Amigo *et al.,*

1992). Soluble fibers reduce the level of blood cholesterol by 5% to 15% in experimental animals and in humans (Behall, 1997), through a number of mechanism. One possible mechanism may be by the effect of fiber on enterohepatic circulation through sequestration and binding of bile acids. Another possible mechanism is that soluble fibers are fermented in the colon into short chain fatty acids (SCFA) that inhibit the amount of cholesterol produced by the liver (Cummings & Macfartene, 1991).

After thirty days of feeding high cholesterol diet, the triglyceride level increased significantly ($p<0.05$). Replacement of this diet with normal diet and graded levels of AYB flour for another thirty days caused a significant ($p<0.5$) decrease in the triglyceride values of the test groups. The decreases in triglyceride concentration of the rats were a function of supplementation. This supports the report of Frota *et al.* (2008) who found out that cowpea whole seed decreased triglyceride level of hypercholesterolemic rats. This is in line with the study of Oboh and Omofoma (2008), where there was a significant decrease in the triglyceride value of hypercholesterolemic rats fed heat treated lima beans for thirty days. Rotimi (2013), also reported AYB to have reduced the triglyceride level of diabetic rats. The low serum triglyceride level of rats fed rat chow and 5% AYB appears to indicate that at this lower level, supplementation was much more efficacious relative to the other levels of supplementation.

The low density lipoprotein (LDL) cholesterol level for all the rats fed rat chow with AYB supplementation (5% - 20%) had values lower than half the standard value of <130mg/dl. There was a significant ($p<0.05$) decrease after feeding with graded levels of AYB. The lower value might be associated with increase in HDL concentration that might have caused LDL removal from the liver into bile salts for excretion. The result agrees with the work on other legumes. According to Oboh and Omofoma (2008), feeding of hypercholeesterolemic rats with heat-treated lima beans for thirty days significantly reduced the LDL-cholesterol.

Also a study on cowpea and its protein isolate produced a reduction in LDL-cholesterol after feeding hypercholesterolemic hamsters with the legume (Frota *et al.,* 2008).

There was a significant ($p<0.05$) increase in high density lipoprotein (HDL) level after feeding of graded levels of AYB. This is in line with the study where the incorporation of 12.5% and 25% cluster beans increased the concentration of HDL associated cholesterol (Pande, Platel & Srinivasan, 2012). The study by Rotimi (2013) also had increased HDL cholesterol after feeding of AYB to diabetic rats. The high levels of high density lipoprotein of rats fed rat chow with various levels of AYB has both nutrition and health implications. It is known that high HDL cholesterol concentration for animals and humans has some advantages over other cholesterol. HDL is synthesized in the liver from where it goes to all parts of the body specifically GIT to collect low density lipoprotein and other lipid fragments (Kwiterovich, 1997). These fragments are injurious to health and could precipitate cardiovascular problems. Furthermore, HDL collects these lipids and sends them to the liver where they will be turned into bile salts. These bile salts are excreted into the urine or feces and prevent development of cardiovascular disease.

5.2 Conclusion

This study showed that the proximate and phytochemical composition of African yam bean is comparable with those of other legumes that have been used in the management of hypercholesterolemia. The hypocholesterolemic effect of AYB could be due to the combined effects of different components of the legume (fiber, saponin, phytic acid, protein, carbohydrate). This suggests that AYB may be effective in lowering total cholesterol, triglyceride, low density lipoprotein and increasing high density lipoprotein, thereby promoting cardiovascular health.

5.3 Recommendations

Based on the findings of this study, the following recommendations are made:

- With proper dissemination of these findings to medicals and para-medics, African yam bean may be used in dietary management of non-communicable diseases.
- Nutrition education should be done by dietitians to make people aware of the health benefits of African yam bean consumption.
- There is need for the incorporation of African yam beans into recipes by the dietitians so as to improve the nutrient composition of their diet.
- Further studies are needed on human volunteers in order to ascertain the effect of African yam bean in the management of hypercholesterolemia in humans.

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