The Influences of High Saturated Fats versus High Cholesterol Diets on Rats

Blood Cholesterol

By

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DEDICATION

This work is dedicated to:

- The soul of my dearest father who was so eager to see this work done but passed away untimely...
- My dearest mother...
- My wife and my childs...
- My brothers...
- My sister...
- My friends...
- And every one who helped me with love...

Khalid
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Abstract

This study was conducted in the department of Biochemistry faculty of Veterinary Medicine University of Khartoum, to evaluate the influences of feeding high saturated fat diet versus feeding high cholesterol diet on blood cholesterol and to detect the presence of atherogenic changes in tissues of heart and Liver in rats. Thirty Wister albino rats were used in this study. The rats were divided into three groups named A, B and C of ten rats each. Group A was given basal diet only and served as control. Group B received the rats basal diet mixed homogeneously with cholesterol powder as 2% of the diet. Group C received saturated fats mixed as 14% of the basal diet. The experimental period extended for three months.

Blood samples were collected after the first, second and third month from all groups, to estimate the levels of cholesterol and its fractions together with the triglycerides concentration. Then all rats were scarified and tissues were collected for histopathology.

When the three groups were compared together, group B showed significantly (P> 0.05) higher levels of total cholesterol and LDL-C compared to the control group for all readings. Whereas, VLDL-C and TAG reported significantly (p> 0.05) Lower levels compared to the control group for all readings. The HDL-C showed significantly (P> 0.05) higher values after the first and third month but after the second month it showed significantly (P> 0.05) Lower value compared to the control group.
In group C the levels of total cholesterol, LDL–C, HDL-C, VLDL-C and TAG were non–significantly different for the three readings, with numerically higher values for the total cholesterol and the LDL-C in all readings compared to the control group. The results obtained from this work, indicated that high dietary cholesterol affect plasma lipid profile concentration while feeding high saturated fats showed non significant changes on lipid profile of the plasma.

For the histopathological findings in group B the heart showed edema of cardiac muscles, zenker necrosis, hyalinization and thickness of blood vessels and presence of cholesterol cleft while heart in group C showed hyalinization of cardiac muscles fiber, edema, infiltration of RBCs in the heart muscle, blood vessels thickness and mild monocular cellular infiltration with zenker necrosis.

In group B, the liver showed vacuolization , dilatation of sinusoid, necrosis and disorganization of hepatocytes while liver in group C showed vacuolization of hepatic cytoplasm, congestions of veins, of sinusoids, bile duct proliferation, kuffer cells were prominent, congestion of central veins and extra hepatic hemopoisis and focal infiltration of inflammatory cells and necrosis. So histopathological findings indicated that both high dietary cholesterol and high dietary saturated fats showed clear histopathological changes in tissues of the heart and liver.

The histopathological changes observed for both treated groups, recorded more signs of inflammation in the group received high saturated fat diet. The control showed healthy histopathology tissues.

The present study concluded that though high cholesterol diet significantly influenced the blood cholesterol and triglyceride levels,
whereas histopathological changes showed that both high cholesterol diet and high saturated fat diet resulted in clear changes to both treated groups with more inflammatory signs in group received saturated fats. So future studies includes more number of animals and to be subjected to more experimental period may give clear picture about the actual effect of both treatments also other atherogenic indices should be measured to evaluate which is the most responsible of inducing atherosclerosis is it the high cholesterol diet or the high saturated fat diet.
المستخلص

تم انجاز هذه الدراسة بقسم الكيمياء الحيوية في كلية الطب البيطري جامعة الخرطوم، وقد هدفت هذه الدراسة لتقييم آثر الكوليسترول والدهون المشبعة في الغذاء على مستويات الكوليسترول الكلي، الپوليبروتين منخفض الكثافة، الپوليبروتين عالي الكثافة، والجلسريدات الثلاثية في دم الفئران. هذا بالإضافة لدراسة التغيرات المرضية على بنية القلب والكبد.

ثلاثون فأرا تم تقسيمهم إلى ثلاث مجموعات (أ)، (ب)، (ج) وكل مجموعة تحتوي على عشر فئران حيث كانت المجموعة (أ) تتمثل مجموعة التحكم وأعطيت هذه المجموعة الوجبة الأساسية، والمجموعة (ب) أعطيت الوجبة الأساسية بالإضافة 2% كوليسترول، والمجموعة (ج) أعطيت الوجبة الأساسية بالإضافة 14% دهون مشبعة وامتدت فترة التجربة لثلاثة أشهر.

أخذت عينات من الدم بعد شهر، شهرين وثلاثة أشهر لكل المجموعات بغرض قياس نسبة الكوليسترول الكلي ومستوى الكوليسترول في البروتينات الدهنية بالإضافة لقياس مستوي الجلسيريدات الثلاثية. تم ذبح جميع الفئران وجمع العينات من أنسجة القلب والكبد بغرض دراسة التغيرات المرضية فيها.

عند مقارنة الثلاث مجموعات مع بعضها وجد أن مستويات الكوليسترول الكلي وكوليسترول الپوليبروتين منخفض الكثافة قد سجلت زيادة معنوية (P<0.05) في المجموعة (ب) بالمقارنة مع مجموعة التحكم لكل القراءات، وسجل مستوى الكوليسترول في الپوليبروتين المنخفض الكثافة جدا والجلسريدات الثلاثية لنفس المجموعة ارتفاعا معنوي (P<0.05) بالمقارنة مع الپوليبروتين عالي الكثافة زيادة معنوية (P<0.05) في الشهر الأول والثالث وسجل ارتفاعا معنوي (P<0.05) بعد الشهر الثاني مقارنة مع مجموعة التحكم.

في المجموعة (ج) لم يكن هناك فرق معنوي في مستويات الكوليسترول الكلي وكوليسترول الپوليبروتينات والجلسريدات الثلاثية للقراءات الثلاث بالمقارنة مع مجموعة التحكم مع وجود زيادة في مستويات الكوليسترول الكلي والكوليسترول في الپوليبروتين عالي الكثافة لكل
القراءات بالمقارنة مع مجموعة التحكم. ويستنتج من هذا أن زيادة الكوليسترول في الغذاء تؤثر
على مستويات الدهون في الدم بينما نجد أن زيادة الدهون المشبعة لا تؤثر.
أما بالنسبة للتغييرات في أنفسة القلب والكبد فقد تلاحظ وجود تغيرات مرادية واضحة في مجموعتي المعالجة، ففي المجموعة (ب) أظهرت عينات القلب وجود استتقاء في العضلة،
نخر، تغش شفافة النسيج، زيادة سمك جدران الأوعية الدموية وتصدع كوليسترولي، بينما
تلاحظ في المجموعة (ج) وجود تغش شفافية ألياف نسيج القلب، استتقاء عضلة القلب، تسرب
كريات الدم الحمراء في العضة، زيادة سمك جدران الأوعية الدموية، نخر بالإضافة لتسرب
خلايا أحادية النواة في العضلة.
وفي المجموعة (ب) أظهرت عينات الكبد تكون فجوات، تمد المنحنيات الجببية، نخر واحتلال
نظام خلايا الكبد، بينما في المجموعة (ج) فقد تلاحظ تكون فجوات في سبيتولاتم الخلايا
الكبدية، تمدد في المنحنيات الجببية، احتقان الأروقة، تشبع القناة الصغراء، وبروز في خلايا
كوفر، نخر وارتفاع خلايا مظلمة.
ولهذا فإن هنالك تغيرات مرادية واضحة في أنفسة القلب والكبد لكلا المجموعتين المعالجة مع
وجود علامات مرادية بصورة أوضح في المجموعة المعالجة بالدهون المشبعة بينما أظهرت
الدراسة سلامة الأنسجة في مجموعة التحكم.
خلصت هذه الدراسة إلى أن زيادة الكوليسترول في الغذاء تؤثر تأثيرا معنويًا على مستويات
الكوليسترول والجسيميات الثلاثية في الدم، وأن زيادة الكوليسترول والدهون المشبعة في الغذاء
تحدث تغيرات نسيجية مرادية وقد كانت هذه التغيرات بصورة أوضح في المجموعة التي
أعطيت دهون مشبعة.
كما أظهرت الدراسات المستقبلية يمكن فيها زيادة عدد حيوانات التجربة وإخفاءها لفترة تجربة
أطول لأن ذلك ربما يعطي نتائج أكثر وضوحا عن الأثر الحقيقي للمعالجتين كذلك فإنه بالإمكان
قياس مثاقف أخرى مع الدهون لمعرفة أيهما أكثر تحسنا لتصلب الشرايين، زيادة
الكوليسترول أم زيادة الدهون المشبعة في الغذاء.
INTRODUCTION

Lipids play many roles in biological systems. Like carbohydrates they are important sources of energy. In addition, they are essential components of membranes, they function as hormones, and they can serve as padding and thermal insulator. Lipids are complex and heterogeneous groups of compounds that they defy rational classification. However, despite their difference in structure, they share the property of being insoluble in water. Indeed they are so insoluble that they require special water-soluble lipid-protein complex (lipoprotein) for transport in the blood stream (Murray et al., 2003).

Dyslipidaemia, which can range from hypercholesterolemia to hyperlipoproteinemia, is one of the many modifiable risk factors for coronary artery disease (CAD), stroke and peripheral vascular disease (Chong and Bachenheimer, 2000). Atherosclerosis is a disease of the intima of large and medium-sized arteries. Prominent among resultant intimal changes is the focal accumulation of lipid, particularly cholesterol and cholesteryl esters, in fibro fatty plaques known as atheroma (Varsheny and Sharma, 1996). The atheroma narrows the arterial lumen, damage the underlying media and frequently become ulcerated and/or calcified, thus further narrowing the arterial lumen. In humans, this disease most commonly affects the abdominal aorta, coronary, iliac, femoral and cerebral arteries (Yoshikawa et al., 1997).

Many researchers have suggested that the blood lipids play a key role in the immune defense system (Gallin et al., 1996). There is also a growing understanding that an inflammatory response of the arterial intima to injury is a crucial step in the genesis of atherosclerosis, and that infections may be one type of such injury (Ross, 1986). These two concepts are
difficult to harmonize with the low-density-lipoprotein (LDL) receptor hypothesis, according to which high LDL cholesterol is the most important cause of atherosclerosis. However, the many observations that conflict with the LDL receptor hypothesis, may be explained by the idea that high serum cholesterol and/or high LDL is protective against infection and atherosclerosis. Diets high in saturated fat are correlated with an increased incidence of atherosclerosis and coronary heart disease according to a number of studies (Kromhout et al., 1995). Controlled experimental studies have found that people consuming high saturated fat diets experience negative cholesterol profile changes (Lapinleimu et al., 1995). A 2003 meta-analysis published in the American Journal of Clinical Nutrition concluded that diets high in saturated fat negatively affected cholesterol profiles — predictors of a heart attack and other cardiovascular diseases (Abbey et al., 1994).

Since the relation between dietary fat and atherosclerosis is a contentious field and there is no research indicating whether or not there is direct association between dietary cholesterol and dietary saturated fats with atherosclerosis, therefore, the aim of this study is to evaluate and compare the role of high dietary cholesterol and high dietary saturated fats in the development of atherosclerotic plaques by measuring the changes in the total blood cholesterol and its fractions together with the triacylglycerol in rats subjected to two types of treatments. Also the histopathological changes were planned to be examined for both cases in comparison to control.
CHAPTER ONE

LITERATURE REVIEW

1.1. Lipids:

Plasma lipids are derived from food (exogenous) or synthesized in the body (endogenous). Lipids are relatively insoluble in water; they are carried in body fluids as soluble protein complexes known as lipoproteins (Murray et al., 2003).

Four main classes of lipids can be recognized, from a metabolic standpoint. These are cholesterol and its esters, triglycerides, phospholipids and fatty acids (Forrester et al., 1987). An understanding of the pathophysiology of plasma lipid metabolism is based on the concept of lipoproteins, the form in which lipids circulate in plasma. The principle functions of lipids are to act as energy stores and to serve as important structural component of cells. To fulfill these functions, lipids have to be transported in plasma from one tissue to another, from the intestine or the liver to other tissues such as muscular or adipose tissue, or from the other tissues to the liver (Bishop et al., 2000). There are complex mechanisms that control the release of lipids from tissues into plasma, and the uptake of lipids by the tissues from the plasma. Abnormalities of these mechanisms may be associated with the development of disease, particularly ischemic heart disease (Champe and Harvey, 1994).

1.1.1 Cholesterol:

Cholesterol is a lipid found in the cell membrane of all animal tissues, and it is transported in the blood plasma of all animals.
Cholesterol is also a sterol (a combination of steroid and alcohol). Because cholesterol is synthesized by all eukaryotes, most of the cholesterol is synthesized by the body and some has dietary origin. Cholesterol is more abundant in tissues which either synthesize more or have more abundant densely-packed membrane, for example, the liver, spinal cord and brain. It plays a central role in many biochemical processes, such as the composition of cell membrane and the synthesis of steroid hormones (Stryer, 1995).

Cholesterol from the liver and intestine is transported in plasma. About 75% esterified with fatty acids and the rest unesterified. It is taken up from plasma by different tissues. Its main route of metabolism is to bile acids, which are secreted into bile as conjugated with glycine or taurine. Unesterified cholesterol is also secreted into bile, and both undergo an entero-hepatic-circulation with some loss of cholesterol and bile acids occur daily in the faeces. Unlike that of triglycerides, plasma concentration of cholesterol does not rise after a fatty meal (Johnson et al., 1991).

Since cholesterol is insoluble in blood, it is transported in the circulatory system within lipoproteins, which are complex spherical particles which have an exterior composed mainly of water-soluble proteins; fats and cholesterol are carried internally (Brunzell, 2008).

1.1.2. Lipoproteins

Lipoproteins are complexes of lipids and proteins held together by non-covalent bonds. The core is of insoluble (non-polar) cholesterol esters and triglycerides, surrounded by proteins (known as apolipoproteins), phospholipids and free cholesterol with their water soluble (polar) groups facing outwards. Clinically, the most important are the lipoproteins of the
plasma, which function as major transporters of lipids. Lipoproteins are classified based on their density to five fractions including high density lipoproteins (HDL), low density lipoproteins (LDL), intermediate density lipoproteins (IDL), very low density lipoproteins (VLDL) and chylomicrons (Davidson and Sittman, 1994).

Chylomicrons are derived from the intestinal absorption of triglycerides. The VLDL is synthesized in the liver for exportation of triglycerides to the extra-hepatic tissues; LDL is the final stage in the catabolism of VLDL; IDL is a transient lipoprotein formed during the conversion of VLDL to LDL, it contains both triglycerides and cholesterol, IDL is usually undetectable in normal plasma (Zilva et al., 1994). HDL is involved in VLDL and chylomicrons metabolism and also in the transport of cholesterol to the liver (Murry et al., 2003).

1.1.2.1 Low-density lipoprotein (LDL):

Low-density lipoprotein (LDL) is a lipoprotein that transports cholesterol and triglycerides from the liver to peripheral tissues. LDL also regulates cholesterol synthesis at these sites. It commonly appears in the medical setting as part of a cholesterol blood test, and since high levels of LDL-cholesterol can signal medical problems like cardiovascular disease, it is sometimes called "bad cholesterol". Each native LDL particle contains a single apolipoprotein B-100 molecule (Apo B-100, a protein with 4536 amino acid residues) that circulated the fatty acids, keeping them soluble in the aqueous environment. In addition, LDL has a highly-hydrophobic core consisting of polyunsaturated fatty acid known as linoleate and about 1500 esterified cholesterol molecules (Segrest, 2001). Conditions with elevated concentrations of oxidized LDL particles, especially "small dense LDL" (sdLDL) particles, are associated with
atheroma formation in the walls of arteries, a condition known as atherosclerosis, which is the principal cause of coronary heart disease and other forms of cardiovascular disease (Lewington et al., 2007).

1.1.2.2. High-density lipoprotein (HDL):

HDL is the smallest of the lipoproteins. It is involved in the transport of cholesterol from the peripheral tissues to the liver. They are the densest because they contain the highest proportion of protein. They contain the A class of apolipoproteins. The liver synthesizes these lipoproteins as complexes of apolipoproteins and phospholipids, which resemble cholesterol-free flattened spherical lipoprotein particles. They are capable of picking up cholesterol, carried internally, from cells they interact with. A plasma enzyme called lecithin-cholesterol acyltransferase (LCAT) converts the free cholesterol into cholesteryl ester (a more hydrophobic form of cholesterol) which is then sequestered into the core of the lipoprotein particle eventually making the newly synthesized HDL spherical (Kwiterovich, 2000). They increase in size as they circulate through the bloodstream and incorporate more cholesterol molecules into their structure. High concentrations of functional HDL, which can remove cholesterol from cells and atheroma, offer protection against atherosclerosis hence termed good cholesterol (Durrington, 2003).

1.1.3 Triglycerides:

Triglycerides are fatty acid esters of glycerol, each containing three fatty acids. They are transported as lipoproteins from the intestine and the liver to various tissues, such as adipose tissue. Following hydrolysis, fatty acids are taken up, re-esterified and stored as triglycerides. Plasma
triglyceride concentrations rise after a fatty meal and remain increased for several hours (Stein and Gary, 1994). Triglycerides are absorbed from the jejunum and transferred into the intestinal lymph and then to the systemic circulation. Liver and intestine are also the major sites of triglycerides synthesis. Those formed in the liver, normally being secreted into plasma, and those in adipose tissue are either stored locally or reconverted to fatty acids and glycerol prior to re-entry into the circulation. They are important storage forms of energy (Whitby et al., 1987).

1.2 Atherosclerosis and coronary heart disease (CHD):

Atherosclerosis develops from low-density lipoprotein molecules (LDL) becoming oxidized (ldl-ox) by free radicals, particularly oxygen free radicals. Blood in arteries contains plenty of oxygen and is where atherosclerosis develops. Blood in veins contains little oxygen where atherosclerosis rarely develops. When oxidized LDL comes in contact with an artery wall, a series of reactions occurs to repair the damage to the artery wall caused by oxidized LDL. The LDL molecule is globular shaped with a hollow core for carrying cholesterol throughout the body for making brain tissue, vitamin D, etc. Cholesterol does not dissolve in water. Blood is 70% water. The only way cholesterol can move in the blood stream is to be carried by LDL. 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. Unfortunately, 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, reduces the blood flow and increases blood pressure (Maton et al., 1993).

Some researchers believe that atherosclerosis may be caused by an infection of the vascular smooth muscle cells. Chickens, for example, develop atherosclerosis when infected with the Marek's disease herpesvirus (Fabricant, 1999). Herpesvirus infection of arterial smooth muscle cells has been shown to cause cholesteryl ester (CE) accumulation. Cholesteryl ester accumulation is associated with atherosclerosis (Hsu et al., 1995).

Atherosclerosis typically begins in early adolescence, and is usually found in most major arteries, yet is asymptomatic and not detected by most diagnostic methods during life. The stage immediately prior to actual atherosclerosis is known as subclinical atherosclerosis. The majority of the process leading to subclinical atherosclerosis can happen without our knowing it, especially given the large variety of risk factors (Tuzcu et al., 2001). Autopsies of healthy young men who died during the Korean and Vietnam Wars showed evidence of the disease (Michael and Fishbein, 2007). It most commonly becomes seriously symptomatic when interfering with the coronary circulation supplying the heart or cerebral circulation supplying the brain, and is considered the most important underlying cause of strokes, heart attacks, various heart diseases including congestive heart failure, and most cardiovascular diseases, in general. Atheroma in arm, or more often in leg arteries, which produces decreased blood flow, is called peripheral artery occlusive disease (PAOD) (Bhatt and Topol, 2002).

Atherogenesis is the developmental process of atheromatous plaques. It is characterized by a remodeling of arteries involving the concomitant
accumulation of fatty substances called plaques. One recent theory suggests that, for unknown reasons, leukocytes, such as monocytes or basophils, begin to attack the endothelium of the artery lumen in cardiac muscle. The ensuing inflammation leads to formation of atheromatous plaques in the arterial tunica intima, a region of the vessel wall located between the endothelium and the tunica media. The bulk of these lesions is made of excess fat, collagen, and elastin. At first, as the plaques grow, only wall thickening occurs without any narrowing, stenosis of the artery opening, called the lumen; stenosis is a late event, which may never occur and is often the result of repeated plaque rupture and healing responses, not just the atherosclerosis process by itself (Glagov et al., 1987).

The first step of atherogenesis is the development of fatty streaks, which are small subendothelial deposits of oxidized cholesterol and monocyte-derived macrophages. The exact cause for this process is unknown, and fatty streaks may appear and disappear. LDL in blood plasma poses a risk for cardiovascular disease when it invades the endothelium and becomes oxidized. A complex set of biochemical reactions regulates the oxidation of LDL, chiefly stimulated by presence of free radicals in the endothelium or blood vessel lining. The initial damage to the blood vessel wall results in a "call for help," an inflammatory response. Monocytes (a type of white blood cell) enter the artery wall from the bloodstream, with platelets adhering to the area of insult. This may be promoted by redox signaling induction of factors such as VCAM-1, which recruit circulating monocytes. The monocytes differentiate macrophages, which ingest oxidized LDL, slowly turning into large "foam cells" – so-described because of their changed appearance resulting from the numerous internal cytoplasmic vesicles and resulting high lipid content. Under the microscope, the lesion now appears as a fatty streak. Foam cells eventually
die, and further propagate the inflammatory process. There is also smooth muscle proliferation and migration from tunica media to intima responding to cytokines secreted by damaged endothelial cells. This would cause the formation of a fibrous capsule covering the fatty streak (Maseri and Fuster, 2003).

1.2.1 Atherosclerosis and cholesterol consumption:

According to the lipid hypothesis, abnormally high cholesterol levels (hypercholesterolemia), or, more correctly, higher concentrations of low density lipoprotein cholesterol (LDL-c) and lower concentrations of functional high density lipoprotein cholesterol (HDL-c) are strongly associated with cardiovascular disease because these promote atheroma development in arteries (atherosclerosis). This disease process leads to myocardial infarction (heart attack), stroke and peripheral vascular disease. Since higher blood LDL-c, especially higher LDL-c particle concentrations and smaller LDL-c particle size, contribute to this process more than the cholesterol content of the LDL-c particles (Brunzell, 2008). LDL-c particles are often termed "bad cholesterol" because they have been linked to atheroma formation. On the other hand, high concentrations of functional HDL-c, which can remove cholesterol from cells and atheroma, offer protection. These balances are mostly genetically determined but can be changed by body build, medications, food choices and other factors (Durrington, 2003).

Individuals with familial hypercholesterolaemia (FH) have very high LDL-C and t-C due to LDL-receptor deficiency. One of the main arguments for the LDL-receptor hypothesis is that members of such families run a great risk of dying from coronary heart disease at an early age (Scientific steering committee, 1991).
Experiments on the induction of atherosclerosis in animals indicate a wide species variation in susceptibility. The rabbit, pig, monkey, and humans are species in which atherosclerosis can be induced by feeding cholesterol (Murray et al., 2003).

In 1936, Lande and Sperry noted that the degree of aortic atherosclerosis at autopsy of healthy individuals, who had died violently, was independent on their blood cholesterol concentration analyzed immediately after death (Lande and Sperry, 1936). Their finding was similar to results obtained by Mathur and others (Paterson et al., 1960). The objection that an analysis of cholesterol after death may not reflect its concentration during life was met by Mathur who found that the cholesterol concentration was almost constant up to 16 hours after death (Cabin and Roberts, 1982). The problem is bypassed by comparing the degree of atherosclerosis at death with the individuals’ cholesterol measured previously on several occasions. In all these studies, plots of blood cholesterol concentrations vs. the lipid content of the aorta or the coronary arteries were widely scattered (Shrrett, 1993).

According to the prevailing paradigm, high LDL cholesterol (LDL-C) is said to promote atherosclerosis growth, which explains why it is a risk factor for cardiovascular disease. There is much contradictory evidence. It is true that high total cholesterol (t-C) is a risk factor for coronary heart disease, but mainly in young and middle-aged men. If high t-C or LDL-C were the most important cause of cardiovascular disease, it should be a risk factor in both sexes, in all populations, and in all age groups. But in many populations including women (Jacobs et al., 1992), Canadian and Russian men (Dagenais et al., 1990), Maoris (Beaglehole et al., 1980), Patients with diabetes (Jarrett, 1992) and patients with the nephrotic syndrome (Ravnskov, 1994) the association between t-C and mortality is
absent (Jacobs et al., 1992) or inverse (Shestov et al., 1993) or increasing t-C is associated with low coronary and total mortality (Anderson et al., 1987). Most strikingly, in most cohort studies of old people, high LDL-C or t-C does not predict coronary heart disease (Lin et al., 2000) or all-cause mortality (Krumholz et al., 1994) in several of these studies the association between t-C and mortality was inverse (Raiha et al., 1997) or high t-C was associated with longevity (Jonsson et al., 1997). These associations have mostly been considered as a minor aberration from the LDL-receptor hypothesis, although by far the highest mortality and the greatest part of all cardiovascular disease are seen in old people (Abbott et al., 2002).

More recent autopsy studies have found weak or inconsistent correlations between LDL-cholesterol or total cholesterol and various measures of atherosclerosis (Sharrett, 1993). For instance, the most severe degree of atherosclerosis was found mainly in individuals with extremely high cholesterol, whereas small differences were seen in the rest (Solberg et al., 1985). A correlation was found in White men, but not in Black men (Oalmann et al., 1981) in men but not in women (Feinleib et al., 1979) in individuals below, but not above age 80 years (Sadoshima et al., 1980) and in the coronary arteries, but not in the thoracic or abdominal aorta (Reed et al., 1989).

The weak and unpredictable correlations probably reflect bias, because most of the studies were performed on selected individuals. In such large projects, the main object of which was to study risk factors for cardiovascular disease, individuals with such diseases, or with high cholesterol, were preferred for post-mortem examination (Sorlie et al., 1981) which means that the proportion of individuals with familial hypercholesterolaemia must have been much larger than in the general
population. As such patients have very high cholesterol and are more prone to vascular changes, their inclusion automatically creates a correlation between degree of atherosclerosis and LDL or total cholesterol (Solberg et al., 1977). It is questionable if the vascular changes seen in familial hypercholesterolaemia are synonymous with atherosclerosis (Stehbens and Martin, 1991). Therefore, to prove that the concentration of LDL-cholesterol has importance in the general population, it is necessary to exclude individuals with familial hypercholesterolaemia (Stehbens, 2001).

The lack of an association in these studies may be explained by an influence of other important risk factors. A more reliable parameter is exposure-response. If the amount of circulating cholesterol has any importance, sequential changes of its concentrations should be followed by parallel changes of atherosclerosis growth (Hecht and Superko, 2001). In a few observational studies with coronary angiography, the correlation of this parameter, graded as continuous variables, was analyzed. In three studies, no correlation was found (Bruschke et al., 1989) in two others; progression of atherosclerosis was associated with a decrease in cholesterol, not an increase (Shub et al., 1981).

In many trials exposure response was analyzed, several explanations were offered: most commonly that other lipids or lipid combinations explained the findings. However, Δhigh-density lipoprotein (HDL) cholesterol was analyzed in twelve studies (Niebauer et al., 1996) Δtriglycerides in ten (Sacks et al., 1994) Δapo-lipoprotein B in six (Blankenhorn et al., 1990) Δapo-lipoprotein A1 in three (Sutherland et al., 1998) Δvery-low-density-lipoprotein cholesterol in three and Δsmall, dense LDL-cholesterol in one study but none of them were associated with atherosclerosis growth (Ruotolo et al., 1998). In an early trial using visual evaluation of the
angiographic findings (Krauss et al., 1987) and intermediate-density lipoprotein cholesterol was associated with atherosclerotic progression, but in two others using computer-assisted analysis (Sutherland et al., 1998) no association was found. In three trials, the ratio total cholesterol/HDL cholesterol was inversely associated with atherosclerotic progression (Niebauer et al., 1996) but in one it was seen only in the placebo group, and in another the analysis was not corrected for other risk factors (Quinn et al., 1994).

1.2.2 Atherosclerosis and saturated fats consumption:

Fat that occurs naturally in living matter contains varying proportions of saturated and unsaturated fat. Foods that contain a high proportion of saturated fat are dairy products (especially cream and cheese but also butter and ghee), animal fats such as suet, tallow, lard and fatty meat, coconut oil, cottonseed oil, palm kernel oil and chocolate, and some prepared foods (U.S Department of Agriculture, 2007). There are several kinds of naturally occurring saturated fatty acids, their only difference being the number of carbon atoms - from 1 to 24. Saturated fatty acids have no double bonds between the carbon atoms of the fatty acid chain; hence, they are fully saturated with hydrogen atoms. While nutrition labels usually lump them together, the saturated fatty acids appear in different proportions among food groups. Lauric and myristic acids are most commonly found in "tropical" oils (e.g. palm kernel, coconut) and dairy products. The saturated fat in meat, eggs, chocolate and nuts is primarily palmitic and stearic acid (Beegom and Singh, 1997).
Table (1) Some common examples of saturated fatty acids:

<table>
<thead>
<tr>
<th>Food</th>
<th>Lauric acid</th>
<th>Myristic acid</th>
<th>Palmitic acid</th>
<th>Stearic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coconut oil</td>
<td>47%</td>
<td>18%</td>
<td>9%</td>
<td>3%</td>
</tr>
<tr>
<td>Butter</td>
<td>3%</td>
<td>11%</td>
<td>29%</td>
<td>13%</td>
</tr>
<tr>
<td>Ground beef</td>
<td>0%</td>
<td>4%</td>
<td>26%</td>
<td>15%</td>
</tr>
<tr>
<td>Dark chocolate</td>
<td>0%</td>
<td>0%</td>
<td>34%</td>
<td>43%</td>
</tr>
<tr>
<td>Salmon</td>
<td>0%</td>
<td>1%</td>
<td>29%</td>
<td>3%</td>
</tr>
<tr>
<td>Eggs</td>
<td>0%</td>
<td>0%</td>
<td>27%</td>
<td>10%</td>
</tr>
<tr>
<td>Cashews</td>
<td>2%</td>
<td>1%</td>
<td>10%</td>
<td>7%</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>0%</td>
<td>0%</td>
<td>11%</td>
<td>4%</td>
</tr>
</tbody>
</table>

- Lauric acid with 12 carbon atoms (contained in coconut oil, palm oil and breast milk),
- Myristic acid with 14 carbon atoms (contained in cow milk and dairy products),
- Palmitic acid with 16 carbon atoms (contained in palm oil, hence the name, and meat),
- Stearic acid with 18 carbon atoms (also contained in meat and cocoa butter).

Harvard Nurses’ Health Study found that diets lower in carbohydrate and higher in protein and animal fat are associated with a statistically insignificant decrease in risk of coronary heart disease in women. When vegetable sources of fat and protein are chosen, these diets
may moderately reduce the risk of coronary heart disease (Halton et al., 2006).

Diets high in saturated fat are correlated with an increased incidence of atherosclerosis and coronary heart disease according to a number of studies, both in African green monkeys (Wolfe et al., 1994) and humans, such as a study of infant diets (Lapinleimu et al., 1995) hypercholesterolemic men (Francisco et al., 2001). Some studies have suggested that diets high in saturated fat increase the risk of heart disease and stroke. Epidemiological studies have found that those whose diets are high in saturated fatty acids, including lauric, myristic, palmitic, and stearic acid, had a higher prevalence of coronary heart disease (Kromhout et al., 1995). Additionally, controlled experimental studies have found that people consuming high saturated fat diets experience negative cholesterol profile changes (Mendis et al., 2001). A 2003 meta-analysis published in the American Journal of Clinical Nutrition concluded that diets high in saturated fat negatively affected cholesterol profiles — predictors of a heart attack and other cardiovascular diseases (U.S. Department of Health and Human Services, 2005).

Experiments in which subjects were randomly assigned to either a control or Mediterranean diet (which replaces saturated fat with mono and polyunsaturated fat) showed that subjects assigned to a Mediterranean diet exhibited a significantly decreased likelihood of suffering a second heart attack, cardiac death, heart failure or stroke (Lorgeril et al., 1999).

Epidemiological studies of heart disease have implicated the four major saturated fatty acids to varying degrees. The World Health Organization has determined that there is "convincing" evidence that myristic and palmitic acid intake increases the probability, "possible" risk from lauric
acid, and no increased risk at all from stearic acid consumption (World Health Organization).

A 2004 statement released by the Centers for Disease Control (CDC) determined that "Americans need to continue working to reduce saturated fat intake. Additionally, reviews by the American Heart Association led the Association to recommend reducing saturated fat intake to less than 7% of total calories according to its 2006 recommendations (Lichtenstein et al., 2006). This concurs with similar conclusions made by the World Health Organization (WHO) and the US Department of Health and Human Services, both of which determined that reduction in saturated fat consumption would positively affect health and reduce the prevalence of heart disease (U.S. Department of Health and Human Services, 2005).

The World Health Organization (WHO) has concluded that saturated fats negatively affect cholesterol profiles, predisposing individuals to heart disease, and recommends avoiding saturated fats in order to reduce the risk of a cardiovascular disease (WHO).

Another confounding issue may be the formation of exogenous (outside the body) advanced glycation end products (AGEs) and oxidation products generated during cooking, which it appears some of the studies have not controlled for. It has been suggested that, "given the prominence of this type of food in the human diet, the deleterious effects of high-(saturated)fat foods may be in part due to the high content in glycotoxins, above and beyond those due to oxidized fatty acid derivatives. The glycotoxins, as he called them, are more commonly called AGEs (Koschinsky et al., 1997).

In a study of 235 postmenopausal women, the study subjects established coronary artery disease. Most were hypertensive and many had diabetes (19–31%). Their body mass index ranged from 29 to 30 and their lipid
profile indicated combined hyperlipidemia. These combined characteristics are consistent with metabolic syndrome. Coronary angiography was employed to examine 2,243 coronary artery segments; once at the start of the study and once more at its conclusion. The study concluded that "in postmenopausal women with relatively low total fat intake, a greater saturated fat intake is associated with less progression of coronary atherosclerosis." The same study revealed similarly surprising results when it was disclosed that a greater consumption of polyunsaturated oils "was also associated with greater progression of atherosclerosis (Mozaffarian et al., 2004) (Knopp and Retzlaff, 2004).

A study of 297 acute myocardial infarction (MI) cases in Portuguese males, published in February 2007, concluded that, "Total fat intake, lauric acid, palmitic acid, and oleic acid were inversely associated with acute MI" and that, "Low intake of total fat and lauric acid from dairy products was related to acute MI". The researchers also stated, in revealing the results of this study that some prospective studies show that replacing saturated fat with unsaturated fat is more effective in lowering CHD risk than reducing total fat consumption (Carla Lopes et al., 2007).

Fulani of northern Nigeria get around 25% of energy from saturated fat, yet their lipid profile is indicative of a low risk of cardiovascular disease. This finding is likely due to their high activity level and their low total energy intake (Glew et al., 2001).

A 2004 article in The American Journal of Clinical Nutrition raised the possibility that the supposed causal relationship between saturated fats and heart disease may actually be a statistical mistake because of the greater precision with which saturated fats have been measured (Journal of Clinical Nutrition, 2004).
1.3 Histopathology of atherosclerosis

Cholesterol enrichment of arteries may induce biochemical and structural abnormalities in vascular smooth muscle resulting in increased arterial contractile sensitivity. We studied the effects of a high-cholesterol diet on arterial structural properties and vascular reactivity in young rabbits. In vivo measurements of aortic intimal-plus-medial thickness using high resolution ultrasound imaging were obtained before and after 3 weeks of a high-cholesterol diet in 12 rabbits (group 2) and compared to data from 12 animals a cholesterol-free diet fed (group 1). Six rabbits (group 3) were studied before and after a 3-week, high-cholesterol diet and after a subsequent 13-week, cholesterol-free recovery diet. In groups 2 and 3, high dietary cholesterol caused an increase in intimal-plus-medial thickness from 0.31 mm and 0.33 mm to 0.88 mm and 0.89 mm, respectively. (Ludwig et al., 1991).

Heart sections of rats fed saturated medium chain triglycerides, when stained with Light Green SF Yellowish revealed areas of muscle fibrils that did not accept the stain, probably as a consequence of cellular damage. (Hans and Ruth, 1973)

The liver plays an important role at the cholesterol metabolism in S. Dawley rats (Dietschy et al., 1993). Hepatic fibrosis was reported to be induced by Cholesterol supplemented diet (Hausner et al., 1995). It has been shown that it causes Cholesterol accumulation in cells by high Cholesterol diet (Higley and Taylor, 1984). Most forms of chronic liver disease are associated with the development of the fibrosis (Britton and Bacon, 1994). It has been stated that, the stimulation of non parenchyma cells, i.e., myofibroblasts, could participate in the mechanism of the hepatic fibrosis (Friedman, 1993).
A computed diet based on cereals and spices incorporated with saturated fats fed to albino rats, histopathological examination of liver tissue in particular showed mild cytoplasmic vacuolation (Ramachandran et al., 2006). When male rats fed saturated medium chain triglycerides, many livers revealed marked proliferation of bile ducts (Hans and Ruth, 1973).
2.1. Experimental details

These experiments were designed to investigate the effect of feeding high levels of cholesterol or saturated fats on formation of atherosclerotic plaques in Wistar albino rats.

2.1.1. Experimental animals

Thirty Wistar albino rats obtained from the University of Khartoum and National Center for Research, Khartoum were used in this study. The rats were housed identically in stainless steel cages in an air room under suitable conditions. All of the rats were initially fed a standard laboratory diet for 15 days to acclimatize to the laboratory. Tap water was freely available.

2.1.2. The experimental diet

The rats were divided into three groups and given a basal diet which fulfilled their requirement. The composition of the basal diet for all groups according to NRC (National Research Council, 1996) was as follows:

Group "A" (the control) received the basal diet. Each kg contains

Wheat flour 692g  
Dry meat 165g  
Sodium chloride 3g  
Oil 140g
Group "B" (cholesterol treated) received cholesterol powder supplemented to the basal diet of the rats, according to (Sharma, 1984). Each kg contains

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat flour</td>
<td>672g</td>
</tr>
<tr>
<td>Dry meat</td>
<td>165g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3g</td>
</tr>
<tr>
<td>Oil</td>
<td>140g</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>20g</td>
</tr>
</tbody>
</table>

Group "C" (saturated fats treated) received 14 % cow tallow supplemented to the basal diet of the rats. Each kg contains

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat flour</td>
<td>692g</td>
</tr>
<tr>
<td>Dry meat</td>
<td>165g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3g</td>
</tr>
<tr>
<td>Saturated fats</td>
<td>140g</td>
</tr>
</tbody>
</table>

2.2. Equipment used during the study

- Heparinized capillary tubes were obtained from Umedic Company.
- Heparinized blood containers were obtained from Umedic Company.
- Plane blood containers were obtained from plastilab.
- Centrifuge.
- Automatic pipettes.
- Colorimeter.

2.2.1. Chemicals

- Cholesterol powder was obtained from Himedia Company.
2.3. Experimental procedure

The animals were divided into three groups of ten animals each. These groups were named as A, B, and C. Group A was given the basal diet and served as control, group B received 2% cholesterol added to the basal diet. Group C received 14% saturated fats. Blood samples were collected after one, two and three months following treatment for the determination of the levels of the cholesterol and its fractions and the triacylglycerols. All animals were sacrificed by the end of the experiment and heart and liver samples were collected for histopathology.

2.3.1. Blood sampling

One and half milliliter of blood was collected from the rats orbital plexus after an overnight fast by capillary tubes and was put in heparinized containers; the blood was centrifuged at 5000 rpm for 10 minutes. Then the plasma was placed into plane containers and used immediately.

2.4. Analytical methods

2.4.1. Colorimetric analysis

All cholesterol fractions and triacylglycerols were estimated using the colorimeter.

2.4.2. Total cholesterol estimation

Principle of the method was carried according to (Richmond, 1973)

In the presence of cholesterol esterase, the cholesterol esters in the sample are hydrolyzed to cholesterol and free fatty acids. The cholesterol produced is oxidized by cholesterol oxidase to Quinoneimine and hydrogen peroxide. Hydrogen peroxide is detected
by chromogenic oxygen acceptor, phenol – ampyrone, in the presence of peroxidase. The red quinine formed is proportional to the amount of cholesterol present in the sample.

\[
\text{Cholesterol ester} + \text{H}_2\text{O} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acid}
\]

\[
\text{Cholesterol} + \frac{1}{2} \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{cholesterol oxidase}} \text{Cholestenone} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 4–\text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{cholesterol oxidase}} \text{Quinoneimine} + 4\text{H}_2\text{O}
\]

**Contents and composition of reagents**

<table>
<thead>
<tr>
<th>Reagent (1)</th>
<th>Buffer pH 6.9</th>
<th>90 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>26 mmol/L</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reagent (2)</th>
<th>Cholesterol esterase (CHO)</th>
<th>300 U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol oxidase (CHOD)</td>
<td>300 U/L</td>
<td></td>
</tr>
<tr>
<td>Phenol- ampyrone/ peroxidase</td>
<td>1250 U/L</td>
<td></td>
</tr>
<tr>
<td>4 – Aminoantipyrine</td>
<td>0.4 mmol/L</td>
<td></td>
</tr>
</tbody>
</table>

Cholesterol Standard 200 mg/dl.

**Procedure:**

1- The working reagent was prepared by dissolving one vial of Reagent (2) in one bottle of Reagent (1).

2- The working reagent was brought to room temperature.

3- Into labeled test tubes the following were pipette:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol standard</td>
<td>___</td>
<td>10 µl</td>
<td>___</td>
</tr>
<tr>
<td>Sample</td>
<td>___</td>
<td>___</td>
<td>10 µl</td>
</tr>
<tr>
<td>Working reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>
4- The tubes were incubated for 10 minutes at room temperature (37º C).
5- The absorbance of both standard and samples were measured at 500 nm against the blank.

**Calculations:**

The cholesterol concentration in the samples was calculated by the following general formula:

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

**2.4.3. Low density lipoprotein-cholesterol (LDL-C) estimation**

**Principle of the method was carried according to (Assmann et al., 1984)**

LDL-c particles in the sample are precipitated with polyvinyl sulphate. Their concentration is calculated from the difference between the serum total cholesterol and the cholesterol in the supernatant after centrifugation. The cholesterol is spectrophotometrically measured by means of the coupled reactions as described for cholesterol in page 19.

**Contents and composition of reagents**

1- Reagent (1): polyvinyl sulphate 3g/L, polyethylene glycol 3g/L.
2- Biosystem cholesterol kits (cod. 11805, 11505, 11506, 11539).

**Procedure:**

1- Into labeled test tubes the following were pipette:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>0.4 ml</td>
</tr>
<tr>
<td>Reagent B</td>
<td>0.2 ml</td>
</tr>
</tbody>
</table>
2- The tubes were let to stand for 10 minutes at room temperature.
3- The tubes were centrifuged at 4000 r.p.m for 15 minutes.
4- The supernatant was collected carefully.
5- The cholesterol kit was brought to room temperature.
6- Into labeled test tubes the following were pipette:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>20 µl</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>Cholesterol standard</td>
<td>___</td>
<td>20 µl</td>
<td>___</td>
</tr>
<tr>
<td>Sample supernatant</td>
<td>___</td>
<td>___</td>
<td>20 µl</td>
</tr>
<tr>
<td>Cholesterol reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

7- The tubes were mixed and incubated at room temperature for 30 minutes.
8- The absorbance of both sample and standard were measured at 500 nm against the blank.

**Calculations:**

Since the dilution factor of the sample in the precipitation is 1.5 the concentration of cholesterol was calculated as follow:

\[
\text{Cholesterol in the supernatant (mg/dl) = Absorbance of sample} \times 200 \times 1.5 \quad \text{Absorbance of standard}
\]

**2.4.4. High density lipoprotein-cholesterol (HDL-C) estimation:**

**Principle of method was carried according to (Bachorik, 1997)**

Very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in the sample are precipitated with phosphotungstate and magnesium ions. The supernatant contains HDL-c. The HDL-c is then
spectrophotometrically measured by means of coupled reactions described for total cholesterol in page 19.

**Contents and composition of reagents**

1- Reagent (B): 50 ml, contains phosphotungstate (0.4 m mol/L) and magnesium chloride (20 m mol/L).

2- HDL-c standard concentration = 40 mg/dl.

3- Reagent (A)-Biosystem cholesterol kits (cod. 11805, 11505, 11506, 11539).

**Procedure:**

1- Into labeled centrifuge tubes the following were pipette:

<table>
<thead>
<tr>
<th></th>
<th>Sample</th>
<th>Reagent B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.2 ml</td>
<td>0.5 ml</td>
</tr>
</tbody>
</table>

2- The tubes were let to stand for 10 minute at room temperature.

3- The tubes were centrifuged at 4000 r.p.m for 10 minutes.

4- The supernatant were collected carefully.

5- The cholesterol reagent brought to room temperature.

6- Into labeled test tubes the following were pipette:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>100 µl</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>HDL –cholesterol standard</td>
<td>___</td>
<td>100 µl</td>
<td>___</td>
</tr>
<tr>
<td>Sample supernatant</td>
<td>___</td>
<td>___</td>
<td>100 µl</td>
</tr>
<tr>
<td>Reagent (A)(cholesterol kit)</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

7- The tubes were incubated for 10 minutes at 37°C.

8- The absorbance of both sample and standard were measured at 500 nm against the blank.
Calculations:

The HDL cholesterol concentration in the samples was calculated by the following general formula:

\[
\text{Concentration of HDL cholesterol (mg/dl)} = \frac{\text{Absorbance of sample}}{40 \times 3.5} \times \text{Absorbance of standard}
\]

2.4.5. Very Low Density Lipoproteins-cholesterol (VLDL-C) calculation according to Friedewald, (1972)

According to the Friedewald calculation, VLDL cholesterol is calculated as triglycerides divided by a factor of 5 when lipids are measured in milligrams per deciliter and by a factor of 2.22 when measured in millimoles per liter. The triglycerides/5 ratio as a proxy for VLDL cholesterol is based on the observation that the ratio of the mass of triglycerides to that of cholesterol in VLDL is relatively constant, approximately 5:1 in healthy individuals. However, this means of estimating VLDL cholesterol introduces the well-known limitations of the Friedewald equation. The triglyceride/cholesterol ratio in VLDL will be greater than 5:1 at triglyceride concentrations more than 400 mg/dl and the VLDL cholesterol concentration will consequently be overestimated. Since triglyceride concentrations in this experiment were less than 400 mg/dl, VLDL cholesterol was calculated as triglycerides divided by a factor of 5.
2.4.6. Triglycerides estimation

Principle of the method was according to (Cole et al., 1997)

Triglycerides in the sample originate, by means of coupled reactions described below, a colored complex that can be measured by spectrophotometry.

\[
\text{Triglyceride} + \text{H}_2\text{O} \rightarrow \text{Glycerol} + \text{fatty acids.}
\]

\[
\text{Glycerol} + 3\text{ATP} \rightarrow \text{Glycerol-3-P} + 3\text{ADP.}
\]

\[
\text{Glycerol-3-P} + \text{O}_2 \rightarrow \text{Dihydroxyacetone-P} + \text{H}_2\text{O.}
\]

\[
2\text{H}_2\text{O} + 4\text{-Aminoantipyrine} + 4\text{-Chlorophenol} \rightarrow \text{Quinoneimine} + 4\text{H}_2\text{O.}
\]

Contents and composition of reagents

1- Reagent (A) contains:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>45 mmol/L</td>
</tr>
<tr>
<td>Chlorophenol</td>
<td>6 mmol/L</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>5 mmol/L</td>
</tr>
<tr>
<td>Lipase</td>
<td>&gt;100 u/ ml</td>
</tr>
<tr>
<td>Glycerol kinase</td>
<td>&gt;1.5 u/ml</td>
</tr>
<tr>
<td>Glycerol-3- oxidase phosphate</td>
<td>&gt;4 u/ ml</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>&gt;0.8 u/ml</td>
</tr>
<tr>
<td>4- aminoantipyrine</td>
<td>0.75 mmol/L</td>
</tr>
<tr>
<td>ATP</td>
<td>0.9 mmol/L</td>
</tr>
<tr>
<td>PH</td>
<td>7.0</td>
</tr>
</tbody>
</table>

2- Triglycerides Standard: Glycerol equivalent to 200 mg/dl triolein.
Procedure:

1- Reagents were brought to room temperature.
2- Into labeled test tubes the following were pipette:

<table>
<thead>
<tr>
<th></th>
<th>Sample</th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>10 ml</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>Triglyceride standard</td>
<td>___</td>
<td>10 ml</td>
<td>___</td>
</tr>
<tr>
<td>Reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

3- The tubes were incubated for 15 minutes at room temperature.
4- The absorbance of both sample and standard were measured at 500 nm against the blank.

Calculations:

The triglycerides concentration in the samples was calculated by the following general formula:

\[
\text{Concentration of triglyceride (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 200
\]

2.5 Histopathological diagnosis according to (Drury and Wallington, 1980)

2.5.1 Collection of samples

Heart and liver samples were collected immediately after scarifying the rats and put in a fixative solution before any of the post mortem changes take place.

2.5.2 Fixation

Fixation is the preservation of cells and tissues constituents as life-like manner as possible. The aim of fixation is to stop autolysis (intracellular enzyme reaction) and stop bacterial decomposition. The
volume of the fixative is ten times to that of sample. 10% neutral formalin was used as fixative agent. Neutral formalin prepared as follow:

Formalin = 100 ml  
Distilled water = 900 ml  
Disodium hydrogen orthophosphate = 6.5 gm  
Sodium dihydrogen orthophosphate = 4.0 gm

2.5.3 Dehydration and clearing

Dehydration means removal of water from tissues. First the tissues were cut into small square pieces about one cubic centimeter and labeled with a pencil then washed in running tap water for 15 minutes to remove the fixing agent. The following different concentrations of alcohol were used to remove water from tissues:

Sections were put in 50% alcohol, 60% alcohol, 70% alcohol, 80% alcohol, 90% alcohol, 95% alcohol and 100% alcohol (absolute alcohol).

Clearing is to make the sections transparent so it can be examined microscopically. Chloroform and zylene were used as clearing agents.

2.5.4 Impregnation, blocking and section cutting

Melted paraffin wax was used to remove the clearing agent from the tissues and penetrate the tissues to fill the intracellular space to facilitate cutting. Then sections were cut with aid of rotary microtome into 5—6 microns (one layer thickness).

2.5.5 Staining

Before staining process sections were treated as follow:

1- Zylene was used to remove wax from the sections (10 minutes).
2- Absolute alcohol was used to remove zylene (two changes 5 minutes each).
3- The sections are then hydrated [return water to sections] as follow:
    Rinse in 90% alcohol for 5 minutes, 70% alcohol for 5 minutes and distilled water for 5 minutes.

2.5.5.1 Preparation of stains
A- Haematoxyline:
Haematoxyline powder = 2 gm
Absolute alcohol = 100 ml
Glycerin = 100 ml
Distilled water = 100 ml
Glacial acetic acid = 100 ml
Ammonium = 14 gm

B- Eosin (1%):
    1 gm of eosin powder dissolved in 100 ml of distilled water.

C- Acid alcohol (1%):
    1 ml of concentrated HCL and 99 ml of 70% alcohol mixed together.

2.5.5.1.1 Staining procedure
1- Sections were stained in haematoxyline for 10 minutes.
2- Sections then washed in running tap water for several times.
3- Then differentiated in 1% acid alcohol.
4- Sections rinsed in running tap water for at least 10 minutes.
5- Counter stain with eosin 2-3 minutes.
6- Then rinsed quickly in tap water.
7- The sections were then dehydrated in 70%, 90% and absolute alcohol.
8- After removal of excess alcohol sections were cleared in zylene.
2.5.6 Mounting

The sections was covered with a cover glass (cover slip) using a suitable mounting medium (Canada balsam). Sections were ready to examine microscopically after overnight drying at room temperature.

2.6. Statistical analysis

Results were analyzed using SPSS version 11. The significance was determined at 5% level by using the t-test.
CHAPTER THREE

RESULTS

3.1 The effect of feeding high cholesterol diet or high saturated fats diet on blood cholesterol level in rats

3.1.1 Total cholesterol (TC)

The effect of feeding high saturated fats or high cholesterol diet to albino rats during three months on total cholesterol (TC) is represented in table (2) and figure (1).

After the first month the level of the total cholesterol showed similar levels in the group fed high saturated fats diet (group C) and the control (group A), but the group fed high cholesterol diet (group B) presented significantly (P < 0.05) higher total cholesterol level compared to other groups.

This effect was continued throughout the experimental period but with increasing values, except in group B which showed lower level after the second month compared to the level after one month. But after three months it scored very high level compared to other groups and it was highly significant (P < 0.05).

After three months the level of total cholesterol in all groups showed higher levels compared to the level after one month, and it scored very highly significant (P < 0.05) level in group B compared to the control group and to the level after one month in the same group. The difference between the level of cholesterol after one month and after three months in this group was also highly significant (P < 0.05).
Table (2)

Cholesterol level after supplementation of 2% cholesterol (group B) and 14% saturated fats (group C) compared to control (group A)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Months</th>
<th>Total cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Group A (control)</td>
<td>107.5 ±4.42&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>129.35±12.77&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group B (high cholesterol)</td>
<td>327.5±43.29&lt;sup&gt;bA&lt;/sup&gt;</td>
<td>270.31±10.28&lt;sup&gt;bA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group C (high saturated fats)</td>
<td>111.25±6.02&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>135.14±10.83&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means ± SE within the same column having different superscript small letters are significantly different at (P < 0.05).

Means ± SE within the same row having different superscript capital letters are significantly different at (P < 0.05).

(P < 0.05)* Significant
(P < 0.01)** Highly significantly
(P < 0.001)** Very highly significantly
The level of cumulative value of total cholesterol in group (B) and group (C) compared to group (A) after supplementation of 2% cholesterol powder and 14% saturated fatty acid mixed with the basal diet respectively.

- Bars having different superscript small letters are significantly different at (P < 0.05) based on t-test.

3.1.2 LDL-cholesterol (LDL-C)

The effect of feeding high saturated fats or high cholesterol diet to albino rats for three months on LDL-cholesterol (LDL-C) is represented in table (3) and figure (2).

After the first month the level of the LDL-cholesterol showed similar levels in the group fed high saturated fats diet (group C) and the control...
(group A), but the group fed high cholesterol diet (group B) presented significantly at \( P < 0.05 \) higher LDL-C level compared to other groups. This effect showed similar results throughout the next two months with increasing LDL-C values, except in both group A and group C which showed lower levels after the third month compared to the levels after the second month.

After second and third month LDL-C level in group A and group C showed significantly \( P < 0.01 \) higher LDL-C compared to LDL-C level after one month but after three months LDL-C level in group B scored very high level compared to such group and other groups and it was highly significant \( P < 0.01 \).

**Table (3)**

Cumulative value of LDL-c level after supplementation of 2% cholesterol (group B) and 14% saturated fats (group C) compared to control (group A):

<table>
<thead>
<tr>
<th>Groups</th>
<th>LDL-C (mg/dl)</th>
<th>Months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Group A (control)</td>
<td>61.13±3.44 (^a_A)</td>
<td>93.10±8.18 (^a_B**)</td>
</tr>
<tr>
<td>Group B (high cholesterol)</td>
<td>240.67±32.93 (^b_A)</td>
<td>244.25±19.21 (^b_A)</td>
</tr>
<tr>
<td>Group C (high saturated fats)</td>
<td>66.56±7.55 (^a_A)</td>
<td>100.80±8.87 (^a_B**)</td>
</tr>
</tbody>
</table>

Means ± SE within the same column having different superscript small letters are significantly different at \( P < 0.05 \) based on t-test.
Means ± SE within the same row having different superscript capital letters are significantly different at (P < 0.05).

(P < 0.05)* Significant
(P < 0.01)** Highly significantly
(P < 0.001)*** Very highly significantly

Fig. (2)
The level of cumulative value of LDL-C in group (B) and group (C) compared to group (A) after supplementation of 2% cholesterol powder and 14% saturated fatty acid mixed with the basal diet respectively

- Bars having different superscript small letters are significantly different at (P < 0.05) based on t-test.
3.1.3 HDL-cholesterol (HDL-C)

The effect of feeding high saturated fats or high cholesterol diet to albino rats for three months on HDL-cholesterol (HDL-C) is represented in table (4) and figure (3).

After the first month from starting the experiment the level of HDL-cholesterol (HDL-C) showed significantly high level at (P < 0.05) in the group fed high cholesterol diet (group B) compared to the control group, while HDL-C levels in the group fed high saturated fats diet (group C) and the control (group A) were showed similar results.

This effect was continued throughout the experimental period showed similar results but with decreasing HDL-C values in the second month. After the third month the levels increased compared to the level after one month and it scored very highly significant (P < 0.001) level in group B.

**Table (4)**

**HDL-C level after supplementation of 2% cholesterol (group B) and 14% saturated fats (group C) compared to control (group A)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>HDL-C (mg/dl)</th>
<th>Months</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>41.36±4.81a</td>
<td>29.05±1.89b</td>
<td>53.39±7.07aA</td>
</tr>
<tr>
<td>Group A (control)</td>
<td></td>
<td></td>
<td>41.36±4.81a</td>
<td>29.05±1.89B**</td>
<td>53.39±7.07aA</td>
</tr>
<tr>
<td>Group B (high cholesterol)</td>
<td></td>
<td></td>
<td>64.10±4.35b</td>
<td>20.36±1.11b</td>
<td>90.69±12.91aB**</td>
</tr>
<tr>
<td>Group C (high saturated fats)</td>
<td></td>
<td></td>
<td>43.71±4.85a</td>
<td>33.34±2.04aA</td>
<td>52.71±5.40aA</td>
</tr>
</tbody>
</table>
Means ± SE within the same column having different superscript small letters are significantly different at (P < 0.05) based on t-test.

Means ± SE within the same row having different superscript capital letters are significantly different at (P < 0.05).

- (P < 0.05)* Significant
- (P < 0.01)** Highly significantly
- (P < 0.001)*** Very highly significantly

Fig. (3)
The level of cumulative value of HDL-C in group (B) and group (C) compared to group (A) after supplementation of 2% cholesterol powder and 14% saturated fatty acid mixed with the basal diet respectively

- Bars having different superscript small letters are significantly different at (P < 0.05) based on t-test.
3.1.4 VLDL-cholesterol (VLDL-C)

The effect of feeding high saturated fats or high cholesterol diet to albino rats for three months on VLDL-cholesterol (VLDL-C) is represented in table (5) and figure (4).

After the first month from starting the experiment the level of VLDL-cholesterol (VLDL-C) showed significantly lower level (P < 0.05) in the group fed high cholesterol diet (group B) compared to the other groups, while VLDL-C levels in the group fed high saturated fats diet (group C) and the control (group A) showed similar results.

This effect was continued for the second month but with small decreasing VLDL-C values in group A and B. After the third month very significantly (P < 0.001) high level were scored in all groups compared to levels after the first month.

Table (5)

VLDL-C level after supplementation of 2% cholesterol (group B) and 14% saturated fats (group C) compared to control (group A)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Months</th>
<th>VLDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Group A (control)</td>
<td></td>
<td>16.63 ± 1.70 (\text{A}^a)</td>
</tr>
<tr>
<td>Group B (high cholesterol)</td>
<td></td>
<td>8.00±1.03 (\text{B}^a)</td>
</tr>
<tr>
<td>Group C (high saturated fats)</td>
<td></td>
<td>15.16±1.53 (\text{A}^a)</td>
</tr>
</tbody>
</table>
Means ± SE within the same column having different superscript small letters are significantly different at (P < 0.05) based on t-test.

Means ± SE within the same row having different superscript capital letters are significantly different at (P < 0.05).

(P < 0.05) Significant
(P < 0.01) Highly significantly
(P < 0.001) Very highly significantly

Fig. (4)
The level of cumulative value of VLDL-C in group (B) and group (C) compared to group (A) after supplementation of 2% cholesterol powder and 14% saturated fatty acid mixed with the basal diet respectively

- Bars having different superscript small letters are significantly different at (P < 0.05) based on t-test.
3.2 The effect of feeding high cholesterol diet or high saturated fats diet on blood triacylglycerol level in rats

3.2 Triacylglycerol (TAG)

The effect of feeding high saturated fats or high cholesterol diet to albino rats for three months on triacylglycerol (TAG) is represented in table (6) and figure (5).

After the first month from starting the experiment the level of TAG showed significantly lower level (P < 0.05) in the group fed high cholesterol diet (group B) compared to the control group, while TAG levels in the group fed high saturated fats diet (group C) and the control (group A) showed similar results.

This effect continued for the second month but with small increasing TAG values in group C. After the third month very significantly (P < 0.001) high levels were scored in all groups compared to levels after the first month.

Table (6)

TAG level after supplementation of 2% cholesterol (group B) and 14% saturated fats (group C) compared to control (group A)

<table>
<thead>
<tr>
<th></th>
<th>Months</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Group A (control)</td>
<td></td>
<td>83.16±8.52</td>
<td>73.69±11.64</td>
<td>149.47±10.02</td>
</tr>
<tr>
<td>Group B (high cholesterol)</td>
<td>40.00±5.16</td>
<td>44.08±11.77</td>
<td>136.72±11.63</td>
<td></td>
</tr>
<tr>
<td>Group C (high saturated fats)</td>
<td>75.79±7.66</td>
<td>83.16±17.36</td>
<td>169.47±6.60</td>
<td></td>
</tr>
</tbody>
</table>
Means ± SE within the same column having different superscript small letters are significantly different at (P < 0.05) based on t-test.

Means ± SE within the same row having different superscript capital letters are significantly different at (P < 0.05).

(P < 0.05)*  Significant
(P < 0.01)**  Highly significantly
(P < 0.001)*** Very highly significantly

Fig. (5)
The level of cumulative value of TAG in group (B) and group (C) compared to group (A) after supplementation of 2% cholesterol powder and 14% saturated fatty acid mixed with the basal diet respectively

- Bars having different superscript small letters are significantly different at (P < 0.05) based on t-test.
3.3 Histopathological Findings

In group (B) which treated with 2% cholesterol for three months the heart showed edema of the cardiac muscles (fig.6) and zenker necrosis, hyalinization and there was increased thickness of the blood vessels (fig.7) and presence of cholesterol cleft (fig.8). The liver in group (B) showed vacuolization (fig.9), dilatation of sinusoid, necrosis and disorganization of hepatocytes (fig.10).

In group (C) which treated with 14% saturated fats for three months heart showed hyalinization of cardiac muscles fiber (fig.11), edema and infiltration of RBCs in the heart muscle (fig.12). The wall of the blood vessels were thickened (fig.13). There was mild mononuclear cellular infiltration with zenker necrosis (fig.14).

The liver in group (C) showed vacuolization of hepatic cytoplasm, congestions of veins and dilatation of sinusoids (fig.15). Also there was bile duct proliferation (fig.16), kuffer cells were prominent (fig.17) and congestion of central veins and extra hepatic hemopoisis were evident (fig.18). In addition there was focal infiltration of inflammatory cells and necrosis.
Fig (6): The heart from rats in group (B) showed edema of cardiac muscle (1) and zenker necrosis (2).

Fig (7): Cardiac muscle from rats in group (B) showed increased thickness of blood vessels.

Fig (8): The heart from rats in group (B) showed cholesterol cleft.
Fig (9): The liver from rats in group (B) showed vacuolization.

Fig (10): The liver from rats in group (B) notice sinusoid dilatation (1), necrosis (2) and disorganization of cells.

Fig (11): Cardiac muscles fiber from rats in group (C) notice hyalinization and loss of nuclei.
Fig (12): Heart muscle from rats in group (C) notice edema (1), necrosis(2) and infiltration of RBCs (3).

Fig (13): Cardiac muscles from rats in group (C) notice the wall of the blood vessels were thickened (1), zenker necrosis (2) and edema of muscles (3).

Fig (14): Heart muscles from rats in group (C) notice the mild mononuclear cellular infiltration (1) with zenker necrosis (2).
Fig (15): Liver from rats in group (C) showed vacuolization (1) and congestion (2).

Fig (16): Liver from rats in group (C) notice bile duct proliferation.

Fig (17): The liver from rats in group (C) showed prominent kuffer cells.
Fig (18): liver from rats evident in group (C) notice congestion of central veins (1) and extra hepatic hemopoisis (2).
CHAPTER FOUR
DISSCUSION

This study was carried out to evaluate the effect of feeding cholesterol versus feeding saturated fats on plasma levels of total cholesterol, low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), very low density lipoprotein-cholesterol (VLDL-C) and triglycerides in Westar albino rats and also to evaluate histopathological changes on heart and liver.

4.1 The effect of feeding high cholesterol diet or high saturated fats diet on blood cholesterol level in rats

4.1.1 Plasma total cholesterol (TC)

The results showed that the plasma total cholesterol level reported significantly higher level (P< 0.05) following administration of 2% cholesterol powder mixed with the basal diet after first, second and third month in group B compared to group A (control group). These results were in agreement with the study of Sharma (1984), who reported that administration of 1% cholesterol powder resulted in an increase in total cholesterol. Also the results were in line with the result obtained by Shela et al. (2005) who reported that administration of 1% cholesterol powder to rabbits resulted in an increase total cholesterol.

The group fed 14% saturated fats mixed with the basal diet (group C) showed no significant differences in the total cholesterol after first, second and third month compared to group A (control group). These results were in agreement with the study of Bonanome and Grundy
(1988), who reported that there was inability of stearic acid (saturated FA) to raise serum cholesterol concentration. In contrast to insignificant effect of feeding saturated fats on total cholesterol in this study Cater et al. (1997) reported that palm oil and oleic acid raise serum cholesterol concentration. Also Mensink et al. (2003) reported that saturated fats boost total cholesterol by elevating harmful LDL. A 2003 meta-analysis published in the American Journal of Clinical Nutrition concluded that diets high in saturated fat negatively affected cholesterol profiles (Abbey et al., 1994). Sylven and Borgstrom (1969) reported that the effects of a diet of 10% homogeneous 12 to 18-carbon chain saturated fatty acids (lauric, myristic, palmitic and stearic acid) on plasma cholesterol was significantly increased only with lauric acid and the variation in these results may be due to that long chain saturated fatty acid has no effect on plasma cholesterol. This might suggest that fats used in the present study are rich in long chain saturated fatty acid, so plasma cholesterol of the rats was not altered.

### 4.1.2 Plasma LDL-cholesterol (LDL-C)

The plasma LDL-C levels were significantly increased (P< 0.05) following administration of 2% cholesterol powder mixed with the basal diet after the first, second and third month in group B compared to group A (control group). These results were in agreement with the study of Sharma (1984), who reported that administration of 1% cholesterol powder resulted in an increase in LDL-C. In support of the present results, Shela et al. (2005) reported that administration of 1% cholesterol powder to rabbits resulted in an increase LDL-C. On the other hand plasma LDL cholesterol showed no significant differences following administration of 14% saturated fats mixed with the
basal diet after first, second and third month in group C compared to 
group A (control group). These results were in agreement with the study 
of Bonanome and Grundy (1988), who said that there was inability of 
stearyic acid (saturated FA) to raise serum LDL-C concentration. In 
contrast to insignificant effect of feeding saturated fats on LDL-C in this 
study Cater et al (1997) reported that palm oil and oleic acid raise humans 
serum LDL-C concentration. Also Mensink et al (2003) reported that 
saturated fats elevate harmful LDL in humans. 
The variation in the results of LDL-C in the studies mentioned above may 
be due to that carbon chain length of a saturated fatty acid has long been 
suspected to be an important determinant of its cholesterol raising 
potential (Keys et al; 1965). Since in the present study plasma LDL-
cholesterol was not altered, then the fats used could be rich of long chain 
saturated fatty acid.

4.1.3 Plasma HDL- cholesterol (HDL-C)

The results in the present work showed that the plasma HDL-C 
levels were significantly decreased (P< 0.05) following administration of 
2% cholesterol powder mixed with the basal diet after the second month 
in group B compared to group A (the control group). These results were in 
agreement with the study of Sharma (1984), who reported that 
administration of 1% cholesterol powder resulted in decrease in LDL-C. 
In support of the present results, Shela et al. (2005) reported that 
administration of 1% cholesterol powder to rabbits resulted in decrease in 
HDL-C. 
However after the first and third month results reported levels of the 
plasma HDL-C significantly (P< 0.05) higher than the control group. 
Since rats in the present study were subjected to relatively long period of
treatment, three months, then one can suggest that the time factor is very important when data is evaluated for the effect of feeding high cholesterol diet to an experimental animal on HDL-C level.

The plasma HDL cholesterol showed no significant differences following administration of 14% saturated fats mixed with the basal diet after first, second and third month in group C compared to group A (the control group). These results were in agreement with the study of Bonanome and Grundy (1988), who said that there was inability of stearic acid (saturated FA) to raise serum HDL-C concentrations. However compared to the insignificant effect of feeding saturated fats on HDL-C in this study, Schaefer et al (1981) have presented evidence that an extremely low fat diet caused significant (P < 0.01) decreases in plasma HDL-C in normolipidemic young subjects. Also these results disagree with Mensink et al. (2003) who concluded that long chain saturated fatty acids such as lauric acid increase HDL cholesterol levels.

The variation in the results of HDL-C in the studies mentioned above may be due to chain length of a saturated fatty acid which has long been suspected to be an important determinant of its cholesterol raising potential (Keys et al; 1965). Since in this study long chain saturated fatty acid could be used, then plasma HDL-cholesterol was not altered.

4.1.4 Plasma VLDL-cholesterol (VLDL-C)

Plasma VLDL-C levels after the first and the second month showed significantly (P< 0.05) lower levels following administration of 2% cholesterol powder mixed with the basal diet in group B compared to group A (the control group). These results were in agreement with the study of Jelske et al (2009) who reported that mice under condition of
feeding a cholesterol-enriched diet (1%) for two weeks resulted in lower levels of VLDL cholesterol. However the levels in group (B) returned to a similar value of the control group after three months. Whereas administration of 14% saturated fats mixed with the basal diet resulted in no significant differences after first, second and third month in group C compared to group A (the control group). These results were in line with the result obtained by Mattson and Grundy (1985) who reported that VLDL cholesterol was not altered after consuming a liquid diet in which the predominant fatty acids were saturated.

4.2 The effect of feeding high cholesterol diet or high saturated fats diet on blood triglycerides level in rats:

The plasma TAG levels were significantly (P< 0.05) decreased following administration of 2% cholesterol powder mixed with the basal diet in group B compared to group A (the control group) this observed after tow months, but after the third month the level in group (B) scored similar level to the control group. These results were in line with those obtained by Grefhorst et al. (2002) who observed that the hepatic free cholesterol and cholesteryl ester accumulation in cholesterol-fed mice was accompanied by extremely low plasma levels of triglycerides. But these results disagree with Conner (1961) who said that our results showed a lack of any significant effect of dietary cholesterol on plasma lipids in normolipidemic men. The differences reported concerning the changes in the TAG in previous studies, could be due to the difference in the length of the periods of application of treatment. The plasma TAG levels showed no significant differences following administration of 14% saturated fats mixed with the basal diet after first, second and third month in group C compared to group A (the control
These results were consistent with result reported by Mattson and Grundy (1985) who said that twenty patients consumed a liquid diet in which the predominant fatty acids was saturated, twelve of the patients had normal TG levels and eight patients had hypertriglyceridemia.

4.3 Histopathological Findings

4.3.1 The effect of feeding high cholesterol diet or high saturated fats diet on heart tissues

The heart samples in group fed 2% cholesterol (group B) when examined, showed zenker necrosis, hyalinization, edema of cardiac muscle, increased thickness of blood vessels and presence of cholesterol cleft. These findings were in agreement with the study of Ludwig et al. (1991) who reported that high dietary cholesterol in young rabbits caused an increase in intimal-plus-medial thickness from 0.31 mm and 0.33 mm to 0.88 mm and 0.89 mm, respectively. Also the findings in the present study were in line with findings obtained by Lutgens et al. (1999) who reported that mice fed high cholesterol develop atherosclerotic lesions resembling those found in humans, depending on their plasma cholesterol levels. Also in a study investigated whether the ACAT inhibitor avasimibe can reduce atherogenesis independently of its cholesterol-lowering effect in ApoE*3-Leiden mice, before start this study, mice were fed high cholesterol diet for 3 weeks and reported accumulation of free cholesterol in atherosclerotic lesions (Dianne et al.; 2001).
Whereas heart samples in the group fed 14% saturated fats (group C) showed hyalinization of cardiac muscles fiber, edema and infiltration of RBCs in the heart muscle, the wall of the blood vessels were thickened and there is mild mononuclear cellular infiltration with zenker necrosis. These findings were in line with those obtained by Hans and Ruth (1973) who observed that some heart sections of rats fed saturated medium chain triglycerides, when stained with Light Green SF Yellowish revealed areas of muscle fibrils that did not accept the stain, probably as a consequence of cellular damage.

4.3.2 The effect of feeding high cholesterol diet or high saturated fats diet on liver

The liver samples in the group fed 2% cholesterol (group B) showed vacuolization, dilatation of sinusoid, necrosis and disorganization of hepatocytes. These findings were in agreement with the study of Wanless et al. (1996) who said Rabbits when given a cholesterol-supplemented diet (1%) developed sinusoidal and portal fibrosis lipid droplets in hepatocytes. Also this findings in the present work were in agreement with the study of Hausner et al. (1995) who showed that cholesterol supplemented diet caused high plasma cholesterol level with hepatic cell degeneration. Also Elaine et al. (1972) reported that histologic examination of sections of livers showed fat infiltration of hepatic cells and a greater damage to the liver cells was noted when cholesterol was added to the basal diet of male rats. Hypercholesterolemia is said to contribute to hepatic injury as a result of cholesterol accumulation. Lack of underlying basement membranes facilitates the flow of LDL cholesterol in circulating plasma between
sinusoidal lumen and hepatocytes, because in normal liver sinusoids are limited by fenestrated endothelial cells (Burt, 1993). The normal liver tissue has cholesterol oxidase. Cholesterol generates cholesterol oxidation products and free radicals by cholesterol oxidase. Cholesterol oxides include cytotoxic and steatotic effects (Aviram, 1992). Lipid accumulation leads to oxidative stress in hepatic tissue. The oxidative stress may contribute to peroxidation of LDL. These peroxidative fatty acids and reactive oxygen species induce hepatic damage (Britton and Bacon, 1994).

Whereas in the group fed 14% saturated fats (group C), showed vacuolization of hepatic cytoplasm, congestions of veins, dilatation of sinusoids, bile duct proliferation, kuffer cells were prominent, congestion of central veins and extra hepatic hemopoisis and focal infiltration of inflammatory cells and necrosis. These findings were in agreement with the study of Ramachandran et al. (2006) who reported that when a computed diet based on cereals and spices incorporated with saturated fats fed to albino rats, histopathological examination of liver tissue in particular showed mild cytoplasmic vacuolation. Also Hans and Ruth (1973) found that when male rats fed saturated medium chain triglycerides, many livers revealed marked proliferation of bile ducts.
CONCLUSIONS AND RECOMMENDATIONS

The results obtained from this work, indicated that high dietary cholesterol affect plasma lipid profile concentration while feeding high saturated fats did not. On the other hand histopathological findings indicated that both high dietary cholesterol and high dietary saturated fats showed clear histopathological changes in tissues of the heart and liver.

As several studies beside this study have confirmed that a high consumption of saturated fatty acids and cholesterol have a high incidence of changes in liver and heart, there is an official recommendation for a minimum daily intake of cholesterol and saturated fats (intake levels that meet the needs of health).
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