EFFECT OF DIFFERENT VEGETABLE FATS ON LIPID PROFILE AND RISK OF ATHEROSCLEROSIS IN RATS

JOANA KOMA AINUSON

(ID: 10050184)

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF PhD DIETETICS DEGREE

JULY 2013

DECLARATION

I, Joana Koma Ainuson, hereby declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy, to the University of Ghana. It has not been submitted before as a part or whole requirement for any degree or examination to this or any other university or institution.

Student:

Signature:……

Joana Koma Ainuson

Principal Supervisor:

Signature:……..…

Prof. Albert G.B. Amoah

Co-supervisor:

Signature:……..

Prof. E.E.K. Wiredu

Co-supervisor:

Signature:……

Dr. George A. Asare

Co-supervisor:

Signature:……

Dr. Phyllis Addo

ABSTRACT

Atherosclerotic vascular diseases (AVDs) are presently increasing rapidly in incidence and have become key contributors to the burden of disease in most developing countries like Ghana. The condition has been projected to more than double by 2025. The type and amount of dietary fat has been associated with several disorders including AVDs and it complications. Diet, as one of the most important modifiable risk factors of Coronary Heart Disease (CHD) modulates the other known risk factors. Excessive intake of dietary saturated fat and cholesterol has been found to increase serum cholesterol, thus leading to a high risk of cardiovascular diseases. Saturated fats, both of animal and vegetable or plant origin, have been discredited. This study was carried out to investigate the effect of vegetable fats including olive oil (OO), red palm oil (RdPO) and refined palm oil or palm olein (RfPO) on lipid profile and risk of Atherosclerosis in rat model. Three months old male Sprague-Dawley (S-D) rats (n=56) were divided into four groups: control, olive, red palm and refined palm oil groups (n=14 per group) received water and feed ad libitum. The controls were fed the standard rat chow whilst the treatment (oil) groups received diet enriched with extra 6% by weight of the corresponding oil. During the 16 weeks of dietary intervention, 7 rats were randomly selected from each group and sacrificed at two months intervals, and blood samples collected for biochemical analysis. Plasma lipid profile comprising of Total Cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), Apolipoprotein-A1 (Apo-A1), and Apolipoprotein-B100 (Apo-B100) were determined at baseline and at the end of every two months by the enzymatic

technique using the Enzyme-Linked Immuno Sorbant Assay (ELISA). Pro-inflammatory markers including Interleukin-2 (IL-2), Interleukin-6 (IL-6), Tumor Necrosis Factor Alpha (TNF-α) and Total Antioxidant Status (TAS) were also determined by the ELISA method at baseline and at 2 and 4 months. There was no significant difference in the lipid profile of the treatment rats compared to the controls. However, there was a significant effect of the intake of the different oils on the inflammatory markers TNF-α and IL-6. There were also significant differences in the TAS of the rats from the different groups at month 2. Olive oil diet caused a significant decrease in the TNF-α and IL-6 mean concentrations (p=0.008) and (0.0041) respectively and an increase in the TAS mean concentrations (p=0.005). The two palm oil diets (RdPO and RfPO) caused an increase in both TNF-α and IL-6 concentrations. The red palm oil diet resulted in an increase in the TAS, whilst the refined palm oil diet caused a decrease in the TAS. However these effects were not statistically significant. The results suggest that the 3 different oils had different effects on the parameters investigated in this study. The increase in levels of the inflammatory indicators and reduction in total antioxidants levels recorded among rats fed RfPO enriched diet shows a likelihood of the oil enhancing inflammation and leading to oxidative stress and subsequent oxidative damage respectively, hence may have a potential of increasing the risk of development of atherosclerosis in rats. The intake of the red palm oil diet, although to some extent improved TAS of the rats, yet was associated with increased inflammation (TNF-α and IL-6). Therefore prolonged use may probably be associated with increased risk of developing atherosclerosis and other inflammatory diseases. On the contrary, intake of olive oil as a major source of fat may reduce the risk of atherosclerotic development due to its

association with significant increase in TAS and a decrease in both IL-6 and TNF-α production in rats. The above findings therefore suggest that, olive oil may be most protective against atherosclerosis compared to the other oils. Also the red palm oil though unrefined, turned out to have a better antioxidant capacity than the refined form.

DEDICATION

To my beloved husband Tony

ACKNOWLEGEMENT

My deepest gratitude to the Almighty God who has enabled me to successfully carryout and complete this thesis. Words cannot express my sincere gratitude to my beloved parents for all the assistance and encouragement. My profound appreciation to my loving husband and children, for their unflinching support, inspiration and advice throughout my studies.

It gives me immense pleasure to express my deep sense of gratitude and sincere thanks to Prof. A. Amoah, Department of Medicine and Therapeutics, KBTH as my principal supervisor and mentor, for his prudent admonitions, guidance, strong commitment, keen and sustained interest during the present investigation and preparation of this thesis. I am especially thankful for the patience and the quality time that was sacrificed for me.

With sincere gratefulness I wish to express my indebtedness and profound thanks to my co-supervisors Prof. E. K. Wiredu, Department of Pathology, KBTH, Dr. George Asare, the Vise Dean, School of Allied health Sciences, UG and Dr. Phyllis Addo, Department of Animal Experimentation, NMIMR: through theirs constant advice and constructive criticisms nourished my interest in the subject and provided a free and pleasant atmosphere to work against all odds.

The research was generously supported by ORID/UG which made it possible to acquire the reagents required for the project. Many thanks to the CHS Grant and Scholarship Department for the bench fees. It is also my privilege to extend

heartfelt thanks to former Director of GAFCO Mr. Kubler, Dr Rule and staff of CSIR, staff of Animal Experimentation Department, Staff of Diabetes Research Lab and the Staff of School of Allied Health Sc. for their affectionate help and moral support in numerous ways.

I am extremely grateful and sincerely acknowledge the following people: Rev. Prof. P. F. Ayeh-Kumi, Prof. D. Sarpong, Prof. A. A. Agyei , Prof. Atoquafio, Prof. Nkyekyer, Dr. Agyemang, Dr. Nortey and Dr. Mettle, Dr. Kwamena Sagoe, Dr. Kumogy, Mr. Nii Dodoo, Mr. and Mrs. David Mensah for their cooperation, inspiration and valuable advice for the success of this research. I owe many thanks to my friends and loved ones, who have been of immense support. Your valuable contribution is inestimable. Thank you so much.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ALA	Alpha-linolenic acid
APO-A1	Apolipoprotein-A1
APO-B100	Apolipoprotein-B100
AVD	Atherosclerotic vascular disease
CAD	Coronary Artery Disease
CEs	Cholesteryl esters
CHD	Coronary heart disease
CRP	C-reactive protein
CRPO	Crude palm oil
CSF	Colony stimulating factors
CSF-1	Colony-stimulating factor 1
CSIR	Center for Scientific and Industrial Research
CVD	Cardiovascular diseases
CVDH	Cardiovascular disease and hypertension
DHA	Docosahexaenoic acid
ELISA	Enzyme-Linked Immuno Sorbant Assay
EPA	Eicosapentaenoic acid
FFA	Free fatty acids
GAFCO	Ghana Agro Food Company
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GSH-Px	Glutathione Peroxidase

GSSG-Rx	Glutathione Reductase
HDL-C	High-density lipoprotein cholesterol
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
IFN	Interferons
IL-2	Interleukin-2
IL-6	Interleukin-6
iTFA	Industrial trans fatty acids
KBTH	Korle-bu Teaching Hospital
LDL-C	Low-density lipoprotein cholesterol
LDL-CR	LDL-C receptor
LMIC	Low- and middle-income countries
MCP-1	Monocyte chemotactic protein 1
MDA	Malondialdehyde
MUFA	Monounsaturated fatty
NCEP	National Cholesterol Education Programme
NMIMR	Noguchi Memorial Institute for Medical Research
PAD	Peripheral artery disease
PUFA	Polyunsaturated fatty
RAS	Renin-angiotensin system
REFPO	Refined palm olein
ROS	Reactive oxygen species
SFA	Saturated fatty acids
SSA	Sub-Saharan Africa
SU	Selective uptake
TAS	Total Antioxidant Status

TC	Total Cholesterol
TGF	Transforming growth factors
TNF	Tumor necrosis factors
TNF-α	Tumor Necrosis Factor Alpha
VCAM-1	Vascular cell adhesion molecule-1
VLDL-C	Very low density lipoprotein
VSMC	Vascular smooth muscle cells

CHAPTER ONE

INTRODUCTION

1.1 Background

It is an established fact that dietary intake influences nutrients and their turnover (Martinez *et al*., 1995). Studies have shown that the type and amount of fat in the diet is associated with changes in the levels, composition and metabolism of serum lipoproteins (Abraham *et al*., 1994), triglycerides and other lipids (Truswell, 1994). Excessive fat and cholesterol consumption as well as lipid structure and configuration affect the lipid profile in plasma (Kritchevsky, 1995).

Experimental and epidemiological studies have demonstrated that elevated levels of LDL-C-cholesterol and cholesterol-enriched very low density lipoprotein (VLDL-C) increase atherosclerosis susceptibility and lead to clinical sequel such as coronary heart disease and stroke which have become the major causes of morbidity and mortality in the world today (Breslow, 1997). In humans, it has been shown that dietary cholesterol and saturated fatty acids (SFA) increase the levels of these atherogenic lipoproteins, and current recommendations include decreasing their intake as part of a heart-healthy diet (Ross, 1993a).

Commonly-used cooking oils in Ghana include palm oil, palm kernel oil, coconut oil, Olive Oil, soybean oil, sunflower oil, groundnut oil, shea butter and cocoa

butter. For many of these oils, their effects on serum lipid profile are still unknown. The link between dietary fats and cardiovascular diseases has necessitated the high research interest in cooking oils.

Different types of fats (e.g. animal fat, vegetable fats/oils) contain various levels of specific fatty acids (saturated fatty acids and unsaturated fatty acids-monounsaturated/ polyunsaturated). The quality and quantity of fat in the diet influence serum lipid concentrations (Denke and Grundy, 1992). Serum lipids play an important role in pathogenesis of many diseases. For instance, excessive intake of cooking oils over a period of time can lead to hyperlipidemia and consequently related conditions such as arteriosclerosis, hypertension, cardiovascular disease (CVD) and certain cancers (Mensink *et al.*, 1992).

Atherosclerosis is a slow progressive disease that can affect the arteries of the brain, heart, kidneys, and the legs. It involves the hardening and narrowing of the arteries resulting from a build-up of fibrolipid plaques beneath the inner lining (the endothelium). This can cause serious diseases and complications including coronary artery diseases (angina, heart attack, and sudden death), cerebrovascular diseases (stroke, transient ischemic attacks (TIA) or "mini strokes") and peripheral arterial diseases (Boushey *et al*., 1995).

Diseases caused by atherosclerosis are the leading cause of illness and death in many countries (Boushey *et al.*, 1995). An estimated 17 million people worldwide die every year from cardiovascular diseases, particularly heart attacks and strokes and projected to cause 25 million deaths in 2020 (WHO, 2008). People who

survive a heart attack or stroke often need long-term medical treatment. These diseases can seriously affect the quality of life of both the patient and his or her family members.

A desirable serum lipid profile is one that has low plasma total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C-C) and a high level of high-density lipoprotein cholesterol (HDL-C). High levels of TC are a risk factor for coronary artery disease and saturated fatty acids have been positively implicated (Knopp and Retzlaff, 2004). These include lauric (C12:0), myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids. Palm kernel and coconut oil contain more than 65% of their fatty acids in the form of lauric and myristic acids, the combination of which have been reported to be atherogenic (Lada and Rudel, 2003).

On the other hand, olive and canola oils, high in monounsaturated fatty acids and corn, soybean and sunflower oil with high polyunsaturated fatty acid, have been shown in both human and animal studies to reduce serum LDL-C cholesterol levels and consequently contribute to reduced risk of atherosclerosis (Rudel *et al.*, 1998). There are many questions and in some cases contradictory reports on dietary vegetable oils: some of these vegetable oils such as the tropical oils (palm oil and coconut oil) are believed to increase the cardiovascular risk while other investigator claim that these rather reduce risk (Kumar, 1997, Zock *et al*., 1994). In this study, the effect of three different dietary fats (Olive Oil, Red Palm oil and oxidized palm oil) on lipid profile and atherosclerosis was assessed.

1.2 Problem Statement

Atherosclerotic vascular disease (AVDs) is becoming a major health problem in most developing countries like Ghana (Cappuccio *et al.*, 2006). AVDs are presently increasing rapidly in incidence and key contributors to the burden of disease. The condition has been projected to more than double by 2025 (WHO, 2008). Dyslipidaemia is a recognized major determinant of the condition.

Dietary intervention is the first step in the treatment of hypercholesterolemia irrespective of the causal factors (Stelmach *et al*., 1993) There is little doubt that saturated fatty acids (SFAs) increase plasma cholesterol concentrations (Morgan *et al*., 1993) and SFA intakes should then be reduced in subjects with dyslipidaemia. The SFAs that have cholesterol-elevating effect are lauric (C 12:0), myristic (C14:0) and palmistic (C 16:0), but Stearic acid (C 18:0) does not raise plasma total cholesterol (TC) (Grande *et al*., 1965).

However, the effect of the different vegetable oils (which have been found to possess varing levels and types of fatty acids), on atherogenesis has been unclear: with some of the reports appearing conflicting especially regarding the impact of certain vegetable oils on atherogenesis (Benson and Devi, 2009, Denke and Grundy, 1992). Currently, there appears to be considerable confusion within the general population with regard to optimal fat and oil consumption (Lichtenstein *et* al., 1993).

The present study was therefore carried out to investigate and compare the effect of some commonly used vegetable oils in Ghana including Olive oil, Red palm oil

and Frytol cooking oil on blood lipid profile and risk of atherosclerosis. Such a study is not easy to perform in humans as various other confounding variables such as level of exercise can affect the results. In this study animal feed was fortified with three different oils (Red Palm oil, oxidized palm oil and Olive Oil) and fed to rats with a control group that was fed the normal unfortified feed. The study provides controlled experimental conditions to tease out the effect of the named oils on lipid profile and consequent risk of atherosclerosis in the rats.

1.3 Aim and Objectives

Aim

To investigate the effects of three common vegetable fats on lipid profile and risk of atherosclerosis in rats**.**

Objective

To investigate the effects of three common vegetable fats (Red palm, refined palm and Olive oil) on lipid profile and other indicators (inflammatory markers and antioxidant levels) of atherosclerotic risk in rats**.**

Specific Objectives

To determine the effect of red palm oil and the refined palm oil compared to the olive oil on

- **lipid profile** : TC, LDL, OxLDL, Apo-B100, Apo-A1;
- **Inflammatory markers**: TNF-α, IL-6 and IL-2;
- **Antioxidant marker**: TAS

1.4 Hypothesis

The effect of the two vegetable oils (red palm oil and refined palm oil) on the selected markers will not be different from that of the olive oil in rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background

2.1.1 Atherosclerotic Vascular Disease

The term "atherosclerosis" was introduced in attempt to describe the association of fatty degeneration and vessel thickening (Cullen *et al*., 2005). Atherosclerosis is a process characterized by patchy intramural thickening of the sub-intima that encroaches on the arterial lumen. It affects medium and large-sized arteries (Stocker and Keaney, 2004). The earliest visible lesion of atherosclerosis is the fatty streak, which is due to an accumulation of lipid-laden cells (foam cells) in the intimal layer of the artery.

This deposition leads to a proliferation of certain cell types within the arterial wall that gradually forms a plague which impinge on the vessel lumen and impede blood flow (Weissberg, 2000). This process may be insidious lasting for decades until an atherosclerotic lesion, becomes disrupted and ruptures leading to thrombosis and compromised oxygen supply to target organs such as the heart and brain, and can precipitate the clinical events such as heart attack and stroke often referred to as coronary artery disease and cerebrovascular disease respectively and collectively term cardiovascular diseases (Ross, 1990).

2.1.2 Epidemiology

2.1.2.1 The Global Burden of Atherosclerotic Disease

Atherosclerosis is considered a public health problem of industrialized countries and is one of the most important and common causes of death and disability in the United States and throughout the world, accounting for an estimated one third of deaths overall (Mahoney *et al.*, 2008). In the United States alone, atherosclerosis reportedly affects one in four persons, causing approximately 42% of all deaths. Approximately half of these are due to atherosclerotic coronary heart disease (CHD) (Faxon et al., 2004b). More than 25 million persons in the United States have at least one clinical manifestation of atherosclerosis and in many more, "atherosclerosis remains an occult but important harbinger of significant cardiovascular events" (Faxon *et al.*, 2004a).

About 60% of deaths in the world are now caused by non-communicable diseases (WHO, 2008). According to WHO report (2011), "Cardiovascular diseases (CVDs) remain the biggest cause of deaths worldwide. There are also new dimensions to this alarming situation. Atherosclerotic CVD now threatens developing countries as well. Thus, the need for public health measures to limit its impact (O'Connor *et al.*, 2001). Over the past two decades, deaths from CVDs have been declining in high-income countries, but have increased at an astonishingly fast rate in low- and middle-income countries (LMIC)". More than 17 million people died from CVDs in 2008 representing 30% of all global deaths of which 80% were from low- and middle-income countries. More than 3 million

of these deaths occurred before the age of 60 and could have largely been prevented (WHO, 2011).

Deaths from CVD are often premature, and millions of nonfatal events result in disability. The percentage of premature deaths from CVDs ranges from 4% in high-income countries to 42% in low-income countries, leading to growing inequalities in the occurrence and outcome of CVDs between countries and populations (Poole-Wilson, 2007). By 2020, studies indicate that mortality from CVD is expected to increase by 120% for women and 137% for men. These findings highlight the need to explore the nature and magnitude of CVDs and other non-communicable diseases in developing countries (Poole-Wilson, 2007).

2.1.2.2 Vascular Disease distribution in Developing Countries

In low income countries, vascular disease accounts for 80% of all deaths (11 million per year). In these regions, cardiovascular disease occurs 1 to 2 decades earlier compared to developed countries. Sadly, because of a high case fatality rate, prevalence of cardiac and cerebrovascular disease in developing areas is lower compared to the developed world. Epidemiological studies from developing regions are scarce and often of limited reliability. Good epidemiology studies are costly, complex and demanding and resources in developing countries are limited (WHO, 2008).

The details on the distribution and characteristics of Vascular Disease in the developing world are needed to identify the areas in greater need to distribute

resources accordingly. In countries such as India and China non communicable vascular diseases has overwhelmed infectious diseases (TB, HIV and malaria) as the number one cause of death; there are about 300 million smokers, 160 million hypertensive people and 20% obese children between 7 and 17 years of age, something unknown to this region in the past (Poole-Wilson, 2007). Cardiovascular diseases (CVD) are increasing in epidemic proportions in developing countries. CVD already accounts for almost 10 percent of the developing world's burden of disease and is likely to become the developing world's leading cause of death (Howson *et al*., 1998).

2.1.2.3 The Burden in Africa

The burden of cardiovascular disease is increasing rapidly in Africa, and it is now a public health problem throughout the African Region. Most important are hypertension, stroke, cardiomyopathies and coronary heart disease. Rheumatic heart disease is still a major concern. One of the reasons for increased cardiovascular diseases (CVDs) worldwide is the increase in aged populations. Another reason is exposure to various modifiable risk factors that are responsible for at least 75% of all the CVDs (Feigin, 2007).

Deaths caused by chronic diseases dominate mortality statistics even in African countries. As with other non-communicable diseases, CVDs are not often given the attention they deserve. As a result, most countries do not have national programmes or strategies to address CVDs. Likewise, surveillance systems for risk factors are almost non-existent in the Region. An important phenomenon of

CVD in developing countries is the trend of complications occurring at younger ages (Popkin *et al.*, 2003). Thus, stroke, cardiac failure and renal failure further fuel the vicious cycle of ill-health and poverty (Feigin, 2007).

2.1.2.4 Sub-Saharan Africa

Sub-Saharan Africa (SSA), countries fully or partially located south of the Sahara Desert, are currently experiencing one of the most rapid epidemiological transitions characterized by increasing urbanization and changing lifestyle factors (Fezeu *et al.*, 2007). which in turn have raised the incidence of Non Communicable Diseases (NCDs), especially CVD (Poole-Wilson, 2007).

Studies indicate that urbanization and economic development have also led to the emergence of a nutritional transition characterized by a shift to a higher caloric content diet and/or reduction of physical activity (Popkin *et al*., 2003). In countries such as Nigeria, Ghana and South Africa, the prevalence of chronic diseases is increasing, while the threat of communicable and poverty-related diseases (malaria, infant mortality, cholera, malnutrition) still exists (Kengne and Awah, 2009). In South Africa, CVD is the second leading cause of death after HIV accounting for up to 40% of deaths among adults (Peer *et al.*, 2008).

This double burden of communicable and chronic NCDs has long-term public health impact as it undermines healthcare systems. Sub-Saharan African countries, similar to most developing countries, often do not have the public health infrastructure and finances to address both communicable and poverty-

related illness and behavior/chronic related illnesses (Yach *et al.*, 2004). In addition, there is reluctance on the part of health funding agencies and policy makers to divert scarce resources away from communicable diseases into other areas of disease burden, such as NCDs (Kengne and Awah, 2009).

However throughout SSA, NCDs such as CVD are anticipated to soon eclipse communicable and poverty-related diseases as the leading cause of mortality and disability (Kengne *et al.*, 2005). Also, evidence suggests that the increasing burden of chronic diseases has grave consequences because very few people will seek treatment, leading to high morbidity and mortality rates from potentially preventable diseases (Duda *et al*., 2007).

2.1.2.5 Prevalence Rate of Atherosclerosis to Countries and Regions

The following table (2.1) attempts to extrapolate the prevalence rate for Atherosclerosis to the populations of various countries and regions. These prevalence extrapolations for Atherosclerosis are only estimates, based on applying the prevalence rates from the US (or a similar country) to the population of other countries. This extrapolation calculation is automated and does not take into account any genetic, cultural, environmental, social, and racial or other differences across the various countries and regions for which the extrapolated statistics refer to. These extrapolations only give a general indication as to the actual prevalence or incidence of Atherosclerosis in that region.

Table 2.1: Prevalence Rate of Atherosclerosis by Countries and Regions

Country/Region	Extrapolated Prevalence	Population Estimate Used
North America		
USA	4,992,142	293,655,4051
Canada	552,633	32,507,8742
Mexico	1,784,313	104,959,5942
Central America		
Belize	4,640	272,9452
Guatemala	242,770	14,280,5962
Nicaragua	91,115	5,359,7592
Caribbean		
Puerto Rico	66,265	3,897,9602
South America		
Brazil	3,129,718	184,101,1092
Chile	269,007	15,823,9572
Colombia	719,283	42,310,7752
Paraguay	105,253	6,191,3682
Peru	468,253	27,544,3052
Venezuela	425,295	25,017,3872
North Africa		
Egypt	1,293,996	76,117,4212
Libya	95,736	5,631,5852
Sudan	665,518	39,148,1622
West Africa		
Congo Brazzaville	50,966	2,998,0402

Ghana	352,869	20,757,0322
Liberia	57,640	3,390,6352
Niger	193,129	11,360,5382
Nigeria	301,756	12,5750,3562
Senegal	184,486	10,852,1472
Sierra Leone	100,026	5,883,8892
Central Africa		
Central African	63,622	3,742,4822
Republic		
Chad	162,155	9,538,5442
Congo kinshasa	991,389	58,317,0302
Rwanda	140,057	8,238,6732
Eastern Africa		
Ethiopia	1,212,721	71,336,5712
Kenya	560,695	32,982,1092
Somalia	141,178	8,304,6012
Tanzania	613,203	36,070,7992
Uganda	448,634	26,390,2582
Southern Africa		
Angola	186,635	10,978,5522
Botswana	27,866	1,639,2312
South Africa	755,624	44,448,4702
Swaziland	19,877	1,169,2412
Zambia	187,436	11,025,6902
Zimbabwe	62,421	1,2671,8602

Source: US Census Bureau Population Estimates (International Data Base, 2004).

2.1.3 Risk Factors for Atherosclerosis

The major modifiable risk factors for the CVD include dyslipidemia, hypertension, cigarette smoking and diabetes. Increasing age, male gender, family history and genetics form the major non-modifiable risk factors. Other risk factors include, obesity, alcohol intake, stress, trans unsaturated fat intake and homocysteine (Lichtenstein *et al*., 2006). Physical inactivity or sedentary lifestyle has also been associated with increased risk of death from cardiovascular disease and an increased CHD risk, and conversely, a high level of physical activity is followed by a reduction of the CHD risk and favorable effects on overweight and plasma lipids and lipoproteins (Wannamethee *et al*., 1998).

2.1.3.1 Traditional Risk Factors and Vascular Risk

The current approach to atherosclerotic vascular disease (AVD) risk estimation involves the assessment of factors such as age, family history, gender, blood pressure, cigarette smoking, dyslipidemia and diabetes status. According to Mitchell (2007) the findings of the Framingham Heart Study (1948) and similar trials have been used to develop risk equations for CHD, intermittent claudication, and stroke. A slightly different set of variables is predictive of outcomes for the various vascular beds. For example, the effects of cigarette smoking and diabetes mellitus are especially important for the development of peripheral artery disease (PAD) and intermittent claudication. The use of risk scores provides a reliable risk estimate, leading to targeted therapies and reduction in AVD events. The effect of risks is multiplicative, with two factors increasing the risk of

atherosclerosis fourfold. Hyperlipidemia, hypertension and cigarette smoking together increase the risk seven times (Mitchell *et al*., 2007).

Age

Age is among the most important risk factors for predicting incident cardiovascular disease. This concept is, perhaps, best illustrated if one considers the risk of developing cardiovascular disease over a 10-year period. Based on experience in the United States, the average risk of developing cardiovascular disease for a 30- to 34-year-old male is ~3% (Wilson and Culleton, 1998). This number rises some sevenfold to 21% for a comparable individual aged 60–64 yr. Prediction of coronary heart disease uses risk factor categories The exact magnitude of age-related risk compared with other cardiovascular disease risk factors is illustrated by work from the Framingham Heart Study that has resulted in a 14-point scoring system to predict incident 10-yr cardiovascular disease.

In this system, increasing risk is characterized by a higher score, and up to 7 points can be attributed to age alone. Thus age is an overriding risk factor for incident cardiovascular disease (Wilson and Culleton, 1998). Clinical manifestations and prognosis of these cardiovascular diseases likely become altered in older persons with advanced age because interactions occur between age-associated cardiovascular changes in health and specific pathophysiologic mechanisms that underlie a disease. A fundamental understanding of age-associated changes in cardiovascular structure and function ranging in scope from

humans to molecules is required for effective and efficient prevention and treatment of cardiovascular disease in older persons (Lakatta, 2002).

Gender

Variations in the prevalence of lower-extremity vascular disease have been related to gender and ethnicity. Numerous observational studies have indicated that males exhibit excess risk for cardiovascular disease compared with age-matched women (Barrett-Connor and Bush, 1991). There has been considerable speculation that estrogens offer a "protective" effect to women, as cardiovascular disease accelerates in women after menopause. However, this speculation has been difficult to substantiate, as the treatment with estrogen has not reduced the incidence of cardiovascular disease of postmenopausal women (Hulley *et al*., 1998). Alternatively, some of this apparent protection could be due to the fact that women exhibit relatively higher concentrations of high-density lipoprotein (HDL) cholesterol than do age-matched men. Nevertheless, incident cardiovascular disease is less common in premenopausal women than their age-matched male counterparts (Hulley *et al*., 1998).

The incidence and the progression rate of cardiovascular disease and hypertension (CVDH) are markedly higher in men than in age-matched, premenopausal women. However, after menopause, this relationship no longer exists, and the incidence as well as the rate of progression of CVDH is very similar in women and men (Reckelhoff, 2001). Sex differences in CVDH have also been reported in animal models, including the spontaneously hypertensive rat and Dahl salt-

sensitive rat (Rowland and Fregly, 1992). The mechanisms underlying these sex differences in the incidence and progression of CVDH are largely unknown, the role of sex hormones in modulating the activity of several regulatory systems, including the renin-angiotensin system (RAS), has been suggested. In addition, genetic differences, especially with respect to the RAS, have also been implicated in mediating sex differences in the incidence and progression of CVDH (Reckelhoff, 2001).

Hypertension

Hypertension, defined as a systolic blood pressure in excess of 140 mmHg or a diastolic blood pressure above 90 mmHg (Barrett-Connor and Bush, 1991), is a main physiological risk factor for other CVDs. The current estimates indicate that the elderly are particularly predisposed to hypertension, with up to 75% of people over 75 years of age qualifying for this diagnosis (Barrett-Connor and Bush, 1991). It is estimated that more than 20 million people are affected in the African Region, mainly in urban areas. Prevalence ranges from 25% to 35% in adults aged 25 to 64 years. Some studies (Amoah, 2003) reveal a clear relationship between level of blood pressure, salt and fat consumption, and body weight.

Prevalence rates for hypertension vary across and within regions in Sub-Saharan Africa (SSA). An analysis of all national data in Zimbabwe in the 1990s found that between 1990 and 1997, the national crude prevalence of hypertension increased from 1% to 4% (Mufunda *et al*., 2006). Study in the Niger Delta region of Nigeria found the prevalence of hypertension to be 16% and 12% for males and

females respectively (Ofuya, 2007). A study in an urban area of Nigeria in the 1990s found that among more than 10,000 adults, the crude prevalence of hypertension (blood pressure > 160/95 mm Hg) was 12.4 percent with an age-adjusted rate of 7.4 percent (Lawoyin *et al.*, 2002).

Regardless of gender or type of community, advancing age is associated with an increased prevalence of hypertension, and this implies greater burden of hypertension as population aging occurs in SSA and failure to address the problem may impose significant burden for the health sector and the economy of sub-Saharan African countries (Asfaw, 2005)

Diabetes mellitus

Approximately 17 million people in the United States, or 6.2% of the population, carry the diagnosis of diabetes mellitus (Cowie *et al.*, 2006). In patients with diabetes, the risk of coronary atherosclerosis is three- to fivefold greater than in non-diabetics after controlling for other risk factors. A number of other known risk factors for coronary disease such as hypertension and abnormal lipids are also more common in diabetics than the general population (Bierman, 1992), but despite this association, no more than 25% of the excess coronary atherosclerosis risk from diabetes can be attributed to these known risk factors (Nishigaki *et al.*, 1981).

Thus diabetes represents a major contributing factor to atherosclerosis. According to International Diabetes Federation (IDF), the current estimated prevalence rate

of type 2 diabetes in Africa is about 2.8%. Countries such as Malawi and Ethiopia have rates under 2%, whereas Ghana, Sudan and South Africa have prevalence rates over 3% (Levitt *et al*., 1993). Regarding urban areas the crude prevalence of type 2 diabetes ranges from 1.3% in Sudan to 6.3% in Cameroon (Elbagir *et al*., 1998).

Cigarette smoking

The notion that cigarette smoking is linked to heart disease dates back to a series of studies that unequivocally linked smoking and the incidence of myocardial infarction (Hammond and Horn, 1958). There is now considerable confidence that smoking is causally related to coronary artery disease, as smoking cessation is quite effective in lowering the future risk of the disease. In fact, the risk of heart attack in ex-smokers approaches that of nonsmokers in only 2 years (Gaziano, 1996).

Tobacco use remains one of the most serious epidemiological risk factors in terms of prevalence of coronary artery disease (Verdier and Fourcade, 2007) and smoking prevalence is increasing among men and women in SSA. A review of tobacco use and smoking research showed that males are more likely to smoke than females, and older males (age 30-49) are more likely to use tobacco products than younger males. The prevalence of smoking also increased among women with age (Camargo *et al*., 1997b).

Alcohol

Camargo *et al*. (1997) indicated that, moderate amounts of alcohol reduce the risk of CVD including mortality, hospitalizations and angina pectoris both in men and women (Camargo *et al.*, 1997a). Moderate alcohol consumption also acts in secondary prevention for those who suffered previous myocardial infarct, reducing new coronary events and mortality. According to Daluz and Coimbra (2001) alcohol is protective in men as well as in women. The Nurses' Health Study, based on data from 87.562 women, aged 34-59 years and 200 CVD cases found a decreased risk of events in those taking alcoholic beverages, after adjustment for other risk factors (daLuz and Coimbra, 2001).

The Framingham Study, also evaluated the clinical manifestations of CVD in 22 years of follow-up; there was an inverse relationship between alcohol consumption and symptoms of CAD (angina, myocardial infarction, sudden death) and death from CVD (Wright *et al*., 1983). In a multicenter study carried out in Hawaii, the protective effect of alcohol in women and Asian men was smaller than in Caucasian men.

On the other hand abstainers and those who take more than 2 drinks per day have increased total mortality and non-cardiac deaths, as well as higher blood pressure. Alcohol abuse has been linked to heart failure, ischemic stroke, heart disease, and acute myocardial infarction (daLuz and Coimbra, 2001). A study by Ormel *et al* (2007) examining the global burden of substance abuse, found that Nigerian patients with alcohol dependency were two times more likely to have heart

disease compared to Nigerians who did not suffer from alcohol abuse (Ormel *et al.*, 2007).

Similarly, in Nigerian patients being seen for heart failure treatment in a teaching hospital in Jos, more than 24% of heart failure patients reported regular alcohol intake (Laabes *et al.*, 2008). Alcohol consumption is also correlated with increased risk for glucose intolerance (GI) and diabetes. Puepet and colleagues (2008) conducted a study to identify risk factors for type 2 diabetes in Jos, Nigeria, and found that alcohol consumption was highly prevalent in a random sample of 250 households. More than 50% of patients consumed alcohol regularly. In a prospective cohort study among 320 Cameroonian adults, alcohol consumption was related to increased probability of cardiovascular death and all-cause death (Puepet and Ohwovoriole, 2008).

Obesity

Overweight/obesity is a major and well-known modifiable risk factor for CVD. The prevalence of overweight and obesity is growing in SSA, while the competing epidemic of malnutrition still exists (Agyemang *et al.*, 2009). There is now a growing appreciation that obesity, defined as an excess body weight with an abnormal high preponderance of body fat. A number of other risk factors for cardiovascular disease, such as hypertension, low HDL cholesterol, and diabetes mellitus, often coexist with obesity (Wilson, 1999). This relation between obesity and cardiovascular disease has become of considerable concern as the prevalence of obesity in the developed world is increasing at an alarming rate.

Abdominal obesity or increased waist-to-hip ratio puts one at particularly high risk for CVD. For example, in a meta-analysis of obesity among West African populations, the prevalence of obesity was 10.0% (95% CI, 6.0-15.0) (Abubakari and Bhopal, 2008). A study in Benin (Ntandou *et al*., 2009) found that abdominal obesity was positively associated with increased probability of metabolic syndrome. Abdominal obesity also proved to be an important risk factor for heart failure among adults in Congo, where adults with increased waist-to-hip ratios had increased risk of heart failure (Longo-Mbenza *et al*., 2008).

Across many sub-Saharan African countries, obesity has been linked to both urban residence and wealth - the more wealth a person has, the more likely he or she is to be overweight or obese due to nutritional transition (Ntandou *et al*., 2009), transitions in energy expenditure due to urbanization and other unknown factors (Joubert *et al*., 2007). Duda and colleagues (2007) found similar results in a sample of Ghanaian women. However, another study by Duda et al in Accra, Ghana in 2006 found that overweight women would be willing to reduce their body size in order to improve their health status (Duda *et al*., 2007).

Dyslipidemia

A high concentration of plasma total cholesterol is a major risk factor for coronary artery disease and responsible for one third of all CVDs in the world (Smith and Pekkanen, 1992). This risk is mediated through the major cholesterol-carrying lipoprotein, LDL-C, which is considered the major atherogenic lipoprotein (Law and Wald, 1994). In a study by Norman and colleagues, high

LDL-C cholesterol level (≥ 3.8 mmol/l) accounted for 59% of ischemic heart disease and 29% of ischemic stroke burden in adults age 30 and over. Several theories exist concerning the mechanisms by which LDL-C produces atherosclerosis (Neaton *et al*., 1992). The concentration, the size and the chemical modification of LDL-C are important for atherogenesis. Clinical trials have shown that a reduction of total and LDL-C cholesterol is followed by a regression of atherosclerotic manifestations (Nguyen and Brownell, 1998).

HDL particles are the smallest lipoproteins and therefore enter and also leave the artery wall easily. The plasma concentrations of HDL cholesterol are inversely associated with the risk of CHD (Natarajan *et al*., 2010). The plasma concentrations of Apo-AI, the structural protein of HDL, correlate strongly with HDL cholesterol levels and are also inversely associated with the CHD risk. That HDL cholesterol and Apo-AI are anti-atherogenic, have been shown by animal studies and genetic human studies and the effect have been explained, at least partly, by the reverse cholesterol transport (Barter and Rye, 1996).

In this mechanism, free cholesterol is transferred from peripheral cells to an acceptor HDL subpopulation. The other potential anti-atherogenic mechanisms of HDL and Apo-AI include the protection of LDL-C from oxidation (Banka, 1996), the protection of endothelial cells from the cytotoxic effect of LDL-C, and the stimulation and stabilization of the vasodilator prostacyclin (Barter and Rye, 1996). The goal for plasma HDL cholesterol concentration is > 1.0mmol/l (Stefanick *et al*., 1998).

Although hypertriglyceridemia has been statistically associated with CHD, the independent association of plasma triglycerides with the CHD risk is less certain than that with LDL-C, and triglycerides may not be causally related to the development of atherosclerosis (Kesaniemi, 1998). Population-based studies in Tanzania and Gambia showed elevated total serum cholesterol level of >5.2mmol/l in up to 25% of people age > 35 years (Njelekela *et al*., 2009). Elevated cholesterol was more prevalent in urban than rural areas in the Gambian study. A Nigerian study among diabetics also demonstrated high prevalence of dyslipidemia among type 2 diabetics (Bonilla *et al.*, 2006).

A community-based study of healthy adults in Port Harcourt, Nigeria, found that more than 30% of the 92 participants had elevated LDL-C-levels. Additionally, LDL-C and total cholesterol increased with increasing social-class (Akpa *et al*., 2006). In a study of 248 diabetic patients attending a hospital in an urban community in Ghana, the distribution of dyslipidemia were as follows: 45% had total cholesterol above 5.2mmol/L, 30.5% had HDL-cholesterol below 1.03mmol/L, and 72.4% had high LDL-C-cholesterol. Thus, prevalence of abnormal cholesterol levels among the diabetic patients was high (Eghan and Acheampong, 2003).

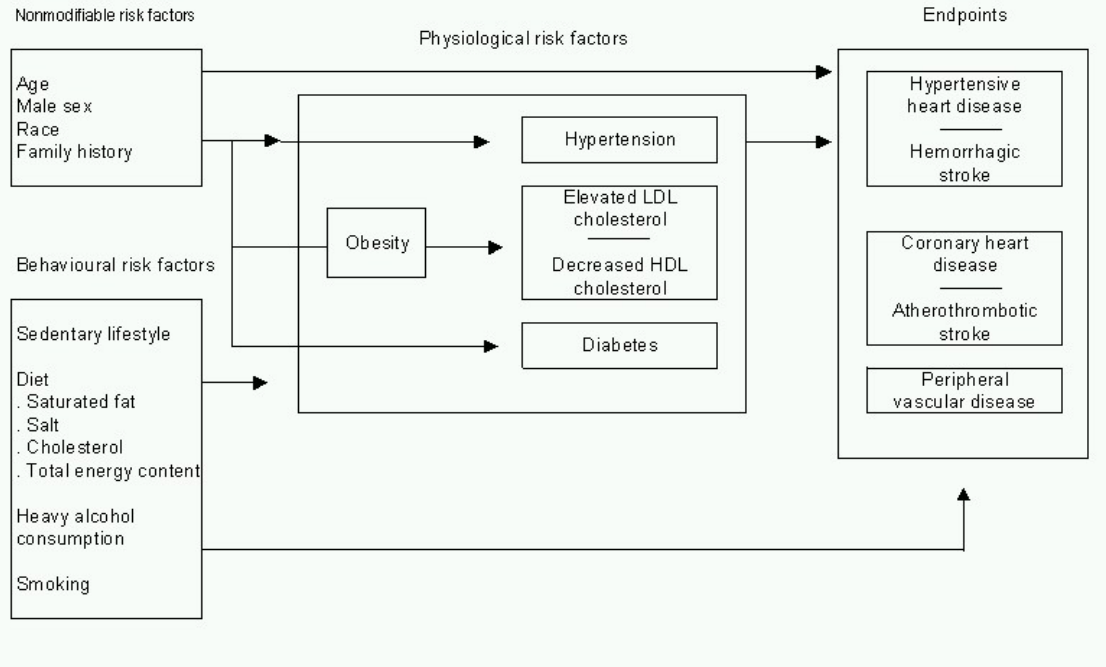

Figure 2.1: Summary of Risk Factors of Atheroslerosis

Two major risk factors: modifiable (behavioral) and non modifiable may lead to physiological risk factors and eventually may develop into the disease state (end points). Source: (Wong et al., 2005).

2.1.3.2 Novel Risk Factors for AVD

New factors, especially those that determine risk associated with cardiovascular disease but have low correlations with existing factors, may be key to improving the current risk estimation approach. The compelling evidence for the role of inflammation in atherothrombotic vascular disease has fueled investigation of novel risk factors (Faxon *et al.*, 2004b). Several novel markers for AVD are under investigation. These include lipoprotein (a) Lp(a), apolipoprotein-A1 (Apo-A1), apolipoprotein-B100 (Apo-B100), high-sensitivity C-reactive protein (hs-CRP), fibrinogen, and homocysteine. Lp(a) concentration, correlate with the CHD risk and may be directly involved in the atherogenic process and, furthermore,

interfere with fibrinolysis (Kronenberg *et al.*, 1999). Lp(a) is largely resistant to modifications, only dietary trans fatty acids and possibly alcohol consumption may alter Lp(a) concentrations (Paassilta *et al*., 1998).

Nevertheless, Lp(a) can be used to identify subjects with increased risk of CHD. Apo-B is the major protein component of LDL-C, IDL, VLDL-C and chylomicrons, and all apo-B containing lipoproteins are atherogenic. Since there is one ApoB molecule per each lipoprotein particle, the Apo-B concentration is a good indicator of the risk of atherosclerosis and CHD (Sniderman *et al*., 1980). In the evaluation of any such novel risk factor or marker, it is very important that its additive effect be directly confirmed in the presence of other established risk factors in a given population to determine its true value.

Studies among both men and women provide information about the additive value of certain novel risk factors. In a comparison of lipid and non-lipid risk factors for AVD prospectively among apparently healthy middle-aged women, the addition of hs-CRP to screening based on standard lipid levels improved prediction of increased risk for stroke and myocardial infarction. In men, a study comparing 11 biomarkers associated with the development of PAD revealed the ratio of total cholesterol to HDL cholesterol to be the strongest lipid predictor of risk (Lackner *et al.*, 1993). The addition of either CRP or fibrinogen to the lipid screening significantly improved predictive value, whereas the addition of Apo-B100, Lp(a), or homocysteine did not add significant value (Banka, 1996).

Although homocysteine and Lp(a) do not seem to add significant clinical predictive value in the general population for screening, they may be useful in the setting of premature or accelerated AVD and probably reducing homocysteine may be associated with improved outcomes (Paassilta *et al.*, 1998). With regard to inflammatory markers, the Center for Disease Control and AHA scientific statement specifies that hs-CRP has the analysis and assay characteristics most conducive to use in clinical practice. The statement recognized hs-CRP as an independent marker of cardiovascular risk that might be used at the discretion of the physician to assist in global risk prediction (Paassilta *et al.*, 1998).

2.1.3.3 Genetic Risk Factors

Evidence from several lines of research indicates that atherosclerosis is, at least partially, genetically determined. Findings from the Framingham Heart Study indicate that up to 50% of the variation in abdominal calcification, another surrogate marker of atherosclerosis is determined by familial factors (Karvonen *et al.*, 1998). Blood-derived inflammatory factors also play a key role. It has been postulated that common polymorphisms with frequent alleles probably account for most of the genetic component of atherosclerosis and CHD (Tymchuk *et al.*, 2006) and rare monogenic disorders associated with a high absolute risk for CHD, such as familial hypercholesterolemia, are only observed in a small proportion of the patients with CHD.

The rare genetic disorders associated with myocardial infarction or atherosclerosis at young age, such as familial hypercholesterolemia and familial defective Apo B-

100, are relatively well documented (Funke and Assmann, 1999). Apo-B, which is an essential lipoprotein for lipid metabolism and also for the absorption of dietary fats and fat-soluble vitamins have many common polymorphic variations, some of them associating with increased plasma total and LDL-C cholesterol concentrations and CHD (Plump *et al.*, 1992).

Apo E polymorphism is an example of a common polymorphism with three major isoforms: apo E2, Apo E3 and Apo E4. Apo E2 differs from E3 by a cysteine for arginine substitution at amino acid residue 158, whereas Apo E4 differs from E3 by an arginine for cysteine substitution at residue 112. Collectively, variation in 1 or more of these genes may be etiologically relevant to the initiation or progression of atherosclerosis, although there is little known about the independent or interactive effects of these genes as risk factors for atherosclerosis in the population or differences in the frequencies of the at-risk alleles of these genes in different ethnic groups. Research is needed to understand the importance of these genes, alone or in combination, in defining AVD in clinical and population settings (Faxon *et al*., 2004a).

2.1.4 Pathogenesis of Atherosclerosis

The pathogenesis of atherosclerosis involves endothelium dysfunction, insudation of lipoproteins into the vessel wall, adhesion and infiltration of monocytes, activation of monocytes into macrophages, and smooth muscle cell migration and proliferation, formation of lesions and plaques and plaque rapture (Ross, 1993a). Molecular changes on the endothelial surface are considered important

contributors to the initiation, progression, and thrombotic complications of atherosclerosis. Desquamation of the endothelium after injury was once considered a contributing mechanism in atherogenesis; however, it is now accepted that atheroma form in the absence of endothelial cell detachment (Ross, 1993b).

Studies in animals and humans have shown that hypercholesterolemia causes focal activation of endothelium in large and medium-sized arteries. The infiltration and retention of LDL-C in the arterial intima initiate an inflammatory response in the artery wall (Steinberg, 2002). Modification of LDL-C, through oxidation or enzymatic attack in the intima, leads to the release of phospholipids that can activate endothelial cells, preferentially at sites of hemodynamic strain. Patterns of hemodynamic flow typical for atherosclerosis-prone segments (low average shear but high oscillatory shear stress) cause increased expression of adhesion molecules and inflammatory genes by endothelial cells (Ross, 1999). The platelet is the first blood cell to arrive at the scene of endothelial activation. Its glycoproteins Ib and IIb/IIIa engage surface molecules on the endothelial cell, which may contribute to endothelial activation. Inhibition of platelet adhesion reduces leukocyte infiltration and atherosclerosis in hypercholesterolemic mice.

Activated endothelial cells express several types of leukocyte adhesion molecules, which cause blood cells rolling along the vascular surface to adhere at the site of activation. Since vascular cell adhesion molecule-1 (VCAM-1) is typically up-regulated in response to hypercholesterolemia, cells carrying counter-receptors for VCAM-1 (i.e., monocytes and lymphocytes) preferentially adhere to these sites

(Nakashima *et al.*, 1998). Once the blood cells have attached, chemokines produced in the underlying intima stimulate them to migrate through the inter-endothelial junctions and into the subendothelial space. M-CSFr produced in the inflamed intima induces monocytes to enter the intima and differentiate into macrophages. This step is critical for the development of atherosclerosis (Carlos and Harlan, 1994).

Infiltrating macrophages contribute in several ways to the local inflammation. Mature macrophages rely on pattern recognition receptors that discriminate between self and non-self to remove selectively modified forms of LDL-C but not native LDL-C (Greaves and Gordon, 2005). Although the removal of cytotoxic and pro-inflammatory LDL-C particles may initially be atheroprotective, the progressive accumulation of lipid-laden macrophages or foam cells ultimately leads to the formation of atherosclerotic lesions (Greaves and Gordon, 2005).

The secretion of reactive oxygen species by the inflammatory cells may further contribute to the oxidation of LDL-C retained in the intima (Greaves and Gordon, 2005). The production of growth factors and cytokines propagates the inflammatory response in an autocrine or paracrine manner, and chemokines facilitate a persistent influx of monocytes. Various mediators of inflammation including adhesion molecules, cytokines, and chemoattractant factors, have been shown to initiate the extravasation of leukocytes (Greaves and Gordon, 2005).
It is well recognized that leukocytes continually interact with the endothelium as part of the immune surveillance.

A T-cell infiltrate is always present in atherosclerotic lesions. T-cell cytokines cause the production of large amounts of molecules downstream in the cytokine cascade. As a result, elevated levels of interleukin-6 and C-reactive protein may be detected in the peripheral circulation. In this way, the activation of a limited number of immune cells can initiate a potent inflammatory cascade in the lesions and eventual formation of plaques (Li *et al*., 1993c). These inflammatory mediators, in addition to oxidized LDL-C, can stimulate the expression of matrix metalloproteases in macrophages, contributing to lesion remodeling and plaque rupture (Fukumoto *et al.*, 2004).

Doran *et al*., (2008) emphasized the important role of vascular smooth muscle cells (VSMCs) in the initiation and progression of atherosclerosis. Serveral studies have reported that VSMCs derived from the outer medial layer migrate, proliferate, and synthesize extracellular matrix components on the luminal side of the vessel and thereby leading to neointimal hyperplasia (Charo and Taub, 2011).

VSMCs are the major producers of extracellular matrix within the vessel wall and in response to atherogenic stimuli can modify the type of matrix proteins produced. Margariti *et al* (2006) also stated that, VSMCs migrate from the media into the intima, where they contribute to neointimal formation by turning into foam cells and producing extracellular matrix. It has also been found that, the type of matrix present can affect the lipid content of the developing plaque and the proliferative index of the cells that are adherent to it (Margariti *et al.*, 2006). VSMCs are also capable of functions typically attributed to other cell types. Like

macrophages, smooth muscle cells (SMCs) can express a variety of receptors for lipid (Doran *et al*., 2008).

In a recent article by Park *et al*. (2011) it was stated that Cholesterol accumulation in VSMCs and monocytes/macrophages leads to the formation of lipid-loaded cells, a key step in the process of atherosclerosis. It is considered that macrophages taking up oxidized low-density lipoprotein cholesterol (ox-LDL-C) could modify the production of cytokines and other factors through oxidative-sensitive signaling pathways, and then initiate and promote atherosclerosis (Park *et al.*, 2011).

2.1.5 Hypotheses of Atherogenesis

These hypotheses of atherosclerosis are not mutually exclusive but rather emphasize different concepts as the necessary and sufficient events to support the development of atherosclerotic lesions. Three distinct hypotheses have emerged that are currently under active investigation: 1) the response-to-injury, 2) the response-to-retention, and 3) oxidative modification.

The Response-to-Injury Hypothesis

In this hypothesis, the proposed initial step in atherogenesis is endothelial denudation leading to a number of compensatory responses that alter the normal vascular homeostatic properties. For example, injury enhances endothelial adhesiveness for leukocytes and platelets and alters the local vascular

anticoagulant milieu to a procoagulant one (Ross and Glomset, 1976). Recruited leukocytes and platelets then release cytokines, vasoactive agents, and growth factors that promote an inflammatory response that is characterized by migration of VSMCs into the intima and their proliferation to form an intermediate lesion (Wang *et al*., 2011).

Another component of the inflammatory response is the recruitment of macrophages into the arterial wall. These macrophages take up deposited LDL-C lipid to form lipid-laden "foam cells," the hallmark of an early atherosclerotic lesion. The process of lipid accumulation and foam cell formation perpetuates an inflammatory response that enables macrophage and lymphocyte recruitment (Park *et al.*, 2011). Continued inflammation allows for cellular necrosis, with a concomitant release of cytokines, growth factors, and proteolytic enzymes that sets the stage for autocatalytic expansion of the lesion to form a space-occupying collection in the intima not unlike an abscess that would form in other tissues. As the lesion enlarges it begins to encroach upon the lumen and, ultimately, blood flow is impaired (Xie *et al*., 2011).

This response-to-injury hypothesis was originally based on the notion of endothelium desquamation as a principal event initiating atherosclerosis (Ross and Glomset, 1976). More recently, it has become clear that endothelial desquamation is not common and that an intact endothelial cell layer covers developing atherosclerotic lesions. These facts, among others, promoted refinement of the initial hypothesis such that endothelial dysfunction is sufficient

to initiate atherogenesis through increased endothelial permeability to atherogenic lipoproteins (Ross, 1999) (Fig. 2.2).

However, normal artery segments exhibit rates of LDL-C entry that exceed the rate of LDL-C accumulation suggesting that atherogenic lipoprotein entry into the arterial wall may not depend on endothelial dysfunction. In fact, the rate of LDL-C entry into the arterial wall is rather uniform, but the accumulation of atherogenic lipoproteins is concentrated in areas that are predisposed to future lesion development (Schwenke and Carew, 1989). Such lesion-prone sites tend rather to demonstrate an enhanced retention of atherogenic apolipoprotein B-containing lipoproteins (Simionescu and Simionescu, 1993). Such observations have prompted alternative hypotheses for the initiation of atherosclerosis.

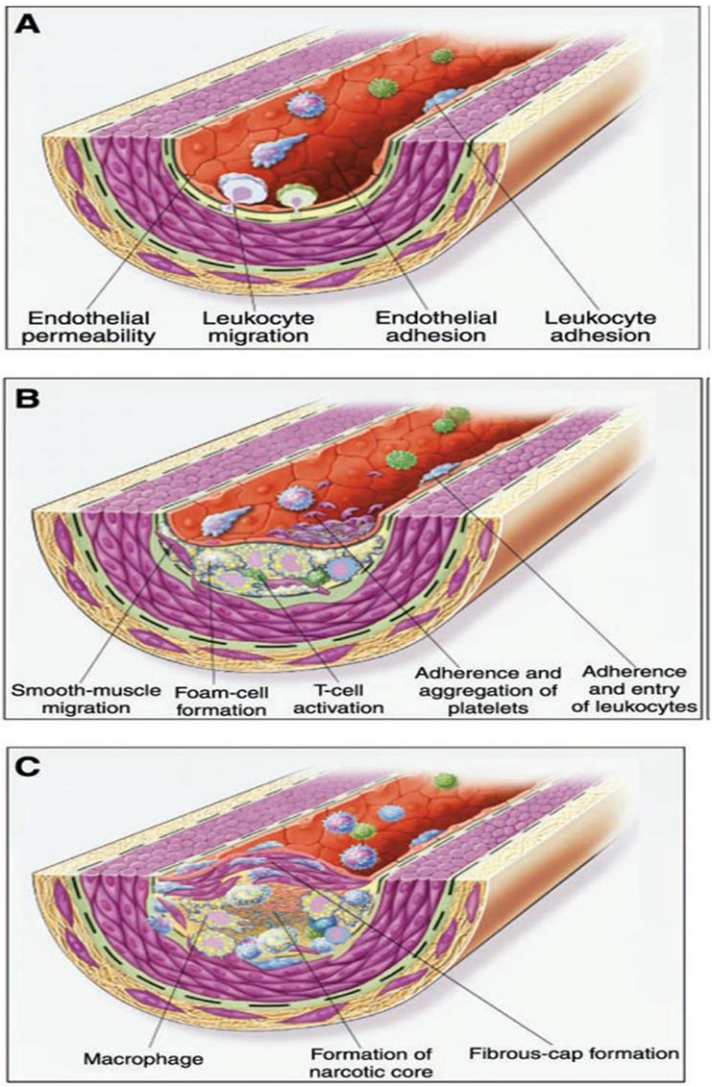

Figure 2.2: Response-to-injury hypothesis of atherosclerosis

In this hypothesis proposed by Ross (1999), atherosclerosis begins with endothelial injury or dysfunction (A) that is characterized by enhanced endothelial permeability and low-density lipoprotein (LDL-C) deposition in the subendothelial space. This is followed by leukocyte adhesion and transmigration across the endothelium. In intermediate stages (B), atherosclerosis is characterized by foam cell formation and an inflammatory response including T-cell activation, the adherence and aggregation of platelets, and further entry of leukocytes into the arterial wall along with migration of smooth muscle cells into the intima. Finally, advanced atherosclerosis (C) is characterized by continued macrophage accumulation, fibrous cap formation, and necrosis in the core of the lesion. [From Ross (1999), Massachusetts Medical Society.]

The Response-to-Retention Hypothesis

This hypothesis submits that the lipoprotein retention is the inciting event for atherosclerosis (Fig. 2.3). Within 2 hours of injecting LDL-C into rabbits, arterial retention of LDL-C and its microaggregates can be observed (Olsson *et al*., 1993). It is estimated that ~85% of sub-endothelial lipoprotein delivery is the result of transcytosis, and this `process is restricted to particles <70 nm in diameter (Simionescu and Simionescu, 1993).

This size restriction is important as it suggests that lipoprotein lipase activity is needed for triacylgycerol-rich lipoproteins to reach the sub-endothelial space (Wallin *et al.*, 1993). The retention of lipoproteins within the arterial wall, however, appears tightly linked to components of the extracellular matrix. Apolipoprotein-B100, the single protein associated with LDL-C, is retained within the arterial wall in close association with arterial proteoglycans (Camejo *et al.*, 1993).

This interaction is mediated by specific residues (3359–3369) that, when mutated, protect experimental animals against the development of atherosclerosis (Boren *et al.*, 1998a). Apolipoprotein-B containing lipoproteins in the early stages of atherosclerosis (Boren *et al.*, 1998b). In addition to proteoglycan binding, lipolytic and lysosomal enzymes in the extracellular matrix also appear to play a role. For example, lipoprotein lipase enhances the adherence of LDL-C in vitro and this effect is independent of enzymatic activity (Williams *et al*., 1992).

Once retained within the arterial wall, LDL-C can form microaggregates, through the action of secretory sphingomyelinase (Xu and Tabas, 1991), an enzyme that also generates ceramides that mediate apoptosis and mitogenesis, as well as lysosomal enzymes such as lysosomal acid lipase (Hakala *et al.*, 2003). Most importantly, aggregated LDL-C is avidly taken up by macrophages and VSMCs and thus can support foam cell formation. Thus many features of atherosclerosis can be attributed to enhanced retention of LDL-C within the arterial wall and its association with proteoglycans (Vijayagopal *et al.*, 1992).

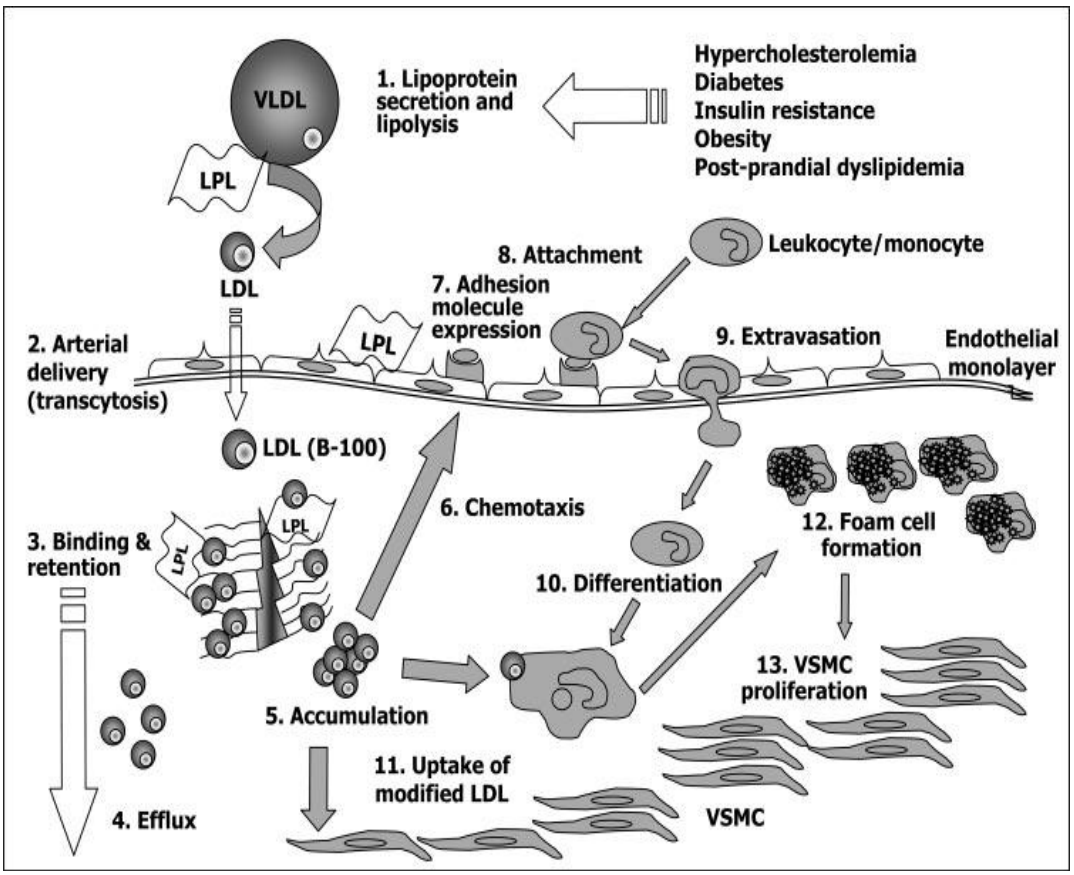

Figure 2.3: Response-to-retention hypothesis of atherosclerosis

According to the original hypothesis (Williams and Tabas, 1995), mild to moderate hyperlipidemia causes lesion development only in specific sites within the arterial tree characterized by local synthesis of apolipoprotein B-retentive molecules such as biglycan and

decorin. The diagram shows the initial stages of arterial lipoprotein delivery, retention, and efflux (1–5). Accumulation (5) is thought to result from both apolipoprotein B-100 motifs that mediate proteoglycan binding and arterial factors such as secretory sphingomyelinase that facilitate lipoprotein aggregation. The accumulation of apolipoprotein B-100-containing lipoproteins within the arterial wall is thought to further trigger a proinflammatory cascade (6–13). Lipoprotein oxidation (not shown) may or may not be part of these responses. [Modified (Proctor et al., 2002)].

The Oxidative-Modification Hypothesis of Atherosclerosis

Oxidative modification of LDL-C is probably the most important and widely regarded critical event in the atherogenic process (Leake, 1993). The relationship between hypercholesterolemia, elevated level of low-density lipoprotein (LDL-C) and premature atherosclerosis is now firmly established (Parthasarathy *et al.*, 1990). The cholesterol that accumulates in atherosclerotic lesions in vessel walls is derived primarily from lipoproteins, predominantly LDL-C (Khoo *et al*., 1990).

Post-secretory modifications in the structure of lipoproteins significantly affect their atherogenicity. Current studies suggest that oxidative stress, particularly the oxidation of LDL-C, represents a risk factor and plays a key role at several steps of atherosclerosis, according to the oxidative-modification hypothesis of atherosclerosis (Westhuyzen, 1997). This hypothesis suggests that oxidatively modified LDL-C (ox-LDL-C), but not native (unmodified) LDL-C, is taken by scavenger receptors on monocytes, VSMCs and macrophages in the intima of blood vessel, by an unregulated process leading to the formation of lipid-laden foam cells (Fig. 2.4).

According to this hypothesis, LDL-C initially accumulates in the extracellular sub endothelial space of arteries because of the augmented permeability of the endothelial cells secondary to any injury that leads to endothelial dysfunction (Westhuyzen, 1997). Through the action of resident vascular cells, LDL-C is oxidized to a form known as minimally modified LDL-C (mm-LDL-C). This mm-LDL-C induces local vascular cells to produce monocyte chemotactic protein 1 (MCP-1), granulocyte-macrophage colony-stimulating factor (GM-CSF) and colony-stimulating factor 1 (CSF-1), which stimulate monocyte recruitment and differentiation to macrophages in arterial walls (Parhami *et al*., 1993).

The accumulating activated monocytes and macrophages stimulate further peroxidation of LDL-C. This completely oxidized LDL-C is recognized by scavenger receptors on macrophages and internalized leading to formation of foam cells (Quinn *et al.,* 1987). In contrast to the uptake of native LDL-C by the LDL-C receptor on macrophages and other cells, the uptake of oxidized LDL-C by the scavenger-receptor pathway is not subject to negative-feedback regulation and thus results in massive uptake of cholesterol by macrophages (Palinski *et al*., 1989).

The oxidative-modification hypothesis is supported by evidence that LDL-C oxidation occurs in vivo. In fact, antibodies raised against oxidized LDL-C react with atherosclerotic lesions but not with normal arterial segments (Yla-Herttuala *et al.*, 1989) Patients with carotid atherosclerosis have also higher levels of auto antibodies to oxidized LDL-C than normal subjects (Salonen *et al*., 1992). This link between LDL-C oxidation and atherogenesis provides a convenient and

simple rationale for the potential beneficial effects of antioxidants on atherosclerosis disease (Stephens *et al*., 1996).

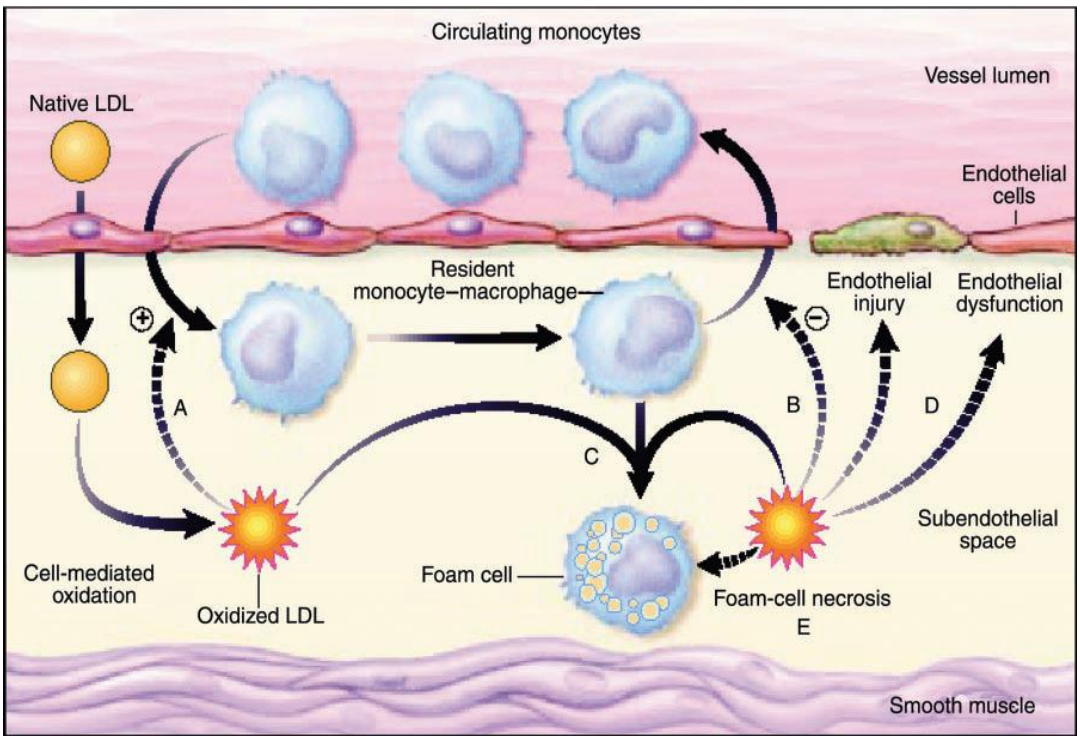

Figure 2.4: Oxidative modification hypothesis of atherosclerosis

LDL-C becomes entrapped in the subendothelial space where it is subject to oxidative modification by resident vascular cells such as smooth muscle cells, endothelial cells, and macrophages. Oxidized LDL-C stimulates monocyte chemotaxis (A), prevents monocyte egress (B), and supports foam cell formation (C). Once formed, oxidized LDL-C also results in endothelial dysfunction and injury (D), and foam cells become necrotic due to the accumulation of oxidized LDL-C (E). Source: (Diaz et al., 1997).

2.1.6 Selective Uptake

Although the LDL-C receptor (LDL-CR) is a major pathway for systemic LDL-C clearance and for cholesterol delivery to many cell types (Brown and Goldstein,

1986), cholesterol accumulation in the arterial wall with limited LDL-CR expression indicates that cholesterol delivery is mediated by mechanisms independent of the LDL-CR..

Pathologic arterial lipid accumulation, an important contributor to atherogenesis, is linked to a number of pathways involving CD36 or other scavenger receptors that bind to normal or modified LDL-C (Rigotti *et al.,* 1995) to promote the progression of atherosclerosis (Handzha, 1997). Moreover, cell surface proteoglycans that are ubiquitously expressed in cells have been shown to mediate LDL-C uptake via low-affinity but high-capacity processes, which leads to substantial cholesterol accumulation in cells. These processes have generally been studied by evaluating apo-B uptake, an indicator for LDL-C cholesterol uptake (Seo and St Clair, 1997) .

LDL-C cholesteryl esters (CEs) can be independently delivered to cells without concomitant uptake of the whole LDL-C particle, a process named "selective uptake" (SU) (Rinninger *et al.*, 1995). SU leads to accumulation of cholesterol in cells and tissues that exceed cholesterol delivery accounted for by whole-particle uptake. SU from HDL via scavenger receptor type B-I (SR-BI) has been well characterized (Krieger and Kozarsky, 1999) and is linked to steroidogenesis (Temel *et al*., 1997) and reverse cholesterol transport (Seo *et al*., 2002).

Unlike those of HDL SU, the pathways and physiological significance of LDL-C SU are less defined. However, studies by Green and Pittman (Green *et al.*, 1991) indicated that LDL-C SU occurs in lipoprotein lipase–rich (LPL-rich) tissues such as muscle, heart, and adipose tissues. In keeping with these observations, LDL-C-

SU in muscle is significantly increased in mice overexpressing human LPL in muscle (Green *et al*., 1997).

Seo *et al* (2002) previously demonstrated that non–SR-BI–mediated SU in cultured macrophages was markedly enhanced by free fatty acids (FFAs). Thus, it is possible that alterations in plasma FFA levels and/or composition, induced by diet or by disease (e.g., diabetes), lead to increased cholesterol delivery via SU to the arterial wall. FFAs and cholesterol often interact and influence systemic lipid homeostasis and LDL-C cholesterol metabolic pathways (Green *et al*., 1997).

2.1.7 Infections and Coronary Artery Disease (CAD)

Several studies have linked infections to atherosclerosis and CAD. Elevated titers of antibodies against chlamydia were found in patients with CAD, and it was speculated that this microbe caused atherosclerosis. However, Chlamydia pneumoniae infection does not cause atherosclerosis in animals, although it may stimulate disease progression and plaque activation (Grayston *et al.*, 1997). This could be due either to a direct action in plaques or to remote signaling by inflammatory mediators.

Molecular mimicry between C. pneumoniae antigens and human molecules may contribute to the activation of inflammation (Maass and Gieffers, 1997). Herpes family viruses may also contribute to CAD. Cytomegalovirus is found in lesions, can modulate immune-cell as well as vascular-cell activity, and increases experimental atherosclerosis. Clinical data imply an important role for

cytomegalovirus in transplantation-related arteriosclerosis causing graft rejection (Dobrilovic *et al.,* 2001). More studies will be needed to determine whether the virus is involved in more common forms of CAD. Since several types of pathogens may contribute to CAD, it is unlikely that a single microbe causes atherosclerosis. Instead, the total burden of infection at various sites may affect the progression of atherosclerosis and elicit clinical manifestations (Peterson and Noskin, 2001).

2.1.8 Morphological Features of Atherosclerosis

The normal artery

The arterial wall normally consists of three well-defined concentric layers that surround the arterial lumen, each of which has a distinctive composition of cells and extracellular matrix. The layer immediately adjacent to the lumen is called the intima, the middle layer is known as the media, and the outermost layer comprises the arterial adventitia. These three layers are demarcated by concentric layers of elastin, known as the internal elastic lamina that separates the intima from the media, and the external elastic lamina that separates the media from the adventitia (Wittram, 2003).

Intima

The intima is composed of a single contiguous layer of endothelial cells lining the luminal surface of arteries. These cells sit on a basement membrane of extracellular matrix and proteoglycans that is bordered by the internal elastic

lamina. Although smooth muscle cells are occasionally found in the intima, endothelial cells are the principal cellular component of this anatomic layer and form a physical and functional barrier between flowing blood and the stroma of the arterial wall. Endothelial cells regulate a wide array of processes including thrombosis, vascular tone, and leukocyte trafficking among others (Kumar *et al*., 1992).

Media

Progressing outwards from the internal elastic lamina, the media consists principally of smooth muscle cells arranged in layers, the number of layers depending on the arterial size. An extracellular matrix consisting largely of elastic fibers and collagen with a lesser content of proteoglycan holds the smooth muscle cells together (Kumar *et al*., 1992). The outer limit of the media of most arteries is marked by a well-defined external elastic lamina. In large and medium sized arteries, smooth muscle layers of the media near the vessel lumen depend on the direct diffusion of oxygen from the vessel lumen for their nutritional needs (Ross, 1993b). Diffusion is facilitated by fenestrations in the internal elastic lamina. Small arterioles (vasa vasorum) arising from outside the vessel perforate the external elastic membrane and sends twigs into the outer 2/3rds of the media because diffusion from the lumen provides inadequate oxygen to the outer portion of the large and medium-sized vessels. In the large or elastic arteries, the media is rich in elastic fibers, disposed in fairly compact layers separated by and alternating with layers of smooth muscle cells. The elastic component of the aorta

allows it to expand during systole therefore storing some of the energy of each heart beat (Ross, 1993b).

Adventitia

The adventitia is the outermost layer of the artery and typically consists of a loose matrix of elastin, smooth muscle cells, fibroblasts, and collagen. It is a layer of connective tissue in which nerve fibers and vessels that nourish the vasa vasorum are dispersed (Kumar *et al*., 1992). Most of the neural input into blood vessels also traverses through the adventitia. At one time, the adventitia was considered inactive with respect to vascular homeostasis; however, recently it has become clear that the adventitia, through the production of reactive oxygen species (ROS), may play an important role in controlling vascular remodeling and nitric oxide (NO) bioactivity (Ross, 1993b).

2.1.8.1 Cells Involved in Atherogenesis

Endothelial Cells

The vascular endothelium is capable of producing numerous bioactive molecules, thereby acting as an autocrine, paracrine, and endocrine organ. In a normal system, endothelial cells maintain vascular tone via endothelium derived relaxing factors including NO, prostacyclin, and endothelium-derived hyperpolarizing factors (Jiang and Dusting, 2001). in an integrated balance with sympathetic and myogenic tone as well as parenchymal cell influences. These molecules help to

regulate the homeostasis of the vascular system by adjusting to a number of systemic demands on blood flow, coagulation, inflammation, platelet aggregation, and signal transduction, with any decay in efficacy considered as dysfunction (O'Riordan *et al.*, 2005). Atherosclerotic lesions begin to develop under an intact but leaky, activated, and dysfunctional endothelium. Later, endothelial cells may vanish and de-endothelialized (denuded) areas appear over advanced lesions, with or without platelets adhering to the exposed subendothelial tissue.

Depending on size and concentration, plasma molecules and lipoprotein particles extravasate through the leaky and defective endothelium into the subendothelial space, where potentially atherogenic lipoproteins are retained and modified (e.g., oxidized) and become cytotoxic, proinflammatory and proatherogenic (Dilaveris *et al.*, 2007). The mechanisms responsible for the atherogenic modification of LDL-C are not clear but could include oxidation mediated by myeloperoxidase, 15-lipoxygenase, and/or nitric oxide synthase (NOS). Nitric oxide (NO), synthesized from the amino acid L-arginine through the enzyme nitric oxide synthase (NOS), has been widely considered as an endothelium-dependent regulator of vascular tone, with additional roles in preventing platelet activation, inhibiting oxidative stress, cell growth, and inflammation, among others (Aggarwal *et al.*, 2001).

Nitric oxide is a potent oxidant produced by both endothelial cells and macrophages that appears to exert both protective and atherogenic effects, depending on its source of production. Nitric oxide produced by endothelial NOS has vasodilator function and is potentially atheroprotective (Ogita *et al.*, 2001). In

contrast, nitric oxide produced via the much higher capacity inducible-NOS in macrophages, serving antimicrobial functions based on its potent oxidative properties, is potentially proatherogenic.

In addition, activated macrophages secrete inflammatory cytokines, such as TNF-α, that contribute to the induction of the expression of monocyte chemotactic protein-1 (MCP-1), macrophage colony stimulating factor (M-CSF), intercellular adhesion molecule-1 (ICAM1), and vascular cell adhesion molecule-1 (VCAM1) in human aortic endothelial cells (HAECs) (Schleser *et al*., 2006). The endothelium becomes activated by atherogenic and proinflammatory stimuli, and the expression of adhesion molecules, primarily VCAM-1, are up-regulated, and monocytes and T cells are recruited. Besides VCAM-1, other adhesion molecules, such as ICAM1, E selecton, and P selecton, probably contribute to the recruitment of blood borne cells to the atherosclerotic lesion (Hansson, 2005).

Leukocytes

One of the earliest cellular responses in atherogenesis is the local recruitment of circulating monocytes and, to a lesser extent, T lymphocytes (Libby, 2002a). The persistence of this cellular response seems to underlie disease progression. The mere adhesion to the endothelium is not enough for blood-borne cells to arrive in the lesion, as transendothelial migration also is required. Experimental studies indicate that the most important atherogenic chemoattractants are oxidized LDL-C and MCP-1 (Libby, 2002a). Cytokines (e.g., interleukin-8) also may play a role in monocyte-macrophage trafficking (Glass and Witztum, 2001).

Within intima, the monocytes differentiate into macrophages and internalize the atherogenic lipoproteins via so called scavenger receptors, of which SR-A and CD36 have been demonstrated to play quantitatively significant roles in experimental atherosclerosis (Falk, 2006). Aside from their scavenger function, macrophages also possess destabilizing and thrombogenic properties by expressing matrix-degrading proteolytic enzymes (e.g., matrix metalloproteinases) and tissue factor (Libby, 2002a).

Thus, these innate protective cells initially recruited to combat cytotoxic lipids, turn into devastating "friendly fire" during the progression of atherothrombosis (Hansson, 2005). There are a number of candidate antigens in the lesion that could be responsible for immune activation, including modified LDL-C, heat-shock proteins, beta-2-glycoprotein I, and microbial antigens. Of these, the most extensive data support an important role for oxidized LDL-C, which is abundantly present in atherosclerotic plaques, where it is recognized by T cells (Hansson, 2005).

Smooth muscle cells

Only endothelial cells, macrophages, and a few T cells participate in the development of the early and asymptomatic foam-cell lesion, the fatty streak. In disease progression, the immunoinflammatory response is joined by a fibroproliferative response mediated by intimal smooth muscle cells. These cells are responsible for healing and repair after arterial injury. If the atherogenic stimuli persist over the course of years, as they often do, the reparative responses may become so voluminous and dominating that lumen is lost, blood flow is

reduced, and ischemia sets in (Kragel *et al*., 1989b). Nevertheless, smooth muscle cells and the collagen-rich matrix they produce do confer stability to plaques, protecting them against much more ominous consequences; plaque rupture and thrombosis. The smooth muscle cell is the principal connective tissue producing cell in the normal and diseased (atherosclerotic) intima (Schwartz *et al*., 2000).

2.1.8.2 Morphology of Atherosclerotic Lesions

Atherosclerosis manifests itself as arterial lesions known as plaques that have been extensively characterized (Stary, 1990) into six major types of lesions that reflect the early, developing, and mature stages of the disease. In lesion-prone arterial sites, adaptive thickening of the intima is among the earliest histological changes. As macrophages accumulate lipid, type II lesions form as nodular areas of lipid deposition that are also known as “fatty streaks”.

Continued foam cell formation and macrophage necrosis can produce type III lesions that contain small extracellular pools of lipid. Types II and III lesions are readily apparent through the use of fat-soluble dyes that stain cholesterol esters accumulated in macrophages and the extracellular space. These early lesions are often evident by age 10 (Stary *et al*., 1995a) and can occupy as much as one-third of the aortic surface by the third decade.

Developing lesions represent the next two types of lesions and, are characterized by significant areas of extracellular lipid that represents the "core" of the atherosclerotic lesion. Type IV lesions are defined by a relatively thin tissue

separation of the lipid core from the arterial lumen, whereas type V lesions exhibit fibrous thickening of this structure, also known as the lesion "cap." These type IV and V lesions can be found initially in areas of the coronary arteries, abdominal aorta, and some aspects of the carotid arteries in the third to fourth decade of life (Stary *et al.*, 1995b). Mature type VI lesions exhibit architecture that is more complicated and characterized by calcified fibrous areas with visible ulceration.

These types of lesions are often associated with symptoms or arterial embolization. It was once thought that end-organ damage and infarction were due to gradual advancement of these lesions, but we now know the processes involved in precipitating heart attack and stroke are considerably more complex (Lusis and Navab, 1993).

Plague Morphology

Plaques contain a central lipid core that is most often hypocellular and may even include crystals of cholesterol that have formed in the aftermath of necrotic foam cells (Guyton *et al.*, 1990). The lipid-rich atheromatous core is a vascular, soft-like gruel, and totally devoid of supporting collagen. Its size is, of course, critical for the stability of a plaque (Fig. 2.5). In this late stage of lesion development, residual foam cells may be difficult to see but have often left the core with an abundant quantity of tissue factor (Wilcox *et al.*, 1989) an important activator of the clotting cascade.

This lipid core is separated from the arterial lumen by a fibrous cap (fig. 2.6) and myeloproliferative tissue that consists of extracellular matrix and smooth muscle cells. The junction between the cap and the morphologically more normal area of artery is known as the "shoulder" region of the atherosclerotic plaque. This area is typically more cellular than other areas of the plaque and may contain a variable composition of smooth muscle cells, macrophages, and even T cells. The shoulder region is most prone to rupture (Lendon *et al.,* 1991).

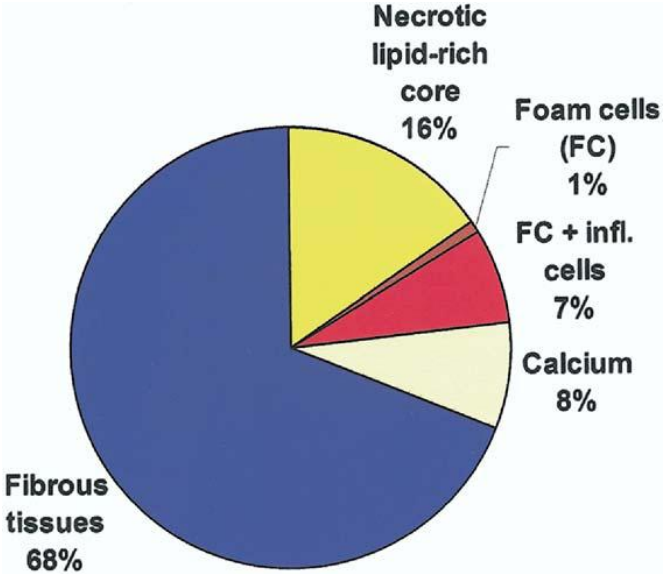

Figure 2.5: Average composition of advanced coronary plaque

Pie diagram illustrating the average composition of advanced atherosclerotic plaques (75% stenosis by histology) in the coronary artery tree in fatal myocardial infarction (Kragel et al., 1989a).

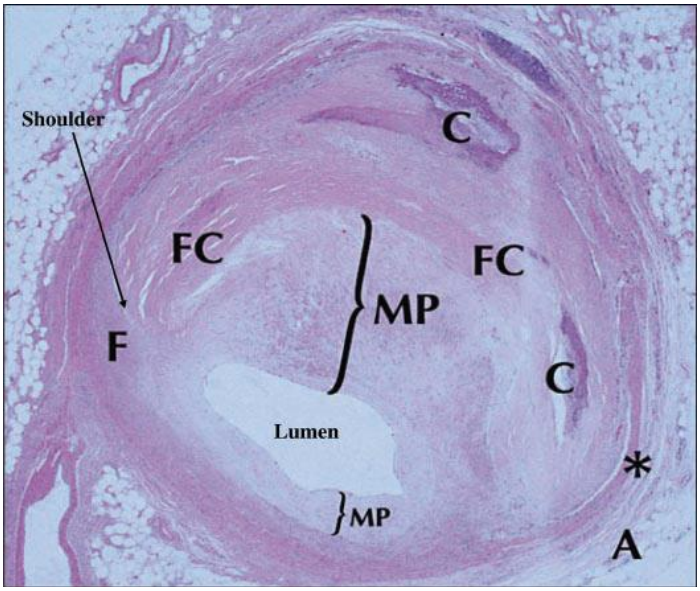

Figure 2.6: Light micrograph of a fibrofatty plaque in the coronary artery

*The lumen is eccentric and separated from the fibrous cap (FC) by myeloproliferative (MP) tissue. Under the fibrous cap are calcified (C) areas of the plaque indicative of advanced lesions. The fibrous cap is separated from the more normal area of the artery by the "shoulder" region that, in this plaque, demonstrates evidence of a healed fissure (F). The adventitia (A) is the outermost area of the artery, and its border with the media is defined by the external elastic lamina *. Source (Gravanis, 1994).*

2.1.8.3 Plaque Progression in Atherosclerosis

Cell Death

During the progression of atherosclerosis, endothelial cells, macrophages, and smooth muscle cells die by apoptosis or necrosis. Disintegration of foam cells and loss of smooth muscle cells may have detrimental consequences, leading to the formation of a destabilizing lipid-rich core and a fragile and rupture prone fibrous cap (Geng and Libby, 2002). Furthermore, apoptosis contributes dramatically to

the high tissue factor activity and thrombogenicity of the lipid-rich core (Tedgui and Mallat, 2001). During regression of atherosclerosis induced by cholesterol lowering in animals, inflammatory cells (macrophages) disappear, but their fate remains elusive. Many macrophages appear to die within the lesion, but others probably emigrate from regressive plaques (Llodra *et al.*, 2004).

Calcification

Focal calcification in atherosclerotic plaques is very common and increases with age (Hoffmann *et al.*, 2003). The total amount of calcification the coronary artery calcium score is a genuine marker of coronary plaque burden and provides prognostic information beyond that provided by traditional risk factor scoring (Pletcher *et al.*, 2004). Plaque calcification is to some extent active and controlled, resembling calcification in bone, and both lipid and connective tissue may calcify. In coronary arteries, calcification is almost always caused by atherosclerosis; medial calcification (Mönckeberg's calcinosis) is exceedingly rare. Clinical observations suggest that culprit lesions responsible for acute coronary syndromes generally are less calcified than plaques responsible for stable angina, and the pattern of plaque calcification also differs (Ehara *et al.*, 2004).

Neovascularization of plaque

Endothelial proliferation and sprouting usually originates from vasa vasorum in adventitia and extends through media into the base of the plaque, where neovascularization is most conspicuous. The new microvessels are fragile, are leaky, and express cellular adhesion molecules (VCAM-1, intercellular adhesion molecule-1), resulting in local extravasation of plasma proteins, erythrocytes, and inflammatory cells. Thus, angiogenesis and inflammation often coexist and could mediate rapid plaque progression (Casscells *et al.*, 2003). Regardless of the integrity of the plaque surface, extravasated erythrocytes are common in neovascularized areas, but there is no convincing evidence that these low-pressure bleedings may precipitate rupture of the plaque surface and/or acute luminal thrombosis (Davies and Thomas, 1984).

2.1.8.4 Mechanisms of Plaque Rupture

Plaque rupture

Rupture of the plaque surface is followed by amounts of hemorrhage into the plaque and luminal thrombosis, causing sudden and rapid but often clinically silent progression of the lesion. It is probably the most important mechanism underlying the episodic (versus linear) progression of coronary lesions observed by serial angiography (Burke *et al*., 2001).

During atherogenesis, multiple sites of endothelial denudation and plaque rupture develop and heal. When sub-endothelial tissue is exposed, platelets adhere and fibrin forms. The magnitude of this thrombotic response depends on the thrombogenic stimulus; plaque rupture probably is much more thrombogenic than plaque erosion. In the pathogenesis of arterial thrombosis, platelet aggregation is responsible for the initial flow obstruction but fibrin formation is necessary for the subsequent stabilization of the platelet-rich thrombosis. Thus, both platelets and fibrin may accumulate over ruptured and eroded plaques (Falk *et al*., 2007).

2.1.9 Treatment and Management

The prevention and treatment of atherosclerosis requires Control of the known modifiable risk factors for this disease. Treatment involves aggressive modification of risk factors to slow progression and induce regression of existing plaques.

2.1.9.1 Lifestyle changes

Lifestyle changes include diet modification, smoking cessation, and regular participation in physical activity. Medical treatment of hypertension, dislipidemia, diabetes mellitus are often required. These lifestyle changes and drugs directly or indirectly improve endothelial function, reduce inflammation, and improve clinical outcome. The statins can decrease atherosclerosis-related morbidity and mortality even when serum cholesterol is normal or slightly high. Antiplatelet

drugs help all atherosclerotic patients. Patients with CAD may benefit additionally from ACE inhibitors and β-blockers (Hauer *et al.,* 2005).

In general, the group of medications referred to as statins has been the most popular and are widely prescribed for treating atherosclerosis. They have relatively few short-term or longer-term undesirable side-effects, and multiple comparative treatment/placebo trials have fairly consistently shown strong effects in reducing atherosclerotic disease events (Maseri, 2003).

2.1.9.2 Surgical intervention

Other physical treatments, helpful in the short term, include minimally invasive angioplasty procedures that may include stents to physically expand narrowed arteries (Huang *et al.,* 2007) and major invasive surgery, such as bypass surgery, to create additional blood supply connections that go around the more severely narrowed areas.

2.1.9.3 Prophylaxis

Patients at risk for atherosclerosis-related diseases are increasingly being treated prophylactically with low-dose aspirin and a statin. The high incidence of cardiovascular disease led Wald and Law to propose a Polypill, a once-daily pill containing these two types of drugs in addition to an ACE inhibitor, diuretic, beta blocker, and folic acid. They maintain that high uptake by the general population by such a Polypill would reduce cardiovascular mortality by 80%. It must be

emphasized however that this is purely theoretical, as the Polypill has not been tested in a clinical trial (Wald and Law, 2003).

2.2 The Role of Lipids in Atherogenesis

2.2.1 Background

Lipids generally refer to a major class of tissue components or naturally occurring molecules (750 to 1500 in molecular weights) which are hydrophobic in nature They encompass diverse group of chemical compounds that share the common characteristic of being insoluble in water but soluble in organic solvents and include fats and oils, waxes, steroids and other related compounds (Edidin, 2003). Lipids are produced, transported, and recognized by the concerted actions of numerous enzymes, binding proteins, and receptors. Lipids that are solid at room temperature (25^{o}C) are known as fats, and those that are liquid at room temperature are known as oils, although they are also generically referred to as fats (Fahy *et al*., 2005).

Fats and oils

Fats are naturally occurring complex mixtures of triacylgycerol molecules solid at room temperature. A molecule of true fat is formed when three fatty acid molecules are covalently link with a molecule of glycerol by means of three ester bonds. Glycerol (glycerine) is a trihydric alcohol, containing three alcoholic or hydroxyl (OH) groups to which the three fatty acids link one after the other to form triglyceride or triacylglycerol (TAG). Triglycerides have lower densities

than water, and at normal room temperatures may be solid or liquid. When solid, they are called "fats" or "butters" and when liquid they are called "oils" (Bender and Bender, 2005).

Triglycerides constitute about 98% of total dietary lipids; the remaining 2% consisting of phospholipids, cholesterol and its esters (Fahy *et al.*, 2005). Nearly all the commercially important fats and oils of animal and plant origin consist almost exclusively of triacylglycerols (Laakso and Voutilainen, 1996). This includes all the vegetable oils, such as those from corn (maize), olive, palm, and sunflower, and animal fats, such as tallow, lard and butter (Laakso and Voutilainen, 1996).

The more abundant animal triacylglycerols are depot fats (from adipose tissue) or milk fats, where their main function may be as a store of energy, but some triacylglycerols (e.g. those of plasma or liver) may have a more dynamic role (Andrikopoulos, 2002). Similarly, seed oils serve as a source of energy and structural fatty acids for the developing embryo. In animals, adipocytes contain very large quantities of triglycerides in the form of fat droplets, which fill almost the entire cell volume (Fahy *et al*., 2005).

Oils are also naturally occurring complex mixtures of triglycerole molecules, liquid at room temperature (25ºC). They are typically obtained from plant sources although there are also fish oils. The major oilseeds are soybeans, rapeseed, cottonseed, sunflowerseed, groundnut, palm, copra, sesame, linseed & castorseed, maize oil, coconut oil palm kernel (seed, meal, oil) palm oil and Olive Oil (Laakso and Voutilainen, 1996).

Cholesterol

Cholesterol is a wax-like substance made by the liver and occurs either free or as fatty esters in all animal cells. It is the principal sterol of higher animals and is especially abundant in nerve tissues and in some gallstones (Bolton-Smith *et al.*, 1991). Cholesterol has both hydrophilic properties, due to its hydroxyl end, and hydrophobic properties, due to its hydrocarbon side-chain. It is commonly found in the lipid bilayer of cell membranes (Loose-Mitchell *et al.*, 1991).

Normal healthy adults synthesize cholesterol at a rate of approximately 1 g/day and consume approximately 0.3 g/ day. A relatively constant level of cholesterol in the body (150 – 200 mg/dL) is maintained primarily by controlling the level of de novo synthesis and that makes up about 85% of blood cholesterol, while only about 15% comes from dietary sources (Ravnskov *et al.*, 2006, Ravnskov, 2005).

The level of cholesterol synthesis is regulated in part by the dietary intake of cholesterol. Exogenous cholesterol down-regulates cholesterol synthesis in the liver to maintain cholesterol balance (Omoigui, 2007). Cholesterol also contributes to the development and working of the central nervous system, and it has major functions in signal transduction and sperm development (Dart *et al.*, 2004).

Table 2.2: Percentage of Specific Types of Fat in Common Oils and Fats

Cooking Oils	Saturated	Mono-unsaturated	Poly-unsaturated	Trans
Canola	7	58	29	0
Safflower	9	12	74	0
Sunflower	10	20	66	0
Corn	13	24	60	0
Olive	13	72	8	0
Soybean	16	44	37	0
Peanut	17	49	32	0
Palm	50	37	10	0
Coconut	87	6	2	0
Cooking Fats				
Shortening	22	29	29	18
Lard	39	44	11	1
Butter	60	26	5	5
Margarine/Spreads				
70% Soybean Oil, Stick	18	2	29	23
67% Corn & Soybean Oil	16	27	44	11
Spread, Tub				
48% Soybean Oil Spread, Tub	17	24	49	8
60% Sunflower, Soybean, and Canola Oil Spread, Tub	18	22	54	5

**Values expressed as percent of total fat; data are from analyses at Harvard School of Public Health Lipid Laboratory and U.S.D.A. publications.*

2.2.2 Lipid Transport

Lipids are insoluble in water but are soluble in alcohol and other solvents. When dietary fats are digested and absorbed into the small intestine, they eventually re-form into triglycerides, which are then packaged into lipoproteins (Birtcher *et al.*, 2000).

Dietary fats, including cholesterol, are absorbed from the small intestines and transported into the liver by lipoproteins called chylomicrons. Chylomicrons are large droplets of lipids with a thin shell of phospholipids, cholesterol, and protein. Once chylomicrons enter the bloodstream, an enzyme called lipoprotein lipase breaks down the triglycerides into fatty acid and glycerol. After a 12- to 14-hour fast, chylomicrons are absent from the bloodstream. Thus, individuals who are having a lipid profile done are advised to fast overnight to ensure that chylomicrons have been cleared (Borgman and Wardlaw, 1975).

The liver removes the chylomicron fragments, and the cholesterol is repackaged for transport in the blood in very low-density lipoproteins (VLDL-Cs), which eventually turn into low-density lipoproteins (LDL-C). LDL-C cholesterol (LDL-C-C) consists mainly of cholesterol. Most LDL-C particles are absorbed from the bloodstream by receptor cells in the liver (Fielding *et al.*, 1995). Cholesterol is then transported throughout the cells. Diets high in saturated fats and cholesterol decrease the uptake of LDL-C particles by the liver. LDL-C particles are also removed from the bloodstream by scavenger cells, or macrophages, which are white blood cells that bury themselves in blood vessels such as arteries. Scavenger cells prevent cholesterol from reentering the bloodstream, but they

deposit the cholesterol in the inner walls of blood vessels, eventually leading to the development of plaque (Havel and Hamilton, 2004).

2.2.2.1 Apolipoproteins

The protein moieties of lipoproteins are called apolipoproteins. Presently, 16 apolipoproteins have been isolated and characterized: apolipoprotein (Apo) A-I, apo A-II, Apo A-IV, apo(a), Apo B, Apo C-I, Apo C-II, Apo C-III, Apo C-IV, Apo D, Apo E, Apo F, Apo G, Apo H, Apo I, and Apo J (Alaupovic, 1996). The apolipoproteins solubilize highly hydrophobic lipids, permitting their transport in blood. Certain apolipoproteins function as ligands for specific receptors that facilitate the uptake and removal of lipoprotein particles or regulate the movement of particular lipids into and out of specific target cells and tissues (Murdoch and Breckenridge, 1995). Other apolipoproteins serve as cofactors for enzymes important in lipoprotein metabolism (Mowri *et al*., 1996). The following are the various types with their main (but not exclusive) lipoprotein associations, molecular weights and broad functions are listed in table 2.5.

Dietary triacylglycerol, cholesteryl ester, and phospholipids are emulsified in the intestine by bile salts, their fatty acids are hydrolyzed by pancreatic lipases, and the resultant molecules (fatty acids, 2 monoacylglycerols, and cholesterol) are taken up by intestinal cells. Triacylglycerol and cholesteryl ester are reformed in the intestinal cells and packaged into chylomicrons for transport in lymph and then blood (Alaupovic, 1996). Apo B-48, A-1, and A-IV are on the surface of chylomicrons. Apo B-48 is essential for secretion of chylomicrons from the intestine. Once in the circulation, Apo C-II, a cofactor for lipoprotein lipase (LPL)

is transferred from HDLs to chylomicrons, a process facilitated by Apo A-IV (Rader et al., 1993b).

Triacylglycerols in chylomicrons are hydrolyzed within minutes by LPL, which is located on the surface of endothelial cells lining the capillaries of adipose and other peripheral tissues, such as muscle. As the triacylglycerols are hydrolyzed, chylomicron remnants are produced, which are rapidly taken up by the liver through the interaction of Apo E on the surface of the chylomicron remnant with the chylomicron receptor. The released fatty acids are either taken up and stored in adipose tissue for future use or utilized by muscle for energy (Rader *et al*., 1993a).

Inside the liver, the cholesteryl esters in the chylomicron remnants are hydrolyzed and the liberated unesterified cholesterol down-regulates the genes for both the LDL-C receptor and the rate-limiting enzyme of cholesterol biosynthesis, hydroxymethylglutaryl-CoA (HMG-CoA) reductase. Thus, the potential effect of dietary cholesterol to increase LDL-C concentrations is offset by the inhibition of hepatic cholesterol synthesis (Young *et al*., 1987).

The triacylglycerol-rich VLDL-Cs are assembled and secreted as particles containing triacylglycerol and cholesteryl ester in the core, surrounded by Apo B-100, E, C-I, C-II, and C-III. The fatty acids attached to the glycerol moiety of the triacylglycerol may be derived from either the endogenous synthesis of fatty acids from acetyl CoA or the mobilization of fatty acid from adipose tissue back to the liver (Dang *et al.*, 1988). Acetyl CoA derived from amino acids, sugars, and fatty acid oxidation can be used to synthesize fatty acids.

Apo B-100 is essential for the secretion of VLDL-C. Once in the circulation, the triacylglycerol in VLDL-C is hydrolyzed by LPL and apo C-II, producing fatty acids and the VLDL-C remnant (Steinberg *et al.*, 1999). The triacylglycerol in the VLDL-C remnant can be further hydrolyzed to smaller particles called intermediate density lipoproteins (IDLs), which are either taken up by the interaction of apo E with the LDL-C (B,E) receptor on the surface of the liver or converted by the action of hepatic lipase (hepatic triacylglycerol lipase) into the cholesteryl ester–rich LDL-C. About two-thirds of LDL-C is removed by the liver and the rest by peripheral tissues. LDL-C also serves as the major carrier of vitamin E (Steinberg *et al.*, 1999).

2.2.2.2 Reverse cholesterol transport and the role of CETP

Cholesterol that is synthesized or deposited in peripheral tissues is returned to the liver in a process referred to as reverse cholesterol transport in which HDL plays a central role (Fielding and Fielding, 1995). The nascent HDL-C particles, consisting of phospholipid and apolipoprotein A-I, interacts with peripheral cells, such as macrophages, to facilitate the removal of excess free cholesterol, a process facilitated by the ATP-binding cassette protein 1 (ABC1) gene. HDL is then converted into mature cholesterol ester-rich HDL as a result of the plasma cholesterol-esterifying enzyme lecithin-cholesterol acyltransferase (LCAT), which is activated by ApoA-I (Weinstock *et al.*, 1995).

Cholesterol esters may be removed by several different pathways, including selective uptake by the liver i.e., the removal of lipid without the uptake of HDL

proteins. Selective uptake appears to be mediated by the scavenger receptor class-B, type I (SR-BI), which is expressed in the liver and has been shown to be a receptor for HDL (Stevenson *et al*., 1993). Cholesterol esters derived from HDL contributes to the hepaticcholesterol pool used for bile acid synthesis. Cholesterol is eventually excreted from the body either as bile acid or as free cholesterol in the bile (Tall, 1993).

2.2.2.3 The Role of lipoproteins in Atherogenesis

LDL-C

Research has shown that LDL-C particles play a vital role in the formation of plaques; increased levels are associated with atherosclerosis (Roe *et al*., 2008). Increasing evidence has revealed that the concentration and size of the LDL-C particles relate to the degree of atherosclerosis progression more than the concentration of cholesterol contained within all the LDL-C particles (Weber and Noels, 2011). The healthiest pattern, though relatively rare, is to have small numbers of large LDL-C particles and no small particles. High concentrations of small LDL-C particles (even though potentially carrying the same total cholesterol content as a low concentration of large particles) correlates with much faster growth of atheroma, progression of atherosclerosis and earlier and more severe cardiovascular disease events and death (Superko *et al.*, 2002).

Modification of LDL-C particles within the blood vessel walls (oxidized by free radicals) has been found to play a major role in atherogenesis (Sacks and Campos,

2003). It is therefore postulated that ingesting antioxidants and minimizing free radical exposure may reduce LDL-C's contribution to antioxidants, though results are not conclusive over time, with more clinical research, LDL-C reduction has been the most effective strategy for reducing free radical death rates in large double blind, randomized clinical trials; far more effective than coronary angioplasty/stenting or bypass surgery (Tornvall *et al.,* 1991).

The Heart Protection Study demonstrated that LDL-C cholesterol reduction to levels as low as 1.7mmol/l was associated with significant clinical benefit in a wide range of high-risk individuals, irrespective of baseline cholesterol levels, with no apparent threshold level for LDL-C cholesterol with respect to cardiovascular risk. The Heart Protection Study also demonstrated that the benefits of LDL-C cholesterol reduction extend into peripheral vascular disease and cerebrovascular disease prevention (Heart Protection Study Collaborative Group., 2002).

HDL-C

The development of human atherosclerosis is inversely related to the concentration of the high density lipoproteins (HDL). High and low concentrations of plasma HDL are associated with decreased and increased risk of developing premature atherosclerotic cardiovascular disease, respectively. It has been proposed that a 1% increase in the concentration of HDL would lead to a 3% reduction in risk of developing clinical atherosclerosis in man (Eriksson *et al.*, 1999). There is increasing epidemiological evidence to show that high levels of

HDL are protective against coronary heart disease (CHD). A low serum HDL cholesterol concentration (less than 35 mg/dl) is associated with a significant increase in coronary risk in both men and women (Garg *et al*., 1991).

According to Wolf (1996) earlier work with human subjects showed that a low-fat, low-cholesterol diet lowered plasma high-density-lipoprotein-cholesterol (HDL-C) and the production rate of apolipoprotein A-I (Apo A-I). More recent research with transgenic mice demonstrated that a high-fat, high-cholesterol diet raised plasma HDL-C and the production rate of Apo A-I by a mechanism involving the regulation of translation of the Apo A-I mRNA (Wolf, 1996).

In attempt to study the effect of a high-fat, high-cholesterol diet raising plasma HDL cholesterol, Wolf (1996) concluded that the rise induced in HDL-C by a high-fat, high-cholesterol diet is defensive and therefore, should not be interpreted as a desirable dietary change. Garg (1991) explained that, the changes in plasma HDL in response to alterations in dietary intake of fat have been largely attributed to changes in fractional clearance of HDL. The reduced cholesterol content of HDL accelerates its clearance. The lack of compensatory increase in HDL production in the face of increasing HDL clearance causes decreased plasma levels of HDL. In contrast, the increased HDL clearance in subjects with familial hypercholesterolemia is associated with compensatory increase in HDL production. Thus, it is possible that low-fat diets may also interfere with HDL production (Eriksson *et al.*, 1999).

2.2.3 Effect of Fatty Acids on Blood Cholesterol Levels

Fatty acids modulate lipid metabolism and other physiological systems that affect risk factors for chronic diseases. Whether these effects on health outcomes are beneficial or harmful depend on the specific fatty acids and the mix of fatty acids in the diet and the body. Individual fatty acids are present in foods as mixtures. Different foods are rich sources of specific fatty acids (Clarke, 2004).

Research on dietary fats by Hegsted and others has shown that myristic acid (C14:0), and palmitic acid (C16:0) increase cholesterol levels, whereas polyunsaturated fats such as linoleic acid (C18:2) reduce cholesterol levels (Zamora and Hidalgo, 1995). High blood serum cholesterol levels are associated with increased risk of cardiovascular diseases (Hegsted *et al.*, 1993).

It has been found that, blood cholesterol levels can be lowered by reducing the sources of dietary cholesterol, increasing the amount of fiber in the diet, and by consuming oils high in polyunsaturated fatty acids while reducing the intake of saturated fats. Animal fats and tropical oils which are high in myristic acid and low in linoleic acid increase cholesterol levels (Hegsted *et al.*, 1965).

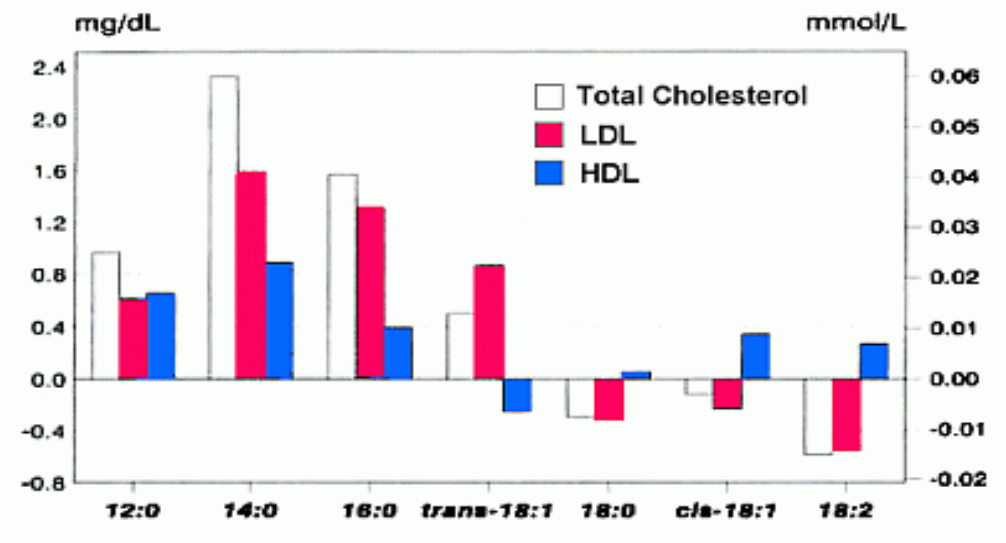

Figure 2.7: Effect of Fatty Acid Type on Blood Cholesterol

The chart above shows the effects of individual dietary fatty acids on Total Serum Cholesterol, LDL-C cholesterol and HDL cholesterol when l% of the energy from carbohydrates in the diet is replaced by 1% of energy of the specific fatty acids. The chart shows cholesterol increases from lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0) which are found in coconut oil, palm oil, and butter. Elaidic acid (trans-C18:1), which is present in hydrogenated fats, is the worst because it increases LDL-C and decreases HDL. The saturated fatty acid stearic acid (C18:0), the monounsaturated oleic acid (C18:1), and the polyunsaturated linoleic acid (C18:2) decrease LDL-C and increase HDL to various degrees (Katan et al., 1994).

2.2.3.1 Effect of Dietary Fat on Lipoproteins

Previous reports have shown that different class of dietary lipids may promote beneficial or detrimental health conditions (Adams *et al.*, 2008, Mukherjee *et al.*, 2009) by their capacity to alter blood lipid profile**.** A critical health issue related to dietary fat is the quality of fat in the diet. The composition of the diet

influences the concentration of cholesterol and lipoproteins in the blood of man and experimental animals. The consumption of certain fats, such as saturated fatty acids (SFA) and trans fatty acids, is associated with a poor lipid/lipoprotein profile and increased risk of CVD (Mensink and Katan, 1990). The plasma lipoprotein that is mostly found to affect CVD risk is elevated LDL-C-cholesterol concentrations (Mensink and Katan, 1992).

It has been established that it is the type of fat, rather than total fat intake that affects common intermediate risk factors, such as serum lipid and lipoprotein levels (Hu *et al.,* 2001) some studies in humans and animals have demonstrated that oils containing saturated fatty acids (SATS) raise serum total cholesterol (TC) and, in particular, low density lipoprotein cholesterol (LDL-C-C) levels (Keys and Grande, 1957, Keys *et al.*, 1957), while those enriched in unsaturated fatty acids) lower LDL-C-C when replacing saturated fat (Brousseau *et al.*, 1995).

When a relatively high level of a trans fatty acid, 11% of energy as elaidic acid (18:1 trans 9), was substituted for a cis fatty acid, oleic acid (18:1 cis 9), or a saturated fatty acid, stearic acid (18:0), total and low-density lipoprotein (LDL-C) cholesterol levels increased, whereas high-density lipoprotein (HDL) levels were comparable to levels obtained when the subjects consumed the oleic- or stearic acid–enriched diets whilst HDL levels were lower in subjects who consumed the elaidic acid–enriched diet (Mensink and Katan, 1990).

Isocaloric substitution of polyunsaturated fat for saturated fat reduces concentrations of total plasma cholesterol (Cortese *et al.,* 1983) and high density

lipoproteins (HDL) in nonhuman primates (Sorci-Thomas *et al.*, 1989, Rudel *et al.*, 1995b, Rudel *et al.*, 1995a). The biochemical mechanisms through which polyunsaturated fat lowers plasma HDL concentrations are not well understood but must involve changes in HDL production or HDL clearance from plasma, or both postulated that serum hypercholesterolemia accelerates atherogenesis by augmenting cholesterol accumulation in the arterial intima (Shepherd *et al.*, 1980b, Shepherd *et al*., 1980a).

The predictive equations of Keys et al. and Hegsted et al. in general have demonstrated that the fatty acid components and cholesterol in the diet are the primary determinants of diet induced hypo- or hypercholesterolemia (Kris-Etherton and Yu, 1997, Kris-Etherton *et al.*, 1997).

However, a review of several studies has also indicated a hypocholesterolemic effect of some unsaponifiables, in particular, plant sterol components. Several investigators have reported that not only can plant sterols significantly lower LDL-C-C levels, even at relatively low intakes (Lees *et al.*, 1977), but also that some plant sterols are more active than others.

In addition, cholesterol-lowering effects of other unsaponifiables such as tocotrienols, an analog of tocopherol found in rich concentrations in palm oil and oryzanol, a ferulic acid ester of phytosterols and tritrepene alcohols found in soybean oil, have been reported (Elson and Qureshi, 1995).

2.2.3.2 Effects of Saturated Fat on LDL-C and HDL Size and Composition

Smaller and denser LDL-C particles have been implicated as being more strongly involved in atherosclerotic CVD than larger LDL-C particles. The reduction in LDL-C cholesterol known to occur with a decreased saturated fat intake appears to be specific to larger more buoyant particles (Obarzanek *et al*., 2001). In persons placed on a baseline high-saturated-fat diet and then switched to a diet high in monounsaturated or polyunsaturated fat, a small but significant reduction in LDL-C particle size was observed (Dreon *et al.*, 1998).

Furthermore, It was recently showed that the lower concentrations of small, dense LDL-C particles resulting from a reduced carbohydrate intake (26% compared with 54% of energy) were similar with diets high (15%) or low (8%) in saturated fat derived primarily from dairy products. In contrast, the higher saturated fat intake raised concentrations of larger, more cholesterol-enriched LDL-C particles, thus offsetting the reduction in total LDL-C concentrations that was observed with lower saturated fat intake (Siri and Krauss, 2005).

2.2.3.3 Factors Affecting Variation in Lipoprotein Response to Saturated Fat

There is considerable inter individual variability in the lipoprotein response to variations in saturated fat intake, and this is related to some extent to variation in response to dietary cholesterol, which suggests a role for intrinsic differences in the regulation of lipid metabolism (Wiberg *et al.*, 1997). Baseline LDL-C-cholesterol concentrations appear to be strongly related to dietary responsiveness

and it was reported that this may be related to differences in the fractional catabolic rates of LDL-C (Katan *et al.*, 1988).

Other factors that have been reported to be associated with a reduced LDL-C response to reductions in saturated fat include decreased BMI, insulin resistance, and female sex (Lefevre *et al.*, 2005). A relation between triglyceride metabolism and the LDL-C response to diet is supported by the finding that saturated fats increase LDL-C cholesterol in normotriglyceridemic but not in hypertriglyceridemic persons (Rudel *et al.*, 1997, Weggemans *et al.*, 1999). Low birth weight has been associated with reduced HDL cholesterol in response to saturated fat in men (Robinson *et al.*, 2006).

Genetic factors may also contribute to variability in the dietary response to saturated fat (Krauss and Dreon, 1995). Among these, the apoE4 isoform, which is associated with increased plasma LDL-C cholesterol in comparison with the more common apoE3 isoform, has been most consistently found to be predictive of a greater LDL-C-cholesterol reduction in response to diet (Williams *et al.*, 1995, Dreon *et al*., 1995).

2.2.3.4 Saturated Fat and CVD Risk

Reduced intake of saturated fat and cholesterol is associated with reduced plasma cholesterol content of various lipoproteins including low-density lipoprotein (LDL-C), very low-density lipoprotein (VLDL-C), and HDL (Jain *et al.*, 1991).

More recently, some researchers showed positive associations between saturated fat in plasma phospholipids and CHD mortality (Clarke and Armitage, 2009). However, these fatty acids are not necessarily valid biomarkers for dietary saturated fat, because they can be endogenously synthesized (Sun *et al.*, 2007).

There is evidence from other studies (Cui *et al.*, 2007, Iso *et al.*, 2003) that saturated fat intake may be inversely related to ischemic and/or hemorrhagic stroke, but a meta-analysis including results from 6 other studies (Fehily *et al.*, 1993, Leosdottir *et al.*, 2005) did not yield a statistically significant risk reduction (Xu et al., 2006). Overall, despite the perception that reduced dietary saturated fat intake is beneficial for cardiovascular health, the evidence for a positive, independent association is lacking (Xu *et al.*, 2006). These conclusions are consistent with a recent review of the relation between dietary patterns and nutrient factors and CVD risk (Sauvaget *et al.*, 2004).

Presently, there is general acceptance that dietary SFA are positively associated with atherosclerosis due to increased concentration of blood cholesterol. However, the type of SFA should be distinguished when considering these atherogenic effects. Medium-chain SFA (8:0 to 10:0) have no effect, stearic acid (18:0), initially considered as being neutral, decreases plasma CHL level (Bonanome *et al*., 1992, Bonanome and Grundy, 1989) and only 12:0, 14:0 and 16:0 appear to elevate plasma CHL level (Bonanome and Grundy, 1988, Denke and Grundy, 1992).

2.2.3.5 Lipid Profile and CVD Risk

High TC and LDL-C-C have been correlated with the increased risk of atherosclerosis (Martin *et al.*, 1986). Triglyceride-rich lipoproteins of both intestinal and liver origin are considered to be atherogenic factors (Zilversmit, 1995). Hypertriglycerideia has been associated with an increased risk of coronary heart disease (Hokanson and Austin, 1996).The triglyceride (TG) level is one of several lipid parameters that can aid prediction of coronary heart disease (CHD) risk. An elevated plasma TG level is strongly associated with an increased risk of CHD. Raised TG levels can be present in individuals at risk for CHD when the total cholesterol is normal.

However, not all individuals with raised TG levels have increased risk of CHD (Charland *et al*., 2008). Factors such as: diet, age, lifestyle, and a range of medical conditions, drug therapy and metabolic disorders, can all affect the TG level. In some of these circumstances, other factors protect against the risk of CHD, and can minimise or negate the effect of the risk factors present. Although TG reducing therapy has been shown to be associated with an improved clinical outcome, more research is needed to determine whether this is an independent effect of TG reduction or an effect of normalising the overall lipid profile in hypertriglyceridemic patients (Cziraky *et al*., 2008).

In addition to the critical role that LDL-C-C plays, recent studies have shown the contribution of other lipid fractions, such as high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG), to overall cardiovascular health (Cziraky *et al.*,

2008). Also, a substantial body of evidence suggests that remnant lipoproteins IDL, and smaller VLDL-C, share with LDL-C the potential for promoting atherosclerosis. However, very large VLDL-C and chylomicrons do not seem to have this effect (Eriksson *et al*., 1999).

Although low-density lipoprotein cholesterol (LDL-C) remains the primary target for coronary heart disease (CHD) prevention in the latest guidelines of the National Cholesterol Education Program (USA), many individuals who have CHD do not have substantially elevated LDL-C but have derangement of other lipid fractions, most commonly low levels of high-density lipoprotein cholesterol (HDL-C) (Campbell *et al.*, 2007). In the guidelines, HDL-C is important in risk stratification in primary prevention, influencing the need for and intensity of treatment of LDL-C, and both HDL-C and triglyceride are defined as risk factors for the metabolic syndrome, a secondary target of therapy (Freiberg *et al.*, 2008).

Triglyceride level also determines in which individuals' non-HDL-C should be a secondary target of therapy. Risk assessment that takes into account the entire lipid profile will identify more high-risk individuals than evaluating LDL-C alone (Singh *et al.*, 2009). Some epidemiologic data suggest that instead of measuring the cholesterol in LDL-C or HDL, measuring their respective apolipoproteins, apolipoprotein (apo) B-100 and Apo A-I may improve CHD risk assessment, and in some observational and interventional studies, ratios of lipids and/or apolipoproteins have been better predictors of CHD risk than levels of any one lipid fraction (Singh *et al.*, 2009, Barter *et al.*, 2007).

Trials of lipid-modifying therapy also suggest that apolipoproteins and ratios may provide improved targets for therapy beyond LDL-C, but optimal values have not been established. Because lipid-modifying therapy affects multiple components of the lipid profile, the effect on all lipid parameters should be considered when selecting the most appropriate agent (Shahar *et al.*, 2003). Arsenault et al in a recent study discovered that independently of their plasma LDL-C levels, participants with high non-HDL-C levels, high TG levels, or with an elevated TC/HDL-C ratio were at increased CHD risk (Arsenault *et al.*, 2009).

2.2.3.6 Cholesterol Lowering and Atherosclerosis

Both cholesterol level and prevalence of coronary heart disease are influenced by environmental factors, including diet. It has been found that individuals who immigrate from countries where the prevalence of coronary heart disease and the serum cholesterol levels are low to a country with a high prevalence of coronary heart disease often have increases in both serum cholesterol levels and rates of coronary heart disease (Blankenhorn *et al.*, 1987). The evidence that decreasing serum cholesterol levels with cholesterol-lowering drugs or dietary modification slows or reverses the progression of coronary atherosclerosis and reduces coronary events comes from many randomized trials that include more than 40,000 subjects (Yusuf et al., 2009, Yusuf and Anand, 1996). Lowering the serum cholesterol level with diet or drug therapy also slows the progression of angiographically documented coronary atherosclerosis in patients with arterial bypass grafts (Blankenhorn *et al.*, 1987).

Modifying several risk factors, such as lowering the serum cholesterol level, the blood pressure, and the levels of LDL-C cholesterol and by cessation of smoking, reduces the risk of ischemic heart disease (Sacks *et al.*, 1996). Aggressive lowering of the serum cholesterol level in patients with recent MI results in a rapid decrease in the risk of subsequent ischemic cardiac complications, the need for surgical revascularization, and death rates.

This effect occurs even when the total cholesterol level falls within the upper range of normal (5.5–5.8 mmol/L, 213–310 mg/dl) (Pedersen, 1995). As high total cholesterol levels are considered to be a major independent risk factor for development of PVD and CAD, considerable attention has been directed toward evaluating the impact and mechanisms of cholesterol lowering therapies and interventions for cardiovascular outcomes (Selvin and Erlinger, 2004).

For adults, age 20 years or over, the most recent federal guidelines--from the National Cholesterol Education Program--recommend the following target levels:

Total cholesterol less than 200 milligrams per deciliter (mg/dl)

HDL cholesterol levels greater than 40 mg/dl

LDL-C cholesterol levels less than 100 mg/dl

Table 2.3: Main Sources of Dietary Fats and Their Effect on Cholesterol Levels.

Type of Fat	Main Source	State at Room Temperature	Effect on Cholesterol Levels
Mono-unsaturated	Olives; Olive Oil, canola oil, peanut oil; cashews, almonds, peanuts, and most other nuts; avocados.	Liquid	Lowers LDL-C; raises HDL
Poly-unsaturated	Corn, soybean, safflower, and cottonseed oils; fish	Liquid	Lowers LDL-C; raises HDL
Saturated	Whole milk, butter, cheese, and ice cream; red meat; chocolate; coconuts, coconut milk, and coconut oil.	Solid	Raises both LDL-C and HDL
Trans	Most margarines; vegetable shortening; partially hydrogenated vegetable oil; deep-fried chips; many fast foods; most commercial baked goods	Solid or semi-solid	Raises LDL-C

A display of the various types of fat, their main sources, state at room temperature and their effect on cholesterol levels.

2.3 Oxidation and Inflammation

2.3.1 Background

Atherosclerosis is known to have oxidative and inflammatory components playing major roles in its cause. Aside genetic factors, environmental factors with particular emphasis on nutrition appear to be responsible for these aberrant oxidative and inflammatory components and the lipid abnormalities associated with the disease (Osorio *et al.*, 2011, Ortega *et al.*, 2011).

Much of the progress in understanding atherosclerosis over the past years has depended on the lipid hypothesis with LDL-C cholesterol contributing importantly to atherosclerosis in many cases. However, it is important to note that most individuals with proven coronary artery disease have 'average' levels of cholesterol (absence of abnormalities in the lipoprotein profile). In many cases, LDL-C levels often remain in the average range, although the particles may have qualitative alterations that render them small and dense, making them particularly prone to oxidation and hence evoking inflammation (Han et al., 2002).

Thus, in addition to LDL-C, many putative non-traditional factors may aggravate atherogenesis by promoting inflammation. It is therefore imperative to identify individuals at risk for concerted intervention before problems manifest. Based on the evidence supporting a role for inflammation in the pathogenesis of atherosclerosis, serum markers of inflammation have garnered substantial interest as markers of atherosclerotic risk; these add to the information available from

traditional measures such as the lipid profile (Osorio *et al.*, 2011, Ortega *et al.*, 2011).

Therefore in this study, in addition to lipid profile, measurements of important markers of inflammation (TNF-α, IL-2 and 6) as well as the antioxidant capacity of the rats were determined to assess the atherosclerotic risk in the rats. The quality of dietary fat has been shown to affect immune responses (Han et al., 2002). Data from experiments in macrophage cell lines from animals (Siri-Tarino et al., 2010b) and humans have shown that saturated fats can induce the activation of inflammatory markers, including interleukin-6 and tumor necrosis factor-α (TNF-α) an effect thought to be mediated by the Toll-like receptor (Siri-Tarino *et al.*, 2010b, Siri-Tarino *et al.*, 2010a).

It is now clear that inflammatory mediators are intimately involved in atherogenic process (Libby, 2002b, Libby and Aikawa, 2002), thereby demonstrating atherosclerosis as an inflammatory disease (Burger-Kentischer *et al.*, 2006) . Excessive inflammation leads to pathological situation that stimulate leucocytes migration into the inflammatory site. Numerous mediators are involved in development of vascular atherosclerosis augmenting the inflammatory process, with leucocytes and ECs playing major cellular roles (Zakynthinos and Pappa, 2009). ECs has been shown to coordinate the recruitment of inflammatory leucocytes to sites of vascular injury that consequently result in production and release of inflammatory cytokines (Akira *et al.*, 1990a). Among these are the pro-inflammatory cytokines, tumour necrosis factor (TNF) and interleukins (1L)-1, 2 and 6 (Kunkel *et al.*, 1990).

2.3.1 Role of Antioxidants in the Development of Atherosclerosis

Lipid oxidation is initiated by reactive oxygen species (ROS) affecting polyunsaturated fatty acids, which comprise about 50% of the lipid moiety in LDL-C. The initiation reaction is followed by oxygen addition and a propagation reaction resulting in lipid radicals. Lipid radicals can react further or can be scavenged by antioxidants such as tocopherol. Alternatively they may react with other lipid radicals (termination reaction) (Esterbauer *et al.*, 1993, Esterbauer, 1993).

One LDL-C particle contains between 5 and 12 molecules of tocopherol, whereas concentrations of other antioxidants (eg. tocopherol, carotene, ubiquinone-10, and lycopene) are less than 1 molecule per LDL-C particle. However, the concentration of these antioxidants in the lipid moiety of LDL-C poorly reflects their antioxidant potential with respect to the oxidation of LDL-C in vitro (Jessup *et al*., 2004, Stocker and Keaney, 2004).

In contrast, water soluble antioxidants (e.g. vitamin C) cannot scavenge lipid radicals in the propagation reaction, but they are able to regenerate oxidized vitamin E and to preserve carotene levels during oxidative stress. Vitamin C is the major water-soluble antioxidant and is able to scavenge free radicals in the cytoplasm (Mason and Diamond, 2001). Vitamin E reduces oxidation of LDL-C by means of CuSO4 ex vivo and by means of ROS synthesized from different cells in vitro (Carr *et al.*, 2000b). In addition, vitamin E inhibits oxLDL-C-induced formation of thrombin in vitro (Rota *et al.*, 1998).

Impaired endothelial functions (eg, increased expression of cellular adhesion molecules, cytokines, and growth factors; variations in the synthesis of arachidonic acid metabolites and prostacycline [PGI2] and variations of synthesis and stability of endothelium- derived relaxing factor (Nitrous oxide, NO) play a central role in the development of atherosclerosis and are also in part induced by ROS (Fig. 2.21) (Drexler, 1999). Like other factors influencing the bioavailability of NO, ROS are able to inhibit the synthesis of NO or to inactivate the molecule after its synthesis and release (Dembinska-Kiec and Gryglewski, 1986).

However, the effect of ROS is very complex, and the bioavailability of NO in endothelium can be reduced by three different mechanisms (Landmesser *et al.*, 2004). In the first one, O_2 rapidly reacts with NO to form peroxynitrite anions (ONOO-), causing a diminished bioavailability of NO. Another mechanism is the redox-dependent inhibition of the enzyme dimethylarginine dimethylaminohydrolase (DDAH) by ROS. The inhibition of this enzyme is followed by an increased concentration of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of the endothelial NO synthase (eNOS), which in consequence is followed by a reduced synthesis of NO. In the third mechanism, tetrahydrobiopterin (H4B), which is an essential cofactor of eNOS, is subject to oxidative degradation by ROS (Landmesser *et al.*, 2004).

The resulting H4B deficiency causes an “uncoupling” of the enzyme, which is followed by a decrease of NO synthesis and an increase of O_2 production (Landmesser *et al.*, 2004). All three mechanisms reduce the bioavailability of NO which is followed by the development of endothelial dysfunction and

morphological changes of the vascular wall (Berliner *et al.*, 1995). Different antioxidants, especially vitamins C and E, may protect endothelial function from ROS (Chan, 1998).

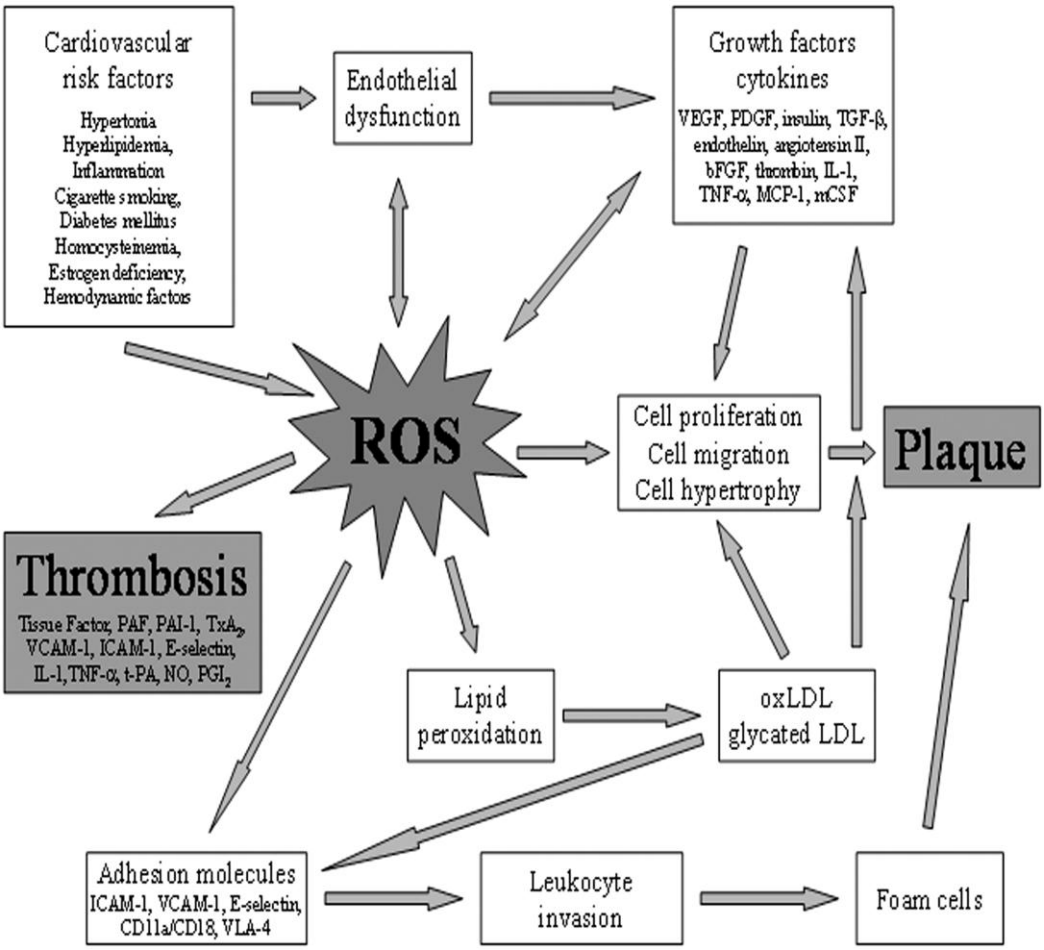

Figure 2.8: Role of ROS in the development of atherosclerosis.

Basic fibroblast growth factor (bFGF); ICAM-1 intercellular adhesion molecule 1; IL-1 interleukin 1; MCP-1 monocyte chemotactic protein 1; M-CSF monocyte colony-stimulating factor; NO nitrogen oxide; PAF platelet activating factor; PAI-1 plasminogen-activator-inhibitor 1; PDGF platelet-derived growth factor; PGI2 prostacycline; TGF-tissue growth factor beta; TNF-tumor necrosis factor-alpha; t-PA tissue-type plasminogen activator; TxA2 thromboxane A2;

VCAM-1 vascular cell adhesion molecule 1; VEGF vascular endothelial growth factor; VLA-4 very late antigen (Ruef et al., 1999).

2.3.2 Inflammation and Atherogenesis

Traditionally, plasma lipoprotein profiles and lipid accumulation in the blood vessel wall have been the major focus of research related to atherogenesis. More recently, a preponderance of evidence from clinical and experimental data suggests that inflammatory responses play a critical role (Willerson and Ridker, 2004).

2.3.2.1 Role of Endothelial Activation, Adhesion Molecules, and Chemokines

Despite the current appreciation that inflammatory cells are involved in atherosclerosis (Gimbrone *et al.*, 1990), the specific mechanisms whereby vascular inflammation is initiated remains elusive. In 1991, Cybulsky and Gimbrone made a seminal observation that early rabbit atherosclerosis was associated with induction of a leukocyte adhesion molecule on the endothelium that was homologous to human vascular cell adhesion molecule-1. It is known that the recruitment of inflammatory cells is dependent on cellular adhesion molecules (Cybulsky and Gimbrone, 1991).

Adhesion molecules are a diverse group of surface proteins that are divided, among others, into selectins, integrins, and immunoglobulin superfamily members. Vascular inflammation begins with leukocyte rolling on the endothelium that is facilitated by endothelial expression of P-selectin and its interaction with leukocyte P-selectin ligand-1 (Bevilacqua and Nelson, 1993).

Firm leukocyte adhesion requires interaction between leukocyte B1- and B2-integrins and endothelial immunoglobulin superfamily members such as vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, respectively (Zimmerman *et al.*, 1992). Once firm adhesion is established, leukocytes may then transmigrate across the endothelium along a chemotactic gradient such as that produced by monocyte chemotactic protein-1 (Cybulsky and Gimbrone, 1991).

Cellular adhesion molecules are an important component in atherosclerosis and the response to vascular injury. Histological studies demonstrate increased endothelial expression of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 in developing and established atherosclerotic lesions (Li *et al.*, 1993b, Li *et al.*, 1993a). Early atherosclerotic events and the initiation of lesion formation appear particularly dependent on vascular cell adhesion molecule-1 (Hwang *et al.*, 1997). Human studies have demonstrated that plasma levels of intercellular adhesion molecule-1 and E-selectin correlate with the clinical manifestations of coronary atherosclerosis (Ridker, 1998). Thus adhesion molecules modulate the biologic response to vascular injury, with atherosclerosis and plaque activation representing two prominent examples of vascular injury.

2.3.2.2 Role of Macrophage Inflammation in the Developing Plaque

Macrophage scavenger receptor 1 (MSR1) and CD36 are two scavenger receptors involved in cholesterol influx (Moore and Freeman, 2006). Two macrophage membrane receptors involved in cholesterol efflux are ATP-transporter cassette A1 (ABCA1) and scavenger receptor B class 1 (SR-B1) (Baranova *et al.*, 2002). When macrophage cholesterol influx is greater than efflux, cholesterol homeostasis is disturbed and cholesteryl ester (CE) accumulates in cytoplasmic droplets. The resulting macrophage-derived foam cells secrete pro-inflammatory factors, which amplify the local inflammatory reaction and produce reactive oxygen species, which in turn modify lipoproteins (Baranova *et al.*, 2002).

2.3.2.3 Inflammatory Markers

Established risk factors for CHD do not fully account for all risk of disease or adequately explain the etiology of the disease process. Therefore, identifying novel risk factors and markers warrants continued investigation. Among the inflammatory markers for CHD risk that have received attention are cytokines and CRP (Tabas, 2005). Cytokines are grouped into several classes: interleukins (33 have been identified to date), tumor necrosis factors (TNF), interferons (IFN), colony stimulating factors (CSF), transforming growth factors (TGF), and chemokines. They are especially important for regulating inflammatory and immune responses and have crucial functions in controlling both innate and adaptive immunity (Willerson and Ridker, 2004).

The predominant actors in adaptive immunity, helper-T (Th) cells, have been categorized on the basis of the pattern of cytokines that they can secrete, resulting in either a cell-mediated immune response (Th1) associated with IL-2 and IFN-γ secretion, or a humoral immune response (Th2), associated with IL-4, IL-5, IL-10, and IL-13 secretion (Tedgui and Mallat, 2006).

Cytokines are categorized according to the structural homology of their receptors as class I or class II cytokines (Boulay *et al*., 2003). Most ILs, CSFs, and IFNs belong to one of these two classes of cytokines. Three other major cytokine families encompass the IL-1 family (including IL-1α, IL-1β, IL-1ra, and IL-18), TNF family, and TGF-β superfamily. IL-1 and TNF family members activate the nuclear factor-κB (NF-κB) and mitogen-activated protein (MAP) kinase signaling pathways, while TGF-β superfamily members activate signaling proteins of the Smad family (Tedgui and Mallat, 2006). Cytokines are often classified according to their pro- (TNF, IL-1, IL-12, IL-18, IFN-γ) or anti-inflammatory (IL-4, IL-10, IL-13, TGF-β) activities (Boulay *et al.*, 2003).

2.3.2.4 Proinflammatory Cytokines

Cytokines can affect endothelial permeability, the expression of adhesion molecules, scavenger receptors (SR), lipid metabolism, and proliferation and migration of smooth muscle cells (SMCs) and endothelial cells (ECs) (Canault et al., 2008). Macrophages, the first inflammatory cells to be associated with atherosclerosis, participate in lipid retention and vascular cell remodeling, and

express scavenger receptors (SRs), Toll-like receptors (TLRs), and other receptors for pathogen-associated molecular patterns (PAMPs). Engagement of these receptors results in release of proinflammatory cytokines IL-1, IL-6, IL-12, IL-15 etc (Tabas, 2005).

An increase in macrophage apoptosis in early lesions appears to cause the attenuation of atherogenesis, whereas impairment in macrophage apoptosis in the late stage may contribute to secondary necrosis, leading to increased proinflammatory responses and further apoptotic signals for SMCs, ECs, and leukocytes within the plaques (Tabas, 2005).

Under conditions of hyperlipidemia, macrophages produce TNF-α, IL-1, IL-6, IL-12, IL-15, and IL-18 but also the anti-inflammatory cytokines IL-10 and TGF-β. Proatherogenic mast cells generate IL-6 and IFN-γ that are crucial in the induction of mast cell–dependent acceleration of atherosclerosis (Canault *et al.*, 2008).
Among the inflammatory pathways the cytokines are central players. Plasma levels of cytokines and related proteins, such as CRP, have been investigated in cardiovascular patients (Zhang *et al.*, 2006). Consistent with these findings the generation of cytokine-deficient animals has provided direct evidence for a role of cytokines in atherosclerosis (Weber, 2008).

In vitro cell culture experiments further support the suggestion that cytokines and other innate mechanisms contribute to atherogenesis. Among the initiation pathways of atherogenesis are innate mechanisms, such as toll-like-receptors (TLRs), including the endotoxin receptor TLR4. On the other hand, innate

cytokines, such as IL-1 or TNF, or even autoimmune triggers may activate the cells. Cytokines potently activate multiple functions relevant to maintain or spoil homeostasis within the vessel wall (Loppnow *et al.*, 2008).

Key cytokines responsible for the coordination of both immune and inflammatory responses include tumor necrosis factors (TNF-α and TNF-β), interleukins (ILs), and interferons (IFN-α, -β, and -γ) (Gabay and Kushner, 1999). Serum TG levels are increased by multiple cytokines, including TNF, IL-1, IL-2, IL-6, leukemia inhibitory factor (LIF), ciliary neurotropic factor (CNTF), nerve growth factor (NGF), keratinocyte growth factor (KGF), platelet-activating factor (PAF), and parathyroid hormone-related protein (PTHrP) (Argiles and Lopez-Soriano, 1998, Nonogaki *et al.*, 1995a, Nonogaki *et al.*, 1995b).

The cytokine, tumor necrosis factor (TNF), has been shown to rapidly increase serum triglyceride levels (Feingold *et al.*, 1988, Krauss *et al.*, 1990). Studies have shown that TNF decreases the activity of lipoprotein lipase (LPL) in cultured fat cells and in the epididymal fat pad of rats treated in vivo. A decrease in adipose tissue LPL activity could produce hypertriglyceridemia by decreasing the clearance of triglyceride-rich lipoproteins (Semb and Olivecrona, 1987).

Recent studies indicate that TNF increases serum triglyceride levels in rodents primarily by stimulating hepatic lipid secretion. Firstly, TNF increases de novo hepatic lipogenesis before the increase in serum triglyceride levels (Feingold and Grunfeld, 1987, Grunfeld *et al.*, 1988). Secondly, the increase in serum triglycerides precedes the decrease in LPL activity in the epididymal fat pad. In

addition, little or no decrease is seen in LPL activity in several other sites of adipose tissue or muscle in vivo (Grunfeld *et al.*, 1988, Feingold and Moser, 1987).

In fact, LPL activity is increased in the post heparin plasma of TNF-treated animals. These observations indicate that TNF stimulation of hepatic triglyceride production is the major cause of the hyperlipidemia that follows TNF administration. Studies by others (Starnes *et al.*, 1988) have demonstrated a decreased deposition of dietary fat in adipose tissue in TNF-treated animals, suggesting that under some experimental conditions, the TNF inhibition of adipose tissue LPL may play a role in hypertriglyceridemia. The two major cytokines released by stimulated macrophages are TNF and interleukin-1 (IL-1), and there is extensive but not complete overlap in the immunological properties of TNF and IL-1 (Nathan and Sieff, 1987, Nathan, 1987).

2.3.2.5 Predictive Value of Inflammatory Markers

Interleukin-6 (IL-6), a circulating cytokine, has been identified as a marker of inflammation in coronary atherosclerotic plaques. Serum levels of IL-6 increase in response to acute MI, unstable angina, percutaneous coronary intervention, and late restenosis (Schieffer *et al*., 2004) IL-6 stimulates platelet aggregation and the expression of tissue factor, macrophage LDL-C receptors, CRP, and fibrinogen. IL-6 also regulates the expression of other inflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) (Ikeda *et al*., 2001). CRP, one of many human acute-phase reactants, is produced in the liver in response to

IL-6, IL-1, and TNF. It activates the classic complement cascade, mediates phagocytosis, regulates inflammation, and is a nonspecific but sensitive marker of infection and tissue inflammation (Mortensen, 2001).

Prospective studies have shown that an elevated level of CRP is associated with increased risk for cardiovascular events in apparently healthy persons (Ridker, 2001). Studies also support the prognostic value of CRP in patients with a prior history of cardiovascular disease (Ridker, 2001). CRP might directly promote vascular disease; alternatively, it may indicate the presence of a chronic infection associated with an increased risk for CHD or an already identified CHD risk factor such as smoking, obesity, or preexisting atherosclerosis (Mortensen, 2001). Because of their interrelationship, CRP could simply be a surrogate marker for IL-6 or some other factor (Ridker, 1997).

IL-6 also promotes the synthesis of acute-phase reactants and monocyte chemoattractant protein-1 (MCP-1) (Fiotti *et al.*, 1999). MCP-1 and its receptor, CC chemokine receptor 2 (CCR2), direct the migration of monocytes into the intima. Subsequent exposure of the monocytes to macrophage colony-stimulating factor promotes their differentiation to macrophages (Willerson and Ridker, 2004). Macrophages avidly take up modified apolipoprotein (apo) B-containing lipoproteins, resulting in the formation of macrophage-derived foam cells (Haddy *et al.*, 2003).

These cells secrete pro-inflammatory factors that amplify the local inflammatory response and produce reactive oxygen species, which modify lipoproteins

resulting in increased uptake by scavenger receptors. Overexpression of MCP-1 is positively associated with accumulation of monocytes in fatty streaks (Vita *et al.*, 2004). The inflammatory process plays a major role in the development of atherosclerotic plaque (Paoletti *et al.*, 2004). IL-6, MCP-1 and TNF-α are important inflammatory biomarkers for atherosclerosis (Brueckmann *et al.*, 2004).

2.4 Interplay between Lipids, Inflammation and Oxidation in Atherogenesis

2.4.1 Background

The inflammatory response is a series of local cellular and vascular responses which are triggered when the body is injured, or invaded by pathogens with the attempt to remove the injurious stimuli and to initiate the healing process (Grimble, 1996). Inflammatory and immune processes are mediated and controlled by a diverse range of molecules.

Among these molecules are: proteins; the pro-inflammatory cytokines, tumour necrosis factor (TNF) and interleukins (1L)-1 and -6; derivatives from membrane phospholipids; the eicosanoids (prostaglandins (PG), leukotrienes (LT)); diacylglycerol and ceramide; miscellaneous compounds such as inositol phosphates and reactive oxygen species (Akira *et al.*, 1990b).

The pro-inflammatory cytokines are predominantly products of the immune system; Biologically, TNF acts as a trigger which activates a cascade of cytokine production. The molecule is released rapidly in response to inflammatory and

infective agents, and induces production of a large number of other cytokines, including IL-1 and IL-6 (Akira *et al.*, 1990a).

2.4.2 Dietary lipids and the inflammatory response

In theory, fats may influence inflammation by altering the production of cytokines and other inflammatory mediators, or by changing the sensitivity of target tissues to inflammatory mediators, or by acting at both levels. Cholesterol may exert a pro-inflammatory effect by enhancing cytokine production. Cholesterol may also exert a more generalized pro-inflammatory effect (Hughes *et al.*, 1992). In summary, it seems that the intensity of the multiplicity of metabolic changes, which are part of the inflammatory process, is influenced by the unsaturated fatty acid and cholesterol content of the diet. While n-6 PUFA and cholesterol exert a pro inflammatory influence, n-3 PUFA and monounsaturated fatty acids exert the opposite effect (Hughes *et al.*, 1992).

Recent studies are now demonstrating that IL-6 and TNF alpha are stronger predictors of cardiovascular disease than C-reactive protein and other inflammatory markers (Grimble, 1998a, Grimble, 1998b). Tappia and Grimble (1994) examined the ability of TNF to induce IL- 1 and IL-6 production by peritoneal macrophages from rats fed for 4 and 8 weeks on a range of fats representing the wide range of types encountered in human diets. The fats studied were maize, olive, coconut and fish oils and butter; chow-fed animals were included in the studies.

Complex modulation occurred, after 4 weeks fish and olive oils suppressed IL-1 production (relative to chow-fed animals). However, after 8 weeks, while fish and coconut oils suppressed IL-1 production, olive oil and maize oil enhanced production. After 4 weeks IL-6 production was enhanced by fish and olive oils, and after 8 weeks all the fats except coconut oil had resulted in enhanced production. However, despite the complexity of these effects, after 8 weeks of receiving the diets, there was a positive relationship between production of IL-1 and the n-6 PUFA intake of the animals and between IL-6 production and the total intake of unsaturated fatty acids (Tappia and Grimble, 1994).

2.4.2.1 Interleukin 6

The interleukin-6 (IL6) family comprises interleukin (IL) - 6, IL-11, leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor and cardiotrophin-1. Among its many functions, IL-6 plays an active role in inflammation, immunology, bone metabolism, reproduction, arthritis, neoplasia, and aging. IL-6 expression is regulated by a variety of factors, including steroidal hormones, at both the transcriptional and post-transcriptional levels (Zhao and Brinton, 2004). The Interleukin-6 family of cytokines, signaling through the common receptor subunit (glycoprotein) subsequently activates signal transducers and activators of transcription (STAT3), mitogen-activated proteinkinase (MAPK), and phosphatidylinositol 3-kinase (PI3K) (Zhao and Brinton, 2004). Elevated levels of IL-6 are associated with the highest risks for subclinical cardiovascular disease as well as for clinical cardiovascular disease in older men and women (Cesari *et al.*, 2003b).

2.4.2.2 TNF-α

It has been shown that circulating TNF-α levels are significantly increased in patients with premature coronary heart disease compared with age-matched healthy Controls (Jovinge *et al.*, 1998). In addition, Solheim *et al.* (2001) reported that hypercholesterolemic individuals treated with pravastatin, a cholesterol lowering agent, showed a significant reduction in the level of circulating TNF-α compared with placebo. It has been shown that TNF-α induces arteriosclerosis-like lesions in coronary arteries in vivo (Solheim *et al.*, 2001, Fukumoto *et al.*, 1997), and promotes in vitro vascular calcification by increasing osteoblastic differentiation of vascular cells (Fukumoto *et al.*, 1997).

According to Popa *et al*. (2007) TNF-α among inflammatory markers, seems to be a critical element in the pathogenesis of these conditions. Although lipid changes are beneficial to the host in the case of acute circulatory TNF-α-increasing conditions, prolonged TNF-α has been found to induce lipid modifications and increase cardiovascular risk and subsequent morbidity and mortality (Popa *et al.*, 2007, Ohta *et al.*, 2005). In this light, chronic inflammation in general and TNF-α in particular are likely to represent the driving force connecting atherosclerosis, and the impaired insulin sensitivity that may occur simultaneously in an individual (Hotamisligil *et al.*, 1993).

2.4.3 Influence of Lipids on Oxidation

In recent years some studies have emphasized the role of Mediterranean diet in prevention of some diseases including atherosclerosis, attributing the positive

influence of such diets to their low saturated and high monounsaturated fatty acids content (Spiller, 1991, Mattson and Jandacek, 1985). It has established that atherosclerotic plaque lipids are derived from plasma oxidized low-density lipoprotein cholesterol, thus prevention of atherosclerosis involves a fight against low-density lipoprotein (LDL-C) oxidation (Harris, 1992).

It is known that diets low in saturated fatty acids and high in monounsaturated fatty acids are effective in controlling blood lipid levels (Manson *et al*., 1993, Hennekens and Gaziano, 1993). For instance, when a typical diet high in saturated fat is replaced with a southern Mediterranean-type diet, plasma cholesterol levels were decreased.

Several studies have shown through laboratory animal Experimentation that, nutritional antioxidants and, especially phenolic substances, prevent lipid peroxidation (Frankel *et al.,* 1993). Some authors have also reported that antioxidant capacity of oils in vitro is directly connected to their phenolic content (Espin *et al.,* 2000). Some inverstigators observed that certain vegetable oils such as the olive oil, affect lipid peroxidation and antioxidant parameters, and lead to favorable changes in the plasma lipid status (Gustafsson *et al*., 1994, Scaccini *et al*., 1992, Visioli *et al*., 1995).

It was demonstrated that, .LDL-C-C oxidation was inhibited by olive oil constituents. Furthermore, it has been seen that, phenolics of olive oils inhibit platelet aggregation (Petroni *et al.*, 1995). For these reasons most investigations claim that olive oils are preferable to other vegetable oils (Gorinstein *et al*., 2002).

2.4.3.1 Dietary antioxidants

Several epidemiological studies have reported an inverse relationship between intake of foods rich in antioxidant vitamins including vitamins C and E and β-carotene, and risk for CVD (Gaziano, 1999). The protective effects of these antioxidant vitamins on atherosclerosis have been intensively investigated in animal and human studies. According to the oxidative modification hypothesis, oxidized LDL-C is immunogenic and atherogenic and LDL-C oxidation triggers atherosclerotic processes. Therefore, the protection of LDL-C from oxidation may be crucial to the prevention of atherosclerosis; the antioxidant components of LDL-C may prevent LDL-C oxidation (Bazzano *et al*., 2002).

Vitamin E is the generic term for all tocopherol and tocotrienol derivatives that exhibit the biological activity of α-tocopherol. There are eight naturally occurring isoforms synthesized in plants. α-Tocopherol is the most biologically and chemically active form of vitamin E. The hydroxyl groups at the C-6 position of tocopherols enable them to scavenge free radicals and superoxide (Munteanu *et al*., 2006). α-Tocopherol is the major antioxidant in LDL-C and one LDL-C particle contains approximately six molecules of α-tocopherol. α-Tocopherol in LDL-C plays a role in preventing LDL-C oxidation. Vitamin E depletion in LDL-C may trigger LDL-C oxidation; and the addition of micromolar concentrations of vitamin E inhibits LDL-C oxidation (Carr *et al*., 2000a).

All other antioxidants, such as γ-tocopherol, carotinoids, and ubiquinol-10, are present in much smaller amounts than α-tocopherol. In contrast to α-tocopherol,

carotenoids play only a minor or no role in LDL-C protection (Esterbauer *et al.*, 1992a, Esterbauer *et al.*, 1992c). However, many clinical studies have failed to demonstrate the protective effects of vitamin E. One explanation may be that vitamin E exhibits prooxidant activity in the absence of co-antioxidant compounds capable of reducing the tocopherol radical (Esterbauer *et al.*, 1992b). A similar situation may occur with other antioxidants, such as β-carotene (Zhang and Omaye, 2001). Depending on the concentrations, environmental conditions and presence of oxygen or other oxidants, compounds with antioxidant properties may exhibit prooxidant or other non-antioxidant properties (Brown *et al*., 1998).

2.4.4 Inflammation and Lipid metabolism

In several animal models of atherosclerosis, signs of inflammation occur hand-in-hand with incipient lipid accumulation in the artery wall. For example, blood leukocytes, mediators of host defenses and inflammation, localize in the earliest lesions of atherosclerosis, not only in experimental animals but in humans as well (Ross, 1979).

In a review by Libby *et al*. (2002) it was explained further that, though for many years lipids have been regarded as the sine qua non of atherosclerosis, over the last few decades, a plausible model linking lipids and inflammation to atherogenesis has emerged. According to the oxidation hypothesis, low-density lipoprotein (LDL) retained in the intima, in part by binding to proteoglycan, undergoes oxidative modification (Libby, 2002b, Bots *et al.*, 2007).

These modified lipids can induce the expression of adhesion molecules, chemokines, proinflammatory cytokines, and other mediators of inflammation in macrophages and vascular wall cells. The apoprotein moieties of the lipoprotein particles can also undergo modification in the artery wall, rendering them antigenic and capable of inciting T-cell responses, thus activating the antigen-specific adaptive limb of the immune response (Croce and Libby, 2007). In some experimental situations, administration of antioxidants can retard the progression of atherosclerotic lesions that develop in the face of hyperlipidemia.

However, even though attractive, theoretically compelling, and supported by a considerable body of experimental evidence, the relevance of the LDL oxidation hypothesis to human atherosclerosis remains unproven (Croce and Libby, 2007). Chemical analysis of the types of modified lipids and proteins extracted from human atheroma do not necessarily correspond to the compounds derived from lipoproteins oxidized in vitro that have furnished much of the evidence linking oxidized lipoproteins to inflammation.

Inflammation contributes across the spectrum of cardiovascular disease, including the earliest steps in atherogenesis. This recognition has had a profound impact on our understanding of atherothrombosis as more than a disease of lipid accumulation, but rather as a disorder characterized by low-grade vascular inflammation (Libby *et al.*, 2002). Practically, this concept can be used to predict future cardiovascular risk. To date, elevated levels of several inflammatory mediators among apparently healthy men and women have proven to have predictive value for future vascular events. In particular, prospective

epidemiological studies have found increased vascular risk in association with increased basal levels of cytokines such as IL-6 and TNF-α (Libby, 2006).

2.5 Characteristics of the Different Vegetable Fats and their Effect on Lipid Profile

2.5.1 Background

Approximately 75% of the world's production of oils and fats come from plant sources (Okpuzor *et al.*, 2009). Research has shown that different fatty acids occur at various levels or concentrations in different types of vegetable fats. Specific fatty acid types may affect vascular wall integrity: by affecting lipoprotein concentration, composition and degree of oxidation and consequently the process of atherosclerosis and its related cardiac complications (Penalva *et al.*, 2008).

2.5.2 Crude Palm oil

Palm oil is produced from the fruit of the Elaeis guineensis. It is a vegetable oil and therefore does not contain cholesterol. It has been used for thousands of years as a nutritious source of oil surpassing soybean oil as the most widely produced vegetable oil in the world. Palm oil is extracted from the fleshy mesocarp of palm fruit and the kernel or seed of the fruit generates Palm Kernel Oil (PKO). These two oils have different fatty acid compositions. The mesocarp comprises about 70 - 80% by weight of the fruit and about 45 -50% of this is oil. The mesocarp oil is

rich in palmitic acid (C 16.0) which may constitute up to 44% of the oil although it also contains oleic acid (C 18.1) about 39% and contains mainly long chain fatty acids (Pereira *et al.*, 1990a, Edem, 2002).

The extracted oil is known as crude palm oil (CPO). The rest of the fruit comprises the shell, kernel, moisture and other non fatty fiber. In the kernel, the oil consists mainly of lauric acid (C12.0.) about 50%, while myristic (C14.0) and oleic acids (C18:1) constitute about 16% each. The kernel contains a very high proportion of medium chain fatty acids. A physical barrier, the shell, separates these two tissues in the fruit (the mesocarp and the kernel) (Edem *et al*., 2002).

Crude palm oil is consumed refined at various stages of oxidation. It is an energy source for the body (Ladeia *et al*., 2008). Refined palm olein (REFPO) is the light-colored liquid fraction obtained from the refining, bleaching and deodorization of the crude palm oil. There are a few varieties of the palm tree including Tenera, which is a hybrid of the Dura and the Pisifera (Edem, 2002). Palm oil is used worldwide (more than 100 countries) for cooking and other food preparations. Palm oil is one of the most widely consumed vegetable oils in West Africa, Central Africa and South East Asia. It has been used in domestic cuisines and food processing industries. It is a bio-effective source of vitamin A. It is consumed as a cooking oil, in margarines and shortening, and as an ingredient in fat blends and a vast array of food products and is currently the number two edible oil in the world (Zeba *et al.*, 2006, Edem, 2002, Edem *et al.*, 2002).

2.5.2.1 Red Palm oil

The Red Palm oil (virgin Red Palm oil) is a minimally processed palm oil that naturally contains extremely rich source of Vitamin E and carotenoids (vitamin A) which gives the oil its red color and makes it a good source of provitamin A (Zeba *et al*., 2006).

Major Composition

Palm oil consists of 50% saturated and 50% unsaturated fatty acids. The saturated fatty acids are made up of 44% palmitic acid and 5% stearic acid. The unsaturated fatty acids consist of 39% oleic acid (monounsaturates) and 10% linoleic acid (polyunsaturates) as shown below (Oguntibeju *et al.*, 2009).

C12:0	Lauric	- 0.2%
C14:0	Myrstic	- 1.1%
C16:0	Palmitic	- 44.0%
C18:0	Stearic	- 4.5%
C18:1	Oleic	- 39.2%
C18:2	Linoleic	- 10.1%
Others		- 0.9%

Minor Component

These are classified into one category because they are fatty in nature but are not really oils. They are referred to as unsaponifiable matter and they include the following: antioxidants, carotineoids, vitamin e, phytonutrients, sterols, polar lipids, impurities (Bonnie and Choo, 1999).

Palm Kernel Oil (PKO) is composed vertually of esterified saturated fatty acid (approximately 80%) and there are contradictory research finding on the health implications as a result of the consumption of PKO and PO (Gustafsson *et al.*, 1994). The saturated fatty acids that have been shown through various epidemiological studies, to increase LDL-C cholesterol level are the C12 Lauric and C14 Myristic saturated fatty acid. These two saturated fatty acids are abundantly found in coconut oil and palm kernel oil (Tholstrup *et al.*, 1994, Vessby *et al.*, 1994).

Palmitic acid in palm oil is predominantly placed within the triglyceride molecule on the Sn1 and Sn3 positions. In contrast, the majority of palmitic acid from animal sources is placed in the Sn2 position. Consequently, research has shown that this unique structure of palmitic acid in palm oil influences the way it is metabolized, resulting in a neutral effect on cholesterol levels (Kritchevsky *et al.*, 2002, Kritchevsky, 2002). Palm Oil contains a very rich supply of powerful natural antioxidants. It also contains significant amounts of lycopene and coenzyme Q10. Coupled with this, it contains extremely low and only a moderate proportion of linolenic and linoleic acid respectively, the two components of oils

that are most readily oxidised (Kritchevsky and Sundram, 2002, Lietz *et al*., 2001).

Antioxidant Component of Red Palm oil

Vitamin E is a general term used for all tocopherols and their derivatives. There are eight different forms of Vitamin E found in nature. These include alpha, beta, gamma, and delta-tocopherols and alpha, beta, gamma and delta-tocotrienols. With the exception of beta tocopherol, Virgin Red Palm Oil contains all of the Vitamin E derivatives as shown below (Patel *et al*., 2006).

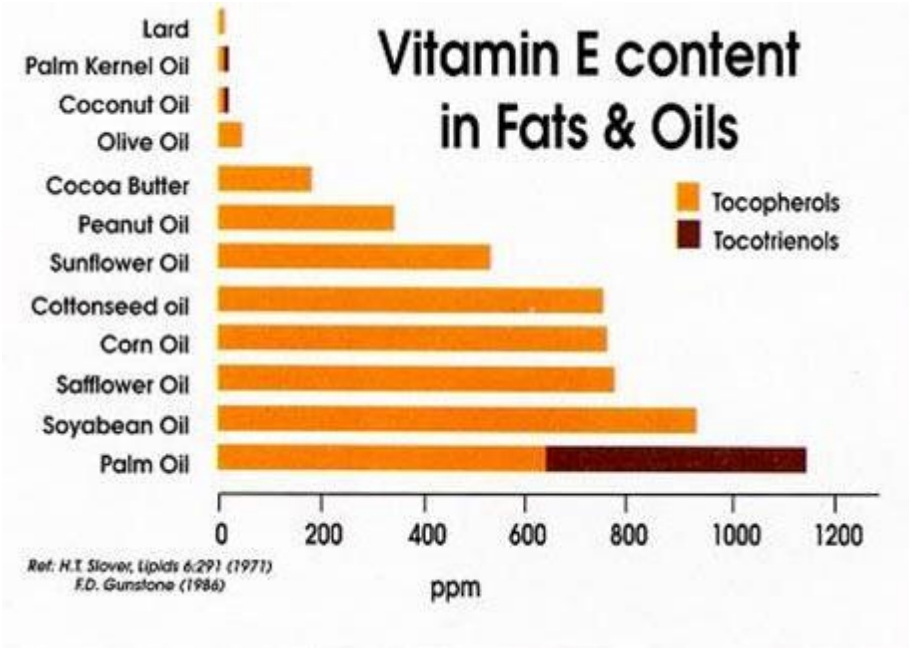

Figure 2.9: Vitamine E Content in Fats and Oils

Levels of tocopherol and tocotrienol of various oil types in ppm. The palm oil has been found to contain substantial amounts of both forms of vitamin E.

Vitamin A is produced in the body from beta carotene and other carotenoids; it can also be consumed in a preformed state known as retinol. Palm oil is considered the richest natural source of bioavailable carotenoids in terms of retinol (provitamin A) equivalent, (500 – 700 mg / L) in the crude oil (Rao and Sharma, 2001, Ong and Goh, 2002) with concentrations of 700-1000 ppm. A single tablespoon of Virgin Red Palm Oil contains 1,200 µg of Vitamin A in the form of provitamin A carotenoids. Red Palm oil, by virtue of its B-carotene content, may protect against vitamin A deficiency and certain forms of cancer (Edem, 2002).

It is generally believed that diets high in saturated fatty acids elevate serum cholesterol (Hegsted *et al.*, 1965) and promote thrombosis and hence atherogenesis, palm oil (PA) is considered to exert the untoward effects on these measures. The high saturated fat content in palm oil has been a major source of objection to it use (Cottrell, 1991). Palm oil has also been shown to increase cholesterol levels, and thus considered worse than animal fat, mainly due to the growing concern over the relationship between atherosclerosis and high cholesterol levels (Anderson *et al.*, 1976).

However, several years later other inverstigators, have indicated that palm oil is hypocholesteremic, increases protective High density lipoprotein (HDL), and does not cause atherogenesis, in spite of its low P/S ratio (0.31) and low linoleic acid content (10%) (Vles *et al.*, 1964, Abdellatif *et al.*, 1972). Animal experiments have also shown that Palm oil is similar to other oils in absorption, feed efficiency ratio (FER) and growth promotion. In humans, it not only decreases cholesterol,

(Rand et al., 1988), but also increases HDL and decreases apo B, relative to the control diets (Sundram *et al*., 1990a, Sundram *et al.*, 1992).

Subsequent studies by have unequivocally demonstrated that palm oil though low in linoleate content, is not hypercholesterolemic because of: (a) the equal distribution of saturated and unsaturated fatty acids in it, and (b) the high content of oleic acid (18:1, n-9) which has been proved to reduce cholesterol levels (Mattson and Jandacek, 1985, Grundy *et al.*, 2009, Grundy, 1994).

Effect of red palm oil on cholesterol

Vitamin E has been shown to help reduce LDL-C cholesterol and triglycerides, raise HDL cholesterol, reduce risk of heart attack, boost the immune system, fight cancer and lower the risk of developing cataracts (Walton *et al.,* 2002). Tocotrienols have also been found to significantly inhibit HMG-CoA reductase (the enzyme that Controls the rate at which cholesterol is synthesized), which ultimately results in lower cholesterol (Qureshi *et al.,* 1986). This effect has been observed in people after they ingested palm oil.

Moreover, it was reported in a recent review (Edem, 2002) that although palm oil-based diets induce a higher blood cholesterol level than do corn, soybean, safflower seed, and sunflower oils, the consumption of palm oil causes the endogenous cholesterol level to drop due to the presence of the tocotrienols and the peculiar isomeric position of its fatty acids.

Again, red or unrefined palm oil at moderate levels in the diet of experimental animals has been found to promote efficient utilization of nutrients, favorable body weight gains, induction of hepatic drug metabolizing enzymes, and adequate hemoglobinization of red cells and improvement of immune function (Edem, 2002).

Sundram *et al.,* (1992) performed a dietary intervention study on a free-living Dutch population which normally consumes diets high in fats. Using a double blind cross-over study design consisting of two periods of six weeks of feeding, the normal fat intake of a group of 40 male volunteers was replaced with 70% of palm oil. The palm oil diet did not raise serum total cholesterol and LDL-C-cholesterol, and caused a significant increase in the HDL-cholesterol and a significant reduction in LDL-C-triglycerides.

In another study the effect of palm olein and canola oil on plasma lipids was examined in double blind experiments in healthy Australian adults. Palm oil performed better than canola oil in raising the HDL-cholesterol (Truswell, 2000, Choudhury *et al.*, 1995).

Furthermore, studies conducted by researchers from China compared the effects of palm oil, soybean oil, peanut oil and lard (Zhang *et al.*, 2003, Zhang *et al.*, 1997). They showed that palm oil had the effect of decreasing total blood cholesterol and LDL-C-cholesterol and increasing the level of HDL-cholesterol. Soya bean oil and peanut oil had no effect on the blood cholesterol but lard increased the cholesterol levels. Another study conducted on healthy Indian

subjects (Ghafoorunissa *et al.*, 1995) showed that palm olein and groundnut oil have comparable effects. Both oils do not induce hypercholesterolemia.

Some studies have shown that palm oil does not promote arteriosclerosis and arterial thrombosis in spite of it high saturated fat content (Pereira *et al.*, 1990a). On the contrary, it has been found to improve cholesterol values. In a another study, researchers demonstrated a 10 percent decrease in total cholesterol in 36 hypercholesterolemic (high cholesterol) subjects given palm oil capsules for four weeks. A follow-up study of 16 subjects resulted in a 13 percent lowering of total cholesterol (Qureshi *et al.*, 1995).

Again, palm oil supplement was administed daily to 31 subjects for 30 days. The results showed that palm oil supplementation lowered both total cholesterol and LDL-C cholesterol in all the volunteers. The magnitude of reduction of total cholesterol ranged from 5 to 35.9 percent and the reduction of LDL-C cholesterol ranged from 0.9 to 37 percent. The cholesterol ratio was reduced in 78 percent of the subjects, demonstrating a highly significant and favorable response to supplementation (Tan *et al*., 1991).

Tocotrienols also strengthen the heart so that it can better withstand stress. Researchers can purposely induce heart attacks in lab animals by cutting off blood flow to the heart. This causes severe injury and death. However, if the animals are fed palm oil the survival rate is greatly increased, injury is minimized, and recovery quicker (Esterhuyse *et al.*, 2005b, Esterhuyse *et al.*, 2005a).

.

Eidangbe *et al.* (2010) compared the effect of palm oil (rich in saturated fatty acid) and egusi melon oil (rich in unsaturated fatty acids) on serum and liver lipids and their antioxidant status. Lipid profiles, Malondialdehyde (MDA) levels and activities of Glutathione Peroxidase (GSH-Px) and Glutathione Reductase (GSSG-Rx) were determined in serum and liver of rats fed a high cholesterol diet for 6 weeks. It was found that palm oil and egusi melon fed rats exhibited improved serum and liver lipid profiles and had a more significant reduction in serum and liver MDA levels as compared to Control rats (Eidangbe *et al.*, 2010). Similarly, GSH-Px and GSSG-Rx activities were significantly higher in rats fed palm oil and egusi melon oil compared to Control. It was concluded that palm oil and egusi melon improve serum and liver lipid profile in rats fed a high fat diet, their consumption could thus offer protection against lipid-related disorders (Elson, 1992).

Palm oil contains negligible amounts (less than 1.5%) of the hypercholesterolemic saturated fatty acids, namely lauric acid (12:0) and myristic acid (14:0). it has moderately rich amounts of the hypocholesterolaemic, monounsaturated oleic acid (18:1, omega-9) and adequate amounts of linoleic acid. (18:2, omega-6). The major saturated fatty acid, palmitic acid (16:0) has recently been shown to be neutral in its cholesterolaemic effect, particularly in situations where the LDL-C receptors have not been down-regulated by dietary means or through a genetic effect (Chong and Ng, 1991).

Moreover, it contains minor components such as the vitamin E tocotrienols which are not only powerful antioxidants but are also natural inhibitors of cholesterol

synthesis (Chong and Ng, 1991). Feeding experiments in various animal species and humans have proven that palm oil is not atherogenic. Palm oil consumption has been found to reduce blood cholesterol contrary to what is normally observed in the traditional sources of saturated fats such as coconut oil, dairy and animal fats. In addition, palm oil consumption has been found in some researches to raise HDL levels and reduce platelet aggregability (Chong and Ng, 1991).

Effect of palm oil on Atherogenesis and CVD

In a study, in which 1899 men with a diet rich in fats were surveyed, it was concluded that those with the highest levels of carotenes in their blood, had 36% less risk of contracting cardiovascular disease. Kritchevsky of the Wister Institute in Philadelphia (US) demonstrated that when consuming Red Palm oil the risk of atherosclerosis is lowest in comparison with refined, bleached and deodorized palm oil. The protection against atherosclerosis is a result of the high levels of antioxidants in Red Palm oil, specifically red carotenes and tocotrienols. The risk of atherosclerosis reduces as the levels of alpha-carotene intake increase (Kritchevsky *et al*., 2002).

Recent studies have shown that dietary red palm oil (RPO) supplementation improves functional recovery following ischaemia/reperfusion in isolated hearts. Bester *et al* (2010) investigated the effects of dietary RPO supplementation on myocardial infarct size after ischaemia/reperfusion injury. Dietary RPO-supplementation was found to be more effective than Sunflower oil-supplementation in reducing myoreperfusion injury (Bester *et al*., 2010).

2.5.2.2 Oxidized palm oil

Palm oil has been used in the fresh state and/or at various levels of oxidation. Oxidation is a result of processing the oil for various culinary purposes (Edem, 2002, Edem *et al.*, 2002). Studies have revealed that relative to fresh palm oil, oxidized palm oil induces an adverse plasma lipid profile, free fatty acids, phospholipids and cerebrosides (Ebong *et al.*, 1999).

Additionally, oxidized palm oil induces reproductive toxicity and organotoxicity particularly of the kidneys, lungs, liver and heart. Also, available evidence suggests that impact of the oxidized oil on health reflects in the generation of toxicants due to oxidation. The reduction of the dietary level of oxidized oil and/or the level of oxidation may reduce the health risk associated with consumption of oxidized fats (Ebong *et al.*, 1999).

2.5.2.3 Refined Palm oil (Palm Olein)

Palm oil is usually a semi-solid, in the tropics, but at colder temperatures it is a solid fat. The solid consistency makes the palm oil less acceptable as cooking oil in cold countries. The process of fractionation by cooling leads to the formation of high-melting triglycerides (palm stearin) leaving behind low-melting glycerides (palm olein). Palm olein and palm stearin also differ in fatty acid composition. Palm stearin is characterized by a low Polyunsaturated/Saturated ratio, and is more saturated than palm olein. Palm olein is therefore the liquid fraction

obtained by fractionation of palm oil after crystallization at controlled temperatures. The physical characteristics of palm olein differ from those of palm oil. It is fully liquid in warm climate and has a narrow range of glycerides (Kusum *et al.*, 2011).

In addition to finding uses as in the case of palm oil, palm olein is widely used as a cooking oil. It also blends perfectly with other popular vegetable oils that are traditionally used in many parts of the world. Like palm oil, palm olein is also widely used as a frying oil and much of its popularity is due to its good resistance to oxidation and formation of breakdown products at frying temperatures and longer shelf life of finished products. In fact, palm olein is considered as the gold standard in frying and is perhaps, on its own, the most widely used frying oil worldwide (Kusum *et al.*, 2011).

Refined palm oil may be further refined through treatment with phosphoric acid, followed by de-acidification with alkali, bleaching and deodorization. Melting and degumming removes impurities. The oil is then filtered and bleached. Such refined palm oil is called chemically refined palm oil (CRPO). Next, physical refining removes smells and coloration, to produce "refined bleached deodorized palm oil", or RBDPO (Faessler, 2007). The CRPO has a lower free fatty acid content than the physically refined palm oil (PRPO).

The quantity of palm oil refined by these two methods is commonly judged by chemical and physical criteria (Williams and Padley, 1985, Miyazawa *et al.*, 1994). CRPO does not seem to be nutritionally superior to the physically refined

palm oil, as both these oils produce similar rates of growth in rats. However, chemical refining does not reduce the free fatty acids to lower levels than physical refining (Duff, 1991).

2.5.3 Olive Oil

Olive oil comes in different grades including extra-virgin, fine virgin or virgin and refined. Extra Virgin is the unrefined oil derived from the first pressing of the olives and has the strongest flavor. The extra virgin oils are called cold pressed as they are produced by mechanically squeezing the oil under pressure. The flavor of extra virgin olive oil varies depending on the place where it has been grown and the type of olives. Virgin olive oil is refined oil that is light in color, odor and taste whilst the refined olive oil is mild in flavor. Extra virgin olive oil is a richer source of polyphenols than refined olive or other refined oils and is believed to be the best vegetable oil for heart health (Covas *et al*., 2006).

Virgin also has a higher acidity level than extra virgin olive oil (as well as less phytonutrients and a less delicate taste). Chemically, the difference between extra virgin olive oil and virgin olive oil involves the amount of free oleic acid, which is a marker for overall acidity. According to the standards adopted by the International Olive Oil Council, "virgin" can contain up to 2% free oleic acid, while "extra virgin" can contain up to 0.8% of free oleic acid (Beauchamp *et al.*, 2005).

Among the major components of extra virgin olive oil are antioxidants. Olive oil provides beta carotene (pro-vitamin A) and tocopherol (vitamin E). Extra virgin olive oil contains 88% of its vitamin E in the form of alpha-tocopherol. Olive oil is also rich in vitamins A, D, E and K. Olive oil is one of the few widely used culinary oils that contains about 75% of its fat in the form of oleic acid (a monounsaturated, omega-9 fatty acid). Research has long been clear about the benefits of oleic acid for proper balance of total cholesterol, LDL cholesterol, and HDL cholesterol in the body. Oleic acid is known to be a prime component of the Mediterranean Diet. Olive oil is a natural juice which preserves the taste, aroma, vitamins and properties of the olive fruit. It is the only vegetable oil that can be consumed as it is - freshly pressed from the fruit (Bermudez *et al*., 2011).

The health effects of olive oil are due to both its high content of monounsaturated fatty acid and its high content of antioxidative substances (Coni *et al.*, 2000): other recent studies (Valavanidis et al., 2004, Masella *et al.*, 2001) confirmed that such heart-healthy effects from olive oil are due not only to its high content of monounsaturated fats, but also to its hefty concentration of antioxidants, including chlorophyll, carotenoids and the polyphenolic compounds tyrosol, hydrotyrosol and oleuropein all of which not only have free radical scavenging abilities, but protect the vitamin E (alpha-tocopherol) also found in olive oil.

Olive oil has been shown to have beneficial effects on various aspect of body function, development and maintenance, including brain development, bone structure, digestion, aging process, the condition of skin and hair, metabolism, and on plaque formation in the blood vessels. Studies have shown that olive oil offers

protection against heart disease by Controlling LDL-C cholesterol levels while raising HDL levels (WHO, 1990).

In another study, it was shown that people who consumed 25 milliliters(ml) about 2 tablespoons - of virgin olive oil daily for 1 week showed less oxidation of LDL-C cholesterol and higher levels of antioxidant compounds, particularly phenols, in the blood. It does not disturb the critical omega 6 to omega 3 ratio and most of the fatty acids are actually omega-9 oil which is monounsaturated (http://www.explorecrete.com/nature/olive-oil-health-benefits.html).

2.5.3.1 Effect of Olive Oil on Atherosclerosis

Oleic acid, predominant fatty acid in olive oil and the most common monounsaturated fatty acid found in the diet, is more resistant to free radical or oxidative damage and reduces inflammation (Moreno, 2003). Numerous studies indicate that monounsaturated fat is about as effective as polyunsaturated fat in lowering total blood cholesterol and LDL-C cholesterol, when substituted for saturated fat in the diet.

Moreover, monounsaturated fat does not lower beneficial HDL cholesterol or raise triglycerides, unlike polyunsaturated fat, which, at high intakes, may lower HDL cholesterol (Aguilera *et al.*, 2001). Shad in 2002 compared the effects of PUFA fat (corn oil) and MUFA fat (olive oil) on serum lipid profile. It was seen that both PUFA diet and MUFA diet decreased all the serum lipid fractions; PUFA diet was rather more potent in this regard. The only difference was in

HDL-C fraction. HDL-C was significantly decreased in case of PUFA diet while it was significantly increased in case of MUFA diet. So MUFA diet is more advantageous for patients with atherosclerotic vascular diseases.

A comparative study in young Australian adults showed that the total blood cholesterol, triglycerides and HDL-cholesterol levels of those fed on palm oil (palm olein) and olive oil were lower than those fed on the usual Australian diet (Choudhury *et al.*, 1995). They showed that young Australian adults fed on palm oil diets had the same total blood cholesterol, triglycerides and HDL-cholesterol levels as those fed on olive oil. Also a cross-over feeding study showed that the blood cholesterol, triglycerides, HDL-cholesterol and LDL-C-cholesterol levels of palm olein and olive oil diets were comparable (Ng *et al.*, 1992).

Studies on olive oil and atherosclerosis reveal that particles of LDL-C cholesterol that contain the monounsaturated fats of olive oil are less likely to become oxidized. A recent in vitro study also showed that polyphenolic compounds present in olive oil, including oleuropein, inhibit the adhesion of monocyte cells to the blood vessel lining, a process that is involved in the development of atherosclerosis (Covas *et al*., 2006).

In addition, when people with high cholesterol levels removed the saturated fat from their diets and replaced it with olive oil, their total cholesterol levels dropped on the average of 13.4%, and their LDL-C cholesterol levels dropped by 18%. However, these benefits occurred only when olive oil was used in place of other

fats, rather than simply adding olive oil to a diet high in unhealthy fats (Covas, 2007b).

It was found in another study (Marrugat *et al*., 2004) that the phenols in olive oil have very potent antioxidant effects. The protective effects exerted by extra virgin olive oil biophenols, namely, protocatechuic acid and oleuropein, against LDL-C oxidation included: completely preventing LDL-C's oxidation when placed in a medium containing macrophage-like cells; inhibiting the production of two powerful oxidants that would normally have been produced and would have damaged LDL-C, thus preventing the expected decrease in glutathione, a powerful antioxidant the body produces to disarm oxidants, restoring to normal levels the protective activities of two free radical-disarming enzymes that contain glutathione, glutathione reductase and glutathione peroxidase, inducing higher than normal production and activity of both of these glutathione-containing enzymes.

A review of the research by Covas strongly suggests that diets in which olive oil is the main source of fat can be a useful tool against a wide variety of risk factors for cardiovascular disease (Covas, 2007b). Compared to diets high in saturated fat and low fat, high carbohydrate diets, a number of studies have shown that olive oil-rich diets not only reduce LDL-C cholesterol levels, but also lower blood sugar levels and decrease insulin requirements in persons with type 2 diabetes.

Some investigators discovered that diet rich in olive oil prevented drop in adiponectin. Adiponectin, a hormone produced and secreted by fat cells, regulates

sugar and fat metabolism, improves insulin sensitivity, and has anti-inflammatory effects on the cells lining the blood vessel walls. Low blood levels of adiponectin are a marker for metabolic syndrome, are common in obesity, and are also associated with increased heart attack risk (Paniagua *et al.*, 2007).

El Sewiedy and co examined the effects of unsaturated fatty acid levels on the oxidative susceptibility of lipoprotein, chemokine expressions and their relationship to atherosclerotic lesion development in experimental rats. Male Wistar rats were fed an atherogenic diet for 4 months and the diet was then supplemented with 10% v/w of virgin olive oil group (OO), sunflower oil group (SO) or fish oil group (FO) for 4 and 8 weeks.

Dietary treatment of atherosclerotic rats with OO greatly reduced lipoprotein oxidative susceptibility (LOS) and remarkably increased aortic superoxide dismutase and reduced glutathione contents as compared to the (SO)- and (FO)-treated groups. The FO-supplemented diet had a more pronounced lowering effect on monocyte chemoattractant protein (MCP-1) compared to the OO and SO diets. This study demonstrated a strong relationship between LOS and circulating levels of chemokines. OO was found to be a potent antioxidant and moderate anti-inflammatory, which effectively reduced aortic atherosclerotic lesions more than the SO- or FO (El Seweidy *et al.*, 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Background

This section comprises the study procedures adopted and the various methods employed in conducting this study.

3.2 Experimental Design

Fifty-six Spangue-Dawley (SD) male rats were studied. Animals were obtained from the Department of Animal Experimentation at the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana. They were weight- matched and divided into 4 groups of 14 animals each, comprising 3 Treatment groups and a Control group (C). They were marked, coded and housed in groups (randomly grouped into 4 or 5 per cage depending on the size of the cage) in a controlled environment (ambient temperature of 24-25°, relative humidity of 55-60%, and a 12-hour light/dark cycle).

The animals were given humane care. Ethics clearance was obtained from the Ethics Review Committee of the University of Ghana Medical School, Korle-Bu, with ethics number MS-Et/M.6.1 - P.1/2006-07. The study was conducted over a four-month period, with seven rats from each group ($\sum n=28$) being sacrificed

every two months (i.e., at 2 months and 4 months) to obtain sufficient blood samples for biochemical analysis (Figure 3.1)**.** The animals were weighed weekly for the four- month period.

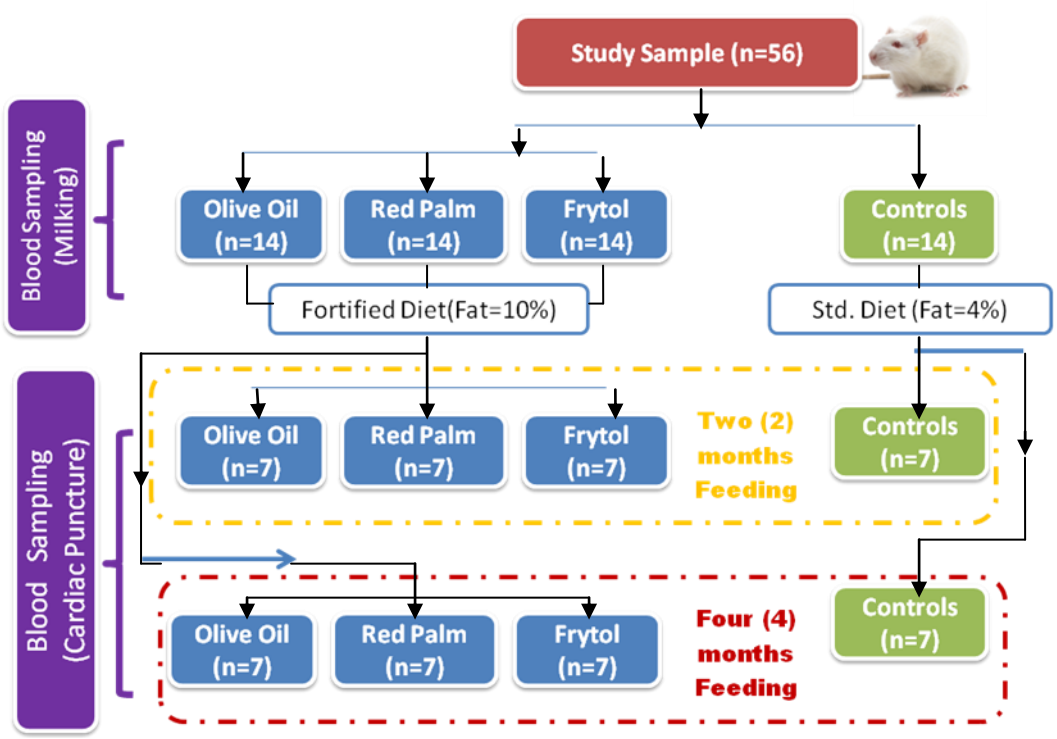

Figure 3.1: Study Design

A total of 56 rats were divide into two main groups: a control group of 14 animals and a treatment group of42 animals which sub divided into 3 groups (representing the oil types) consisting of 14 animals each. The milking method was used for baseline blood sampling of the rats. After feeding commenced, 7 rats were selected from each group and blood samples collected by cardiac puncture every two months for biochemical analysis.

3.2.1 Diet

The animals were provided with feed and water ad libitum. The feed was obtained from the Ghana Agro Food Company (GAFCO) and analyzed at the Center for

Scientific and Industrial Research (CSIR), Accra, to ensure that the composition was up to standard specifications (Table 3.1). The animals were acclimated to their respective cages and groups for one week before the diet treatment. At the end of the first week, the rats were placed on the experimental diet for 16 weeks.

Feeding was carried out with the different dietary formulations: the Control group (C) received the standard diet (~4% fat) while the 3 treatment groups received a fortified diet enriched with additional 6% fat: olive oil, red palm oil and frytol cooking oil respectively (Table 3.2). The choice of the 6% fat was in order to achieve a total of 10% fat in the treatment diets, with the aim of obtaining approximately a double of the recommended amount of fat in rat diet (4-5%) (5001* Laboratory Rodent Diet, 2005 Appendix-A). This is to avoid high fortification which normally affects the palatability texture and intake of the feed by the rats.

Also considering the relatively long duration (16 weeks) of study compared to others 28 days (Edem, 2009), 4-12 weeks (Kamisah *et al.*, 2005), a lower level of fortification was required**.** The diets were prepared according to the recommendations of the International Laboratory Rodent Diet (5001* Laboratory Rodent Diet, 2005 Appendix-A).

Table 3.1: Proximate Analysis of Feed

Parameter	Method				Units	Results
Moisture	AOAC Editions	925.10	(1990)	15th	g/100g	10.8
Ash	AOAC Editions	923.03	(2000)	15th	g/100g	7.6
Fat	AOAC Editions	920.39	(2000)	15th	g/100g	4.2
Protein	AOAC Editions	984.13	(1990)	15th	g/100g	23.8
Carbohydrate	By difference				g/100g	53.6
Energy	At water Factor				KCal/100g	347.4
Calcium	Permanganate Titration				mg/100g	1050.7
Phosphorus	Molybdenum Blue Calorimetric				mg/100g	968.6

The various constituents of recommended rat feed analyzed using appropraite methods displayed, results presented in grams (g).

Table 3.2: Composition of the diets in g/100 (%)

Ingredients	Control group	Red Palm oil group	Ref. palm oil group	Olive oil group
Maize	51.70	51.70	51.70	51.70
Wheatbran	15.95	15.95	15.95	15.95
Fishmeal	13.80	13.80	13.80	13.80
Soyabean Meal	17.10	17.10	17.10	17.10
Salt	0.20	0.20	0.20	0.20
Oyster shells	1.00	1.00	1.00	1.00
Premix (vitamin + Minerals)	0.25	0.25	0.25	0.25
Added oil	0	6.00	6.00	6.00
Water	6.00	0	0	0
Total	100.00	100.00	100.00	100.00

Composition of diet for the different groups: fortified diet with specific oil type for treatment groups and standard diet (normal rat chow- no oil added) for control group (Kamisah et al., 2005).

3.2.2 Blood Sample Collection

Before the commencement of the experiment, blood samples were taken from the tails of all the rats (after overnight fasting) using the milking method, to establish the baseline levels of the studied parameters. Subsequently, blood samples were

collected from 28 overnight fasted rats (seven rats randomly selected from each group) at the 2nd and 4th month before they were sacrificed.

The rats were anesthetized with ethyl ether, and blood was drawn from the heart (cardiac puncture). From each rat, two blood samples were collected. The first blood sample (4ml) was collected into 5ml litium heparin anticoagulant tubes to obtain plasma. The second (for the serum) was put into 5ml (BD Vacutainer® SST™) plain tubes and allowed to clot at room temperature. The samples were centrifuged at 3000 g for 10 min to separate the plasma and sera into Eppendorf tubes, labeled and stored in laboratory freezer at −80°C until analysis. The stored samples (plasma) were thawed to room temperature before assaying. Rats were later euthanized.

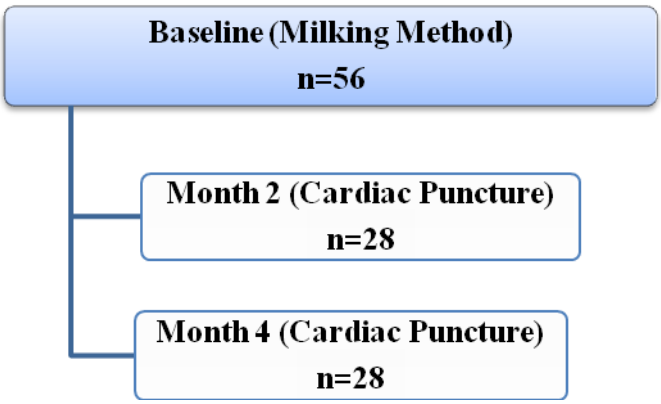

Figure 3.2: ***Blood Sampling Methods***

Shows the two blood sampling methods used. The milking method for baseline blood sampling involving the total number of rats (n=56) and the cardiac puncture method used for 28 rats at both months 2 and 4.

3.2.2.1 Blood Sampling Techniques

Blood sampling methods used were according to standard protocol (Diehl et al., 2001).

Blood draw by Tail Clipping

- This technique was used because it is relatively non-traumatic to allow the animals survive the four months period even though obtainable blood volumes from tail clipping are usually small.
- Extra care was taken during the milking to avoid contamination with tissue and skin products and samples of variable quality.
- Warming of tail was done with the aid of a heat lamp to increase obtainable blood volume.
- Bleeding was done by clipping (i.e., cutting off the tip of the tail) 1-2 mm of the distal tail after animal was effectively restrained.
- The blood was collected by carefully draining the blood along the tail directly into a prepared test tube and labeled immediately.

Blood draw by cardiac puncture

- This technique was used as a terminal procedure to permit the collection of larger amounts of blood from each animal.
- Animals were anesthesised with ethyl ether before the procedure (cardiac puncture).
- 5 ml syringes with 23G1 needles were used.

- Rats were deeply anesthesized and placed on their back, facing away.
- The left index finger was placed at the level of the lowest ribs, without applying any pressure. The heart was gently located ~ 1 cm above this point, slightly to the right.
- Holding the syringe at a 45-degree angle, the needle was inserted between two ribs. A drop of blood entering the needle indicated that the needle was in the heart. Without moving the syringe, the plunger was pulled on to fill the syringe.
- Once the syringe was full, it was carefully disconnected from the needle and emptied into the respective tube and labeled. The syringe was then re-attached to the needle for drawing more blood.
- The rats were immediately euthanized

These techniques were done in the presence of a veterinarian and a veterinary technician who assessed that animals were handled properly and not subjected to unnecessary pain or distress.

3.3 Biochemical Analysis

Plasma lipid profile comprising of Total Cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), Apolipoprotein-A1 (Apo-A1), and Apolipoprotein-B100 (Apo-B100) was determined at baseline and at the end of every two months by biochemical analysis using the Enzyme-Linked Immuno Sorbant Assay (ELISA). Pro-inflammatory markers including Interleukin-2 (IL-2), Interleukin-6 (IL-6), Tumor Necrosis Factor Alpha (TNF-α) were determined. Total Antioxidant Status

(TAS) was also determined by the ELISA method at baseline and at months 2 and 4. All immunoassay kits were obtained from the CUSABIO BIOTECH CO., LTD. (Wuhan, China) with the exception of the Total Antioxidant Assay kit which was imported from CAYMAN (Ann Abor, USA).

3.3.1 Basic Principle of ELISA

Enzyme-linked immunosorbent assays (ELISAs) are plate-based assays designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. ELISAs are typically performed in 96-well (or 384-well) polystyrene plates, which will passively bind antibodies and proteins. Having the reactants of the ELISA immobilized to the microplate surface makes it easy to separate bound from non-bound material during the assay. This ability to wash away nonspecifically bound materials makes the ELISA a powerful tool for measuring specific analytes within a crude preparation.

ELISAs can be performed with a number of modifications to the basic procedure. The key step, immobilization of the antigen of interest, can be accomplished by direct adsorption to the assay plate or indirectly via a capture antibody that has been attached to the plate. The antigen is then detected either directly (labeled primary antibody) or indirectly (labeled secondary antibody). The most powerful ELISA assay format is the sandwich assay.

Source: (Perry, et al., 2002; http://www.piercenet.com).

3.3.1.1 Common ELISA formats

In the assay, the antigen of interest is immobilized by direct adsorption to the assay plate or by first attaching a capture antibody to the plate surface. Detection of the antigen can then be performed using an enzyme-conjugated primary antibody (direct detection) or a matched set of unlabeled primary and conjugated secondary antibodies (indirect detection).

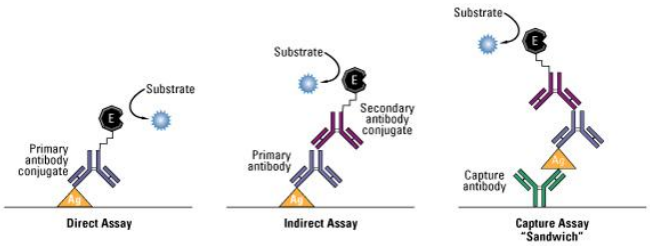

Figure 3.3: Types of Detection Methods

The key step in the detection of antigen involves immobilization of the antigen of interest, which can be accomplished by direct adsorption to the assay plate or indirectly via a capture antibody that has been attached to the plate. The antigen is then detected either directly (labeled primary antibody) or indirectly (labeled secondary antibody). The most powerful ELISA assay format is the sandwich assay. Source: (Perry, et al., 2002; http://www.piercenet.com).

The direct detection method uses a labeled primary antibody that reacts directly with the antigen. Direct detection can be performed with antigen that is directly immobilized on the assay plate or with the capture assay format.

The indirect detection method uses a labeled secondary antibody for detection and is the most popular format for ELISA. The secondary antibody has specificity for

the primary antibody. In a sandwich ELISA, it is critical that the secondary antibody be specific for the detection primary antibody only (and not the capture antibody) or the assay will not be specific for the antigen

For sandwich assays, it is beneficial to use secondary antibodies that have been cross-adsorbed to remove any antibodies that have affinity for the capture antibody. In addition to the individual components and general principles of ELISA, complete kits are available for detection of specific cytokines and other targets, such as interferon gamma (IFN gamma) and interleukin 6 (IL-6) (http://www.piercenet.com).

3.3.2 Lipid Profile Analysis

3.3.2.1 Determination of Rat Cholesterol (TC)

CUSABIO BIOTECH CSB-E11706r (Wuhan, China) Rat Cholesterol kit was used and the manufacturer's instruction followed accordingly.

Principle of the Assay

The microtiter plate provided in the kit was pre-coated with rat cholesterol. Standards or samples are added to the appropriate microtiter plate wells with Horseradish Peroxidase (HRP) – conjugated antibody preparation specific for rat Cholesterol, mix well and incubated. The more the amount of rat Cholesterol in samples, the less Harseradish Peroxidase (HRP) – conjugated antibody

preparation specific for rat Cholesterol is bound by pre-coated rat Cholesterol. Then a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well and the color develops in inverse proportion to the amount of rat Cholesterol in the sample. The color development is stopped and the intensity of the color is measured.

Materials Provided

Reagent	Quantity
Assay plate	1
Standard	6 (0.5 ml)
HRP-conjugate	1 (6 ml)
Sample Diluent	2 (20 ml)
Wash Buffer	1 (20 ml)
TMB Substrate	1 (10 ml)
Stop Solution	1 (10 ml)

Sample Preparation

The suggested 5000-fold dilution was achieved by the addition of 5 µl sample to 45 µl of Sample Diluent, followed by addition of 5 µl of the diluted sample to 95 µl of Sample Diluent. The 5000-fold dilution was completed by adding 5 µl of the solution to 120 µl of Sample Diluent.

Assay Procedure

1. 50 µl of Standard or Sample was added per well. 50 µl HRP – conjugate was immediately added to each well mixed well for 60 seconds and incubated for 60 minutes at 37°C.

2. Each well was aspirated and washed, with 200 µl of Wash Buffer using an auto-washer. The process was repeated for a total of five time washes. After the last wash, remaining Wash Buffer was removed by decanting and blotting against clean paper towels.

3. 90 µl of TMB Substrate was added to each well and incubated for 20 minutes at 37°C away from drafts and other temperature fluctuations in the dark. A blue color developed after 50 µl of Stop solution was added to each well.

4. The optical density of each well was determined within 15 minutes, using a microplate reader set to 450 nm.

Calculation of Results

A calibration curve of absorbance of standards against the Log10 of the concentration was plotted. The concentrations of the various samples were read from the standard curve. Since samples were diluted, the concentration read from the standard curve was multiplied by the dilution factor (x 5000).

Example of Spread Sheet and calibration Curve

Table 3.3: Spread Sheet for Determination of TC Concentration

Samples (STD)	**Abs**	**STD Conc.**	**Log of Conc.**	**TC Conc. (pmol/ml)**
SI	1	0		
S2	0.962	23.44	1.369958	23.44
S3	0.697	93.75	1.971971	93.75
S4	0.394	375	2.574031	375
S5	0.192	1500	3.176091	1500
S6	0.085	6000	3.778151	6000
Test Samples				
CT1T	0.77		1.747312	55.89
CT4B	0.733		1.846774	70.27

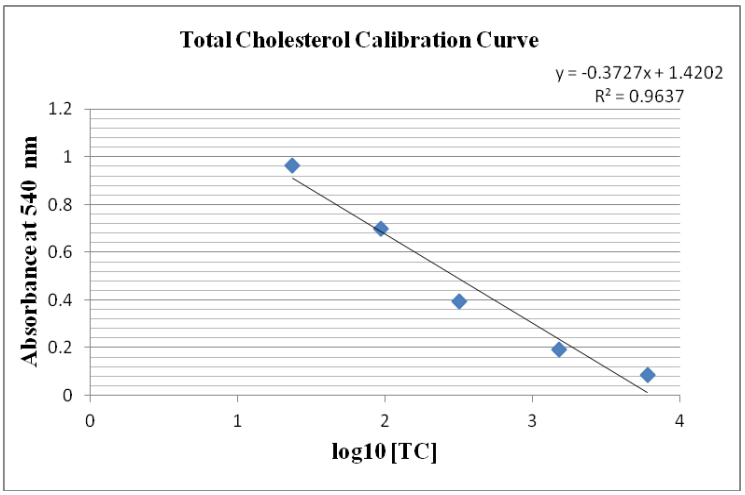

Figure 3.4: Calibration Curve for Determination of TC Concentration

3.3.2.2 Determination of Rat Oxidized Low-density Lipoprotein (OxLDL-C)

CUSABIO BIOTECH CSB-E11706r (WUHAN, CHINA) kit was used according to the manufacturer's instruction.

Principle of the Assay

The microtiter plate provided in the kit was pre-coated with an antibody specific to OxLDL-C. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody preparation specific for OxLDL-C and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is then added to each well. Only those wells that contain OxLDL-C, biotin-conjugated antibody and enzyme- conjugated Avidin will exhibit a change in color, which is directly proportional to the amount of OxLDL-C in the sample. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± nm. The concentration of OxLDL-C in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Materials Provided

Reagent	Quantity
Assay plate	1
Standard	2
Sample Diluent	2 (20 ml)

Biotin-antibody Diluent	1 (10 ml)
HRP-avidin Diluent	1 (10ml)
Biotin-antibody	1 (120µl)
HRP-avidin	1 (120µl)
Wash Buffer	1 (20 ml)
TMB Substrate	1 (10 ml)
Stop Solution	1 (10 ml)

Reagent Preparation

Wash Buffer: 20 ml of Wash Buffer Concentrate was diluted into deionized or distilled water to prepare 500 ml of Wash Buffer.

Standard: The standard vial was centrifuged at 6000 – 10000 rpm for 30 s. The Standard was reconstituted with 1.0 ml of Sample Diluent. This reconstitution produced a stock solution of 400 pg/ml.

The standard was allowed to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The undiluted standard served as the high standard (400 pg/ml). The Sample Diluent served as the zero standards (0 pg/ml).

Biotin-antibody: The vial was centrifuged before opening and diluted to the working concentration using Biotin-antibody Diluent (1.100), respectively.

HRP-avidin: The vial was centrifuged before opening and diluted to the working concentration using HRP-avidin Diluent (1:100), respectively.

Assay Procedure

The suggested 1000-fold dilution was achieved by addition of 5µl sample to 120 µl of Sample Diluent first, then the 1000-fold dilution was completed by addition of 5 µl of the prepare solution to 195µl of Sample Diliuent.

100µl of Standard, Blank, or Sample was add per well, covered with the adhesive strip and Incubated for 2 hours at 37°C. The liquid of each well was removed and 100µl of Biotin-antibody working solution was added to each well. After incubating for 1 hour at 37°C.

Biotin-antibody working solution appeared cloudy. The solution was cooled to room temperature and mixed gently until it appeared uniform.
Each well was aspirated and washed, and the process repeated three times for a total of three washes.

The liquid was removed by flicking the plate over a sink. The remaining drops were removed by patting the plate on a paper towel. Hundred microliters (100) µl of HRP-avidin working solution was added to each well and the microtiter plate was covered with a new adhesive strip and Incubated for 1 hour at 37°C.

The aspiration was repeated and washed five times as step 3. Ninety microliters (90) µl of TMB Substrate was added to each well, incubated for 10-30 minutes at 37°C.

Fifty microliters (50) µl of Stop Solution was added to each well when the first four wells containing the highest concentration of standards develop obvious blue color.

The optical density of each well was determined within 30 minutes, using a microplate reader set to 450 nm.

Calculation of Results

A calibration curve of absorbance of standards against the Log10 of the concentration was plotted. The concentrations of the various samples were read from the standard curve. Since samples were diluted, the concentration read from the standard curve was multiplied by the dilution factor (x 20).

Example of Spread Sheet and calibration Curve

Table 3.4: Spread Sheet for Determination of OxLDL-C Concentration

Samples (STD)	Abs	STD Conc.	Log of Conc.	OxLDL-C Conc. (ng/ml)
SI	0.105	0		
S2	0.424	50	1.69897	50
S3	0.566	100	2	100
S4	0.938	200	2.30103	200
S5	1.23	300	2.477121	300
S6	1.233	350	2.544068	350
S7	1.256	400	2.60206	400

Test Samples			
CT1T	0.727	2.060784	115.02
CT4B	0.68	2.017647	104.15

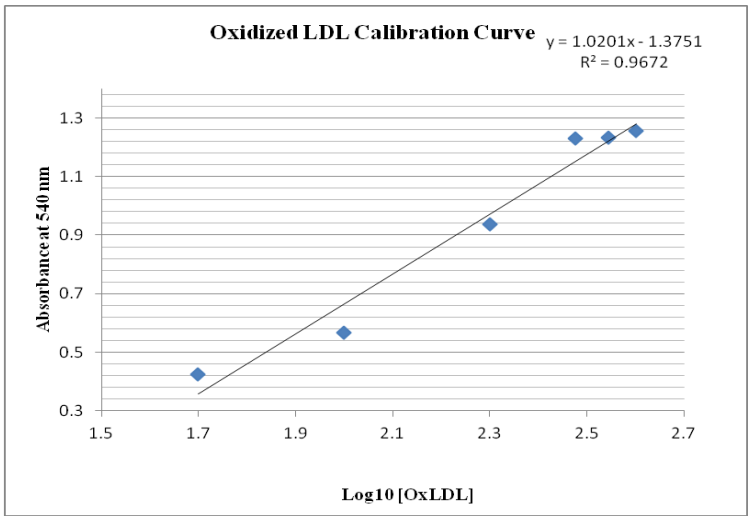

Figure 3.5: Calibration Curve for Determination of OxLDL-C Concentration

3.3.2.3 Determination of Lipid Profile and Inflammatory Markers

The six subsequent tests followed the same principle and procedure as specified above with the exception of the TAS, the various steps carried out have been illustrated in a flow chart below (Fig.3.6). The different spread sheets and caliberation curves are also shown below (Figs.3.7 to 3.13: Tables 3.5 to 3.10).

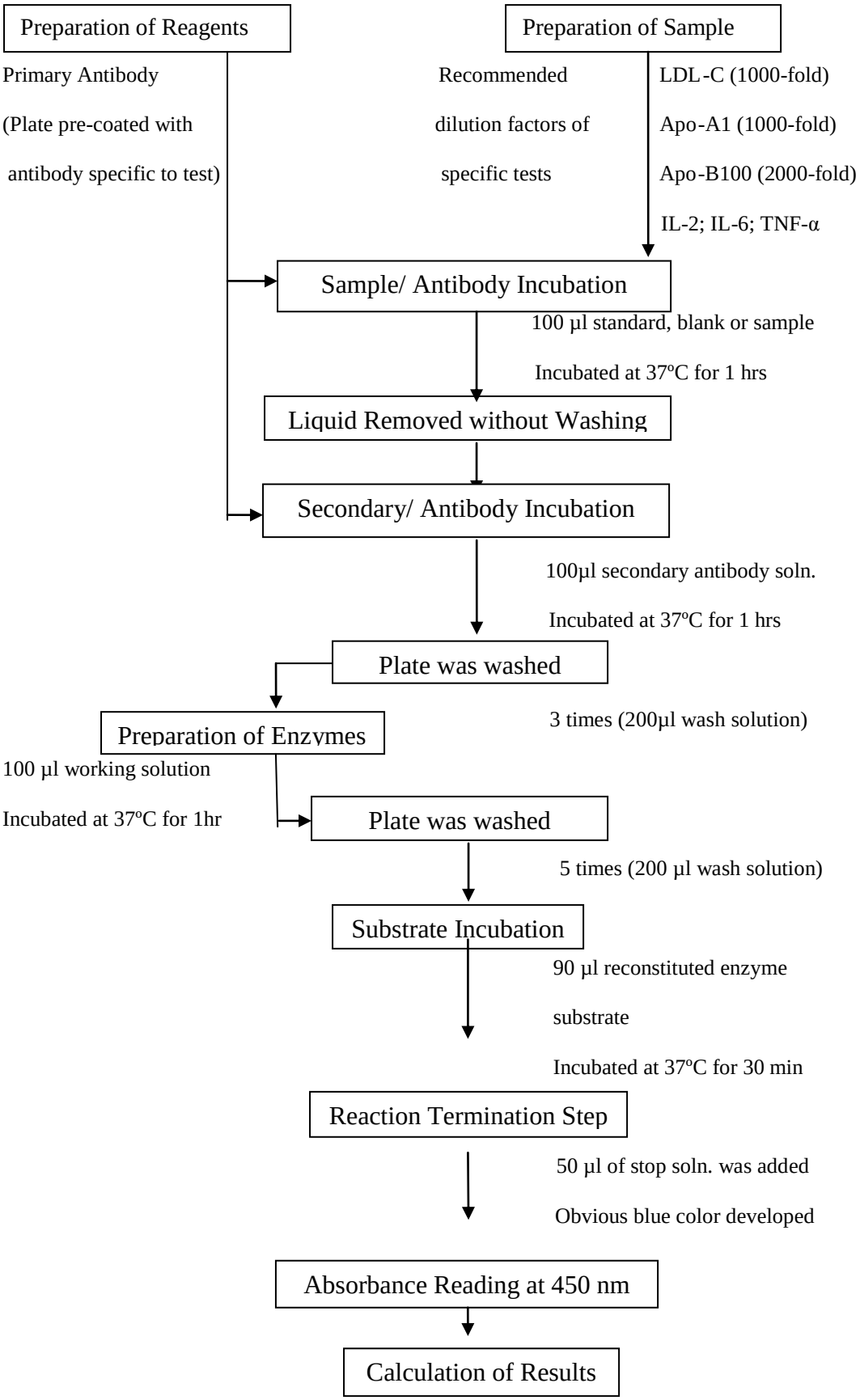

Figure 3.6: Flow Diagram Showing Procedure for Biochemical Analysis.

3.3.2.4 Determination of Rat Low Density Lipoprotein Concentration (LDL-C)

Example of Spread Sheet and calibration Curve

Table 3.5: Spread Sheet for Determination of LDL-C Concentration

Samples (STD)	**Abs.**	**STD Conc.**	**Log Conc.**	**LDL-C Conc. (ng/ml)**
SI	0.007			
S2	0.039	0		
S3	0.135	125	2.09691	125
S4	0.365	250	2.39794	250
S5	0.565	500	2.69897	500
S6	1.52	1000	3	1000
S8	2.091	2000	3.30103	2000
Test Samples				
CT1T	0.043		2.168746	147.48
CT4B	0.088		2.195484	156.85

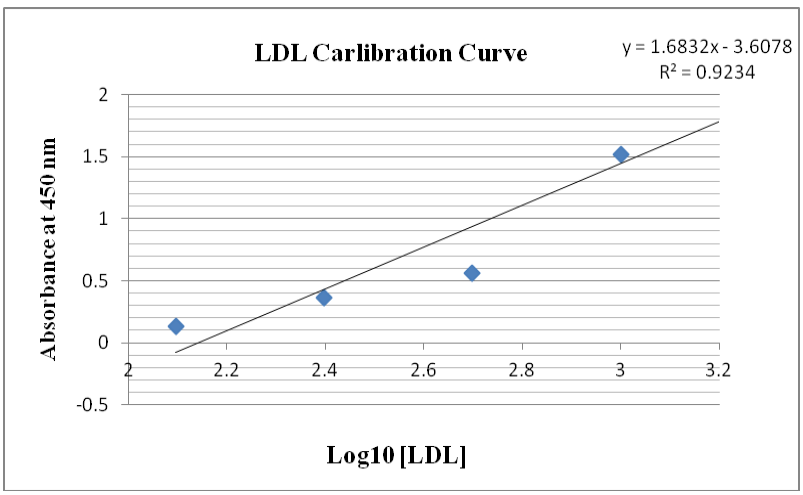

Figure 3.7: Calibration Curve for Determination of LDL-C Concentration

3.3.2.5 Determination of Rat Apolipoprotein-A1 Concentration

Example of Spread Sheet and calibration Curve

Table 3.6: Spread Sheet for Determination of Apo-A1 Concentration

Samples (STD)	Abs	STD Conc.	Log of Conc.	Apo-A1 Conc. (ug/ml)
SI	0.485	2	0.30103	2
S2	0.554	3	0.477121	3
S3	0.692	4	0.60206	4
S4	0.712	5	0.69897	5
S5	0.66	6	0.778151	6
S6	0.707	8	0.90309	8
S7	0.878	9	0.954243	9
Test Samples				
CT3H	0.277		0.50332	3.19
CT2N	0.24		0.426556	2.67

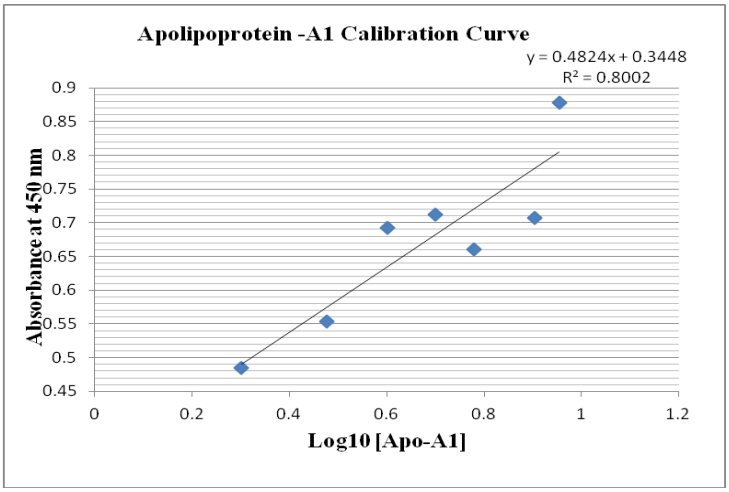

Figure 3.8: Calibration Curve for Determination of Apo-A1 Concentration

3.3.2.6 Determination of Rat Apolipoprotein-B100 Concentration

Example of Spread Sheet and calibration Curve

Table 3.7: Spread Sheet for Determination of Apo-B100 Concentration

Samples (STD)	Abs.	STD Conc.	Log of Conc.	Apo-B100 Conc. (ng /ml)
SI	0.249	1000	3	1000
S2	0.34	2000	3.30103	2000
S3	0.544	3000	3.477121	3000
S4	0.605	4000	3.60206	4000
S5	0.681	5000	3.69897	5000
S6	0.831	6000	3.778151	6000
S7	0.812	7000	3.845098	7000
Test Samples				
CT1T	0.292		3.133242	1359.07
CT3H	0.295		3.137363	1372.03

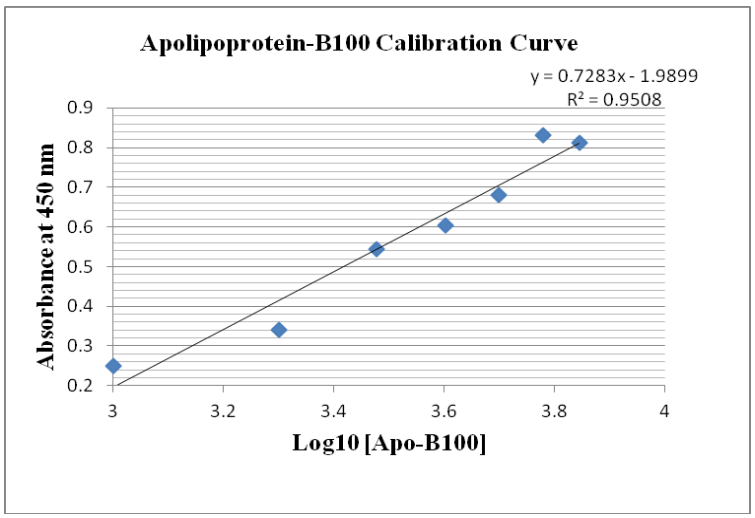

Figure 3.9: Calibration Curve for Determination of Apo-B100 Concentration

3.3.2.7 Determination of Rat Interleukin-2 Concentration

Example of Spread Sheet and calibration Curve

Table 3.8: Spread Sheet for Determination of IL-2 Concentration

Samples (Std)	Abs	STD Conc.	Log of Conc.	Il2 Conc. (pg/ml)
S1	0.126	50	1.69897	50
S2	0.147	100	2	100
S3	0.185	150	2.176091	150
S4	0.205	200	2.30103	200
S5	0.219	250	2.39794	250
S6	0.246	300	2.477121	300
S7	0.254	350	2.544068	350
Test Samples				
CT1T	0.1		1.613924	41.11
CT5N	0.094		1.575949	37.67

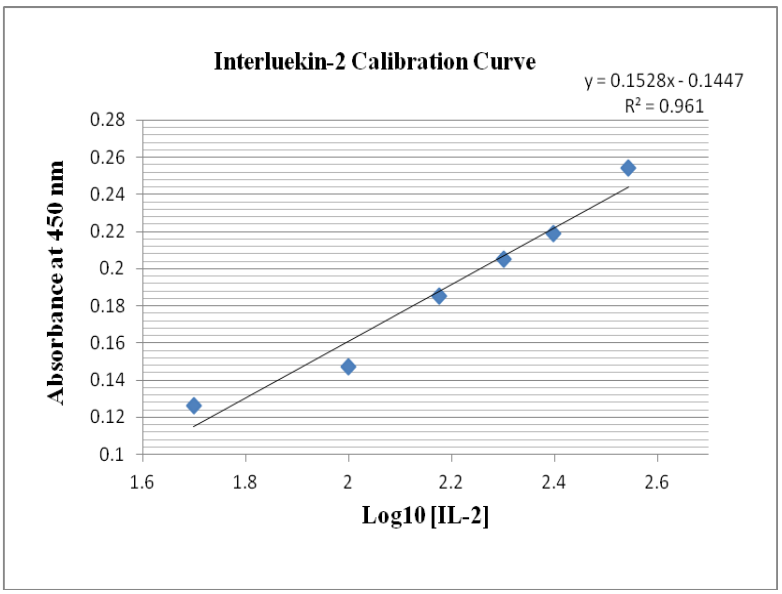

Figure 3.10: Calibration Curve for Determination of IL-2 Concentration

3.3.2.8 Determination of Rat Interleukin-6 Concentration

Example of Spread Sheet and calibration Curve

Table 3.9: Spread Sheet for Determination of IL-6 Concentration

Samples (STD)	Abs.	STD Conc.	Log of Conc.	IL6 Conc. (pg/ml)
S1	0.084	2.5	0.39794	2.5
S2	0.129	5	0.69897	5
S3	0.165	7.5	0.875061	7.5
S4	0.221	10	1	10
S5	0.271	15	1.176091	15
S6	0.283	17.5	1.243038	17.5
S7	0.291	20	1.30103	20
Test Samples				
CT2B	0.39		1.700405	50.17
CT4T	0.466		2.008097	101.88

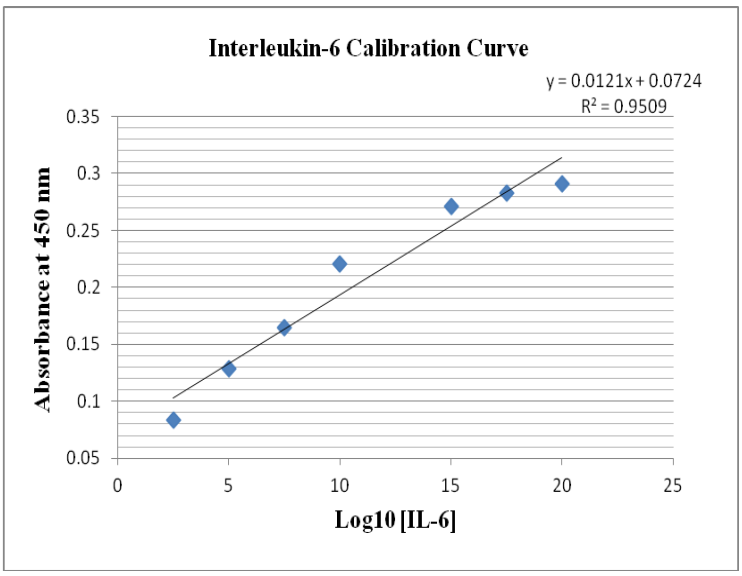

Figure 3.11: Calibration Curve for Determination of IL-6 Concentration

3.3.2.9 Determination of Rat Tumor Necrosis Factor Alpha Concentration

Example of Spread Sheet and calibration Curve

Table 3.10: Spread Sheet for Determination of TNF-α Concentration

Plasma	Abs	Std Conc.	Log Of Conc.	TNF-α Conc. (pg/ml)
S1	0.126	500	2.69897	500
S2	0.188	1000	3	1000
S3	0.213	1500	3.176091	1500
S4	0.273	2000	3.30103	2000
S5	0.324	2500	3.39794	2500
S6	0.34	3000	3.477121	3000
S7	0.459	3500	3.544068	3500
Test Samples				
CT2H	0.107		2.746398	557.70
CT1B	0.155		2.884726	766.88

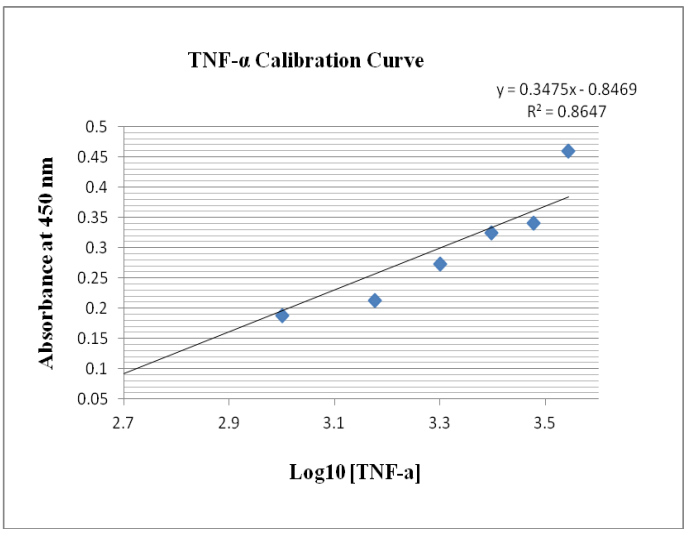

Figure 3.12: Calibration Curve for Determination of TNF-α Concentration

3.3.3 Antioxidant Capacity Analysis

3.3.3.1 Total Antioxidant Status

CAYMAN CHEMICAL COMPANY 709001 (Ann Abor, USA). Antioxidant Assay kits was used and the test was carried out according to the manufacturer's instructions.

Introduction

Reactive oxygen species (ROS) are produced as a consequence of normal aerobic metabolism. Unstable free radical species attack cellular components causing damage to lipids, proteins, and DNA which can initiate a chain of events resulting in the onset of a variety of diseases. Living organisms have developed complex antioxidant systems to counteract ROS and to reduce their damage.

These antioxidant systems include enzymes such as superoxide dismutase, catalase, and glutathione peroxidase; macromolecules such as albumin, ceruloplasmin, and ferritin; and an array of small molecules, including ascorbic acid, α-tocopherol, β-carotene, reduced glutathione, uric acid, and bilirubin. The sum of endogenous and food-derived antioxidants represents the total antioxidant activity of the system.

The cooperation among different antioxidants provides greater protection against overall oxidative capacity and may provide more relevant biological information compared to that obtained by measurement of individual components, as it

considers the cumulative effect of all antioxidants present in plasma and body fluids.

Principle

The assay relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS® (2, 2'- Azino-di [3-ethylbenzthiazoline sulphonate]) to $ABTS^{®+}$ by metmyoglobin. The amount of $ABTS^{®+}$ produced can be monitored by reading the absorbance at 750 nm or 405 nm to a degree which is proportional to their concentration. The capacity of antioxidants in the sample to prevent $ABTS^{®+}$oxidation is compared with that of Trolox, a water-soluble tocopherol analogue, and is quantified as millimolar Trolox equivalents.

Reagents in Kit: The kit contained the following

1. Antioxidant Assay Buffer (10×)
2. Antioxidant Assay Chromogen
3. Antioxidant Assay Metmyoglobin
4. Antioxidant Assay Trolox
5. Antioxidant Assay Hydrogen Peroxide
6. 96-Well Plate (Colorimetric Assay)
7. 96-Well Cover Sheet

Reagent preparation

Antioxidant Assay Buffer (10×): 3 ml of Assay Buffer concentrate was diluted with 27 ml of HPLC-grade water. This diluted Assay Buffer (5 mM potassium phosphate, ph 7.4, containing 0.9% sodium chloride and 0.1% glucose) was used to reconstitute the metmyoblobin.

Antioxidant Assay Chromogen: The chromogen was reconstituted by adding 6 ml of HPLC-grade water to the vial and vortexed well.

Antioxidant Assay Metmyoglobin: The metmyoblobin was reconstituted by the addition of 600 µl of Assay Buffer to the vial and vortexed well.

Antioxidant Assay Trolox: Reconstitution of the Trolox (6-hydroxy-2,5,7,8-tetrathylchroman-2-carboxylic acid) was done by adding 1 ml of HPLC-grade water to the vial and vortexing well.

Antioxidant Assay Hydrogen Peroxide: 10 µl of Hydrogen Peroxide was diluted with 990 µl of HPLC-grade water. Further dilution was done by removing 20 µl and diluting with 3.98 ml of HPLC-grade water to yield a 441 µM working solution.

Performing the Assay

1. The Trolox Standards were prepared according to the manufactures' instruction as follows. Seven clean test tubes were marked A-G. The

amount of reconstituted Trolox and Assay Buffer added to each test tube are indicated in the Table (3.11) below.

Table 3.11: TAS Standard Preparation

Tube	Reconstituted Trolox (µl)	Assay Buffer (µl)	Final Concentration (Mm Trolox)
A	0	1,000	0
B	30	970	0.045
C	60	940	0.090
D	90	910	0.135
E	120	880	0.18
F	150	850	0.225
G	220	780	0.330

Trolox Standards preparation

2. **Trolox Standards Wells** –10 µl of Trolox Standards (tubes A-G), 10 µl Metmyoglobin, and 150 µl of chromogen were added per well in the designated wells on the plate

3. **Sample Wells** –10 µl of sample, 10 µl of Metmyoglobin, and 150 µl of Chromogen were added to two wells.

4. The reaction was initiated by adding 10 µl of Hydrogen Peroxide working solution to all the wells being used as quickly as possible (within one minute as recommended).

5. The plate was covered with the plate cover and incubated on a shaker for five minutes at room temperature. The cover was removed and the absorbance read at 405 nm using a plate reader.

Calculations

The average absorbance of each standard and sample was calculated. The average absorbance of standards plotted as functions of the final trolox concentration (Mm)

The antioxidant concentration of samples using the equation obtained from the linear regression of the standard curve was calculated by substituting the average absorbance values for each sample into the equation.

$$\textbf{Antioxidant (Mm)} = \frac{\text{sample average absorbance} - (\text{y-intercept})}{\text{Slope}} \times \text{dilution}$$

Determination of Rat Total Antioxidant Status

Example of Spread Sheet and calibration Curve

Table 3.12: Spread Sheet for Determination of TAS Concentration

Sample (STD)	T AS Conc (nM)	Abs (A)
SI	0	1.874
S2	0.045	1.619
S3	0.09	1.507
S4	0.135	1.539
S5	0.18	1.479
S6	0.225	1.509
S7	0.33	1.175
Test Samples		
CT1T	1.912	0.230
CT4B	1.713	0.335

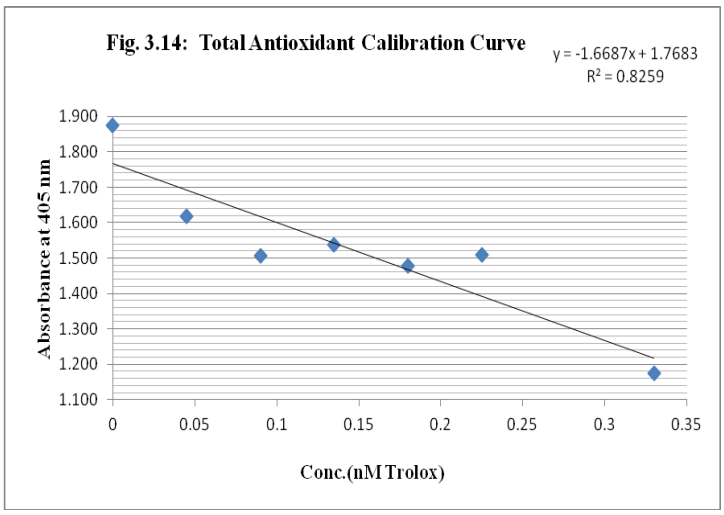

Figure 3.13: Calibration Curve for Determination of TAS Concentration

3.4 Statistical Analysis

The results are expressed as Means ± SEM. Unpaired t-test was used to compare two mean values of continuous variables, One-way ANOVA and Bonferroni post-hoc was used to compare more than two mean values of continuous variables. A level of $p<0.05$ was considered as statistically significant. Graph Pad Prism version 5.00 for windows was used for statistical analysis (Graph Pad soft-ware, San Diego California USA, (www.graphpad.com).

CHAPTER FOUR

RESULTS

4.1 Background

The purpose of this study was to investigate the effect of red palm oil (RdPO), refined palm oil (Palm Olein) (RfPO) and olive oil (OO) on lipid profile in rats and the impact of these oils on indicators (inflammatory markers and antioxidant levels) of the atherosclerotic process in the rats. This chapter is a presentation of the results of laboratory analyses conducted, with the aim of achieving the above. These sections show the trends in Growth, Lipid Profile, Inflammatory Markers and Anti-oxidant Status of the rats over the study period. The sections also include results of statistical analyses to ascertain significant effect of the intake of the different oils (OO, RfPO and RdPO) on growth and the various biochemical indices in the rats. These have been shown in Fig. 4.1 and Tables 4.1 to 4.4).

4.2 Trend in growth (Week one to eight)

The average weight of the rats in the various groups was statistically comparable at the start of the study. After two weeks of feeding, the study observed an increase in weight across the various experimental groups. However the mean

weight in the individual groups remained statistically comparable. With the exception of the seventh week where an average weight loss was recorded among rats in the red palm oil group. The animals gained weight week by week, through to the eighth week. Although no significant difference in weight was recorded among the various treatment groups week by week, the control group recorded the highest mean weight from week four through to week eight, (Table 4.1).

Table 4.1: Mean weights of experimental animal measured from start to the eighth week stratified by treatment groups

Parameter (g)	Control	Refined	Olive	Red Palm	p-Value
Basal Weight	245.7±11.0	245.9±9.4	251.8±7.5	235.4±7.3	0.6306
Week Two	295.3±8.8	288.7±8.2	301.7±6.1	296±9.1	0.7324
Week Three	313.2±8.6	307.7±8.9	313.8±6.6	310±9.6	0.9527
Week Four	329.9±8.5	321.2±9.5	327±6.9	325.1±9.5	0.9121
Week Five	341.6±8.2	336±10.2	341±7.2	329.2±9.5	0.7379
Week Six	357.8±8.1	345.9±10.9	350.4±7.1	348±10.1	0.8113
Week Seven	358.5±7.6	347.1±10.7	352.6±7.1	347.6±10.4	0.8000
Week Eight	369.4±7.8	358.9±11.0	363.3±7.6	359.4±10.4	0.8467

Data is presented as means ± SEM. p is significant at < 0.05

4.2.1 Trend in growth (Week eight to sixteen)

The second half of the experimental period saw a continual increase in weight of the experimental animals fed with only standard food. The rats in the refined palm oil group had their average weight changing from the least, at the end of the first half of the study period to the highest average weight at the start of the second half, till the end of the study period. Weekly incremental mean weights were recorded except in the thirteenth week. In general an average week by week increase in weight was also observed among both the animals fed the olive oil and red palm oil diets, the exceptions though being the decrease in weight recorded in the twelfth and sixteenth weeks for rats in the olive oil group and the thirteenth for those in the red palm oil group. Again as observed in the first half of the experimental period the weekly average weights across the four experimental groups for the second half were found to be statistically insignificant (Table 4.2).

Table 4.2: Measured weight of experimental animal from ninth to the sixteenth week stratified by treatment groups

Parameter (g)	Control	Refined	Olive	Red Palm	p-Value
Week Nine	384.9±8.0	395±17.1	381.7±11.1	373.7±18.0	0.7634
Week Ten	393.3±5.923	402.7±18.3	389.3±13.3	379.9±19.0	0.7573
Week Eleven	394.1±6.2	402.4±18.9	393.3±12.4	384.3±18.8	0.8646
Week Twelve	410.3±7.0	422.3±21.2	390.0±17.5	393.3±19.8	0.5257
Week Fourteen	417.3±7.4	440.7±21.1	405.1±15.3	405.0±20.7	0.4209
Week Fifteen	421.7±9.4	447.7±22.2	415.7±22.9	410.3±21.6	0.5731
Week Sixteen	428.2±9.3	459±26.1	415.1±16.6	416.1±22.8	0.4004

Data is presented as mean ± SEM. p is significant at < 0.05

4.2.2 Effect of Different Vegetable Oils on Growth of Rats

From the average baseline weight of experimental rats (235.4 to 251.8 g), the average weight of the rats after two months of feeding ranged between 329.4 g to 337.9 g (fig. 4.1) and 391.6 g to 429.2 g (fig. 4.1) after four month of feeding. Significant weight increase was recorded among all experimental animal after first half of the experimental period and also from the first to the second half of the study. The magnitude of weight gained after month two ranged from animals fed with red palm oil (95.4 g), standard diet (92.2 g), to olive oil (83.9 g) to refined oil (83.5 g). At the end of the study period however animals fed fortified refined oil had the maximum net weight gained among the groups (183.3 g), followed by animals on red palm oil diet (166.8 g) with animals fed on fortified olive oil diet gaining the least weight among the groups (139.8 g). Animals fed on refined oil also showed the highest weight gained after twelve hours of fasting between the second and fourth months with those fed on olive oil gaining the least weight measured after twelve hours fasting.

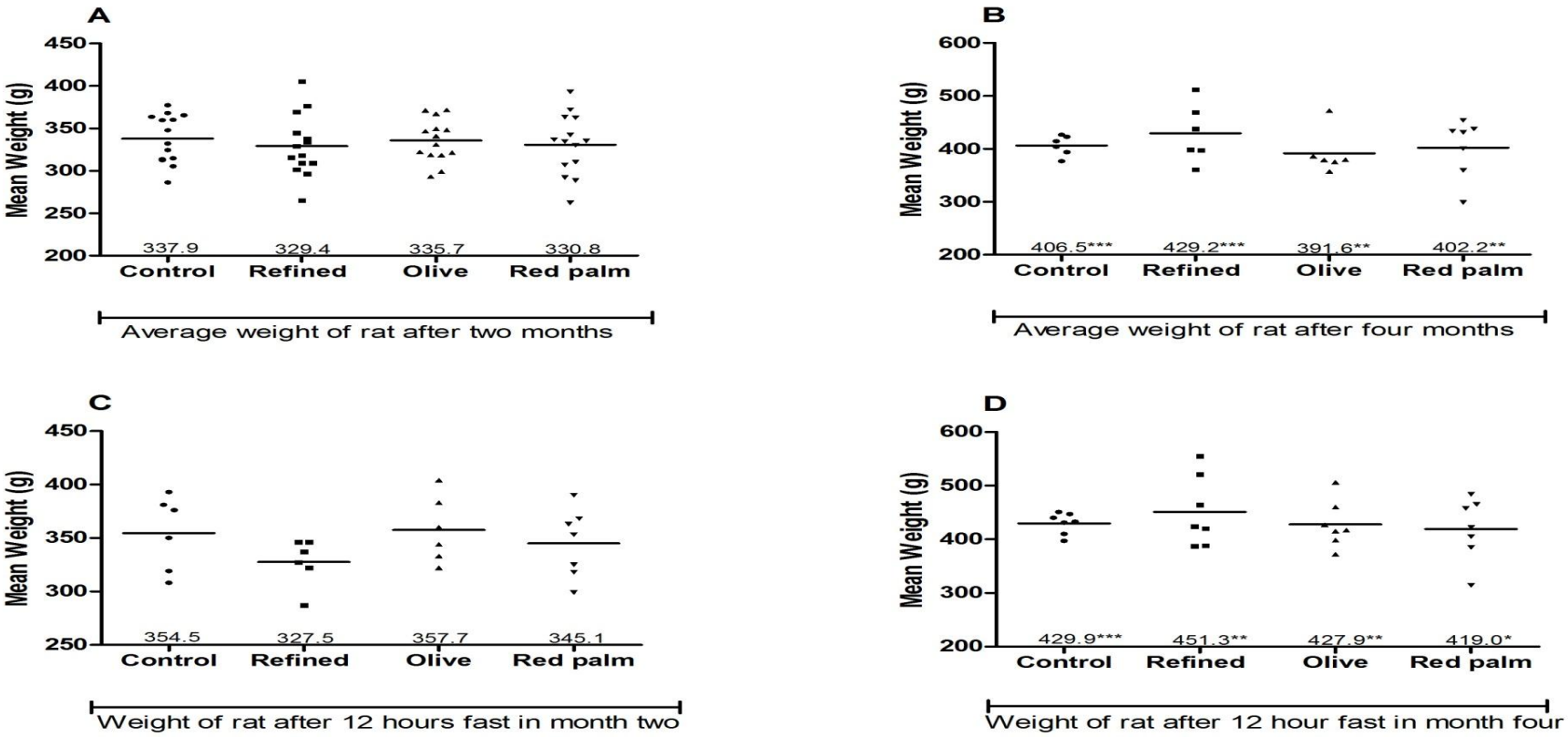

Figure 4.1: Mean and fating weight of experimental animals at the middle and end of study period.

*Charts 'A' and 'B' show the mean weights of the rats in the various groups after feeding (the specified experimental diet) at month two and four respectively whilst 'C' and 'D' shows their mean fasting weights. *p<0.05, **p<0.01, ***p<0.001, p indicates levels of significant when intra group comparism is made between month two and month four.*

4.3 Lipid Profile

The following results show the effect of intake of the three vegetable oils on the Lipid Profile of the rats as shown by the trends and change in levels of biochemical parameters measured. These included Total Cholesterol (TC), Low Density Lipoprotein cholesterol (LDL-C), Oxidized Low Density Lipoprotein (oxLDL-C), Apolipoprotein-A100 (ApoA) and Apolipoprotein-B100 (ApoB).

As seen in table 4.3, In general the levels of the biochemical markers of lipids assayed in this study were found to be statistically comparable among the various treatment groups, at all three stages of measurement. Though not significant, increase in total cholesterol levels of the experimental animals from the baseline through the second to the fourth month was observed in all treatment groups.

With the exception of the rat on the standard feed, the highest levels of LDL-C levels were recorded for all experimental groups in the second month of the study. An increased trend of oxLDL-C, was observed among rats on the standard diet, refined oil fortified diet and to some extend those on the fortified red palm oil diet. However animals fed with feed fortified with olive oil recorded decreasing levels of oxLDL-C (1.34 ± 0.19, 1.19 ± 0.15, 1.15 ± 0.10) µg/ml for the baseline, months two and four respectively.

At the end of the experimental period, marginal increases of Apo–A was seen in the control, as well as the olive oil groups whilst refined oil and red palm oil groups recorded the opposite (decreases). Apo–B levels found at the end of the study was higher than that measured at the start for animals put on standard diets, refined oil fortified diet as well as those on red palm oil fortified diet. Among those on the olive oil diet however decreasing levels of Apo–B were recorded.

Table 4.3: Effect of Different Vegetable Oils on Lipid Profile of S-D Rats before and after Treatment.

Parameter	Control	Refined	Olive	Red Palm	p-Value
TC (umol/ml)					
Baseline	1.07±0.11	1.07±0.14	0.81±0.11	1.05±0.16	0.7101
Month 2	1.09±0.32	1.45±0.33	0.85±0.15	1.08±0.05	0.5064
Month 4	1.17±0.18	1.49±0.43	1.02±0.06	1.14±0.25	0.6552
p1-Value	0.9424	0.3833	0.3835	0.9401	
LDL (ug/ml)					
Baseline	156.0±0.7	154.5±1.4	153.4±0.9	154.8±1.2	0.5627
Month 2	151.7±1.6	156±3.1	153.2±1.5	158.6±6.7	0.4732
Month 4	151.8±3.5	155.9±1.9	152.6±1.6	156.4±1.4	0.4769
p1-Value	0.1787	0.8212	0.9158	0.7533	
Ox LDL (ug/ml)					
Baseline	1.19±0.17	1.03±1.12	1.34±0.19	1.19±0.17	0.6212
Month 2	1.22±0.16	1.34±0.07	1.19±0.15	1.18±0.15	0.8708
Month 4	1.24±0.08	1.37±0.05	1.15±0.10	1.28±0.04	0.2783
p1-Value	0.9805	0.0956	0.6977	0.9228	

Table 4.3 Cont.

Parameter	Control	Refined	Olive	Red Palm	p-Value
Apo-A1(mg/ml)					
Baseline	2.9±0.2	3.1±0.1	3.5±0.2	4.1±0.4	0.0610
Month 2	3.4±0.4	3.4±0.1	2.9±0.2	3.7±0.3	0.3612
Month 4	3.4±0.3	2.7±0.3	3.5±0.4	2.9±0.2	0.2525
p1-Value	0.5247	0.1513	0.3529	0.1181	
Apo -B100 (mg/ml)					
Baseline	2.08±0.08	2.61±0.36	2.91±0.40	2.22±0.26	0.2987
Month 2	2.19±0.26	2.48±0.12	2.12±0.23	2.13±0.20	0.6633
Month 4	2.19±0.27	2.79±0.16	2.08±0.26	2.22±0.20	0.1961
p1-Value	0.8785	0.8936	0.1613	0.9638	

Data is presented as mean ± SEM. p is significant at < 0.05. p- Inter group comparism between controls, refined, olive, red palm oil. p1- Intra group comparism between baseline, months two and four.

4.4 Inflammatory markers and Total Antioxidant Status

The effect of intake of the three vegetable oils on inflammatory markers and total antioxidant levels of the rats, are shown on table 4.4. At the end of the experimental period, marginal reduction in the levels of interleukin 2 (IL-2) was seen in animals put on standard diet, those put on refined oil fortified diet as well as those fed with olive oil fortified diet with these groups recording their lowest levels of IL-2 at the second month of the experimental period. For the animals fed with red palm however marginal increase of IL-2 levels was observed. Inter group analysis revealed no significant difference of IL-2 levels among the various treatment groups at each point of measurement.

The study revealed a significant reduction of interleukin 6 (IL-6) levels at both month two and four in comparison with concentrations recorded at the start of the study for animal fed with standard feed (p-0.0013), animals fed with refined oil (p-0.0311) and animals fed with olive oil (p-0.0041). For animal fed with red palm oil however marginal increases in IL-6 levels were recorded (table 4).

The levels of tumor necrotic alpha (TNF-α) increased among all experimental groups with the exception of the animals fed with the fortified olive oil diet which revealed increased total antioxidant status (TAS). After two months of feeding TAS

of the animals on olive oil diet was found to be significantly lower compared to the control group.

The total antioxidant level measured by the TAS of the rats at baseline was found to be comparable among all experimental groups. After two months of feeding decrease in the TAS was recorded among the control group and animals fed with refined oil. The TAS of animals fed with olive oil and those fed with red palm oil had an increase in their TAS levels. Though after four month of feeding reduction in the TAS of all groups, the reduction was significant in the treatment groups (p-0.0055. 0.0001, <0.0001 for refined, olive and red palm, respectively).

Table 4.4: Effect of Different Vegetable Oils on inflammatory Markers and antioxidant Levels of S-D Rats before and after Treatment.

Parameter	Control	Refined	Olive	Red Palm	p-Value
Interleukin 2 (pg/ml)					
Baseline	37.7±3.7	41.6±2.6	41.9±4.3	36.5±2.8	0.5877
Month 2	31.9±0.8	37.3±1.9	34.1±3.7	37.1±5.5	0.6779
Month 4	32.5±1.5	41.5±5.2	35.5±4.6	38.1±0.9	0.4647
p1-Value	0.2001	0.6223	0.4581	0.9487	
Interleukin 6 (pg/ml)					
Baseline	20.1±2.1	25.9±3.5	19.9±2.0[a]	11.4±1.0	0.0153
Month 2	10.2±1.1[¥¥]	12.9±0.3[¥]	12.9±1.7[¥]	13.5±0.8	0.1783
Month 4	9.3±0.3[ββ]	14.1±1.1	10.3±0.2 [ββ]	16.6±4.6	0.2278
p1-Value	0.0013	0.0311	0.0041	0.4144	
TNF-α (pg/ml)					
Baseline	603.3±32.3	693.2±46.7	668.5±39.4	567.1±31.4	0.0989
Month 2	619.2±74.6	809.2±73.0	565.7±43.6[a]	596.0±15.6	0.0336
Month 4	619.4±8.7	836.2±146.4	540.7±25.3	645.7±9.1	0.0894
p1-Value	0.9491	0.3976	0.059	0.1889	

*Data is presented as mean ± SEM. p is significant at < 0.05. p- Inter group comparism, p1- Intra group comparism. * Comparism between treated groups and control, [a] comparism between refined oil and olive oil. ¥ comparism between baseline and month two. β comparism between baseline and month four and ŧ comparism between month two and month four.*

Table 4.4 (Cont.): Effect of Different Vegetable Oils on and Antioxidant Levels of S-D Rats before and after Treatment.

Parameter	Control	Refined	Olive	Red Palm	p-Value
Total Antioxidant Status (nM) (TAS)					
Baseline	0.32±0.03	0.32±0.03	0.34±0.03	0.31±0.01	0.8817
Month 2	0.25±0.05	0.30±0.03	0.39±0.02*	0.37±0.02	0.0196
Month 4	0.18±0.05	0.21±0.0 ββ†	0.20±0.02 ββ†††	0.19±0.1 βββ†††	0.9266
p1-Value	0.1249	0.0055	0.0001	<0.0001	

*Data is presented as mean ± SEM. p is significant at < 0.05. p- Inter group comparism, p1- Intra group comparism. * Comparism between treated groups and control, [a] comparism between refined oil and olive oil. ¥ comparism between baseline and month two. β comparism between baseline and month four and † comparism between month two and month four.*

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Background

The choice of healthy dietary fat has become a crucial subject because of the association with several disorders. The continuous modernization and technological advancement of the developing world has brought rapid lifestyle changes which has led to the consumption of high fat diets, fast-food, caloric-dense diets coupled with sedentary lifestyle, which are known to have a major impact on the development of cardiovascular and other chronic diseases (Ighosotu and Tonukari, 2010).

Many investigators have pointed out that excessive intake of dietary cholesterol increases the serum cholesterol, thus leading to a high risk of cardiovascular diseases (Liu and Yeh, 2000). All saturated fats, whether animal or vegetable in origin, have been discredited (Ramirez-Tortosa *et al.,* 1999).

Most Ghanaian meals are mainly prepared using red palm oil (RdPO) and processed / refined palm oil (RfPO) or palm olein. Refined palm oil has over the years become

one of the most frequently and commonly used vegetable oils. Whereas the effect of the RfPO on lipid profile and risk of atherosclerosis is not known, information about the red palm oil in this regard is highly controversial which clued-up the choice of these oils for the current study. The inclusion of the Olive oil (OO) was as a result of current recognition of its benefits in the Ghanaian society, making it very popular in spite of its exceptional high cost. The olive oil was also to serve as a second control since results (from studies involving olive oil) are consistent. The present study therefore investigated the effect of the intake of these three important vegetable oils in relation to atherosclerosis, focusing on the lipid hypothesis and the major mechanisms (inflammatory/ oxidative stress) of the disease.

5.2 Effect of the Different Oils on Weight of Rats

The data on the mean body weight indicated accelerated growth in rats for both experimental groups (on 6% oil fortified diets) and the control group at the end of month 2 and 4 (fig 4.1). The amount of food consumed by animals greatly influence growth responses (Fashakin and Unokiwedi, 1993; Edem *et al.*, 2003). When moderate amounts of fat are added to the diet, food consumption is more frequently increased than depressed and growth performance is improved (Lim *et al.*, 2001; Pereira *et al.*, 1990b). Lim, *et al.* (2001) observed that growth performance of African catfish Clarias gariepinus responded significantly in a positive manner to

palm oil additions of up to 8 % and no further improvement in growth was observed with higher levels of palm oil in the diet. It was observed that food intake seems depressed when high amounts of fat are added to the diet (Edem, 2009).

In the present study, the body weights of the rats on the palm oil diets (RdPO and RfPO) at the end of the 4 months showed a higher increase than those in the olive oil and control groups. The OO group showed the lowest mean weight. The increase in mean body weight of rats in the palm oil groups could be attributed to the high total body fat content in the groups fed different palm oil diets in comparison with those on the olive oil and control diets.

This may be the result of higher intake of the RfPO due to it palatability and odorless nature compared to the RdPO and the OO which may have led to a relatively higher accumulation of fat and more weight gain and probably an alteration in lipid metabolism. The OO is noted for its distinct strong odor, this may probably have led to the lower intake. This is consistent with the work carried out by Oluba *et al.* (2008) in which rats that were fed with palm oil enriched diet had a significantly higher body weight. These findings are similar to that of Hamid *et al.* (2010) who reported an increase in the body weight of rats fed palm olein (Dauqan *et al.*, 2011b; Oluba *et al*., 2008). On the other hand, Sundram and co observed the contrary. That study found insignificant difference in body weight of rats fed on palm oil, palm

olein and palm stearin (Sundram *et al.*, 1990b; Karaji-Bani *et al.*, 2006). This may be attributed to difference in study conditions.

5.3 Effect of the Different Oils on Biomarkers in Rats

5.3.1 Effect of the Different Oils on Lipid Profile

After treatment with the different oils (6%) the various changes in the lipid parameters observed in the present study did not show statistical significance, however these changes may be of important biological implications. The experimental diets (olive oil, red palm oil and refined palm oil) did not significantly alter the lipid parameters measured: comprising TC, LDL-C, OxLDL-C, Apo-A and B (Table 4.3). High TC, LDL-C, OxLDL-C and increased levels of Apo-B increased the risk of heart disease and stroke while high levels of HDL-C and Apo-A1 reduce the risk of cardiovascular disease (Birtcher and Ballantyne, 2004, Owolabi *et al*., 2010).

Apo-B and Apo-AI are thought to be better predictors of CHD risk than TC and LDL-C (Walldius *et al.*, 2001). Apo-AI also acts as a cofactor for lecithin cholesterol acyltransferase (LCAT), (Phillips *et al*., 1998) which is an important enzyme involved in removing excess cholesterol from tissues and incorporating into HDL for

reverse cholesterol transport to the liver (Betteridge, 1999). Apo B is synthesized in the liver and is present in LDL, IDL and VLDL particles (Betteridge, 1999), and therefore the total Apo-B concentration indicates the amount of potentially atherogenic lipoproteins in plasma or liver (Genest *et al.*, 1992).

The variations in total cholesterol levels of rats fed the various experimental diets in this study were not suggestive of an atherogenic potential. Results from this study showed no abnormal impact of saturated fatty acids in the blood of the rats: the two palm oil diets used in the present study showed neither hyper or hypo cholestrolemic effect compared to the olive oil diet and the control diet.

Palmitic acid which is known to be the dominant saturated fatty acid present in RdPO did not increase the TC levels significantly in the blood of the RdPO fed group when compared with the control group and the olive oil group. This partly may be due to the cholesterol free nature of the experimental diets. These findings were similar to that of Kamisah *et al.* (2005), which reported that after treatment with 18% fortified diet, the TC levels were unaffected in plasma for rat that were fed different types of palm oil at three different times 4, 8 and 12 weeks (Kamisah *et al.*, 2005).

This result is also in agreement with that of other studies (Khosla and Hayes, 1993; Hayes and Khosla, 2007), which pointed out that, relative to oleic acid, palmitic acid

in palm oil is not hypercholesterolemic in a cholesterol-free diet (Hayes and Khosla, 1992).

The findings are further supported by those of Kritchevsky (Kritchevsky, 2001; Kritchevsky *et al.*, 2002) which revealed that, with the cholesterol free diets there were virtually no difference in the effect of palm oil, corn oil and the mixtures of both on total cholesterol. In another study refined palm oil (palm olein) was demonstrated to have a neutral effect on plasma cholesterol concentration when compared with olive oil (Ng *et al.*, 1992) which was confirmed later in an Australian study (Choudhury *et al.*, 1995).

Moreover, the plasma total cholesterol concentrations in rats fed refined palm oil based diets in the present study were not significantly different from those fed red palm oil diets. From the foregoing, refined palm olein and red palm oil showed no adverse effects on cholesteroleamia over the study period (16 weeks). This may be due to the fact that the main hypercholesterolemic fatty acid in palm oil (both red palm and refined palm) viz. palmitic (C16:0) is mostly in the sn-1, 3 configuration (Edem, 2009), with only 17% in the sn-2 position (Renaud *et al.*, 1995). This is because, it is usually the fatty acids in the sn-2 position (preferentially absorbed) which are able to influence lipaedemia since those in the sn-1, 3 position are normally released in the intestinal tract and partly excreted in the faeces (Aoyama *et al.*, 1996; Amr, 2010).

Furthermore, the similarity in TC values of RfPO fed rats when compared with those of RdPO fed rats is in line with the observations by Khor and Tan (1992) who fed palm oil diets to hamsters.

Again, not only were the plasma total cholesterol levels unaltered but also the apo - lipoprotein levels remained not significantly changed after 16 weeks of treatment with the palm oil diets. Many other studies have demonstrated that when palm oil was used to replace a major part of other fats in a traditional diet, it does not increase serum cholesterol or affect HDL-C (Zhang *et al.,* 1997). Red palm oil (14% diet) was reported not to elevate TC in rabbits fed low cholesterol diet (0.1%), but increased the TC level when the animals were fed a higher percentage of cholesterol (0.2%), compared to refined, bleached and deodorized (RBD) palm oil (Kritchevsky *et al*., 2002). The study also showed that, the red palm oil-fed rabbits had a significantly lower severity of thoracic aorta atherosclerosis compared to the RBD-palm oil group.

Furthermore, the LDL-C and OxLDL-C values obtained like that of TC, were comparable after 16 weeks of feeding with the experimental diet in the current study. There was no significant difference at the eight week and sixteenth week for the two palm oil groups compared to the controls as well as the olive oil group (Table 4.3). A possible explanation may be the potential of the red palm oil to stimulate the

synthesis of protective HDL-C and suppression of harmful LDL-C (Mukherjee and Mitra, 2009).

The presence of high amounts of vitamin E (tocopherols and tocotrienols) as well as high vitamin A (carotenoid) content (300 times higher than tomatoes for equivalent amounts), which are very potent antioxidants in red palm oil coupled with 45% oleic acid may also be a contributing factor in making this oil present as a balanced oil (Jacques, 2005). This result however disagrees with recent findings reported by Dauqan et al. (2011), which observed that rats which received the 15% RPO had higher LDL-C at the 8th week than the control group. Benson et al. (2008) made a similar observation when they reported an increase in the non-HDL-C levels in the PO-treated group, which was supported by an earlier study involving normal and stressed rats (Benson and Devi, 2009).

According to literature, intake of saturated fatty acids (SFA) increased TC, LDL-C and Apo-B while PUFA (rich in Olive oil) in fats decreased these values (Hayes *et al.*, 2007; Hayes and Khosla, 1992). Palm oil (PO), a SFA dietary oil, contains 40% of palmitic acid (C16:0) and only 0.2% lauric acid (Benson and Devi, 2009; Edem *et al.*, 2002). Both lauric and palmitic acids have been found to be hypercholesterolemic (Edem, 2002).

Palmitic acid is believed to increase the cholesterol ester transport protein activity, which is responsible for the transfer of cholesterol from HDL to LDL hence, decreasing the HDL-C and Apo-A1 concentration combined with an increase in LDL-C and apo-B1. Also, palmitic acid is metabolized to palmitoleic acid, leading to reduction in HDL-C (Schwab *et al*., 1996). PO is therefore suspected to possess a hypercholesterolemic effect due to the high palmitic acid content (Denke and Grundy, 1992). On the other hand, results from other investigators have been in contrast to the afore-mentioned. Red palm oil supplementations have been reported by some researcher to rather have beneficial effects on serum total cholesterol despite its high saturated fat content (Kruger *et al.*, 2007).

Dauqan *et al.* (2011) showed that PO rather has a decreasing effect on blood cholesterol: in their study they found that there was a decrease in TC after 8 weeks of feeding rats with different concentrations of RdPO even though there was no effect after 2 and 4 weeks of treatment. The TC in 5%, 10% and 15% RPO treated rat blood was significantly lower than the control group after 8 weeks of treatment (Dauqan *et al.*, 2011a).

A similar observation was made by Jaarin *et al.* (2002) who also observed a reduction in total cholesterol by the red palm oil (Jaarin *et al.*, 2002). This (the hypolipideamic effect of RPO) could to some extent be attributed the high content of antioxidant including β-carotene and vitamin E (tocopherol and tocotrienol) in the

RPO (Qureshi *et al.*, 1991; Sugano and Imaizumi, 1991). Tocotrienols have been shown to inhibit cholesterol biosynthesis and reduce plasma cholesterol levels (Ebong *et al.*, 1999).

Controversy though exists as to the exact effect of palm oil on blood lipids, nonetheless, careful consideration of results from the present study in comparison to previous work seem to divulge that the effect of the palm oil on blood cholesterol probably depends strongly on the period of treatment as well as the level of fortification (% of fat used).

Edem (2009) seem to have come to a similar realization when he concluded after his investigation that, rats can tolerate 20% palm oil in their diets without adverse effect on blood lipid parameters after discovering that biochemical parameters in addition to liver histology were not adversely affected by the inclusion of palm oil at 10 – 20% levels of rat diets (Edem, 2009). On the whole further investigations are warranted with regards to palm oil in terms of period of use, cumulative effect of usage, appropriate level of fortification.

5.3.2 Effect of the Different Oils on Inflammatory Process in Rats

In view of the persuasive evidence implicating inflammation in atherothrombosis, several investigators have examined a variety of circulating markers of inflammation

to predict either the presence of vascular disease or the risk of vascular events (Ridker, 1999).

In this study, three of these important markers were included (IL-6, IL-2 and TNF-α) in the determination of the impact of the oils on risk of atherosclerosis. These among other important proinflammatory cytokines have served as inflammatory markers for evaluation of atherosclerotic risk (Larsson *et al.*, 2005; Tzoulaki *et al.*, 2006). The results showed no significant effect of intake of the different oils on IL-2 mean concentration.

However, there were significant differences in the IL-6 and TNF-α concentration for the various groups. Unlike the control and the olive oil groups where IL-6 levels decreased from baseline through to week 16, the two palm oil diets caused an increase in the IL-6 level from week 8 to week 16. However these were found to be statistically insignificant even though at the end of the first 8 weeks there was a significant decrease in the IL-6 levels for the RfPO group. The results agree partially with that of a recent study which looked at effect of palm oil on inflammation. The study was designed to test the impact of oil composition of high-fat diets on endotoxin metabolism and inflammation in mice (Laugerette *et al.*, 2012). After 8 wk of feeding it was shown in vivo that the palm oil-enriched diet induced higher IL-6 than other diets, including the control diet.

For the measurement of the TNF-α, both the refined and the red palm oil diet caused an increase in the mean concentration, compared to the intake of the olive oil diet which caused a decrease in the TNF-α mean concentration in the rats.

The above findings is in agreement with results from a previous study by EL Wakf *et al*. (2010) which was aimed at determining changes in inflammatory markers, lipid profile and vascular wall integrity, in male rats. An elevation in serum level of the two inflammatory markers measured, TNF-α and fibrinogen was demonstrated, where administration of olive oil to atherogenic rats exhibited ameliorative effect on the inflammatory status (EL Wakf *et al.*, 2010).

The present study also found an improvement in the inflammatory status of the rats by the intake of the olive oil whilst the consumption of the refined palm oil and the red palm oil rather worsened it. This may be due to the phenolic compounds (PhCs) such as oleuropein glycoside, caffiec acid and tyrosol found in OO which are known to generate anti-inflammatory effects, through inhibiting a number of inflammatory mediators released by endothelial cells (Cicerale *et al.*, 2012).

Moreover, evidence from human and animal studies (Miles *et al.*, 2005) have also proven the existence of a strong effect of oleuropein glycocide on production of cytokines including TNF-α. Also, monocyte adhesion to endothelial cells (ECs) can be modulated by OO- PhCs, through inhibiting mRNA expression of several

adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1) (Carluccio *et al.,* 2003). Red palm oil has also been found to contain some amount of phenolic compounds among other anti-oxidants (Atawodi *et al.,* 2011). This may account for the minimal increase in the TNF-α concentration observed compared to the refined palm oil which caused a much higher increase probably due to its deficiency in PhCs thereby increasing its inflammatory tendencies. This is in accordance with results from the study by Martinez-Dominguez *et al (*2001) where the inflammation indices of animals fed on a diet rich in olive oil were lower compared to animals fed with oils high in palm olein. They concluded in their study that olive oil with a higher content of polyphenolic compounds, shows protective effects in inflammation (Martinez-Dominguez *et al.*, 2001). The inflammatory effect of these oils observed in this (present) study is important because of the strong association of TNF-α production with atherogenesis (Libby and Ridker, 1999).

Current literature points out that pro-inflammatory cytokines, including TNF-α, are important in initiating the expression of variety of genes that in turn promote cell adhesion and other processes required for progression of atherosclerosis (Zhang *et al*., 2007). According to Cesari *et al.* (2003) TNF-α has been associated with an increased risk of developing CHD evident in a recent study observation where feeding rats on atherogenic diet induced marked elevation in several inflammatory markers, including total leucocytes, monocytes and lymphocytes, as well as serum level of the pro-inflammatory and pro-atherogenic TNF-α (Cesari *et al.*, 2003a).

In other studies, the interactions between inflammatory mediators including TNF-α promoted smooth muscle cells (SMCs) migration and proliferation. This in turn led to atheroma plaque rupture with subsequent adherence and aggregation of platelets, eventually leading to progression of atherosclerotic injury (Cesari *et al.*, 2003b).

Moreover, a recent review by (Popa *et al.*, 2007), showed further that, among many inflammatory markers, tumor necrosis factor-α (TNF-α) emerged as a key cytokine that influences intermediary metabolism. It has also been discovered that, "in rodents, TNF-α may increase hepatic cholesterol synthesis by stimulating the activity of 5-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) (the rate-limiting enzyme in the cholesterol biosynthetic pathway) (Memon *et al.*, 1993). Despite a marked increase in HMG-CoA reductase activity, TNF-α produces only a modest increase in hepatic cholesterol synthesis and circulating cholesterol concentrations.

This is attributable to an inhibitory effect of TNF-α on the production and activity of squalene synthase (the first major enzyme in cholesterol synthesis) located in the mevalonate pathway (Memon *et al.*, 1997). The enzyme plays an important role in regulating the flux of metabolic intermediates to the sterol pathways. Thus, the effects of TNF-α on the cholesterol biosynthetic pathway are likely to maintain an adequate cholesterol synthesis while redirecting a proportion of the mevalonate metabolites into the nonsteroidal pathways.

Furthermore it is now evident that, whereas the administration of TNF-α in rodents is followed by a delayed increase in serum concentrations of TC and hepatic cholesterol synthesis (Feingold *et al.*, 1998) nonhuman primates and humans show either no change or a decrease in serum cholesterol and LDL-cholesterol levels (Grunfeld and Feingold, 1991b).

The mechanisms underlying this species difference are not known. In primates, TNF-α is indicated to decrease HDL concentrations (Grunfeld *et al.*, 1991). In addition, the composition of lipoproteins can be altered upon the action of TNF-α since the mechanisms through which TNF-α exerts its effects on cholesterol metabolism are complex and takes place at different levels, including the hepatocytes and peripheral cells, such as endothelial cells (Grunfeld and Feingold, 1991b; Grunfeld and Feingold, 1991a).

This, to some extent could be a contributing factor to the neutral effect observed in the lipid profile of the rats in the present study. The increase in the rats TNF-α concentration caused by the two palm oil diet may have probably lead to the delayed change in the lipoproteins and the total cholesterol concentrations (Xu *et al.*, 2011).

5.3.3 Effect of the Different Oils on Antioxidant Status of Rats

Antioxidants have also been implicated to play a vital protective role in the atherogenic process. High antioxidant concentrations have been hypothesized to be protective against atherogenesis (Rimm and Stampfer, 2001). In several epidemiological and clinical studies the preventive potential of antioxidants on coronary heart disease (CHD) has been investigated. A preventive effect of dietary and supplementary vitamin E against CHD has been described in prospective studies (Rimm and Colditz, 1993). Dietary antioxidants include vitamin E, vitamin C and β-carotene (Kushi *et al.*, 1996) are believed to be abundant in some vegetable fats and hence protect against atherogenesis. Both palm oil and olive oil have been shown to have high antioxidant properties.

The present study showed that the olive oil diet significantly increased the mean TAS of the rats relative to the control at week 8, whilst the palm oil diets showed no significant effect. This was consistent with a current study (Ayeleso *et al.*, 2012) which was carried out to evaluate the antioxidant status in male rats following the dietary consumption of red palm oil. It was reported that no significant increase in the TAS in plasma, for the red palm oil group when compared to the control.

The result also agrees with the study by Dauqan *et al.* (2012) which looked at effect of different concentrations of red palm olein and different vegetable oils on

antioxidant enzymes in liver of normal and stressed SD rats (with similar sample size n=6 per group). At both week 4 and 8 there were no significance differences in antioxidant levels (catalase and superoxide dismutase) between the control group and different concentrations groups (5%, 10%, and 15%) of RPO normal rats (Dauqan *et al.*, 2012, Dauqan *et al.*, 2011b).

The significant increase in the TAS observed in the olive oil group may be due to the contribution of the additional antioxidant compounds found in the olive oil aside the polyphenols. These include: Phytosterols (β-Sitosterol, δ-Avenasterol, Campesterol), Tocopherols (α-Tocopherol β-Tocopherol, γ-Tocopherol) and Hydrocarbons (Squalene, Carotene). Moreover, the lower susceptibility of its MUFA content to scavenge free radicals is believed to reduce oxidative stress (Carluccio *et al.*, 2007).

On the other hand the lower antioxidant level observed in the refined palm oil group compared to the olive oil group may be attributed to its lower antioxidant potential. Current literature has shown that, palm olein contains relatively lower amounts of tocopherol and phenolic compounds, than the red palm oil (Ong and Goh, 2002, Amr, 2010, Dauqan *et al.*, 2011a). Additionally, a substantial amount of carotene which gives the red color may be lost during refinery resulting in the light golden color characterizing the palm olein, further reducing its antioxidant capacity.

In the present study a decrease in the antioxidant capacity was recorded among the control rats and those fed with refined palm oil unlike rats fed with olive oil and those fed with red palm oil which had increased TAS. The increase in the total antioxidant level by the red palm oil diet observed at week 8 was in agreement with results of a recent study which examined the effects of palm oil tocotrienol-rich fractions (TRF) on rats, which demonstrated that 8 weeks of supplementation with TRF increased the antioxidant levels (vitamin C and SOD) suggesting that, TRF may improve the antioxidant defense mechanism by decreasing free radical levels and thereby reducing the oxidative damage that occurs in certain disease conditions such as diabetes and atherosclerosis (Budin *et al.*, 2009).

The present study result is further supported by those of a previous study where tocotrienols from palm oil were found to act as potent inhibitors of lipid peroxidation and protein oxidation (Kamat and Devasagayam, 1995).

This may be attributed to its unique antioxidant composition of the red palm oil. A current review by Oguntibeju *et al.* (2009) discussed the possible role of red palm oil in reducing oxidative stress and thus proposed that red palm oil supplementation could sufficiently scavenge free radicals, increase total antioxidant capacity with the potential to reduce disease progression and complications hence alter the prevalence of disease (Oguntibeju *et al.*, 2009).

In another review, Oguntibeju *et al.* (2010) again highlighted similar results from other studies. Many of the studies illustrated that red palm oil contains many beneficial antioxidants/ micronutrient compounds such as tocopherol, tocotrienol, lycopene, squalene, Co-enzyme Q10, physterol, glycolipids, phosphatides, calcium, phosphorus, iron, riboflavin, chlorophyll, xanthophil, flavonoids, phospholipids and carotenoid (Ebong *et al.*, 1999, Edem *et al.*, 2002, Sundram *et al.*, 2003). Some feeding experiments using various animal models also confirmed the protective effects of red palm oil in reducing oxidative stress (Esterhuyse *et al.*, 2005a, 2005b; Kruger *et al.*, 2007) in rats.

Another contributing factor may be the combined effect of the presence of the antioxidants (carotenoid, tocopherols, tocotrienols) and 50% unstauration of the fatty acids (Kamisah *et al.*, 2006), especially its higher vitamin E levels (tocopherol level~ 650ppm and~ 500ppm of tocotrienols) (Bascetta *et al.*, 1983). Tocopherols are considered a major antioxidant in biomembranes, and are considered as general antioxidants for protection of membrane stability, including quenching or scavenging ROS. Out of four isomers of tocopherols (α, β, δ, $_{\gamma}$) found in plants, α-tocopherol has the highest antioxidative activity due to the presence of three methyl groups in its molecular structure (Sarvajeet and Narendra, 2010).

Also, unlike most vegetable oils, palm oil is one of the richest sources of α- and β-carotenes (400-3500 mg/kg), which produce the deep red color and constitute (700-

800ppm) which is more than 80% of the total carotenoids in palm oil (Ming *et al.*, 2009). Palm oil is semi-solid at room temperature; a characteristic brought about by its 50% saturation level (Dauqan *et al.*, 2011a) and has fatty acid composition with low polyunsaturation which contributes further to its superior oxidative stability.

Nevertheless, comparing the effect of the oils on TNF-α and TAS simultaneously, it can be seen that there was a steep rise in the TNF-α for the refined palm oil group up to the second month, and a gradual increase from the second month to the fourth month. On the other hand, there was a gradual fall in TAS from baseline to the second month and a steep fall between the second and fourth month. To some extent, the trends observed in the TNF-α and TAS mirrors each other. For the olive oil group, the mean TNF-α concentration decreased and continued decreasing to the fourth month, whilst the TAS increased at the second month, but between month 2 and 4 there was an unexpected sharp fall in the mean concentration.

In the case of the red palm oil group, there was a gradual rise in TNF-α and a fall in the TAS of the rats. If the rise in the TNF-α was due to inflammation, then one would expect a fall in the TAS in the red palm oil group at second month. However this was not the case. Also between second and fourth month, there was a fall in the TAS for both red palm oil and olive oil groups when rather the fall was expected from only the red palm oil group. This observation is not fully understood and warrants further investigations to establish clarity. Probably other factors may be

contributing to this effect. Other researchers have reported some inconsistencies in the determination of antioxidant activity in relation to fats. The reason for these discrepancies is not clear however some investigators have suggested that different experimental period might lead to different result about the effect of dietary vitamin E (high in palm oil) on the activities of antioxidant enzymes (Fu *et al*., 2007). Fu *et al*. (2007) also mentioned that, although few studies explicitly show the effects of vitamin E on the activities of antioxidant enzymes, there is no consensus on what might be the responses of antioxidant enzymes to vitamin E, partly because of different feeding behavior and other ecological conditions.

Furthermore, in a recent study by Olisekodiaka *et al*. (2012), it was observed that, total antioxidant measurement although known to be the best method for comprehensive assessment of oxidative stress in biological systems, has been associated with some discrepancies. Therefore in order to consolidate the findings on TAS in the present study, additional studies need to be undertaken (Olisekodiaka *et al*., 2012).

5.4 Effect of the Oils on Atherosclerotic Risk

Current research has shown that extensive use of olive oil by people in the Mediterranean region has been associated with reduced incidence of CVD in these

people (Covas, 2007a; Perona *et al*., 2004, 2007). This is believed to be as a result of the high content of MUFAs mainly oleic acid and the biologically active components, such as the phenolic compounds (PhCs) (Miles *et al.*, 2005). Additionally, olive oil rich diets have been acclaimed for their protective effects on several changes associated with atherosclerosis and heart disease. This was demonstrated by Acın *et al.* (2007) in a study where feeding rats on atherogenic diet supplemented with Virgin Olive Oil showed lowered atherogenic hazards (Acin *et al.*, 2007).

EL Wakf *et al.*(2010) further showed that, nutrients including MUFAs may modulate atherosclerosis by affecting vascular endothelium (Covas, 2007b), through increasing the amount of oleic acid in the arterial wall and displacing saturated fatty acids (SFAs), while leaving polyunsaturated fatty acid PUFAs. Thus, oleic acid may contribute to the prevention of atherosclerosis, mainly through a protective effect against atherogenic vascular wall lesions (Perona *et al*., 2006).

The vast knowledge that exists from epidemiological, clinical, experimental animal models and in vitro studies have also indicated that olive oil can be regarded as functional food for its anti-atherogenic properties and that diets enriched with olive oil prevent the development and progression of atherosclerosis and may also play an important role in the regression of the disease (Aguilera *et al*., 2002, Mangiapane *et al.*, 1999).

Carluccio *et al*. (2007) summarized the mechanisms by which olive oil affects the development of atherosclerosis as follows: (1) regulation of cholesterol levels as olive oil decreases LDL-C and increases HDL cholesterol; (2) decreased susceptibility of human LDL-C to oxidation, because of the lower susceptibility of its MUFAs content and to the ability of its polyphenol fraction to scavenge free radicals and reduce oxidative stress; (3) both, oleic acid and olive oil antioxidant polyphenols inhibit endothelial activation and monocyte recruitment during early atherogenesis; (4) decreased macrophage production of inflammatory cytokines, eicosanoid inflammatory mediators derived from arachidonic acids and increased nitric oxide (NO) production, which improves vascular stability; (5) decreased macrophages matrixmetalloproteinases (MMPs) production, which improves plaque stability; (6) oleic acid and olive oil polyphenols are associated with a reduced risk of hypertension; (7) oleic acid and olive oil polyphenols also affect blood coagulation and fibrinolytic factors, thereby reducing the risk of acute thrombotic cardiovascular events (Carluccio *et al.*, 2007).

Moreover, it is evident from literature that, compared to red palm oil; olive oil is richer in polyphenols which strengthens the anti-inflammatory capacity. These anti-inflammatory compounds include at least nine different categories of polyphenols and several other well-researched anti-inflammatory nutrients. There have also been suggestions that, diets rich in olive oil may have anti-inflammatory properties of polyphenols contained in olive oil.

A polyphenol of olive oil, 2-(3, 4-dihydroxyphenil) ethanol (DPE), which possesses both anti-inflammatory and anti-clotting activities, has been shown to reduce the tumor necrosis factor alpha–induced activation of the inflammatory pathway in an animal model (Terzuoli *et al.*, 2010). Research (Castaner *et al*., 2011; Patrick and Uzick, 2001; Bogani *et al*., 2007) has documented a wide variety of anti-inflammatory mechanisms used by olive oil polyphenols to lower the risk of inflammatory problems. These mechanisms include decreased production of messaging molecules that would otherwise increase inflammation (including TNF-α, IL-1b, thromboxane B2, and leukotriene B4); inhibition of pro-inflammatory enzymes like cyclo-oxygenase 1 and cyclo-oxygenase 2; and decreased synthesis of the enzyme inducible nitric oxide synthase. They have also been found to reduce activity in a metabolic pathway called the arachidonic acid pathway, which is central for mobilizing inflammatory processes (Lucas *et al.*, 2011).

Based on results from present study, the above verity has been confirmed, it can be inferred that the olive oil is a better choice of oil than the red palm oil and the refined palm oil. The combined protective effect of the high antioxidant property and the capacity to reduce inflammation may lead to significant reduction in atherosclerotic risk. Therefore in spite of its high cost, it should be encouraged as preferred oil for cooking in view of its health benefits.

Regarding the red palm oil, even though it is cheaper and seems to have a higher antioxidant capacity than the refined palm oil (due to the higher content of carotene and vitamin E and its derivatives), the relatively lower levels of phenolic compounds it contains compared to olive oil reduces its anti-inflammatory properties (Atawodi *et al*., 2011). Hence frequent consumption of high amount of the red palm oil may not be completely safe. Fortification with appropriate anti-inflammatory compounds may improve its quality. The same can also be achieved by blending with other suitable oils. This calls for more research in this area in order to come up with oils of higher quality.

The refined palm oil, as earlier mentioned, is rich in oleic acid (42.7% - 43.9%), and also contain some amount of β-carotene and vitamin E (tocopherols and tocotrienols) (Narang *et al*., 2004). In addition, it is widely used in cooking and locally regarded as a gold standard for frying. However, as revealed by the present study, the chronic intake of this oil may lead to increased risk of atherosclerosis and therefore turns out to be the worse of the three oils examined.

A major contributing factor to this fact is the lack of adequate anti-inflammatory constituents. Although one would have thought that being refined this processed state would be of a higher quality than the unbleached, yet research shows that, the attempt to refine in most cases rather deprives the product of certain essential inherent components. Some protective components end up being lost or removed during the refinery process. In the case of the FPO most of the β-carotene gets

destroyed during processing (Edem and Akpanabiatu, 2006), unlike the red palm oil (RPO) which is obtained before refining. The characteristic red color of RPO is due to the abundance of carotenoids in the crude oil (Edem, 2009).

Similarly research has shown that Extra virgin olive oil (un refined or crude) is of a higher quality than the virgin olive oil (partially refined) and this is also found to be better than the refined olive oil (Salvini et al., 2006, EL Wakf et al., 2010) signifying that refinery may not always be the best option for health reasons.

5.5 Limitations

In attempt to model what pertains to human in a normal or natural situation, the present study doubled the recommended amount of fat for rats in the basal diet, achieving a total of 10% fat as treatment diet. Retrospectively, an increase in the dose could probably have yielded even better results.

Also, the design did not allow the same set of rats to be studied over the period; this could have reduced or minimized the influence of individual variations and genetic differences of the rats.

The study used the exact sample size required to power the study, in retrospect, this number should have been slightly increased to make room for unpredictable loss of samples.

The rats were divided into groups of four and five per cage, depending on the cage size and a non iso-caloric diet was adopted for the rats with reference to design of similar studies. However looking back, more interesting outcomes probably may have resulted if caloric content were adjusted for in the provision of iso-coloric diet, which becomes more relevant when the animals are housed or caged individually.

Blood sample collection by milking method (clipping the distal tail) employed at baseline has a tendency to decrease quality with prolonged bleeding times and "milking" of the tail.

Blood samples collected could not be analyzed immediately due to unexpected breakdown of equipment, and delay in acquisition of reagents which lead to storage of samples. Earlier analysis could have provided preliminary data to better appreciate the direction of the study.

The duration of the experiment in relation to life expectancy of the rats compared to time required for development of atherogenesis in humans could also be a limitation. Probably the use of much older rats could be beneficial since atherosclerosis is associated with increasing age.

5.6 Conclusions

In conclusion, the results suggest that, the 3 different oils had different effects on the parameters investigated in this study. At 6% fortification level, the effect of the oils on Lipid profile was not statistically significant.

Consumption of red palm and refined palm oil both may adversely affect the inflammatory process in atherosclerosis by increasing the production of the pro-inflammatory markers TNF-α and IL-6 in rats and also reducing TAS levels compared to the olive oil and the control. However, the former showed a greater (negative) effect than the later in spite of it refined status.

On the contrary, the intake of the Olive oil as a major source of fat improved both the antioxidant status as well as the inflammatory process significantly by increasing the TAS capacity whilst decreasing the TNF-α and IL-6 production there by reducing the risk of developing atherosclerosis in the rats. On the whole, under the given conditions of the study, the olive oil stood out as the best oil followed by the red palm oil and the refined palm oil respectively.

5.7 Recommendation

Future studies could look at different and higher doses of oil (10%, 15%, and 20%) for comparison over a longer period of study (not less than 12 months), bearing in mind the safety and life span of the laboratory rat. This may provide a wider view and give a broader picture of the impact of the oils.

Additionally other important frequently used cooking oils like the coconut oil, palm kernel oil, shea butter oil etc need to be examined to explore their health benefits.

Further research aimed at exploring the use of TNF-α as a therapeutic target in atherosclerosis is warranted. Moreover additional research may also be required to find other causes that may lead to rise in TNF-α other than inflammation.

It may be necessary to modify the project design by considering using the initial set of animals throughout the study period (ie. avoiding the periodic sampling: in which case method of blood collection may need to be carefully selected), in comparison to the present design.

APPENDIX A

REFERENCES

ABDELLATIF, A. M., STARRENBURG, A. & VLES, R. O. 1972. Effects of hardened palm oil and protein on the pathological and hematological characteristics of ducklings fed rapeseed oil. Nutr Metab, 14**,** 17-27.

ABRAHAM, R., KUMAR, N. S., KUMAR, G. S., SUDHAKARAN, P. R. & KURUP, P. A. 1994. Dietary carbohydrates and synthesis and secretion of apolipoprotein B-containing lipoproteins in rat hepatocytes. Nutrition, 10**,** 138-43.

ABUBAKARI, A. R. & BHOPAL, R. S. 2008. Systematic review on the prevalence of diabetes, overweight/obesity and physical inactivity in Ghanaians and Nigerians. Public Health, 122**,** 173-82.

ACIN, S., NAVARRO, M. A., PERONA, J. S., ARBONES-MAINAR, J. M., SURRA, J. C., GUZMAN, M. A., CARNICER, R., ARNAL, C., ORMAN, I., SEGOVIA, J. C., OSADA, J. & RUIZ-GUTIERREZ, V. 2007. Olive oil preparation determines the atherosclerotic protection in apolipoprotein E knockout mice. J Nutr Biochem, 18**,** 418-24.

ADAMS, M. R., ANTHONY, M. S., CHEN, H. & CLARKSON, T. B. 2008. Replacement of dietary soy protein isolate with concentrates of soy 7S or 11S globulin has minimal or no effects on plasma lipoprotein profiles and biomarkers of coronary risk in monkeys. Atherosclerosis, 196**,** 76-80.

AGGARWAL, A., BROWN, K. A. & LEWINTER, M. M. 2001. Diastolic dysfunction: pathophysiology, clinical features, and assessment with radionuclide methods. J Nucl Cardiol, 8**,** 98-106.

AGUILERA, C. M., RAMIREZ-TORTOSA, M. C., MESA, M. D. & GIL, A. 2001. [Protective effect of monounsaturated and polyunsaturated fatty acids on the development of cardiovascular disease]. Nutr Hosp, 16**,** 78-91.

AGUILERA, C. M., RAMIREZ-TORTOSA, M. C., MESA, M. D., RAMIREZ-TORTOSA, C. L. & GIL, A. 2002. Sunflower, virgin-olive and fish oils differentially affect the progression of aortic lesions in rabbits with experimental atherosclerosis. Atherosclerosis, 162**,** 335-44.

AGYEMANG, C., OWUSU-DABO, E., DE JONGE, A., MARTINS, D., OGEDEGBE, G. & STRONKS, K. 2009. Overweight and obesity among Ghanaian residents in The Netherlands: how do they weigh against their urban and rural counterparts in Ghana? Public Health Nutr, 12**,** 909-16.

AKIRA, S., HIRANO, T., TAGA, T. & KISHIMOTO, T. 1990a. Biology of multifunctional cytokines: IL 6 and related molecules (IL 1 and TNF). FASEB J, 4**,** 2860-7.

AKIRA, S., ISSHIKI, H., SUGITA, T., TANABE, O., KINOSHITA, S., NISHIO, Y., NAKAJIMA, T., HIRANO, T. & KISHIMOTO, T. 1990b. A nuclear factor for IL-6 expression (NF-IL6) is a member of a C/EBP family. EMBO J, 9**,** 1897-906.

AKPA, M. R., AGOMOUH, D. I. & ALASIA, D. D. 2006. Lipid profile of healthy adult Nigerians in Port Harcourt, Nigeria. Niger J Med, 15**,** 137-40.

ALAUPOVIC, P. 1996. Significance of apolipoproteins for structure, function, and classification of plasma lipoproteins. Methods Enzymol, 263**,** 32-60.

AMOAH, A. G. 2003. Hypertension in Ghana: a cross-sectional community prevalence study in greater Accra. Ethn Dis, 13**,** 310-5.

AMR, A. R., A.L. FATMA AND M.A. ABD-ELRAHMAN 2010. Effect of some dietary oils and fats on serum lipid profile; Calcium absorption and bone mineralization in Mice. Pak. J. Nutrition, 9 643-650.

ANDERSON, J. T., GRANDE, F. & KEYS, A. 1976. Independence of the effects of cholesterol and degree of saturation of the fat in the diet on serum cholesterol in man. Am J Clin Nutr, 29**,** 1184-9.

ANDRIKOPOULOS, N. K. 2002. Chromatographic and spectroscopic methods in the analysis of triacylglycerol species and regiospecific isomers of oils and fats. Crit Rev Food Sci Nutr, 42**,** 473-505.

AOYAMA, T., FUKUI, K., TANIGUCHI, K., NAGAOKA, S., YAMAOTO, T. & HASHIMOTO, Y. 1996. Absorption and metabolism of lipids in rats depend on fatty acid isomeric position. J Nutr, 126**,** 225-31.

ARGILES, J. M. & LOPEZ-SORIANO, F. J. 1998. Catabolic proinflammatory cytokines. Curr Opin Clin Nutr Metab Care, 1**,** 245-51.

ARSENAULT, B. J., RANA, J. S., STROES, E. S., DESPRES, J. P., SHAH, P. K., KASTELEIN, J. J., WAREHAM, N. J., BOEKHOLDT, S. M. & KHAW, K.

T. 2009. Beyond low-density lipoprotein cholesterol: respective contributions of non-high-density lipoprotein cholesterol levels, triglycerides, and the total cholesterol/high-density lipoprotein cholesterol ratio to coronary heart disease risk in apparently healthy men and women. J Am Coll Cardiol, 55**,** 35-41.

ASFAW, A. 2005. The effects of obesity on doctor-diagnosed chronic diseases in Africa: empirical results from Senegal and South Africa. . Journal of public health policy, 27**,** 250–264.

ATAWODI, S. E., YUSUFU, L. M. D., ASUKU, O., ATAWODI, S. E. & YAKUBU, O. E. 2011. Phenolic Compounds and Antioxidant Potential of Nigerian Red Palm Oil (Elaeis Guineensis). International Journal of Biology, 3 153-154.

AYELESO, A., OGUNTIBEJU, O. & BROOKS, N. 2012. Influence of dietary red palm oil on antioxidant status in male Wistar rats. BMC Complementary and Alternative Medicine 12**,** 50.

BANKA, C. L. 1996. High density lipoprotein and lipoprotein oxidation. Curr Opin Lipidol, 7**,** 139-42.

BARANOVA, I., VISHNYAKOVA, T., BOCHAROV, A., CHEN, Z., REMALEY, A. T., STONIK, J., EGGERMAN, T. L. & PATTERSON, A. P. 2002. Lipopolysaccharide down regulates both scavenger receptor B1 and ATP binding cassette transporter A1 in RAW cells. Infect Immun, 70**,** 2995-3003.

BARRETT-CONNOR, E. & BUSH, T. L. 1991. Estrogen and coronary heart disease in women. JAMA, 265**,** 1861-7.

BARTER, P. J., PURANIK, R. & RYE, K. A. 2007. New insights into the role of HDL as an anti-inflammatory agent in the prevention of cardiovascular disease. Curr Cardiol Rep, 9**,** 493-8.

BARTER, P. J. & RYE, K. A. 1996. High density lipoproteins and coronary heart disease. Atherosclerosis, 121**,** 1-12.

BASCETTA, E., GUNSTONE, F. D. & WALTON, J. C. 1983. Electron spin resonance study of the role of vitamin E and vitamin C in the inhibition of fatty acid oxidation in a model membrane. Chem Phys Lipids, 33**,** 207-10.

BAZZANO, L. A., HE, J., OGDEN, L. G., LORIA, C. M., VUPPUTURI, S., MYERS, L. & WHELTON, P. K. 2002. Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. Am J Clin Nutr, 76**,** 93-9.

BEAUCHAMP, G. K., KEAST, R. S., MOREL, D., LIN, J., PIKA, J., HAN, Q., LEE, C. H., SMITH, A. B. & BRESLIN, P. A. 2005. Phytochemistry: ibuprofen-like activity in extra-virgin olive oil. Nature, 437**,** 45-6.

BENDER, D. A. & BENDER, A. E. 2005. A dictionary of food and nutrition, Oxford U.K. ; New York, Oxford University Press.

BENSON, M. K. & DEVI, K. 2009. Influence of omega-6/omega-3 rich dietary oils on lipid profile and antioxidant enzymes in normal and stressed rats. Indian J Exp Biol, 47**,** 98-103.

BENSON, M. K., KSHAMA, D. & FATTEPUR, S. R. 2008. Studies on SFA/TFA (saturated/trans fatty acid) rich dietary fats on lipid profile and antioxidant enzymes in normal and stressed rats. Phcog Mag, 4**,** 320-8.

BERLINER, J. A., NAVAB, M., FOGELMAN, A. M., FRANK, J. S., DEMER, L. L., EDWARDS, P. A., WATSON, A. D. & LUSIS, A. J. 1995. Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics. Circulation, 91**,** 2488-96.

BERMUDEZ, B., LOPEZ, S., ORTEGA, A., VARELA, L. M., PACHECO, Y. M., ABIA, R. & MURIANA, F. J. 2011. Oleic acid in olive oil: from a metabolic framework toward a clinical perspective. Curr Pharm Des, 17**,** 831-43.

BESTER, D. J., KUPAI, K., CSONT, T., SZUCS, G., CSONKA, C., ESTERHUYSE, A. J., FERDINANDY, P. & VAN ROOYEN, J. 2010. Dietary red palm oil supplementation reduces myocardial infarct size in an isolated perfused rat heart model. Lipids Health Dis, 9**,** 64.

BETTERIDGE, D. J. 1999. Diabetic dyslipidaemia. Eur J Clin Invest, 29 Suppl 2**,** 12-6.

BEVILACQUA, M. P. & NELSON, R. M. 1993. Endothelial-leukocyte adhesion molecules in inflammation and metastasis. Thromb Haemost, 70**,** 152-4.

BIERMAN, E. L. 1992. George Lyman Duff Memorial Lecture. Atherogenesis in diabetes. Arterioscler Thromb, 12**,** 647-56.

BIRTCHER, K. K. & BALLANTYNE, C. M. 2004. Cardiology patient page. Measurement of cholesterol: a patient perspective. Circulation, 110**,** e296-7.

BIRTCHER, K. K., BOWDEN, C., BALLANTYNE, C. M. & HUYEN, M. 2000. Strategies for implementing lipid-lowering therapy: pharmacy-based approach. Am J Cardiol, 85**,** 30A-35A.

BLANKENHORN, D. H., JOHNSON, R. L., NESSIM, S. A., AZEN, S. P., SANMARCO, M. E. & SELZER, R. H. 1987. The Cholesterol Lowering Atherosclerosis Study (CLAS): design, methods, and baseline results. Control Clin Trials, 8**,** 356-87.

BOGANI, P., GALLI, C., VILLA, M. & VISIOLI, F. 2007. Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. Atherosclerosis, 190**,** 181-6.

BOLTON-SMITH, C., WOODWARD, M., SMITH, W. C. & TUNSTALL-PEDOE, H. 1991. Dietary and non-dietary predictors of serum total and HDL-cholesterol in men and women: results from the Scottish Heart Health Study. Int J Epidemiol, 20**,** 95-104.

BONANOME, A., BENNETT, M. & GRUNDY, S. M. 1992. Metabolic effects of dietary stearic acid in mice: changes in the fatty acid composition of triglycerides and phospholipids in various tissues. Atherosclerosis, 94**,** 119-27.

BONANOME, A. & GRUNDY, S. M. 1988. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. N Engl J Med, 318**,** 1244-8.

BONANOME, A. & GRUNDY, S. M. 1989. Intestinal absorption of stearic acid after consumption of high fat meals in humans. J Nutr, 119**,** 1556-60.

BONILLA, C., PANGULURI, R. K., TALIAFERRO-SMITH, L., ARGYROPOULOS, G., CHEN, G., ADEYEMO, A. A., AMOAH, A., OWUSU, S., ACHEAMPONG, J., AGYENIM-BOATENG, K., EGHAN, B. A., OLI, J., OKAFOR, G., ABBIYESUKU, F., JOHNSON, T., RUFUS, T., FASANMADE, O., CHEN, Y., COLLINS, F. S., DUNSTON, G. M., ROTIMI, C. & KITTLES, R. A. 2006. Agouti-related protein promoter variant associated with leanness and decreased risk for diabetes in West Africans. Int J Obes (Lond), 30**,** 715-21.

BONNIE, T. Y. P. & CHOO, Y. M. 1999 Valuable minor constituents of commercial red palm olein: carotenoids, vitamin E, ubiquinones and sterols. Proceedings of the 1999 PORIM International Palm Oil Congress Chemistry and Technology**,** 97-108.

BOREN, J., OLIN, K., LEE, I., CHAIT, A., WIGHT, T. N. & INNERARITY, T. L. 1998a. Identification of the principal proteoglycan-binding site in LDL. A single-point mutation in apo-B100 severely affects proteoglycan interaction without affecting LDL receptor binding. J Clin Invest, 101**,** 2658-64.

BOREN, J., VENIANT, M. M. & YOUNG, S. G. 1998b. Apo B100-containing lipoproteins are secreted by the heart. J Clin Invest, 101**,** 1197-202.

BORGMAN, R. F. & WARDLAW, F. B. 1975. Serum cholesterol concentrations and cholelithiasis in rabbits as influenced by the form of dietary fat. Am J Vet Res, 36**,** 93-5.

BOTS, M. L., BALDASSARRE, D., SIMON, A., DE GROOT, E., O'LEARY, D. H., RILEY, W., KASTELEIN, J. J. & GROBBEE, D. E. 2007. Carotid intima-media thickness and coronary atherosclerosis: weak or strong relations? Eur Heart J, 28**,** 398-406.

BOULAY, J. L., O'SHEA, J. J. & PAUL, W. E. 2003. Molecular phylogeny within type I cytokines and their cognate receptors. Immunity, 19**,** 159-63.

BOUSHEY, C. J., BERESFORD, S. A., OMENN, G. S. & MOTULSKY, A. G. 1995. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA, 274**,** 1049-57.

BRESLOW, J. L. 1997. Cardiovascular disease burden increases, NIH funding decreases. Nat Med, 3**,** 600-1.

BROUSSEAU, M. E., ORDOVAS, J. M., OSADA, J., FASULO, J., ROBINS, S. J., NICOLOSI, R. J. & SCHAEFER, E. J. 1995. Dietary monounsaturated and polyunsaturated fatty acids are comparable in their effects on hepatic apolipoprotein mRNA abundance and liver lipid concentrations when substituted for saturated fatty acids in cynomolgus monkeys. J Nutr, 125**,** 425-36.

BROWN, B. G., ZHAO, X. Q., CHAIT, A., FROHLICH, J., CHEUNG, M., HEISE, N., DOWDY, A., DEANGELIS, D., FISHER, L. D. & ALBERS, J. 1998. Lipid altering or antioxidant vitamins for patients with coronary disease and very low HDL cholesterol? The HDL-Atherosclerosis Treatment Study Design. Can J Cardiol, 14 Suppl A**,** 6A-13A.

BROWN, M. S. & GOLDSTEIN, J. L. 1986. A receptor-mediated pathway for cholesterol homeostasis. Science, 232**,** 34-47.

BRUECKMANN, M., BERTSCH, T., LANG, S., SUESELBECK, T., WOLPERT, C., KADEN, J. J., JARAMILLO, C., HUHLE, G., BORGGREFE, M. & HAASE, K. K. 2004. Time course of systemic markers of inflammation in patients presenting with acute coronary syndromes. Clin Chem Lab Med, 42**,** 1132-9.

BUDIN, S. B., OTHMAN, F., LOUIS, S. R., BAKAR, M. A., DAS, S. & MOHAMED, J. 2009. The effects of palm oil tocotrienol-rich fraction supplementation on biochemical parameters, oxidative stress and the vascular wall of streptozotocin-induced diabetic rats. Clinics (Sao Paulo), 64**,** 235-44.

BURGER-KENTISCHER, A., GOBEL, H., KLEEMANN, R., ZERNECKE, A., BUCALA, R., LENG, L., FINKELMEIER, D., GEIGER, G., SCHAEFER, H. E., SCHOBER, A., WEBER, C., BRUNNER, H., RUTTEN, H., IHLING, C. & BERNHAGEN, J. 2006. Reduction of the aortic inflammatory response in spontaneous atherosclerosis by blockade of macrophage migration inhibitory factor (MIF). Atherosclerosis, 184**,** 28-38.

BURKE, A. P., KOLODGIE, F. D., FARB, A., WEBER, D. K., MALCOM, G. T., SMIALEK, J. & VIRMANI, R. 2001. Healed plaque ruptures and sudden coronary death: evidence that subclinical rupture has a role in plaque progression. Circulation, 103**,** 934-40.

CAMARGO, C. A., JR., HENNEKENS, C. H., GAZIANO, J. M., GLYNN, R. J., MANSON, J. E. & STAMPFER, M. J. 1997a. Prospective study of moderate alcohol consumption and mortality in US male physicians. Arch Intern Med, 157**,** 79-85.

CAMARGO, C. A., JR., STAMPFER, M. J., GLYNN, R. J., GAZIANO, J. M., MANSON, J. E., GOLDHABER, S. Z. & HENNEKENS, C. H. 1997b. Prospective study of moderate alcohol consumption and risk of peripheral arterial disease in US male physicians. Circulation, 95**,** 577-80.

CAMEJO, G., FAGER, G., ROSENGREN, B., HURT-CAMEJO, E. & BONDJERS, G. 1993. Binding of low density lipoproteins by proteoglycans synthesized by proliferating and quiescent human arterial smooth muscle cells. J Biol Chem, 268**,** 14131-7.

CAMPBELL, C. Y., NASIR, K., SARWAR, A., MENEGHELO, R. S., CARVALHO, J. A., BLUMENTHAL, R. S. & SANTOS, R. D. 2007. Combined effect of high low-density lipoprotein cholesterol and metabolic syndrome on subclinical coronary atherosclerosis in white men without clinical evidence of myocardial ischemia. Am J Cardiol, 100**,** 840-3.

CANAULT, M., PEIRETTI, F., POGGI, M., MUELLER, C., KOPP, F., BONARDO, B., BASTELICA, D., NICOLAY, A., ALESSI, M. C. & NALBONE, G. 2008. Progression of atherosclerosis in ApoE-deficient mice that express distinct molecular forms of TNF-alpha. J Pathol, 214**,** 574-83.

CAPPUCCIO, F. P., KERRY, S. M., MICAH, F. B., PLANGE-RHULE, J. & EASTWOOD, J. B. 2006. A community programme to reduce salt intake and blood pressure in Ghana [ISRCTN88789643]. BMC Public Health, 6**,** 13.

CARLOS, T. M. & HARLAN, J. M. 1994. Leukocyte-endothelial adhesion molecules. Blood, 84**,** 2068-101.

CARLUCCIO, M. A., ANCORA, M. A., MASSARO, M., CARLUCCIO, M., SCODITTI, E., DISTANTE, A., STORELLI, C. & DE CATERINA, R. 2007. Homocysteine induces VCAM-1 gene expression through NF-kappaB and NAD(P)H oxidase activation: protective role of Mediterranean diet polyphenolic antioxidants. Am J Physiol Heart Circ Physiol, 293**,** H2344-54.

CARLUCCIO, M. A., SICULELLA, L., ANCORA, M. A., MASSARO, M., SCODITTI, E., STORELLI, C., VISIOLI, F., DISTANTE, A. & DE CATERINA, R. 2003. Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: antiatherogenic properties of Mediterranean diet phytochemicals. Arterioscler Thromb Vasc Biol, 23**,** 622-9.

CARR, A. C., MCCALL, M. R. & FREI, B. 2000a. Oxidation of LDL by myeloperoxidase and reactive nitrogen species: reaction pathways and antioxidant protection. Arterioscler Thromb Vasc Biol, 20**,** 1716-23.

CARR, A. C., ZHU, B. Z. & FREI, B. 2000b. Potential antiatherogenic mechanisms of ascorbate (vitamin C) and alpha-tocopherol (vitamin E). Circ Res, 87**,** 349-54.

CASSCELLS, W., HASSAN, K., VASEGHI, M. F., SIADATY, M. S., NAGHAVI, M., KIRKEEIDE, R. L., HASSAN, M. R. & MADJID, M. 2003. Plaque blush, branch location, and calcification are angiographic predictors of progression of mild to moderate coronary stenoses. Am Heart J, 145**,** 813-20.

CASTANER, O., FITO, M., LOPEZ-SABATER, M. C., POULSEN, H. E., NYYSSONEN, K., SCHRODER, H., SALONEN, J. T., DE LA TORRE-CARBOT, K., ZUNFT, H. F., DE LA TORRE, R., BAUMLER, H., GADDI, A. V., SAEZ, G. T., TOMAS, M. & COVAS, M. I. 2011. The effect of olive oil polyphenols on antibodies against oxidized LDL. A randomized clinical trial. Clin Nutr, 30**,** 490-3.

CESARI, M., PENNINX, B. W., NEWMAN, A. B., KRITCHEVSKY, S. B., NICKLAS, B. J., SUTTON-TYRRELL, K., RUBIN, S. M., DING, J., SIMONSICK, E. M., HARRIS, T. B. & PAHOR, M. 2003a. Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. Circulation, 108**,** 2317-22.

CESARI, M., PENNINX, B. W., NEWMAN, A. B., KRITCHEVSKY, S. B., NICKLAS, B. J., SUTTON-TYRRELL, K., TRACY, R. P., RUBIN, S. M., HARRIS, T. B. & PAHOR, M. 2003b. Inflammatory markers and

cardiovascular disease (The Health, Aging and Body Composition [Health ABC] Study). Am J Cardiol, 92**,** 522-8.

CHAN, A. C. 1998. Vitamin E and atherosclerosis. J Nutr, 128**,** 1593-6.

CHARLAND, S. L., CZIRAKY, M. J., QUIMBO, R., KARAS, R. H., INSULL, W., JR., DAVIDSON, M. & STANEK, E. J. 2008. Achieving optimal lipid values in patients with dyslipidemia is associated with reduced risk of cardiovascular events. J Clin Lipidol, 2**,** 343-53.

CHARO, I. F. & TAUB, R. 2011. Anti-inflammatory therapeutics for the treatment of atherosclerosis. Nat Rev Drug Discov, 10**,** 365-76.

CHONG, Y. H. & NG, T. K. 1991. Effects of palm oil on cardiovascular risk. Med J Malaysia, 46**,** 41-50.

CHOUDHURY, N., TAN, L. & TRUSWELL, A. S. 1995. Comparison of palmolein and olive oil: effects on plasma lipids and vitamin E in young adults. Am J Clin Nutr, 61**,** 1043-51.

CICERALE, S., LUCAS, L. J. & KEAST, R. S. 2012. Antimicrobial, antioxidant and anti-inflammatory phenolic activities in extra virgin olive oil. Curr Opin Biotechnol, 23**,** 129-35.

CLARKE, R. & ARMITAGE, J. 2009. Greater absolute reductions in LDL cholesterol should yield greater proportional reductions in risk of cardiovascular disease. J Intern Med, 266**,** 499-501.

CLARKE, S. D. 2004. The multi-dimensional regulation of gene expression by fatty acids: polyunsaturated fats as nutrient sensors. Curr Opin Lipidol, 15**,** 13-8.

CONI, E., DI BENEDETTO, R., DI PASQUALE, M., MASELLA, R., MODESTI, D., MATTEI, R. & CARLINI, E. A. 2000. Protective effect of oleuropein, an olive oil biophenol, on low density lipoprotein oxidizability in rabbits. Lipids, 35**,** 45-54.

CORTESE, C., MILLER, N. E., MARENAH, C. B. & LEWIS, B. 1983. HDL cholesterol and LDL receptor activity. Atherosclerosis, 49**,** 215-7.

COTTRELL, R. C. 1991. Introduction: nutritional aspects of palm oil. Am J Clin Nutr, 53**,** 989S-1009S.

COVAS, M. I. 2007a. Benefits of the Mediterranean diet on cardiovascular disease. Future Cardiol, 3**,** 575-8.

COVAS, M. I. 2007b. Olive oil and the cardiovascular system. Pharmacol Res, 55**,** 175-86.

COVAS, M. I., NYYSSONEN, K., POULSEN, H. E., KAIKKONEN, J., ZUNFT, H. J., KIESEWETTER, H., GADDI, A., DE LA TORRE, R., MURSU, J., BAUMLER, H., NASCETTI, S., SALONEN, J. T., FITO, M., VIRTANEN, J. & MARRUGAT, J. 2006. The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. Ann Intern Med, 145**,** 333-41.

COWIE, C. C., RUST, K. F., BYRD-HOLT, D. D., EBERHARDT, M. S., FLEGAL, K. M., ENGELGAU, M. M., SAYDAH, S. H., WILLIAMS, D. E., GEISS, L. S. & GREGG, E. W. 2006. Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population: National Health And Nutrition Examination Survey 1999-2002. Diabetes Care, 29**,** 1263-8.

CROCE, K. & LIBBY, P. 2007. Intertwining of thrombosis and inflammation in atherosclerosis. Curr Opin Hematol, 14**,** 55-61.

CUI, R., ISO, H., TOYOSHIMA, H., DATE, C., YAMAMOTO, A., KIKUCHI, S., KONDO, T., WATANABE, Y., KOIZUMI, A., INABA, Y. & TAMAKOSHI, A. 2007. Serum total cholesterol levels and risk of mortality from stroke and coronary heart disease in Japanese: the JACC study. Atherosclerosis, 194**,** 415-20.

CULLEN, P., RAUTERBERG, J. & LORKOWSKI, S. 2005. The pathogenesis of atherosclerosis. Handb Exp Pharmacol**,** 3-70.

CYBULSKY, M. I. & GIMBRONE, M. A., JR. 1991. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science, 251**,** 788-91.

CZIRAKY, M. J., WATSON, K. E. & TALBERT, R. L. 2008. Targeting low HDL-cholesterol to decrease residual cardiovascular risk in the managed care setting. J Manag Care Pharm, 14**,** S3-28; quiz S30-1.

DALUZ, P. L. & COIMBRA, S. R. 2001. Alcohol and atherosclerosis. An Acad Bras Cienc, 73**,** 51-5.

DANG, A. Q., FAAS, F. H., LEE, J. A. & CARTER, W. J. 1988. Altered fatty acid composition in the plasma, platelets, and aorta of the streptozotocin-induced diabetic rat. Metabolism, 37**,** 1065-72.

DART, A. M., GATZKA, C. D., CAMERON, J. D., KINGWELL, B. A., LIANG, Y. L., BERRY, K. L., REID, C. M. & JENNINGS, G. L. 2004. Large artery

stiffness is not related to plasma cholesterol in older subjects with hypertension. Arterioscler Thromb Vasc Biol, 24**,** 962-8.

DAUQAN, E., SANI, H. A., ABDULLAH, A. & KASIM, Z. M. 2011a. Effect of four different vegetable oils (red palm olein, palm olein, corn oil, coconut oil) on antioxidant enzymes activity of rat liver. Pak J Biol Sci, 14**,** 399-403.

DAUQAN, E. M. A., ABDULLAH, A. & SANI, H. A. 2012. Biochemistry, Genetics and Molecular Biology "Antioxidant Enzyme",. In: EL-MISSIRY, M. A. (ed.) Effect of Different Concentrations of Red Palm Olein and Different Vegetable Oils on Antioxidant Enzymes in Normal and Stressed Rat InTech.

DAUQAN, E. M. A., SANI, H. A., ABDULLAH, A. & KASIM, Z. M. 2011b. Effect of different concentration of red palm olein on blood lipid profile in rat. International Research Journal of Biochemistry and Bioinformatics, 1**,** 184-189.

DAVIES, M. J. & THOMAS, A. 1984. Thrombosis and acute coronary-artery lesions in sudden cardiac ischemic death. N Engl J Med, 310**,** 1137-40.

DEMBINSKA-KIEC, A. & GRYGLEWSKI, R. J. 1986. Contribution of arachidonic acid metabolites to atherosclerosis. Wien Klin Wochenschr, 98**,** 198-206.

DENKE, M. A. & GRUNDY, S. M. 1992. Comparison of effects of lauric acid and palmitic acid on plasma lipids and lipoproteins. Am J Clin Nutr, 56**,** 895-8.

DIAZ, M. N., FREI, B., VITA, J. A. & KEANEY, J. F., JR. 1997. Antioxidants and atherosclerotic heart disease. N Engl J Med, 337**,** 408-16.

DIEHL, K. H., HULL, R., MORTON, D., PFISTER, R., RABEMAMPIANINA, Y., SMITH, D. C., VIDAL, J. M. & VAN DE VORSTENBOSCH, C. 2001. A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes. Journal of Applied Toxicology, 21**,** 15-23.

DILAVERIS, P., GIANNOPOULOS, G., RIGA, M., SYNETOS, A. & STEFANADIS, C. 2007. Beneficial effects of statins on endothelial dysfunction and vascular stiffness. Curr Vasc Pharmacol, 5**,** 227-37.

DOBRILOVIC, N., VADLAMANI, L., MEYER, M. & WRIGHT, C. B. 2001. Chlamydia pneumoniae in atherosclerotic carotid artery plaques: high prevalence among heavy smokers. Am Surg, 67**,** 589-93.

DORAN, A. C., MELLER, N. & MCNAMARA, C. A. 2008. Role of smooth muscle cells in the initiation and early progression of atherosclerosis. Arterioscler Thromb Vasc Biol, 28**,** 812-9.

DREON, D. M., FERNSTROM, H. A., CAMPOS, H., BLANCHE, P., WILLIAMS, P. T. & KRAUSS, R. M. 1998. Change in dietary saturated fat intake is correlated with change in mass of large low-density-lipoprotein particles in men. Am J Clin Nutr, 67**,** 828-36.

DREON, D. M., FERNSTROM, H. A., MILLER, B. & KRAUSS, R. M. 1995. Apolipoprotein E isoform phenotype and LDL subclass response to a reduced-fat diet. Arterioscler Thromb Vasc Biol, 15**,** 105-11.

DREXLER, H. 1999. Nitric oxide and coronary endothelial dysfunction in humans. Cardiovasc Res, 43**,** 572-9.

DUDA, R. B., KIM, M. P., DARKO, R., ADANU, R. M., SEFFAH, J., ANARFI, J. K. & HILL, A. G. 2007. Results of the Women's Health Study of Accra: assessment of blood pressure in urban women. Int J Cardiol, 117**,** 115-22.

DUFF, H. G. 1991. Refining. In Wan PJ (eds.) Introduction to fats and oils Technology. Am. Oil Chem. Soc., Champaign, Illinois,**,** 85.

EBONG, P. E., OWU, D. U. & ISONG, E. U. 1999. Influence of palm oil (Elaesis guineensis) on health. Plant Foods Hum Nutr, 53**,** 209-22.

EDEM 2009. Hematological and Histological Alterations Induced in Rats by Palm Oil – Containing Diets. European Journal of Scientific Research, 32**,** 405-418.

EDEM, D. O. 2002. Palm oil: biochemical, physiological, nutritional, hematological, and toxicological aspects: a review. Plant Foods Hum Nutr, 57**,** 319-41.

EDEM, D. O. & AKPANABIATU, M. I. 2006. Effect of palm oil containing diet on enzyme activities of rats

Pak. J. Nutr, 5**,** 301-305.

EDEM, D. O., EKA, O. U. & UMOH, I. B. 2002. Feeding of red palm oil-supplemented diets to rats may impact positively on vitamin A status. Int J Food Sci Nutr, 53**,** 285-91.

EDEM, D. O., EKA, O. U., UMOH, I. B., UDOH, A. P. & AKPAN, E. J. 2003. Effect of Red Palm Oil and Refined Palm Olein on Nutrient Digestion in the Rat. Pakistan Journal of Nutrition, 2 271-278.

EDIDIN, M. 2003. Lipids on the frontier: a century of cell-membrane bilayers. Nat Rev Mol Cell Biol, 4**,** 414-8.

EGHAN, B. A., JR. & ACHEAMPONG, J. W. 2003. Dyslipidemia in outpatients at General Hospital in Kumasi, Ghana: cross-sectional study. Croat Med J, 44**,** 576-8.

EHARA, S., KOBAYASHI, Y., YOSHIYAMA, M., SHIMADA, K., SHIMADA, Y., FUKUDA, D., NAKAMURA, Y., YAMASHITA, H., YAMAGISHI, H., TAKEUCHI, K., NARUKO, T., HAZE, K., BECKER, A. E., YOSHIKAWA, J. & UEDA, M. 2004. Spotty calcification typifies the culprit plaque in patients with acute myocardial infarction: an intravascular ultrasound study. Circulation, 110**,** 3424-9.

EIDANGBE, G. O., OJIEH, G. C., IDONIJE, B. O. & OLUBA, O. M. 2010. Palm oil and Egusi melon oil lower serum and liver lipids profile and improve antioxidant activity in rats fed a high fat diet.

. Journal of Food Technology, 8 (4)**,** 154-158.

EL SEWEIDY, M. M., EL-SWEFY, S. E., ABDALLAH, F. R. & HASHEM, R. M. 2005. Dietary fatty acid unsaturation levels, lipoprotein oxidation and circulating chemokine in experimentally induced atherosclerotic rats. J Pharm Pharmacol, 57(11), 467-74.

EL WAKF, A. M., EBRAHEEM, H. A., SERAG, H. A., HANAA, A. H. & GUMAIH, H. S. 2010. Association between inflammation and the risk of cardiovascular disorders in atherogenic male rats: Role of virgin and refined olive oil. Journal of American Science, 6, 807-817.

ELBAGIR, M. N., ELTOM, M. A., ELMAHADI, E. M., KADAM, I. M. & BERNE, C. 1998. A high prevalence of diabetes mellitus and impaired glucose tolerance in the Danagla community in northern Sudan. Diabet Med, 15, 164-9.

ELSON, C. E. 1992. Tropical oils: nutritional and scientific issues. Crit Rev Food Sci Nutr, 31, 79-102.

ELSON, C. E. & QURESHI, A. A. 1995. Coupling the cholesterol- and tumor-suppressive actions of palm oil to the impact of its minor constituents on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. Prostaglandins Leukot Essent Fatty Acids, 52, 205-7.

ERIKSSON, L. T., ROSCHER, R., INGEMANSSON, R. & STEEN, S. 1999. Cardiovascular effects of induced hypothermia after lung transplantation. Ann Thorac Surg, 67, 804-9.

ESPIN, J. C., SOLER-RIVAS, C. & WICHERS, H. J. 2000. Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-picrylhydrazyl radical. J Agric Food Chem, 48**,** 648-56.

ESTERBAUER, H. 1993. Cytotoxicity and genotoxicity of lipid-oxidation products. Am J Clin Nutr, 57**,** 779S-785S; discussion 785S-786S.

ESTERBAUER, H., GEBICKI, J., PUHL, H. & JURGENS, G. 1992a. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Radic Biol Med, 13**,** 341-90.

ESTERBAUER, H., PUHL, H., WAEG, G., KREBS, A., TATZBER, F. & RABL, H. 1992b. Vitamin E and atherosclerosis: an overview. J Nutr Sci Vitaminol (Tokyo), Spec No**,** 177-82.

ESTERBAUER, H., WAEG, G., PUHL, H., DIEBER-ROTHENEDER, M. & TATZBER, F. 1992c. Inhibition of LDL oxidation by antioxidants. EXS, 62**,** 145-57.

ESTERBAUER, H., WAG, G. & PUHL, H. 1993. Lipid peroxidation and its role in atherosclerosis. Br Med Bull, 49**,** 566-76.

ESTERHUYSE, A. J., DU TOIT, E. F., BENADE, A. J. & VAN ROOYEN, J. 2005a. Dietary red palm oil improves reperfusion cardiac function in the isolated perfused rat heart of animals fed a high cholesterol diet. Prostaglandins Leukot Essent Fatty Acids, 72**,** 153-61.

ESTERHUYSE, A. J., TOIT, E. D. & ROOYEN, J. V. 2005b. Dietary red palm oil supplementation protects against the consequences of global ischemia in the isolated perfused rat heart. Asia Pac J Clin Nutr, 14**,** 340-7.

FAESSLER, P. K., KARL; SEANG, KEK WAN; LEE, SIANG HUA 2007. "Advanced fractionation technology for the oleochemical industry". : . Asia-Pacific Journal of Chemical Engineering 2 (4)**,** 315-316.

FAHY, E., SUBRAMANIAM, S., BROWN, H. A., GLASS, C. K., MERRILL, A. H., JR., MURPHY, R. C., RAETZ, C. R., RUSSELL, D. W., SEYAMA, Y., SHAW, W., SHIMIZU, T., SPENER, F., VAN MEER, G., VANNIEUWENHZE, M. S., WHITE, S. H., WITZTUM, J. L. & DENNIS, E. A. 2005. A comprehensive classification system for lipids. J Lipid Res, 46**,** 839-61.

FALK, E. 2006. Pathogenesis of atherosclerosis. J Am Coll Cardiol, 47**,** C7-12.

FALK, E., SCHWARTZ, S. M., GALIS, Z. S. & ROSENFELD, M. E. 2007. Neointimal cracks (plaque rupture?) and thrombosis in wrapped arteries without flow. Arterioscler Thromb Vasc Biol, 27**,** 248-9; author reply 250-2.

FASHAKIN, J. B. & UNOKIWEDI, C. C. 1993. Nutritional evaluation of “warankasi” and “waragusi” prepared from cow milk partially substituted with melon milk. Nig. Food J, 11**,** 128-134.

FAXON, D. P., CREAGER, M. A., SMITH, S. C., JR., PASTERNAK, R. C., OLIN, J. W., BETTMANN, M. A., CRIQUI, M. H., MILANI, R. V., LOSCALZO, J., KAUFMAN, J. A., JONES, D. W. & PEARCE, W. H. 2004a.

Atherosclerotic Vascular Disease Conference: Executive summary: Atherosclerotic Vascular Disease Conference proceeding for healthcare professionals from a special writing group of the American Heart Association. Circulation, 109**,** 2595-604.

FAXON, D. P., FUSTER, V., LIBBY, P., BECKMAN, J. A., HIATT, W. R., THOMPSON, R. W., TOPPER, J. N., ANNEX, B. H., RUNDBACK, J. H., FABUNMI, R. P., ROBERTSON, R. M. & LOSCALZO, J. 2004b. Atherosclerotic Vascular Disease Conference: Writing Group III: pathophysiology. Circulation, 109**,** 2617-25.

FEHILY, A. M., YARNELL, J. W., SWEETNAM, P. M. & ELWOOD, P. C. 1993. Diet and incident ischaemic heart disease: the Caerphilly Study. Br J Nutr, 69**,** 303-14.

FEIGIN, V. L. 2007. Stroke in developing countries: can the epidemic be stopped and outcomes improved? Lancet Neurol, 6**,** 94-7.

FEINGOLD, K. R. & GRUNFELD, C. 1987. Tumor necrosis factor-alpha stimulates hepatic lipogenesis in the rat in vivo. J Clin Invest, 80**,** 184-90.

FEINGOLD, K. R., HARDARDOTTIR, I. & GRUNFELD, C. 1998. Beneficial effects of cytokine induced hyperlipidemia. Z Ernahrungswiss, 37 Suppl 1**,** 66-74.

FEINGOLD, K. R. & MOSER, A. H. 1987. Effect of hypocholesterolemia on cholesterol synthesis in small intestine of diabetic rats. Diabetes, 36**,** 1223-9.

FEINGOLD, K. R., SOUED, M. & GRUNFELD, C. 1988. Tumor necrosis factor stimulates DNA synthesis in the liver of intact rats. Biochem Biophys Res Commun, 153**,** 576-82.

FEZEU, L., BALKAU, B., KENGNE, A. P., SOBNGWI, E. & MBANYA, J. C. 2007. Metabolic syndrome in a sub-Saharan African setting: central obesity may be the key determinant. Atherosclerosis, 193**,** 70-6.

FIELDING, C. J. & FIELDING, P. E. 1995. Molecular physiology of reverse cholesterol transport. J Lipid Res, 36**,** 211-28.

FIELDING, C. J., HAVEL, R. J., TODD, K. M., YEO, K. E., SCHLOETTER, M. C., WEINBERG, V. & FROST, P. H. 1995. Effects of dietary cholesterol and fat saturation on plasma lipoproteins in an ethnically diverse population of healthy young men. J Clin Invest, 95**,** 611-8.

FIOTTI, N., GIANSANTE, C., PONTE, E., DELBELLO, C., CALABRESE, S., ZACCHI, T., DOBRINA, A. & GUARNIERI, G. 1999. Atherosclerosis and inflammation. Patterns of cytokine regulation in patients with peripheral arterial disease. Atherosclerosis, 145**,** 51-60.

FRANKEL, E. N., KANNER, J., GERMAN, J. B., PARKS, E. & KINSELLA, J. E. 1993. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. Lancet, 341**,** 454-7.

FREIBERG, J. J., TYBJAERG-HANSEN, A., JENSEN, J. S. & NORDESTGAARD, B. G. 2008. Nonfasting triglycerides and risk of ischemic stroke in the general population. JAMA, 300**,** 2142-52.

FU, J., ZHANG, W., MAI, K., FENG, X. & XU, W. 2007. Effects of vitamin E on antioxidant enzyme activities and fatty acid compostions in juvenile abalone Haliotis discus hannai Ino. . J. Shellfish Res, 26**,** 809-814.

FUKUMOTO, Y., DEGUCHI, J. O., LIBBY, P., RABKIN-AIKAWA, E., SAKATA, Y., CHIN, M. T., HILL, C. C., LAWLER, P. R., VARO, N., SCHOEN, F. J., KRANE, S. M. & AIKAWA, M. 2004. Genetically determined resistance to collagenase action augments interstitial collagen accumulation in atherosclerotic plaques. Circulation, 110**,** 1953-9.

FUKUMOTO, Y., SHIMOKAWA, H., ITO, A., KADOKAMI, T., YONEMITSU, Y., AIKAWA, M., OWADA, M. K., EGASHIRA, K., SUEISHI, K., NAGAI, R., YAZAKI, Y. & TAKESHITA, A. 1997. Inflammatory cytokines cause coronary arteriosclerosis-like changes and alterations in the smooth-muscle phenotypes in pigs. J Cardiovasc Pharmacol, 29**,** 222-31.

FUNKE, H. & ASSMANN, G. 1999. Strategies for the assessment of genetic coronary artery disease risk. Curr Opin Lipidol, 10**,** 285-91.

GABAY, C. & KUSHNER, I. 1999. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med, 340**,** 448-54.

GARG, R. K., ARORA, R. C. & AGARWAL, N. 1991. High density lipoprotein. J Assoc Physicians India, 39**,** 269-71.

GAZIANO, J. M. 1996. Antioxidants in cardiovascular disease: randomized trials. Nutrition, 12**,** 583-8.

GAZIANO, J. M. 1999. Antioxidant vitamins and cardiovascular disease. Proc Assoc Am Physicians, 111**,** 2-9.

GENEST, J., JR., MCNAMARA, J. R., ORDOVAS, J. M., JENNER, J. L., SILBERMAN, S. R., ANDERSON, K. M., WILSON, P. W., SALEM, D. N. & SCHAEFER, E. J. 1992. Lipoprotein cholesterol, apolipoprotein A-I and B and lipoprotein (a) abnormalities in men with premature coronary artery disease. J Am Coll Cardiol, 19**,** 792-802.

GENG, Y. J. & LIBBY, P. 2002. Progression of atheroma: a struggle between death and procreation. Arterioscler Thromb Vasc Biol, 22**,** 1370-80.

GHAFOORUNISSA, REDDY, V. & SESIKARAN, B. 1995. Palmolein and groundnut oil have comparable effects on blood lipids and platelet aggregation in healthy Indian subjects. Lipids, 30**,** 1163-9.

GIMBRONE, M. A., JR., BEVILACQUA, M. P. & CYBULSKY, M. I. 1990. Endothelial-dependent mechanisms of leukocyte adhesion in inflammation and atherosclerosis. Ann N Y Acad Sci, 598**,** 77-85.

GLASS, C. K. & WITZTUM, J. L. 2001. Atherosclerosis. the road ahead. Cell, 104**,** 503-16.

GORINSTEIN, S., LEONTOWICZ, H., LOJEK, A., LEONTOWICZ, M., CIZ, M., KRZEMINSKI, R., GRALAK, M., CZERWINSKI, J., JASTRZEBSKI, Z., TRAKHTENBERG, S., GRIGELMO-MIGUEL, N., SOLIVA-FORTUNY, R. & MARTIN-BELLOSO, O. 2002. Olive oils improve lipid metabolism

and increase antioxidant potential in rats fed diets containing cholesterol. J Agric Food Chem, 50**,** 6102-8.

GRANDE, F., ANDERSON, J. T., CHLOUVERAKIS, C., PROJA, M. & KEYS, A. 1965. Effect of dietary cholesterol on man's serum lipids. J Nutr, 87**,** 52-62.

GRAVANIS, M. B. 1994. Accelerated coronary atherosclerosis in the allograft heart. Postgrad Med J, 70 Suppl 1**,** S93-8.

GRAYSTON, J. T., KUO, C. C., CAMPBELL, L. A., WANG, S. P. & JACKSON, L. A. 1997. Chlamydia pneumoniae and cardiovascular disease. Cardiologia, 42**,** 1145-51.

GREAVES, D. R. & GORDON, S. 2005. Thematic review series: the immune system and atherogenesis. Recent insights into the biology of macrophage scavenger receptors. J Lipid Res, 46**,** 11-20.

GREEN, D., CHAMBERLAIN, M. A., RUTH, K. J., FOLSOM, A. R. & LIU, K. 1997. Factor VII, cholesterol, and triglycerides. The CARDIA Study. Coronary Artery Risk Development in Young Adults Study. Arterioscler Thromb Vasc Biol, 17**,** 51-5.

GREEN, M. S., ETZION, T. & JUCHA, E. 1991. Blood pressure and serum cholesterol among male Ethiopian immigrants compared to other Israelis. J Epidemiol Community Health, 45**,** 281-6.

GRIMBLE, R. F. 1996. Interaction between nutrients, pro-inflammatory cytokines and inflammation. Clin Sci (Lond), 91**,** 121-30.

GRIMBLE, R. F. 1998a. Dietary lipids and the inflammatory response. Proc Nutr Soc, 57**,** 535-42.

GRIMBLE, R. F. 1998b. Nutritional modulation of cytokine biology. Nutrition, 14**,** 634-40.

GROUP., H. P. S. C. 2002. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol-lowering drug therapy and of antioxidant vitamin supplementation in patients at increased risk of coronary heart disease: design, study population and early experience. Eur Heart J(submitted).

GRUNDY, S. M. 1994. Influence of stearic acid on cholesterol metabolism relative to other long-chain fatty acids. Am J Clin Nutr, 60**,** 986S-990S.

GRUNDY, S. M., VEGA, G. L., TOMASSINI, J. E. & TERSHAKOVEC, A. M. 2009. Correlation of non-high-density lipoprotein cholesterol and low-density lipoprotein cholesterol with apolipoprotein B during simvastatin + fenofibrate therapy in patients with combined hyperlipidemia (a subanalysis of the SAFARI trial). Am J Cardiol, 104**,** 548-53.

GRUNFELD, C., DINARELLO, C. A. & FEINGOLD, K. R. 1991. Tumor necrosis factor-alpha, interleukin-1, and interferon alpha stimulate triglyceride synthesis in HepG2 cells. Metabolism, 40**,** 894-8.

GRUNFELD, C. & FEINGOLD, K. R. 1991a. The metabolic effects of tumor necrosis factor and other cytokines. Biotherapy, 3**,** 143-58.

GRUNFELD, C. & FEINGOLD, K. R. 1991b. Tumor necrosis factor, cytokines, and the hyperlipidemia of infection. Trends Endocrinol Metab, 2**,** 213-9.

GRUNFELD, C., VERDIER, J. A., NEESE, R., MOSER, A. H. & FEINGOLD, K. R. 1988. Mechanisms by which tumor necrosis factor stimulates hepatic fatty acid synthesis in vivo. J Lipid Res, 29**,** 1327-35.

GUSTAFSSON, I. B., VESSBY, B., OHRVALL, M. & NYDAHL, M. 1994. A diet rich in monounsaturated rapeseed oil reduces the lipoprotein cholesterol concentration and increases the relative content of n-3 fatty acids in serum in hyperlipidemic subjects. Am J Clin Nutr, 59**,** 667-74.

GUYTON, J. R., KLEMP, K. F., BLACK, B. L. & BOCAN, T. M. 1990. Extracellular lipid deposition in atherosclerosis. Eur Heart J, 11 Suppl E**,** 20-8.

HADDY, N., SASS, C., DROESCH, S., ZAIOU, M., SIEST, G., PONTHIEUX, A., LAMBERT, D. & VISVIKIS, S. 2003. IL-6, TNF-alpha and atherosclerosis risk indicators in a healthy family population: the STANISLAS cohort. Atherosclerosis, 170**,** 277-83.

HAKALA, J. K., OKSJOKI, R., LAINE, P., DU, H., GRABOWSKI, G. A., KOVANEN, P. T. & PENTIKAINEN, M. O. 2003. Lysosomal enzymes are released from cultured human macrophages, hydrolyze LDL in vitro, and are present extracellularly in human atherosclerotic lesions. Arterioscler Thromb Vasc Biol, 23**,** 1430-6.

HAMMOND, E. C. & HORN, D. 1958. Smoking and death rates; report on forty-four monghs of follow-up of 187,783 men. II. Death rates by cause. J Am Med Assoc, 166**,** 1294-308.

HAN, X. T., NOZIERE, P., REMOND, D., CHABROT, J. & DOREAU, M. 2002. Effects of nutrient supply and dietary bulk on O2 uptake and nutrient net fluxes across rumen, mesenteric- and portal-drained viscera in ewes. J Anim Sci, 80**,** 1362-74.

HANDZHA, I. M. 1997. [Atherosclerosis]. Lik Sprava**,** 3-8.

HANSSON, G. K. 2005. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med, 352**,** 1685-95.

HARRIS, W. S. 1992. The prevention of atherosclerosis with antioxidants. Clin Cardiol, 15**,** 636-40.

HAUER, A. D., UYTTENHOVE, C., DE VOS, P., STROOBANT, V., RENAULD, J. C., VAN BERKEL, T. J., VAN SNICK, J. & KUIPER, J. 2005. Blockade of interleukin-12 function by protein vaccination attenuates atherosclerosis. Circulation, 112**,** 1054-62.

HAVEL, R. J. & HAMILTON, R. L. 2004. Hepatic catabolism of remnant lipoproteins: where the action is. Arterioscler Thromb Vasc Biol, 24**,** 213-5.

HAYES, B., LYNCH, B., O'KEEFE, M., MONAVARI, A. A. & TREACY, E. P. 2007. Long chain fatty acid oxidation defects in children: importance of detection and treatment options. Ir J Med Sci, 176**,** 189-92.

HAYES, K. C. & KHOSLA, P. 1992. Dietary fatty acid thresholds and cholesterolemia. FASEB J, 6**,** 2600-7.

HAYES, K. C. & KHOSLA, P. 2007. The complex interplay of palm oil fatty acid on blood lipids European Journal of Lipid Science and Technology, 4**,** 453-464.

HEGSTED, D. M., AUSMAN, L. M., JOHNSON, J. A. & DALLAL, G. E. 1993. Dietary fat and serum lipids: an evaluation of the experimental data. Am J Clin Nutr, 57**,** 875-83.

HEGSTED, D. M., MCGANDY, R. B., MYERS, M. L. & STARE, F. J. 1965. Quantitative effects of dietary fat on serum cholesterol in man. Am J Clin Nutr, 17**,** 281-95.

HENNEKENS, C. H. & GAZIANO, J. M. 1993. Antioxidants and heart disease: epidemiology and clinical evidence. Clin Cardiol, 16**,** I10-3; discussion I13-5.

HOFFMANN, U., BRADY, T. J. & MULLER, J. 2003. Cardiology patient page. Use of new imaging techniques to screen for coronary artery disease. Circulation, 108**,** e50-3.

HOKANSON, J. E. & AUSTIN, M. A. 1996. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk, 3**,** 213-9.

HOTAMISLIGIL, G. S., SHARGILL, N. S. & SPIEGELMAN, B. M. 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science, 259**,** 87-91.

HOWSON, C. P., FINEBERG, H. V. & BLOOM, B. R. 1998. The pursuit of global health: the relevance of engagement for developed countries. Lancet, 351**,** 586-90.

HU, F. B., VAN DAM, R. M. & LIU, S. 2001. Diet and risk of Type II diabetes: the role of types of fat and carbohydrate. Diabetologia, 44**,** 805-17.

HUANG, Y., GLOVICZKI, P., NOEL, A. A., SULLIVAN, T. M., KALRA, M., GULLERUD, R. E., HOSKIN, T. L. & BOWER, T. C. 2007. Early complications and long-term outcome after open surgical treatment of popliteal artery aneurysms: is exclusion with saphenous vein bypass still the gold standard? J Vasc Surg, 45**,** 706-713; discussion 713-5.

HUGHES, D. A., TOWNSEND, P. J. & HASLAM, P. L. 1992. Enhancement of the antigen-presenting function of monocytes by cholesterol: possible relevance to inflammatory mechanisms in extrinsic allergic alveolitis and atherosclerosis. Clin Exp Immunol, 87**,** 279-86.

HULLEY, S., GRADY, D., BUSH, T., FURBERG, C., HERRINGTON, D., RIGGS, B. & VITTINGHOFF, E. 1998. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. JAMA, 280**,** 605-13.

HWANG, S. J., BALLANTYNE, C. M., SHARRETT, A. R., SMITH, L. C., DAVIS, C. E., GOTTO, A. M., JR. & BOERWINKLE, E. 1997. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. Circulation, 96**,** 4219-25.

IGHOSOTU, S. & TONUKARI, N. J. 2010. The influence of dietary intake on the serum lipid profile, body mass index and risk of cardiovascular diseases in adults on the Niger Delta region. Int. J. Nutr. Metabolism, 2**,** 40-44.

IKEDA, U., SHIMPO, M., IKEDA, M., MINOTA, S. & SHIMADA, K. 2001. Lipophilic statins augment inducible nitric oxide synthase expression in cytokine-stimulated cardiac myocytes. J Cardiovasc Pharmacol, 38**,** 69-77.

ISO, H., SATO, S., KITAMURA, A., NAITO, Y., SHIMAMOTO, T. & KOMACHI, Y. 2003. Fat and protein intakes and risk of intraparenchymal hemorrhage among middle-aged Japanese. Am J Epidemiol, 157**,** 32-9.

JAARIN, K., GAPOR, M. T., NAFEEZA, M. I. & FAUZEE, A. M. 2002. Effect of various doses of palm vitamin E and tocopherol on aspirin-induced gastric lesions in rats. Int J Exp Pathol, 83**,** 295-302.

JACQUES, V. R. 2005. Can red palm oil protect the heart? Science in Africa.

JAIN, P. K., ARORA, R. C., AGARWAL, N., GARG, R. K., ARORA, S., GURBAXANI, R. K. & SHARMA, D. 1991. Dietary cholesterol induced changes in lipid profile in patients with nephrotic syndrome and chronic renal failure. J Assoc Physicians India, 39**,** 751-3.

JESSUP, W., KRITHARIDES, L. & STOCKER, R. 2004. Lipid oxidation in atherogenesis: an overview. Biochem Soc Trans, 32**,** 134-8.

JIANG, F. & DUSTING, G. J. 2001. Endothelium-dependent vasorelaxation independent of nitric oxide and K(+) release in isolated renal arteries of rats. Br J Pharmacol, 132**,** 1558-64.

JOUBERT, J., NORMAN, R., LAMBERT, E. V., GROENEWALD, P., SCHNEIDER, M., BULL, F. & BRADSHAW, D. 2007. Estimating the burden of disease attributable to physical inactivity in South Africa in 2000. S Afr Med J, 97**,** 725-31.

JOVINGE, S., HAMSTEN, A., TORNVALL, P., PROUDLER, A., BAVENHOLM, P., ERICSSON, C. G., GODSLAND, I., DE FAIRE, U. & NILSSON, J. 1998. Evidence for a role of tumor necrosis factor alpha in disturbances of triglyceride and glucose metabolism predisposing to coronary heart disease. Metabolism, 47**,** 113-8.

KAMAT, J. P. & DEVASAGAYAM, T. P. 1995. Tocotrienols from palm oil as potent inhibitors of lipid peroxidation and protein oxidation in rat brain mitochondria. Neurosci Lett, 195**,** 179-82.

KAMISAH, J., NORHAYATI, M., NORZANA, G., NOR AINI, U. & IMA-NIRWANA, S. 2006. Effects of heated vegetable oils on serum lipids and aorta of ovariectomized rats. Pakistan J. Nutr, 5 19-29.

KAMISAH, Y., ADAM, W. Z. & WAN NGAH, M. T. E. A. 2005. Chronic intake of red palm olein and palm olein produce benefical effects on plasma lipid profile in rats. Pakistan Journal of Nutrition, 4 89-96.

KARAJI-BANI, M., MONTAZERI, F. & HASHEMI, M. 2006. Effect of palm oil on serum lipid profiles in rats. Pak. J. Nutr., 5 234-236.

KARVONEN, M. K., PESONEN, U., KOULU, M., NISKANEN, L., LAAKSO, M., RISSANEN, A., DEKKER, J. M., HART, L. M., VALVE, R. & UUSITUPA, M. I. 1998. Association of a leucine(7)-to-proline(7) polymorphism in the signal peptide of neuropeptide Y with high serum cholesterol and LDL cholesterol levels. Nat Med, 4**,** 1434-7.

KATAN, M. B., VAN GASTEL, A. C., DE ROVER, C. M., VAN MONTFORT, M. A. & KNUIMAN, J. T. 1988. Differences in individual responsiveness of serum cholesterol to fat-modified diets in man. Eur J Clin Invest, 18**,** 644-7.

KATAN, M. B., ZOCK, P. L. & MENSINK, R. P. 1994. Effects of fats and fatty acids on blood lipids in humans: an overview. Am J Clin Nutr, 60**,** 1017S-1022S.

KENGNE, A. P., AMOAH, A. G. & MBANYA, J. C. 2005. Cardiovascular complications of diabetes mellitus in sub-Saharan Africa. Circulation, 112**,** 3592-601.

KENGNE, A. P. & AWAH, P. K. 2009. Classical cardiovascular risk factors and all-cause mortality in rural Cameroon. QJM, 102**,** 209-15.

KESANIEMI, Y. A. 1998. Relevance of the reduction of triglycerides in the prevention of coronary heart disease. Curr Opin Lipidol, 9**,** 571-4.

KEYS, A., ANDERSON, J. T. & GRANDE, F. 1957. Prediction of serum-cholesterol responses of man to changes in fats in the diet. Lancet, 273**,** 959-66.

KEYS, A. & GRANDE, F. 1957. Role of dietary fat in human nutrition. III. Diet and the epidemiology of coronary heart disease. Am J Public Health Nations Health, 47**,** 1520-30.

KHOO, J. C., MILLER, E., MCLOUGHLIN, P. & STEINBERG, D. 1990. Prevention of low density lipoprotein aggregation by high density lipoprotein or apolipoprotein A-I. J Lipid Res, 31**,** 645-52.

KHOR, H. T. & TAN, D. T. S. 1992. The triglycerides in palm oil are not hypercholesterolemic in the hamster. Nutrition Research, 12 (4-5)**,** 621-628.

KHOSLA, P. & HAYES, K. C. 1993. Dietary palmitic acid raises plasma LDL cholesterol relative to oleic acid only at a high intake of cholesterol. Biochim Biophys Acta, 1210**,** 13-22.

KNOPP, R. H. & RETZLAFF, B. M. 2004. Saturated fat prevents coronary artery disease? An American paradox. Am J Clin Nutr, 80**,** 1102-3.

KRAGEL, A. H., MCINTOSH, C. M. & ROBERTS, W. C. 1989a. Morphologic changes in coronary artery seen late after endarterectomy. Am J Cardiol, 63**,** 757-9.

KRAGEL, A. H., REDDY, S. G., WITTES, J. T. & ROBERTS, W. C. 1989b. Morphometric analysis of the composition of atherosclerotic plaques in the four major epicardial coronary arteries in acute myocardial infarction and in sudden coronary death. Circulation, 80**,** 1747-56.

KRAUSS, R. M. & DREON, D. M. 1995. Low-density-lipoprotein subclasses and response to a low-fat diet in healthy men. Am J Clin Nutr, 62**,** 478S-487S.

KRAUSS, R. M., GRUNFELD, C., DOERRLER, W. T. & FEINGOLD, K. R. 1990. Tumor necrosis factor acutely increases plasma levels of very low density lipoproteins of normal size and composition. Endocrinology, 127**,** 1016-21.

KRIEGER, M. & KOZARSKY, K. 1999. Influence of the HDL receptor SR-BI on atherosclerosis. Curr Opin Lipidol, 10**,** 491-7.

KRIS-ETHERTON, P. M. & YU, S. 1997. Individual fatty acid effects on plasma lipids and lipoproteins: human studies. Am J Clin Nutr, 65**,** 1628S-1644S.

KRIS-ETHERTON, P. M., YU, S., ETHERTON, T. D., MORGAN, R., MORIARTY, K. & SHAFFER, D. 1997. Fatty acids and progression of coronary artery disease. Am J Clin Nutr, 65**,** 1088-90.

KRITCHEVSKY, D. 1995. Dietary protein, cholesterol and atherosclerosis: a review of the early history. J Nutr, 125**,** 589S-593S.

KRITCHEVSKY, D. 2001. Diet and atherosclerosis. J Nutr Health Aging, 5**,** 155-9.

KRITCHEVSKY, D. 2002. Dietary fat and atherosclerosis. J Nutr Biochem, 13**,** 391.

KRITCHEVSKY, D. & SUNDRAM, K. 2002. Palm oil in human nutrition: recent advances. Asia Pac J Clin Nutr, 11 Suppl 7**,** S393.

KRITCHEVSKY, D., TEPPER, S. A., CZARNECKI, S. K. & SUNDRAM, K. 2002. Red palm oil in experimental atherosclerosis. Asia Pac J Clin Nutr, 11 Suppl 7**,** S433-7.

KRONENBERG, F., NEYER, U., LHOTTA, K., TRENKWALDER, E., AUINGER, M., PRIBASNIG, A., MEISL, T., KONIG, P. & DIEPLINGER, H. 1999. The low molecular weight apo(a) phenotype is an independent predictor for coronary artery disease in hemodialysis patients: a prospective follow-up. J Am Soc Nephrol, 10**,** 1027-36.

KRUGER, M. J., ENGELBRECHT, A. M., ESTERHUYSE, J., DU TOIT, E. F. & VAN ROOYEN, J. 2007. Dietary red palm oil reduces ischaemia-reperfusion injury in rats fed a hypercholesterolaemic diet. Br J Nutr, 97**,** 653-60.

KUMAR, P. D. 1997. The role of coconut and coconut oil in coronary heart disease in Kerala, south India. Trop Doct, 27**,** 215-7.

KUMAR, V., COTRAN, R. S. & ROBBINS, S. L. 1992. Basic pathology, Philadelphia, Saunders.

KUNKEL, S. L., STRIETER, R. M., CHENSUE, S. W., BASHA, M., STANDIFORD, T., HAM, J. & REMICK, D. G. 1990. Tumor necrosis factor-alpha, interleukin-8 and chemotactic cytokines. Prog Clin Biol Res, 349**,** 433-44.

KUSHI, L. H., FOLSOM, A. R., PRINEAS, R. J., MINK, P. J., WU, Y. & BOSTICK, R. M. 1996. Dietary antioxidant vitamins and death from

coronary heart disease in postmenopausal women. N Engl J Med, 334**,** 1156-62.

KUSUM, R., BOMMAYYA, H., FAYAZ PASHA, P. & RAMACHANDRAN, H. D. 2011. Palm oil and rice bran oil: Current status and future prospects. International Journal of Plant Physiology and Biochemistry, 3(8)**,** 125-132.

LAABES, E. P., THACHER, T. D. & OKEAHIALAM, B. N. 2008. Risk factors for heart failure in adult Nigerians. Acta Cardiol, 63**,** 437-43.

LAAKSO, P. & VOUTILAINEN, P. 1996. Analysis of triacylglycerols by silver-ion high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. Lipids, 31**,** 1311-22.

LACKNER, K. J., DIEPLINGER, H., NOWICKA, G. & SCHMITZ, G. 1993. High density lipoprotein deficiency with xanthomas. A defect in reverse cholesterol transport caused by a point mutation in the apolipoprotein A-I gene. J Clin Invest, 92**,** 2262-73.

LADA, A. T. & RUDEL, L. L. 2003. Dietary monounsaturated versus polyunsaturated fatty acids: which is really better for protection from coronary heart disease? Curr Opin Lipidol, 14**,** 41-6.

LADEIA, A. M., COSTA-MATOS, E., BARATA-PASSOS, R. & COSTA GUIMARAES, A. 2008. A palm oil-rich diet may reduce serum lipids in healthy young individuals. Nutrition, 24**,** 11-5.

LANDMESSER, U., HORNIG, B. & DREXLER, H. 2004. Endothelial function: a critical determinant in atherosclerosis? Circulation, 109**,** II27-33.

LARSSON, E., HARRIS, H. E., PALMBLAD, K., MANSSON, B., SAXNE, T. & KLARESKOG, L. 2005. CNI-1493, an inhibitor of proinflammatory cytokines, retards cartilage destruction in rats with collagen induced arthritis. Ann Rheum Dis, 64**,** 494-6.

LAUGERETTE, F., FURET, J. P., DEBARD, C., DAIRA, P., LOIZON, E., GELOEN, A., SOULAGE, C. O., SIMONET, C., LEFILS-LACOURTABLAISE, J., BERNOUD-HUBAC, N., BODENNEC, J., PERETTI, N., VIDAL, H. & MICHALSKI, M. C. 2012. Oil composition of high-fat diet affects metabolic inflammation differently in connection with endotoxin receptors in mice. Am J Physiol Endocrinol Metab, 302**,** E374-86.

LAW, M. R. & WALD, N. J. 1994. An ecological study of serum cholesterol and ischaemic heart disease between 1950 and 1990. Eur J Clin Nutr, 48**,** 305-25.

LAWOYIN, T. O., ASUZU, M. C., KAUFMAN, J., ROTIMI, C., OWOAJE, E., JOHNSON, L. & COOPER, R. 2002. Prevalence of cardiovascular risk factors in an African, urban inner city community. West Afr J Med, 21**,** 208-11.

LEAKE, D. S. 1993. Oxidised low density lipoproteins and atherogenesis. Br Heart J, 69**,** 476-8.

LEES, A. M., MOK, H. Y., LEES, R. S., MCCLUSKEY, M. A. & GRUNDY, S. M. 1977. Plant sterols as cholesterol-lowering agents: clinical trials in patients with hypercholesterolemia and studies of sterol balance. Atherosclerosis, 28**,** 325-38.

LEFEVRE, M., CHAMPAGNE, C. M., TULLEY, R. T., ROOD, J. C. & MOST, M. M. 2005. Individual variability in cardiovascular disease risk factor responses to low-fat and low-saturated-fat diets in men: body mass index, adiposity, and insulin resistance predict changes in LDL cholesterol. Am J Clin Nutr, 82**,** 957-63; quiz 1145-6.

LENDON, C. L., DAVIES, M. J., BORN, G. V. & RICHARDSON, P. D. 1991. Atherosclerotic plaque caps are locally weakened when macrophages density is increased. Atherosclerosis, 87**,** 87-90.

LEOSDOTTIR, M., NILSSON, P. M., NILSSON, J. A., MANSSON, H. & BERGLUND, G. 2005. Dietary fat intake and early mortality patterns--data from The Malmo Diet and Cancer Study. J Intern Med, 258**,** 153-65.

LEVITT, N. S., KATZENELLENBOGEN, J. M., BRADSHAW, D., HOFFMAN, M. N. & BONNICI, F. 1993. The prevalence and identification of risk factors for NIDDM in urban Africans in Cape Town, South Africa. Diabetes Care, 16**,** 601-7.

LI, H., CYBULSKY, M. I., GIMBRONE, M. A., JR. & LIBBY, P. 1993a. An atherogenic diet rapidly induces VCAM-1, a cytokine-regulatable mononuclear leukocyte adhesion molecule, in rabbit aortic endothelium. Arterioscler Thromb, 13**,** 197-204.

LI, H., CYBULSKY, M. I., GIMBRONE, M. A., JR. & LIBBY, P. 1993b. Inducible expression of vascular cell adhesion molecule-1 by vascular smooth muscle cells in vitro and within rabbit atheroma. Am J Pathol, 143**,** 1551-9.

LI, H., NEWCOMBE, J., GROOME, N. P. & CUZNER, M. L. 1993c. Characterization and distribution of phagocytic macrophages in multiple sclerosis plaques. Neuropathol Appl Neurobiol, 19**,** 214-23.

LIBBY, P. 2002a. Atherosclerosis: the new view. Sci Am, 286**,** 46-55.

LIBBY, P. 2002b. Inflammation in atherosclerosis. Nature, 420**,** 868-74.

LIBBY, P. 2006. Inflammation and cardiovascular disease mechanisms. Am J Clin Nutr, 83**,** 456S-460S.

LIBBY, P. & AIKAWA, M. 2002. Stabilization of atherosclerotic plaques: new mechanisms and clinical targets. Nat Med, 8**,** 1257-62.

LIBBY, P. & RIDKER, P. M. 1999. Novel inflammatory markers of coronary risk: theory versus practice. Circulation, 100**,** 1148-50.

LIBBY, P., RIDKER, P. M. & MASERI, A. 2002. Inflammation and atherosclerosis. Circulation, 105**,** 1135-43.

LICHTENSTEIN, A. H., AUSMAN, L. M., CARRASCO, W., JENNER, J. L., GUALTIERI, L. J., GOLDIN, B. R., ORDOVAS, J. M. & SCHAEFER, E. J. 1993. Effects of canola, corn, and olive oils on fasting and postprandial plasma lipoproteins in humans as part of a National Cholesterol Education Program Step 2 diet. Arterioscler Thromb, 13**,** 1533-42.

LICHTENSTEIN, A. H., MATTHAN, N. R., JALBERT, S. M., RESTEGHINI, N. A., SCHAEFER, E. J. & AUSMAN, L. M. 2006. Novel soybean oils with different fatty acid profiles alter cardiovascular disease risk factors in moderately hyperlipidemic subjects. Am J Clin Nutr, 84**,** 497-504.

LIETZ, G., HENRY, C. J., MULOKOZI, G., MUGYABUSO, J. K., BALLART, A., NDOSSI, G. D., LORRI, W. & TOMKINS, A. 2001. Comparison of the effects of supplemental red palm oil and sunflower oil on maternal vitamin A status. Am J Clin Nutr, 74**,** 501-9.

LIM, P. K., BOEY, P. L. & NG, W. K. 2001. Dietary palm oil level affects growthperformance, protein retention and tissue vitamin E concentration of African catfish, Clariasgariepinus. Aquaculture 202(1-2)**,** 101-112.

LIU, L. & YEH, Y. Y. 2000. Inhibition of cholesterol biosynthesis by organosulfur compounds derived from garlic. Lipids, 35**,** 197-203.

LLODRA, J., ANGELI, V., LIU, J., TROGAN, E., FISHER, E. A. & RANDOLPH, G. J. 2004. Emigration of monocyte-derived cells from atherosclerotic lesions characterizes regressive, but not progressive, plaques. Proc Natl Acad Sci U S A, 101**,** 11779-84.

LONGO-MBENZA, B., NGIMBI, R. M., NGOMA, D. V., FUELE, S. M. & BUASSA-BU-TSUMBU, B. 2008. [Risk factors of stroke among Congolese black hypertensive diabetics]. Ann Cardiol Angeiol (Paris), 57**,** 37-43.

LOOSE-MITCHELL, D. S., POORMAN, J. A., SMITH, S. A., OVERTURF, M. L., MORRISETT, J. D., GOTTO, A. M., JR. & SOMA, M. R. 1991. Cholesterol metabolism in hypercholesterolemia-resistant rabbits. Atherosclerosis, 87**,** 169-81.

LOPPNOW, H., WERDAN, K. & BUERKE, M. 2008. Vascular cells contribute to atherosclerosis by cytokine- and innate-immunity-related inflammatory mechanisms. Innate Immun, 14**,** 63-87.

LUCAS, L., RUSSELL, A. & KEAST, R. 2011. Molecular mechanisms of inflammation. Anti-inflammatory benefits of virgin olive oil and the phenolic compound oleocanthal. Curr Pharm Des, 17**,** 754-68.

LUSIS, A. J. & NAVAB, M. 1993. Lipoprotein oxidation and gene expression in the artery wall. New opportunities for pharmacologic intervention in atherosclerosis. Biochem Pharmacol, 46**,** 2119-26.

MAASS, M. & GIEFFERS, J. 1997. Cardiovascular disease risk from prior Chlamydia pneumoniae infection can be related to certain antigens recognized in the immunoblot profile. J Infect, 35**,** 171-6.

MAHONEY, E. M., WANG, K., COHEN, D. J., HIRSCH, A. T., ALBERTS, M. J., EAGLE, K., MOSSE, F., JACKSON, J. D., STEG, P. G. & BHATT, D. L. 2008. One-year costs in patients with a history of or at risk for atherothrombosis in the United States. Circ Cardiovasc Qual Outcomes, 1**,** 38-45.

MANGIAPANE, E. H., MCATEER, M. A., BENSON, G. M., WHITE, D. A. & SALTER, A. M. 1999. Modulation of the regression of atherosclerosis in the hamster by dietary lipids: comparison of coconut oil and olive oil. Br J Nutr, 82**,** 401-9.

MANSON, J. E., GAZIANO, J. M., JONAS, M. A. & HENNEKENS, C. H. 1993. Antioxidants and cardiovascular disease: a review. J Am Coll Nutr, 12**,** 426-32.

MARGARITI, A., ZENG, L. & XU, Q. 2006. Stem cells, vascular smooth muscle cells and atherosclerosis. Histol Histopathol, 21**,** 979-85.

MARRUGAT, J., COVAS, M. I., FITO, M., SCHRODER, H., MIRO-CASAS, E., GIMENO, E., LOPEZ-SABATER, M. C., DE LA TORRE, R. & FARRE, M. 2004. Effects of differing phenolic content in dietary olive oils on lipids and LDL oxidation--a randomized controlled trial. Eur J Nutr, 43**,** 140-7.

MARTIN, N. A., HADLEY, M. N., SPETZLER, R. F. & CARTER, L. P. 1986. Management of asymptomatic carotid atherosclerosis. Neurosurgery, 18**,** 505-13.

MARTINEZ-DOMINGUEZ, E., DE LA PUERTA, R. & RUIZ-GUTIERREZ, V. 2001. Protective effects upon experimental inflammation models of a polyphenol-supplemented virgin olive oil diet. Inflamm Res, 50**,** 102-6.

MARTINEZ, J. A., MARCOS, R., MACARULLA, M. T. & LARRALDE, J. 1995. Growth, hormonal status and protein turnover in rats fed on a diet containing peas (Pisum sativum L.) as the source of protein. Plant Foods Hum Nutr, 47**,** 211-20.

MASELLA, R., GIOVANNINI, C., VARI, R., DI BENEDETTO, R., CONI, E., VOLPE, R., FRAONE, N. & BUCCI, A. 2001. Effects of dietary virgin olive

oil phenols on low density lipoprotein oxidation in hyperlipidemic patients. Lipids, 36**,** 1195-202.

MASERI, A. 2003. The transition from stable to unstable coronary artery disease: a key research target. Ital Heart J, 4**,** 345-6.

MASON, R. S. & DIAMOND, T. H. 2001. Vitamin D deficiency and multicultural Australia. Med J Aust, 175**,** 236-7.

MATTSON, F. H. & JANDACEK, R. J. 1985. The effect of a non-absorbable fat on the turnover of plasma cholesterol in the rat. Lipids, 20**,** 273-7.

MEMON, R. A., GRUNFELD, C., MOSER, A. H. & FEINGOLD, K. R. 1993. Tumor necrosis factor mediates the effects of endotoxin on cholesterol and triglyceride metabolism in mice. Endocrinology, 132**,** 2246-53.

MEMON, R. A., SHECHTER, I., MOSER, A. H., SHIGENAGA, J. K., GRUNFELD, C. & FEINGOLD, K. R. 1997. Endotoxin, tumor necrosis factor, and interleukin-1 decrease hepatic squalene synthase activity, protein, and mRNA levels in Syrian hamsters. J Lipid Res, 38**,** 1620-9.

MENSINK, R. P. & KATAN, M. B. 1990. Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. N Engl J Med, 323**,** 439-45.

MENSINK, R. P. & KATAN, M. B. 1992. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. Arterioscler Thromb, 12**,** 911-9.

MENSINK, R. P., ZOCK, P. L., KATAN, M. B. & HORNSTRA, G. 1992. Effect of dietary cis and trans fatty acids on serum lipoprotein[a] levels in humans. J Lipid Res, 33**,** 1493-501.

MILES, E. A., ZOUBOULI, P. & CALDER, P. C. 2005. Differential anti-inflammatory effects of phenolic compounds from extra virgin olive oil identified in human whole blood cultures. Nutrition, 21**,** 389-94.

MING, C. C., CEASAR, M. C. & LIRENY, A. G. G. 2009. Carotenoids concentration of palm oil using membrane technology. Desalination, 246**,** 410-413.

MITCHELL, G. F., VASAN, R. S., KEYES, M. J., PARISE, H., WANG, T. J., LARSON, M. G., D'AGOSTINO, R. B., SR., KANNEL, W. B., LEVY, D. & BENJAMIN, E. J. 2007. Pulse pressure and risk of new-onset atrial fibrillation. JAMA, 297**,** 709-15.

MIYAZAWA, T., REBHUNG, F., FUJIMOTO, K. & KANEDA, T. 1994. Soybean oil supplementation improves growth and prevents docosapentaenoic acid (22:5n-6) accumulation in tissues of rats fed on palm oil diet.

. Biosci. Biotech. Biochem, 58**,** 1794 - 1798.

MOORE, K. J. & FREEMAN, M. W. 2006. Scavenger receptors in atherosclerosis: beyond lipid uptake. Arterioscler Thromb Vasc Biol, 26**,** 1702-11.

MORENO, J. J. 2003. Effect of olive oil minor components on oxidative stress and arachidonic acid mobilization and metabolism by macrophages RAW 264.7. Free Radic Biol Med, 35**,** 1073-81.

MORGAN, R. E., PALINKAS, L. A., BARRETT-CONNOR, E. L. & WINGARD, D. L. 1993. Plasma cholesterol and depressive symptoms in older men. Lancet, 341**,** 75-9.

MORTENSEN, R. F. 2001. C-reactive protein, inflammation, and innate immunity. Immunol Res, 24**,** 163-76.

MOWRI, H. O., PATSCH, J. R., GOTTO, A. M., JR. & PATSCH, W. 1996. Apolipoprotein A-II influences the substrate properties of human HDL2 and HDL3 for hepatic lipase. Arterioscler Thromb Vasc Biol, 16**,** 755-62.

MUFUNDA, J., MEBRAHTU, G., USMAN, A., NYARANGO, P., KOSIA, A., GHEBRAT, Y., OGBAMARIAM, A., MASJUAN, M. & GEBREMICHAEL, A. 2006. The prevalence of hypertension and its relationship with obesity: results from a national blood pressure survey in Eritrea. J Hum Hypertens, 20**,** 59-65.

MUKHERJEE, S. & MITRA, A. 2009. Health effects of palm oil. J. Hum. Ecol, 26**,** 197-203.

MUKHERJEE, S., THAKUR, G., KUMAR, B. D., MITRA, A. & CHAKRABORTY, C. 2009. Long-term effects of a carbohydrate-rich diet on fasting blood sugar, lipid profile, and serum insulin values in rural Bengalis. J Diabetes, 1**,** 288-95.

MUNTEANU, A., TADDEI, M., TAMBURINI, I., BERGAMINI, E., AZZI, A. & ZINGG, J. M. 2006. Antagonistic effects of oxidized low density lipoprotein and alpha-tocopherol on CD36 scavenger receptor expression in monocytes:

involvement of protein kinase B and peroxisome proliferator-activated receptor-gamma. J Biol Chem, 281**,** 6489-97.

MURDOCH, S. J. & BRECKENRIDGE, W. C. 1995. Influence of lipoprotein lipase and hepatic lipase on the transformation of VLDL and HDL during lipolysis of VLDL. Atherosclerosis, 118**,** 193-212.

NAKASHIMA, Y., RAINES, E. W., PLUMP, A. S., BRESLOW, J. L. & ROSS, R. 1998. Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the ApoE-deficient mouse. Arterioscler Thromb Vasc Biol, 18**,** 842-51.

NARANG, D., SOOD, S., THOMAS, M. K., DINDA, A. K. & MAULIK, S. K. 2004. Effect of dietary palm olein oil on oxidative stress associated with ischemic-reperfusion injury in isolated rat heart. BMC Pharmacol, 4**,** 29.

NATARAJAN, P., RAY, K. K. & CANNON, C. P. 2010. High-density lipoprotein and coronary heart disease: current and future therapies. J Am Coll Cardiol, 55**,** 1283-99.

NATHAN, C. F. 1987. Secretory products of macrophages. J Clin Invest, 79**,** 319-26.

NATHAN, D. G. & SIEFF, C. A. 1987. The biological activities and uses of recombinant granulocyte-macrophage and multi-colony stimulating factors. Prog Hematol, 15**,** 1-18.

NEATON, J. D., BLACKBURN, H., JACOBS, D., KULLER, L., LEE, D. J., SHERWIN, R., SHIH, J., STAMLER, J. & WENTWORTH, D. 1992. Serum

cholesterol level and mortality findings for men screened in the Multiple Risk Factor Intervention Trial. Multiple Risk Factor Intervention Trial Research Group. Arch Intern Med, 152**,** 1490-500.

NG, T. K., HAYES, K. C., DEWITT, G. F., JEGATHESAN, M., SATGUNASINGAM, N., ONG, A. S. & TAN, D. 1992. Dietary palmitic and oleic acids exert similar effects on serum cholesterol and lipoprotein profiles in normocholesterolemic men and women. J Am Coll Nutr, 11**,** 383-90.

NGUYEN, T. V. & BROWNELL, W. E. 1998. Contribution of membrane cholesterol to outer hair cell lateral wall stiffness. Otolaryngol Head Neck Surg, 119**,** 14-20.

NISHIGAKI, I., HAGIHARA, M., TSUNEKAWA, H., MASEKI, M. & YAGI, K. 1981. Lipid peroxide levels of serum lipoprotein fractions of diabetic patients. Biochem Med, 25**,** 373-8.

NJELEKELA, M. A., MPEMBENI, R., MUHIHI, A., MLIGILICHE, N. L., SPIEGELMAN, D., HERTZMARK, E., LIU, E., FINKELSTEIN, J. L., FAWZI, W. W., WILLETT, W. C. & MTABAJI, J. 2009. Gender-related differences in the prevalence of cardiovascular disease risk factors and their correlates in urban Tanzania. BMC Cardiovasc Disord, 9**,** 30.

NONOGAKI, K., FULLER, G. M., FUENTES, N. L., MOSER, A. H., STAPRANS, I., GRUNFELD, C. & FEINGOLD, K. R. 1995a. Interleukin-6 stimulates hepatic triglyceride secretion in rats. Endocrinology, 136**,** 2143-9.

NONOGAKI, K., PAN, X. M., MOSER, A. H., STAPRANS, I., FEINGOLD, K. R. & GRUNFELD, C. 1995b. Keratinocyte growth factor increases fatty acid mobilization and hepatic triglyceride secretion in rats. Endocrinology, 136**,** 4278-84.

NTANDOU, G., DELISLE, H., AGUEH, V. & FAYOMI, B. 2009. Abdominal obesity explains the positive rural-urban gradient in the prevalence of the metabolic syndrome in Benin, West Africa. Nutr Res, 29**,** 180-9.

O'CONNOR, S., TAYLOR, C., CAMPBELL, L. A., EPSTEIN, S. & LIBBY, P. 2001. Potential infectious etiologies of atherosclerosis: a multifactorial perspective. Emerg Infect Dis, 7**,** 780-8.

O'RIORDAN, E., CHEN, J., BRODSKY, S. V., SMIRNOVA, I., LI, H. & GOLIGORSKY, M. S. 2005. Endothelial cell dysfunction: the syndrome in making. Kidney Int, 67**,** 1654-8.

OBARZANEK, E., SACKS, F. M., VOLLMER, W. M., BRAY, G. A., MILLER, E. R., 3RD, LIN, P. H., KARANJA, N. M., MOST-WINDHAUSER, M. M., MOORE, T. J., SWAIN, J. F., BALES, C. W. & PROSCHAN, M. A. 2001. Effects on blood lipids of a blood pressure-lowering diet: the Dietary Approaches to Stop Hypertension (DASH) Trial. Am J Clin Nutr, 74**,** 80-9.

OFUYA, Z. M. 2007. The incidence of hypertension among a select population of adults in the Niger Delta region of Nigeria. Southeast Asian J Trop Med Public Health, 38**,** 947-9.

OGITA, H., SHIMONAGATA, T., FUKUNAMI, M., KUMAGAI, K., YAMADA, T., ASANO, Y., HIRATA, A., ASAI, M., KUSUOKA, H., HORI, M. & HOKI, N. 2001. Prognostic significance of cardiac (123)I metaiodobenzylguanidine imaging for mortality and morbidity in patients with chronic heart failure: a prospective study. Heart, 86**,** 656-60.

OGUNTIBEJU, O. O., ESTERHUYSE, A. J. & TRUTER, E. J. 2009. Red palm oil: nutritional, physiological and therapeutic roles in improving human wellbeing and quality of life. Br J Biomed Sci, 66**,** 216-22.

OGUNTIBEJU, O. O., ESTERHUYSE, A. J. & TRUTER, E. J. 2010. Possible role of red palm oil supplementation in reducing oxidative stress in HIV/AIDS and TB patients: A Review. Journal of Medicinal Plants Research, 4 (3)**,** 188–196.

OHTA, H., WADA, H., NIWA, T., KIRII, H., IWAMOTO, N., FUJII, H., SAITO, K., SEKIKAWA, K. & SEISHIMA, M. 2005. Disruption of tumor necrosis factor-alpha gene diminishes the development of atherosclerosis in ApoE-deficient mice. Atherosclerosis, 180**,** 11-7.

OKPUZOR, J., OKOCHI, V. I., OGBUNUGAFOR, H. A., OGBONNIA, S., FAGBAYI, T. & OBIDIEGWU, C. 2009. Estimation of Cholesterol Level in Different Brands of Vegetable Oils. Pakistan Journal of Nutrition 8 (1)**,** 57-62.

OLISEKODIAKA, M. J., IGBENEGHU, C. A., ONUEGBU, A. J., ODURU, R. & LAWAL, A. O. 2012. Lipid, lipoproteins, total antioxidant status and organ

changes in rats administered high doses of cadmium chloride. Med Princ Pract, 21**,** 156-9.

OLSSON, U., CAMEJO, G. & BONDJERS, G. 1993. Binding of a synthetic apolipoprotein B-100 peptide and peptide analogues to chondroitin 6-sulfate: effects of the lipid environment. Biochemistry, 32**,** 1858-65.

OLUBA, O. M., ADEYEMI, O., OJIEH, G. C., ABOLUWOYE, C. O. & EIDANGBE, G. O. 2008. Comparative effect of soybean oil and palm oil on serum lipids and some serum enzymes in cholesterol-fed rats. Eur. J. Sci., 23**,** 559-566.

OMOIGUI, S. 2007. The Interleukin-6 inflammation pathway from cholesterol to aging--role of statins, bisphosphonates and plant polyphenols in aging and age-related diseases. Immun Ageing, 4**,** 1.

ONG, A. S. & GOH, S. H. 2002. Palm oil: a healthful and cost-effective dietary component. Food Nutr Bull, 23**,** 11-22.

ORMEL, J., VON KORFF, M., BURGER, H., SCOTT, K., DEMYTTENAERE, K., HUANG, Y. Q., POSADA-VILLA, J., PIERRE LEPINE, J., ANGERMEYER, M. C., LEVINSON, D., DE GIROLAMO, G., KAWAKAMI, N., KARAM, E., MEDINA-MORA, M. E., GUREJE, O., WILLIAMS, D., HARO, J. M., BROMET, E. J., ALONSO, J. & KESSLER, R. 2007. Mental disorders among persons with heart disease - results from World Mental Health surveys. Gen Hosp Psychiatry, 29**,** 325-34.

ORTEGA, F. J., MORENO-NAVARRETE, J. M., SABATER, M., RICART, W., FRUHBECK, G. & FERNANDEZ-REAL, J. M. 2011. Circulating glucagon is associated with inflammatory mediators in metabolically compromised subjects. Eur J Endocrinol, 165**,** 639-45.

OSORIO, A., ORTEGA, E., DE HARO, T., TORRES, J. M., SANCHEZ, P. & RUIZ-REQUENA, E. 2011. Lipid profiles and oxidative stress parameters in male and female hemodialysis patients. Mol Cell Biochem, 353**,** 59-63.

OWOLABI, M. A., ABASS, M. M., EMEKA, P. M., JAJA, S. I., NNOLI, M. & DOSA, B. O. 2010. Biochemical and histologic changes in rats after prolonged administration of the crude aqueous extract of the leaves of Vitex grandifolia. Pharmacognosy Res, 2**,** 273-8.

PAASSILTA, M., KERVINEN, K., RANTALA, A. O., SAVOLAINEN, M. J., LILJA, M., REUNANEN, A. & KESANIEMI, Y. A. 1998. Social alcohol consumption and low Lp(a) lipoprotein concentrations in middle aged Finnish men: population based study. BMJ, 316**,** 594-5.

PALINSKI, W., ROSENFELD, M. E., YLA-HERTTUALA, S., GURTNER, G. C., SOCHER, S. S., BUTLER, S. W., PARTHASARATHY, S., CAREW, T. E., STEINBERG, D. & WITZTUM, J. L. 1989. Low density lipoprotein undergoes oxidative modification in vivo. Proc Natl Acad Sci U S A, 86**,** 1372-6.

PANIAGUA, J. A., GALLEGO DE LA SACRISTANA, A., ROMERO, I., VIDAL-PUIG, A., LATRE, J. M., SANCHEZ, E., PEREZ-MARTINEZ, P., LOPEZ-

MIRANDA, J. & PEREZ-JIMENEZ, F. 2007. Monounsaturated fat-rich diet prevents central body fat distribution and decreases postprandial adiponectin expression induced by a carbohydrate-rich diet in insulin-resistant subjects. Diabetes Care, 30**,** 1717-23.

PAOLETTI, R., GOTTO, A. M., JR. & HAJJAR, D. P. 2004. Inflammation in atherosclerosis and implications for therapy. Circulation, 109**,** III20-6.

PARHAMI, F., FANG, Z. T., FOGELMAN, A. M., ANDALIBI, A., TERRITO, M. C. & BERLINER, J. A. 1993. Minimally modified low density lipoprotein-induced inflammatory responses in endothelial cells are mediated by cyclic adenosine monophosphate. J Clin Invest, 92**,** 471-8.

PARK, H. W., KIM, W. H., KIM, K. H., YANG, D. J., KIM, J. H., SONG, I. G., KWON, T. G. & BAE, J. H. 2011. Carotid plaque is associated with increased cardiac mortality in patients with coronary artery disease. Int J Cardiol.

PARTHASARATHY, S., KHOO, J. C., MILLER, E., BARNETT, J., WITZTUM, J. L. & STEINBERG, D. 1990. Low density lipoprotein rich in oleic acid is protected against oxidative modification: implications for dietary prevention of atherosclerosis. Proc Natl Acad Sci U S A, 87**,** 3894-8.

PATEL, V., KHANNA, S., ROY, S., EZZIDDIN, O. & SEN, C. K. 2006. Natural vitamin E alpha-tocotrienol: retention in vital organs in response to long-term oral supplementation and withdrawal. Free Radic Res, 40**,** 763-71.

PATRICK, L. & UZICK, M. 2001. Cardiovascular disease: C-reactive protein and the inflammatory disease paradigm: HMG-CoA reductase inhibitors, alpha-tocopherol, red yeast rice, and olive oil polyphenols. A review of the literature. Altern Med Rev, 6**,** 248-71.

PEDERSEN, T. R. 1995. Lowering cholesterol with drugs and diet. N Engl J Med, 333**,** 1350-1.

PEER, P. A., TRIVEDI, P. C., NIGADE, P. B., GHAISAS, M. M. & DESHPANDE, A. D. 2008. Cardioprotective effect of Azadirachta indica A. Juss. on isoprenaline induced myocardial infarction in rats. Int J Cardiol, 126**,** 123-6.

PENALVA, R. A., HUOYA MDE, O., CORREIA, L. C., FEITOSA, G. S. & LADEIA, A. M. 2008. Lipid profile and intensity of atherosclerosis disease in acute coronary syndrome. Arq Bras Cardiol, 90**,** 24-30.

PEREIRA, T. A., SINNIAH, R. & DAS, N. P. 1990a. Effect of dietary palm oil on lipoprotein lipases: lipoprotein levels and tissue lipids in rat. Biochem Med Metab Biol, 44**,** 207-17.

PEREIRA, T. A., SINNIAH, R. & DAS, N. P. 1990b. Effect of dietary palm oil on lipoprotein lipases: Lipoprotein levels and tissue lipids in rat. . Biochemical Medicine and Metabolic Biology 44(3)**,** 207-217.

PERONA, J. S., CABELLO-MORUNO, R. & RUIZ-GUTIERREZ, V. 2006. The role of virgin olive oil components in the modulation of endothelial function. J Nutr Biochem, 17**,** 429-45.

PERONA, J. S., CANIZARES, J., MONTERO, E., SANCHEZ-DOMINGUEZ, J. M., PACHECO, Y. M. & RUIZ-GUTIERREZ, V. 2004. Dietary virgin olive oil triacylglycerols as an independent determinant of very low-density lipoprotein composition. Nutrition, 20**,** 509-14.

PERONA, J. S., VOGLER, O., SANCHEZ-DOMINGUEZ, J. M., MONTERO, E., ESCRIBA, P. V. & RUIZ-GUTIERREZ, V. 2007. Consumption of virgin olive oil influences membrane lipid composition and regulates intracellular signaling in elderly adults with type 2 diabetes mellitus. J Gerontol A Biol Sci Med Sci, 62**,** 256-63.

PETERSON, L. R. & NOSKIN, G. A. 2001. New technology for detecting multidrug-resistant pathogens in the clinical microbiology laboratory. Emerg Infect Dis, 7**,** 306-11.

PETRONI, A., BLASEVICH, M., SALAMI, M., PAPINI, N., MONTEDORO, G. F. & GALLI, C. 1995. Inhibition of platelet aggregation and eicosanoid production by phenolic components of olive oil. Thromb Res, 78**,** 151-60.

PHILLIPS, M. C., GILLOTTE, K. L., HAYNES, M. P., JOHNSON, W. J., LUND-KATZ, S. & ROTHBLAT, G. H. 1998. Mechanisms of high density lipoprotein-mediated efflux of cholesterol from cell plasma membranes. Atherosclerosis, 137 Suppl**,** S13-7.

PLETCHER, M. J., TICE, J. A., PIGNONE, M. & BROWNER, W. S. 2004. Using the coronary artery calcium score to predict coronary heart disease events: a systematic review and meta-analysis. Arch Intern Med, 164**,** 1285-92.

PLUMP, A. S., SMITH, J. D., HAYEK, T., AALTO-SETALA, K., WALSH, A., VERSTUYFT, J. G., RUBIN, E. M. & BRESLOW, J. L. 1992. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. Cell, 71**,** 343-53.

POOLE-WILSON, P. A. 2007. Cardiovascular health in Europe: a global problem. J Am Coll Cardiol, 49**,** 117-9.

POPA, C., NETEA, M. G., VAN RIEL, P. L., VAN DER MEER, J. W. & STALENHOEF, A. F. 2007. The role of TNF-alpha in chronic inflammatory conditions, intermediary metabolism, and cardiovascular risk. J Lipid Res, 48**,** 751-62.

POPKIN, B. M., ZIZZA, C. & SIEGA-RIZ, A. M. 2003. Who is leading the change?. U.S. dietary quality comparison between 1965 and 1996. Am J Prev Med, 25**,** 1-8.

PROCTOR, S. D., VINE, D. F. & MAMO, J. C. 2002. Arterial retention of apolipoprotein B(48)- and B(100)-containing lipoproteins in atherogenesis. Curr Opin Lipidol, 13**,** 461-70.

PUEPET, F. H. & OHWOVORIOLE, A. E. 2008. Prevalence of risk factors for diabetes mellitus in a non-diabetic population in Jos, Nigeria. Niger J Med, 17**,** 71-4.

QUINN, M. T., PARTHASARATHY, S., FONG, L. G. & STEINBERG, D. 1987. Oxidatively modified low density lipoproteins: a potential role in recruitment

and retention of monocyte/macrophages during atherogenesis. Proc Natl Acad Sci U S A, 84**,** 2995-8.

QURESHI, A. A., BRADLOW, B. A., BRACE, L., MANGANELLO, J., PETERSON, D. M., PEARCE, B. C., WRIGHT, J. J., GAPOR, A. & ELSON, C. E. 1995. Response of hypercholesterolemic subjects to administration of tocotrienols. Lipids, 30**,** 1171-7.

QURESHI, A. A., BURGER, W. C., PETERSON, D. M. & ELSON, C. E. 1986. The structure of an inhibitor of cholesterol biosynthesis isolated from barley. J Biol Chem, 261**,** 10544-50.

QURESHI, A. A., QURESHI, N., WRIGHT, J. J., SHEN, Z., KRAMER, G., GAPOR, A., CHONG, Y. H., DEWITT, G., ONG, A., PETERSON, D. M. & ET AL. 1991. Lowering of serum cholesterol in hypercholesterolemic humans by tocotrienols (palmvitee). Am J Clin Nutr, 53**,** 1021S-1026S.

RADER, D. J., IKEWAKI, K., DUVERGER, N., FEUERSTEIN, I., ZECH, L., CONNOR, W. & BREWER, H. B., JR. 1993a. Very low high-density lipoproteins without coronary atherosclerosis. Lancet, 342**,** 1455-8.

RADER, D. J., SCHAEFER, J. R., LOHSE, P., IKEWAKI, K., THOMAS, F., HARRIS, W. A., ZECH, L. A., DUJOVNE, C. A. & BREWER, H. B., JR. 1993b. Increased production of apolipoprotein A-I associated with elevated plasma levels of high-density lipoproteins, apolipoprotein A-I, and lipoprotein A-I in a patient with familial hyperalphalipoproteinemia. Metabolism, 42**,** 1429-34.

RAMIREZ-TORTOSA, M. C., URBANO, G., LOPEZ-JURADO, M., NESTARES, T., GOMEZ, M. C., MIR, A., ROS, E., MATAIX, J. & GIL, A. 1999. Extra-virgin olive oil increases the resistance of LDL to oxidation more than refined olive oil in free-living men with peripheral vascular disease. J Nutr, 129**,** 2177-83.

RAND, M. L., HENNISSEN, A. A. & HORNSTRA, G. 1988. Effects of dietary palm oil on arterial thrombosis, platelet responses and platelet membrane fluidity in rats. Lipids, 23**,** 1019-23.

RAO, M. V. & SHARMA, P. S. 2001. Protective effect of vitamin E against mercuric chloride reproductive toxicity in male mice. Reprod Toxicol, 15**,** 705-12.

RAVNSKOV, U. 2005. [Should cholesterol levels be reduced more aggressively?]. Lakartidningen, 102**,** 2583-4; discussion 2584.

RAVNSKOV, U., ROSCH, P. J., SUTTER, M. C. & HOUSTON, M. C. 2006. Should we lower cholesterol as much as possible? BMJ, 332**,** 1330-2.

RECKELHOFF, J. F. 2001. Gender differences in the regulation of blood pressure. Hypertension, 37**,** 1199-208.

RENAUD, S. C., RUF, J. C. & PETITHORY, D. 1995. The positional distribution of fatty acids in palm oil and lard influences their biologic effects in rats. J Nutr, 125**,** 229-37.

RIDKER 1999. Evaluating novel cardiovascular risk factors: can we do better predict heart attacks? Ann Intern Med, 130**,** 933–937.

RIDKER, P. M. 1997. Fibrinolytic and inflammatory markers for arterial occlusion: the evolving epidemiology of thrombosis and hemostasis. Thromb Haemost, 78**,** 53-9.

RIDKER, P. M. 1998. Intercellular adhesion molecule (ICAM-1) and the risks of developing atherosclerotic disease. Eur Heart J, 19**,** 1119-21.

RIDKER, P. M. 2001. High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. Circulation, 103**,** 1813-8.

RIGOTTI, A., ACTON, S. L. & KRIEGER, M. 1995. The class B scavenger receptors SR-BI and CD36 are receptors for anionic phospholipids. J Biol Chem, 270**,** 16221-4.

RIMM, E. & COLDITZ, G. 1993. Smoking, alcohol, and plasma levels of carotenes and vitamin E. Ann N Y Acad Sci, 686**,** 323-33; discussion 333-4.

RIMM, E. B. & STAMPFER, M. J. 2001. Antioxidants and chronic disease: evidence from observational epidemiology. Bibl Nutr Dieta**,** 80-91.

RINNINGER, F., BRUNDERT, M., JACKLE, S., KAISER, T. & GRETEN, H. 1995. Selective uptake of low-density lipoprotein-associated cholesteryl esters by human fibroblasts, human HepG2 hepatoma cells and J774 macrophages in culture. Biochim Biophys Acta, 1255**,** 141-53.

ROBINSON, S. M., BATELAAN, S. F., SYDDALL, H. E., SAYER, A. A., DENNISON, E. M., MARTIN, H. J., BARKER, D. J. & COOPER, C. 2006.

Combined effects of dietary fat and birth weight on serum cholesterol concentrations: the Hertfordshire Cohort Study. Am J Clin Nutr, 84**,** 237-44.

ROE, M. T., OU, F. S., ALEXANDER, K. P., NEWBY, L. K., FOODY, J. M., GIBLER, W. B., BODEN, W. E., OHMAN, E. M., SMITH, S. C., JR. & PETERSON, E. D. 2008. Patterns and prognostic implications of low high-density lipoprotein levels in patients with non-ST-segment elevation acute coronary syndromes. Eur Heart J, 29**,** 2480-8.

ROSS, R. 1979. The pathogenesis of atherosclerosis. Mech Ageing Dev, 9**,** 435-40.

ROSS, R. 1990. Mechanisms of atherosclerosis--a review. Adv Nephrol Necker Hosp, 19**,** 79-86.

ROSS, R. 1993a. Atherosclerosis: current understanding of mechanisms and future strategies in therapy. Transplant Proc, 25**,** 2041-3.

ROSS, R. 1993b. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature, 362**,** 801-9.

ROSS, R. 1999. Atherosclerosis is an inflammatory disease. Am Heart J, 138**,** S419-20.

ROSS, R. & GLOMSET, J. A. 1976. The pathogenesis of atherosclerosis (second of two parts). N Engl J Med, 295**,** 420-5.

ROTA, S., MCWILLIAM, N. A., BAGLIN, T. P. & BYRNE, C. D. 1998. Atherogenic lipoproteins support assembly of the prothrombinase complex and thrombin generation: modulation by oxidation and vitamin E. Blood, 91**,** 508-15.

ROWLAND, N. E. & FREGLY, M. J. 1992. Role of gonadal hormones in hypertension in the Dahl salt-sensitive rat. Clin Exp Hypertens A, 14**,** 367-75.

RUDEL, L. L., HAINES, J., SAWYER, J. K., SHAH, R., WILSON, M. S. & CARR, T. P. 1997. Hepatic origin of cholesteryl oleate in coronary artery atherosclerosis in African green monkeys. Enrichment by dietary monounsaturated fat. J Clin Invest, 100**,** 74-83.

RUDEL, L. L., JOHNSON, F. L., SAWYER, J. K., WILSON, M. S. & PARKS, J. S. 1995a. Dietary polyunsaturated fat modifies low-density lipoproteins and reduces atherosclerosis of nonhuman primates with high and low diet responsiveness. Am J Clin Nutr, 62**,** 463S-470S.

RUDEL, L. L., KELLEY, K., SAWYER, J. K., SHAH, R. & WILSON, M. D. 1998. Dietary monounsaturated fatty acids promote aortic atherosclerosis in LDL receptor-null, human ApoB100-overexpressing transgenic mice. Arterioscler Thromb Vasc Biol, 18**,** 1818-27.

RUDEL, L. L., PARKS, J. S. & SAWYER, J. K. 1995b. Compared with dietary monounsaturated and saturated fat, polyunsaturated fat protects African green monkeys from coronary artery atherosclerosis. Arterioscler Thromb Vasc Biol, 15**,** 2101-10.

RUEF, J., PETER, K., NORDT, T. K., RUNGE, M. S., KUBLER, W. & BODE, C. 1999. Oxidative stress and atherosclerosis: its relationship to growth factors,

thrombus formation and therapeutic approaches. Thromb Haemost, 82 Suppl 1**,** 32-7.

SACKS, F. M. & CAMPOS, H. 2003. Clinical review 163: Cardiovascular endocrinology: Low-density lipoprotein size and cardiovascular disease: a reappraisal. J Clin Endocrinol Metab, 88**,** 4525-32.

SACKS, F. M., PFEFFER, M. A., MOYE, L. A., ROULEAU, J. L., RUTHERFORD, J. D., COLE, T. G., BROWN, L., WARNICA, J. W., ARNOLD, J. M., WUN, C. C., DAVIS, B. R. & BRAUNWALD, E. 1996. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. N Engl J Med, 335**,** 1001-9.

SALONEN, J. T., YLA-HERTTUALA, S., YAMAMOTO, R., BUTLER, S., KORPELA, H., SALONEN, R., NYYSSONEN, K., PALINSKI, W. & WITZTUM, J. L. 1992. Autoantibody against oxidised LDL and progression of carotid atherosclerosis. Lancet, 339**,** 883-7.

SALVINI, S., SERA, F., CARUSO, D., GIOVANNELLI, L., VISIOLI, F., SAIEVA, C., MASALA, G., CEROTI, M., GIOVACCHINI, V., PITOZZI, V., GALLI, C., ROMANI, A., MULINACCI, N., BORTOLOMEAZZI, R., DOLARA, P. & PALLI, D. 2006. Daily consumption of a high-phenol extra-virgin olive oil reduces oxidative DNA damage in postmenopausal women. Br J Nutr, 95**,** 742-51.

SARVAJEET, S. G. & NARENDRA, T. 2010. Reactive oxygen spicies and antioxidant machinery in abiotic stress tolerant in crop plants. Plant physiol. Bioch., 48**,** 909-930.

SAUVAGET, C., NAGANO, J., HAYASHI, M. & YAMADA, M. 2004. Animal protein, animal fat, and cholesterol intakes and risk of cerebral infarction mortality in the adult health study. Stroke, 35**,** 1531-7.

SCACCINI, C., NARDINI, M., D'AQUINO, M., GENTILI, V., DI FELICE, M. & TOMASSI, G. 1992. Effect of dietary oils on lipid peroxidation and on antioxidant parameters of rat plasma and lipoprotein fractions. J Lipid Res, 33**,** 627-33.

SCHIEFFER, B., SELLE, T., HILFIKER, A., HILFIKER-KLEINER, D., GROTE, K., TIETGE, U. J., TRAUTWEIN, C., LUCHTEFELD, M., SCHMITTKAMP, C., HEENEMAN, S., DAEMEN, M. J. & DREXLER, H. 2004. Impact of interleukin-6 on plaque development and morphology in experimental atherosclerosis. Circulation, 110**,** 3493-500.

SCHLESER, S., RINGSEIS, R. & EDER, K. 2006. Conjugated linoleic acids have no effect on TNF alpha-induced adhesion molecule expression, U937 monocyte adhesion, and chemokine release in human aortic endothelial cells. Atherosclerosis, 186**,** 337-44.

SCHWAB, U. S., MALIRANTA, H. M., SARKKINEN, E. S., SAVOLAINEN, M. J., KESANIEMI, Y. A. & UUSITUPA, M. I. 1996. Different effects of palmitic and stearic acid-enriched diets on serum lipids and lipoproteins and

plasma cholesteryl ester transfer protein activity in healthy young women. Metabolism, 45**,** 143-9.

SCHWARTZ, S. M., VIRMANI, R. & ROSENFELD, M. E. 2000. The good smooth muscle cells in atherosclerosis. Curr Atheroscler Rep, 2**,** 422-9.

SCHWENKE, D. C. & CAREW, T. E. 1989. Initiation of atherosclerotic lesions in cholesterol-fed rabbits. II. Selective retention of LDL vs. selective increases in LDL permeability in susceptible sites of arteries. Arteriosclerosis, 9**,** 908-18.

SELVIN, E. & ERLINGER, T. P. 2004. Prevalence of and risk factors for peripheral arterial disease in the United States: results from the National Health and Nutrition Examination Survey, 1999-2000. Circulation, 110**,** 738-43.

SEMB, H. & OLIVECRONA, T. 1987. Mechanisms for turnover of lipoprotein lipase in guinea pig adipocytes. Biochim Biophys Acta, 921**,** 104-15.

SEO, T. & ST CLAIR, R. W. 1997. Heparan sulfate proteoglycans mediate internalization and degradation of beta-VLDL and promote cholesterol accumulation by pigeon macrophages. J Lipid Res, 38**,** 765-79.

SEO, T., VELEZ-CARRASCO, W., QI, K., HALL, M., WORGALL, T. S., JOHNSON, R. A. & DECKELBAUM, R. J. 2002. Selective uptake from LDL is stimulated by unsaturated fatty acids and modulated by cholesterol content in the plasma membrane: role of plasma membrane composition in regulating non-SR-BI-mediated selective lipid transfer. Biochemistry, 41**,** 7885-94.

SHAHAR, E., CHAMBLESS, L. E., ROSAMOND, W. D., BOLAND, L. L., BALLANTYNE, C. M., MCGOVERN, P. G. & SHARRETT, A. R. 2003. Plasma lipid profile and incident ischemic stroke: the Atherosclerosis Risk in Communities (ARIC) study. Stroke, 34**,** 623-31.

SHEPHERD, J., PACKARD, C. J., GRUNDY, S. M., YESHURUN, D., GOTTO, A. M., JR. & TAUNTON, O. D. 1980a. Effects of saturated and polyunsaturated fat diets on the chemical composition and metabolism of low density lipoproteins in man. J Lipid Res, 21**,** 91-9.

SHEPHERD, J., STEWART, J. M., CLARK, J. G. & CARR, K. 1980b. Sequential changes in plasma lipoproteins and body fat composition during polyunsaturated fat feeding in man. Br J Nutr, 44**,** 265-71.

SIMIONESCU, M. & SIMIONESCU, N. 1993. Proatherosclerotic events: pathobiochemical changes occurring in the arterial wall before monocyte migration. FASEB J, 7**,** 1359-66.

SINGH, P., SINGH, M. & KAUR, T. 2009. Role of apolipoproteins E and A-I: epistatic villains of triglyceride mediation in coronary heart disease. Int J Cardiol, 134**,** 410-2.

SIRI-TARINO, P. W., SUN, Q., HU, F. B. & KRAUSS, R. M. 2010a. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. Am J Clin Nutr, 91**,** 535-46.

SIRI-TARINO, P. W., SUN, Q., HU, F. B. & KRAUSS, R. M. 2010b. Saturated fatty acids and risk of coronary heart disease: modulation by replacement nutrients. Curr Atheroscler Rep, 12**,** 384-90.

SIRI, P. W. & KRAUSS, R. M. 2005. Influence of dietary carbohydrate and fat on LDL and HDL particle distributions. Curr Atheroscler Rep, 7**,** 455-9.

SMITH, G. D. & PEKKANEN, J. 1992. The cholesterol controversy. BMJ, 304**,** 913.

SNIDERMAN, A., SHAPIRO, S., MARPOLE, D., SKINNER, B., TENG, B. & KWITEROVICH, P. O., JR. 1980. Association of coronary atherosclerosis with hyperapobetalipoproteinemia [increased protein but normal cholesterol levels in human plasma low density (beta) lipoproteins]. Proc Natl Acad Sci U S A, 77**,** 604-8.

SOLHEIM, S., SELJEFLOT, I., ARNESEN, H., ERITSLAND, J. & EIKVAR, L. 2001. Reduced levels of TNF alpha in hypercholesterolemic individuals after treatment with pravastatin for 8 weeks. Atherosclerosis, 157**,** 411-5.

SORCI-THOMAS, M., PRACK, M. M., DASHTI, N., JOHNSON, F., RUDEL, L. L. & WILLIAMS, D. L. 1989. Differential effects of dietary fat on the tissue-specific expression of the apolipoprotein A-I gene: relationship to plasma concentration of high density lipoproteins. J Lipid Res, 30**,** 1397-403.

SPILLER, G. A. 1991. Beyond dietary fiber. Am J Clin Nutr, 54**,** 615-7.

STARNES, H. F., JR., WARREN, R. S., JEEVANANDAM, M., GABRILOVE, J. L., LARCHIAN, W., OETTGEN, H. F. & BRENNAN, M. F. 1988. Tumor

necrosis factor and the acute metabolic response to tissue injury in man. J Clin Invest, 82**,** 1321-5.

STARY, H. C. 1990. [Atherosclerotic lesions in the young]. G Ital Cardiol, 20**,** 1056-8.

STARY, H. C., CHANDLER, A. B., DINSMORE, R. E., FUSTER, V., GLAGOV, S., INSULL, W., JR., ROSENFELD, M. E., SCHWARTZ, C. J., WAGNER, W. D. & WISSLER, R. W. 1995a. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation, 92**,** 1355-74.

STARY, H. C., CHANDLER, A. B., DINSMORE, R. E., FUSTER, V., GLAGOV, S., INSULL, W., JR., ROSENFELD, M. E., SCHWARTZ, C. J., WAGNER, W. D. & WISSLER, R. W. 1995b. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Arterioscler Thromb Vasc Biol, 15**,** 1512-31.

STEFANICK, M. L., MACKEY, S., SHEEHAN, M., ELLSWORTH, N., HASKELL, W. L. & WOOD, P. D. 1998. Effects of diet and exercise in men and postmenopausal women with low levels of HDL cholesterol and high levels of LDL cholesterol. N Engl J Med, 339**,** 12-20.

STEINBERG, D. 2002. Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. Nat Med, 8**,** 1211-7.

STEINBERG, S. J., WANG, S. J., KIM, D. G., MIHALIK, S. J. & WATKINS, P. A. 1999. Human very-long-chain acyl-CoA synthetase: cloning, topography, and relevance to branched-chain fatty acid metabolism. Biochem Biophys Res Commun, 257**,** 615-21.

STELMACH, W. J., RUSH, D. R., BRUCKER, P. C., SCHAEFER, E. J., HOLVERSON, H. E., KANE, W. J. & HUFFMAN, B. L., JR. 1993. Diet and exercise and gemfibrozil therapy for the management of dyslipidemia: a CEN study. Clinical Experience Network. J Fam Pract, 36**,** 401-8.

STEPHENS, N. G., PARSONS, A., SCHOFIELD, P. M., KELLY, F., CHEESEMAN, K. & MITCHINSON, M. J. 1996. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). Lancet, 347**,** 781-6.

STEVENSON, S. C., WANG, S., DENG, L. & TALL, A. R. 1993. Human plasma cholesteryl ester transfer protein consists of a mixture of two forms reflecting variable glycosylation at asparagine 341. Biochemistry, 32**,** 5121-6.

STOCKER, R. & KEANEY, J. F., JR. 2004. Role of oxidative modifications in atherosclerosis. Physiol Rev, 84**,** 1381-478.

SUGANO, M. & IMAIZUMI, K. 1991. Effect of palm oil on lipid and lipoprotein metabolism and eicosanoid production in rats. Am J Clin Nutr, 53**,** 1034S-1038S.

SUN, Y., YANG, Y., PEI, W., WU, Y. & ZHAO, J. 2007. Is elevated high-density lipoprotein cholesterol always good for coronary heart disease? Clin Cardiol, 30**,** 576-80.

SUNDRAM, K., HORNSTRA, G., VON HOUWELINGEN, A. C. & KESTER, A. D. 1992. Replacement of dietary fat with palm oil: effect on human serum lipids, lipoproteins and apolipoproteins. Br J Nutr, 68**,** 677-92.

SUNDRAM, K., KHOR, H. T. & ONG, A. S. 1990a. Effect of dietary palm oil and its fractions on rat plasma and high density lipoprotein lipids. Lipids, 25**,** 187-93.

SUNDRAM, K., SAMBANTHAMURTHI, R. & TAN, Y. A. 2003. Palm fruit chemistry and nutrition. Asia Pac J Clin Nutr, 12**,** 355-62.

SUNDRAM, K., T, K. H. & ONG, A. S. H. 1990b. Biochemical and nutrition studies on palm oil. Proc.Mai. Biochem. Soc. Conference, 11**,** 28-31.

SUPERKO, H. R., NEJEDLY, M. & GARRETT, B. 2002. Small LDL and its clinical importance as a new CAD risk factor: a female case study. Prog Cardiovasc Nurs, 17**,** 167-73.

TABAS, I. 2005. Consequences and therapeutic implications of macrophage apoptosis in atherosclerosis: the importance of lesion stage and phagocytic efficiency. Arterioscler Thromb Vasc Biol, 25**,** 2255-64.

TALL, A. R. 1993. Plasma cholesteryl ester transfer protein. J Lipid Res, 34**,** 1255-74.

TAN, D. T., KHOR, H. T., LOW, W. H., ALI, A. & GAPOR, A. 1991. Effect of a palm-oil-vitamin E concentrate on the serum and lipoprotein lipids in humans. Am J Clin Nutr, 53**,** 1027S-1030S.

TAPPIA, P. S. & GRIMBLE, R. F. 1994. Complex modulation of cytokine induction by endotoxin and tumour necrosis factor from peritoneal macrophages of rats by diets containing fats of different saturated, monounsaturated and polyunsaturated fatty acid composition. Clin Sci (Lond), 87**,** 173-8.

TEDGUI, A. & MALLAT, Z. 2001. Apoptosis as a determinant of atherothrombosis. Thromb Haemost, 86**,** 420-6.

TEDGUI, A. & MALLAT, Z. 2006. Cytokines in atherosclerosis: pathogenic and regulatory pathways. Physiol Rev, 86**,** 515-81.

TEMEL, R. E., TRIGATTI, B., DEMATTOS, R. B., AZHAR, S., KRIEGER, M. & WILLIAMS, D. L. 1997. Scavenger receptor class B, type I (SR-BI) is the major route for the delivery of high density lipoprotein cholesterol to the steroidogenic pathway in cultured mouse adrenocortical cells. Proc Natl Acad Sci U S A, 94**,** 13600-5.

TERZUOLI, E., DONNINI, S., GIACHETTI, A., INIGUEZ, M. A., FRESNO, M., MELILLO, G. & ZICHE, M. 2010. Inhibition of hypoxia inducible factor-1alpha by dihydroxyphenylethanol, a product from olive oil, blocks microsomal prostaglandin-E synthase-1/vascular endothelial growth factor expression and reduces tumor angiogenesis. Clin Cancer Res, 16**,** 4207-16.

THOLSTRUP, T., MARCKMANN, P., JESPERSEN, J., VESSBY, B., JART, A. & SANDSTROM, B. 1994. Effect on blood lipids, coagulation, and fibrinolysis of a fat high in myristic acid and a fat high in palmitic acid. Am J Clin Nutr, 60**,** 919-25.

TORNVALL, P., KARPE, F., CARLSON, L. A. & HAMSTEN, A. 1991. Relationships of low density lipoprotein subfractions to angiographically defined coronary artery disease in young survivors of myocardial infarction. Atherosclerosis, 90**,** 67-80.

TRUSWELL, A. S. 1994. Food carbohydrates and plasma lipids--an update. Am J Clin Nutr, 59**,** 710S-718S.

TRUSWELL, A. S. 2000. Comparing palmolein with different predominantly monounsaturated oils: effect on plasma lipids. Int J Food Sci Nutr, 51 Suppl**,** S73-7.

TYMCHUK, C. N., HARTIALA, J., PATEL, P. I., MEHRABIAN, M. & ALLAYEE, H. 2006. Nonconventional genetic risk factors for cardiovascular disease. Curr Atheroscler Rep, 8**,** 184-92.

TZOULAKI, I., MURRAY, G. D., PRICE, J. F., SMITH, F. B., LEE, A. J., RUMLEY, A., LOWE, G. D. & FOWKES, F. G. 2006. Hemostatic factors, inflammatory markers, and progressive peripheral atherosclerosis: the Edinburgh Artery Study. Am J Epidemiol, 163**,** 334-41.

VALAVANIDIS, A., NISIOTOU, C., PAPAGEORGIOU, Y., KREMLI, I., SATRAVELAS, N., ZINIERIS, N. & ZYGALAKI, H. 2004. Comparison of

the radical scavenging potential of polar and lipidic fractions of olive oil and other vegetable oils under normal conditions and after thermal treatment. J Agric Food Chem, 52**,** 2358-65.

VERDIER, F. & FOURCADE, L. 2007. [Changes in cardiovascular risk factors in developing countries]. Med Trop (Mars), 67**,** 552-8.

VESSBY, B., ARO, A., SKARFORS, E., BERGLUND, L., SALMINEN, I. & LITHELL, H. 1994. The risk to develop NIDDM is related to the fatty acid composition of the serum cholesterol esters. Diabetes, 43**,** 1353-7.

VIJAYAGOPAL, P., SRINIVASAN, S. R., RADHAKRISHNAMURTHY, B. & BERENSON, G. S. 1992. Lipoprotein-proteoglycan complexes from atherosclerotic lesions promote cholesteryl ester accumulation in human monocytes/macrophages. Arterioscler Thromb, 12**,** 237-49.

VISIOLI, F., BELLOMO, G., MONTEDORO, G. & GALLI, C. 1995. Low density lipoprotein oxidation is inhibited in vitro by olive oil constituents. Atherosclerosis, 117**,** 25-32.

VITA, J. A., KEANEY, J. F., JR., LARSON, M. G., KEYES, M. J., MASSARO, J. M., LIPINSKA, I., LEHMAN, B. T., FAN, S., OSYPIUK, E., WILSON, P. W., VASAN, R. S., MITCHELL, G. F. & BENJAMIN, E. J. 2004. Brachial artery vasodilator function and systemic inflammation in the Framingham Offspring Study. Circulation, 110**,** 3604-9.

VLES, R. O., BUELLER, J., GOTTENBOS, J. & THOMASSON, H. J. 1964. Influence of Type of Dietary Fat on Cholesterol-Induced Atherosclerosis in the Rabbit. J Atheroscler Res, 4**,** 170-83.

WALD, N. J. & LAW, M. R. 2003. A strategy to reduce cardiovascular disease by more than 80%. BMJ, 326**,** 1419.

WALLDIUS, G., JUNGNER, I., HOLME, I., AASTVEIT, A. H., KOLAR, W. & STEINER, E. 2001. High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. Lancet, 358**,** 2026-33.

WALLIN, B., ROSENGREN, B., SHERTZER, H. G. & CAMEJO, G. 1993. Lipoprotein oxidation and measurement of thiobarbituric acid reacting substances formation in a single microtiter plate: its use for evaluation of antioxidants. Anal Biochem, 208**,** 10-5.

WALTON, T. J., SAMANI, N. J. & ANDREWS, R. 2002. Systemic cholesterol crystal embolisation with pulmonary involvement: a fatal combination after coronary angiography. Postgrad Med J, 78**,** 288-9.

WANG, W. Y., ZHANG, J., WU, W. Y., LI, J., MA, Y. L., CHEN, W. H., YAN, H., WANG, K., XU, W. W., SHEN, J. H. & WANG, Y. P. 2011. Inhibition of lipoprotein-associated phospholipase A2 ameliorates inflammation and decreases atherosclerotic plaque formation in ApoE-deficient mice. PLoS One, 6**,** e23425.

WANNAMETHEE, S. G., SHAPER, A. G. & WALKER, M. 1998. Changes in physical activity, mortality, and incidence of coronary heart disease in older men. Lancet, 351**,** 1603-8.

WEBER, C. 2008. Chemokines in atherosclerosis, thrombosis, and vascular biology. Arterioscler Thromb Vasc Biol, 28**,** 1896.

WEBER, C. & NOELS, H. 2011. Atherosclerosis: current pathogenesis and therapeutic options. Nat Med, 17**,** 1410-22.

WEGGEMANS, R. M., ZOCK, P. L., URGERT, R. & KATAN, M. B. 1999. Differences between men and women in the response of serum cholesterol to dietary changes. Eur J Clin Invest, 29**,** 827-34.

WEINSTOCK, P. H., BISGAIER, C. L., AALTO-SETALA, K., RADNER, H., RAMAKRISHNAN, R., LEVAK-FRANK, S., ESSENBURG, A. D., ZECHNER, R. & BRESLOW, J. L. 1995. Severe hypertriglyceridemia, reduced high density lipoprotein, and neonatal death in lipoprotein lipase knockout mice. Mild hypertriglyceridemia with impaired very low density lipoprotein clearance in heterozygotes. J Clin Invest, 96**,** 2555-68.

WEISSBERG, P. L. 2000. Atherogenesis: current understanding of the causes of atheroma. Indian Heart J, 52**,** 467-72.

WESTHUYZEN, J. 1997. The oxidation hypothesis of atherosclerosis: an update. Ann Clin Lab Sci, 27**,** 1-10.

WHO 1990 Health Organization: Diet, nutrition, and the prevention of chronic diseases. Report of a WHO Study GroupWorld. WHO Technical Report Series 797. Geneva

WHO 2008. The global burden of disease: 2004 update. Geneva: World Health Organization.

WHO 2011. Global Atlas on cardiovascular disease prevention and control In: SHANTHI MENDIS, P. P. A. B. N. (ed.) Geneva: World Health Organization in collaboration with the World Heart Federation and the World Stroke Organization.

WIBERG, E., BANAS, A. & STYMNE, S. 1997. Fatty acid distribution and lipid metabolism in developing seeds of laurate-producing rape (Brassica napus L.). Planta, 203**,** 341-8.

WILCOX, J. N., SMITH, K. M., SCHWARTZ, S. M. & GORDON, D. 1989. Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. Proc Natl Acad Sci U S A, 86**,** 2839-43.

WILLERSON, J. T. & RIDKER, P. M. 2004. Inflammation as a cardiovascular risk factor. Circulation, 109**,** II2-10.

WILLIAMS, K. J., FLESS, G. M., PETRIE, K. A., SNYDER, M. L., BROCIA, R. W. & SWENSON, T. L. 1992. Mechanisms by which lipoprotein lipase alters cellular metabolism of lipoprotein(a), low density lipoprotein, and nascent lipoproteins. Roles for low density lipoprotein receptors and heparan sulfate proteoglycans. J Biol Chem, 267**,** 13284-92.

WILLIAMS, M. G. A. & PADLEY, F. B. 1985. Palm oil: quality requirements from a customer's point of view. . J. Amer. Oil Chem Soc, 62**,** 454-456.

WILLIAMS, P. T., DREON, D. M. & KRAUSS, R. M. 1995. Effects of dietary fat on high-density-lipoprotein subclasses are influenced by both apolipoprotein E isoforms and low-density-lipoprotein subclass patterns. Am J Clin Nutr, 61**,** 1234-40.

WILSON, P. W. 1999. From hypertension to heart failure: what have we learned? Clin Cardiol, 22 Suppl 5**,** V1-10.

WILSON, P. W. & CULLETON, B. F. 1998. Epidemiology of cardiovascular disease in the United States. Am J Kidney Dis, 32**,** S56-65.

WITTRAM, C. 2003. The normal main pulmonary artery diameter. Br J Radiol, 76**,** 79; author reply 79.

WOLF, G. 1996. High-fat, high-cholesterol diet raises plasma HDL cholesterol: studies on the mechanism of this effect. Nutr Rev, 54**,** 34-5.

WONG, T. Y., LARSEN, E. K., KLEIN, R., MITCHELL, P., COUPER, D. J., KLEIN, B. E., HUBBARD, L. D., SISCOVICK, D. S. & SHARRETT, A. R. 2005. Cardiovascular risk factors for retinal vein occlusion and arteriolar emboli: the Atherosclerosis Risk in Communities & Cardiovascular Health studies. Ophthalmology, 112**,** 540-7.

WRIGHT, J. T., WATERSON, E. J., BARRISON, I. G., TOPLIS, P. J., LEWIS, I. G., GORDON, M. G., MACRAE, K. D., MORRIS, N. F. & MURRAY-

LYON, I. M. 1983. Alcohol consumption, pregnancy, and low birthweight. Lancet, 1**,** 663-5.

XIE, W., LIANG, L., ZHAO, L., SHI, P., YANG, Y., XIE, G., HUO, Y. & WU, Y. 2011. Combination of carotid intima-media thickness and plaque for better predicting risk of ischaemic cardiovascular events. Heart, 97**,** 1326-31.

XU, H., YAO, Y., SU, Z., YANG, Y., KAO, R., MARTIN, C. M. & RUI, T. 2011. Endogenous HMGB1 contributes to ischemia-reperfusion-induced myocardial apoptosis by potentiating the effect of TNF-α/JNK. Am J Physiol Heart Circ Physiol, 300**,** H913-21.

XU, J., EILAT-ADAR, S., LORIA, C., GOLDBOURT, U., HOWARD, B. V., FABSITZ, R. R., ZEPHIER, E. M., MATTIL, C. & LEE, E. T. 2006. Dietary fat intake and risk of coronary heart disease: the Strong Heart Study. Am J Clin Nutr, 84**,** 894-902.

XU, X. X. & TABAS, I. 1991. Sphingomyelinase enhances low density lipoprotein uptake and ability to induce cholesteryl ester accumulation in macrophages. J Biol Chem, 266**,** 24849-58.

YACH, D., HAWKES, C., GOULD, C. L. & HOFMAN, K. J. 2004. The global burden of chronic diseases: overcoming impediments to prevention and control. JAMA, 291**,** 2616-22.

YLA-HERTTUALA, S., PALINSKI, W., ROSENFELD, M. E., PARTHASARATHY, S., CAREW, T. E., BUTLER, S., WITZTUM, J. L. & STEINBERG, D. 1989. Evidence for the presence of oxidatively modified

low density lipoprotein in atherosclerotic lesions of rabbit and man. J Clin Invest, 84**,** 1086-95.

YOUNG, N. L., MCNAMARA, D. J. & WILLEMS-DE HARVEN, G. 1987. Regulation of indices of cholesterol synthesis in human mononuclear leukocytes by dietary cholesterol and fat saturation. Atherosclerosis, 68**,** 137-49.

YUSUF, S. & ANAND, S. 1996. Cost of prevention. The case of lipid lowering. Circulation, 93**,** 1774-6.

YUSUF, S., LONN, E. & BOSCH, J. 2009. Lipid lowering for primary prevention. Lancet, 373**,** 1152-5.

ZAKYNTHINOS, E. & PAPPA, N. 2009. Inflammatory biomarkers in coronary artery disease. J Cardiol, 53**,** 317-33.

ZAMORA, R. & HIDALGO, F. J. 1995. Linoleic acid oxidation in the presence of amino compounds produces pyrroles by carbonyl amine reactions. Biochim Biophys Acta, 1258**,** 319-27.

ZEBA, A. N., MARTIN PREVEL, Y., SOME, I. T. & DELISLE, H. F. 2006. The positive impact of red palm oil in school meals on vitamin A status: study in Burkina Faso. Nutr J, 5**,** 17.

ZHANG, C., ZHANG, M. X., SHEN, Y. H., BURKS, J. K., ZHANG, Y., WANG, J., LEMAIRE, S. A., YOSHIMURA, K., AOKI, H., COSELLI, J. S. & WANG, X. L. 2007. TNF-alpha suppresses prolyl-4-hydroxylase alpha1

expression via the ASK1-JNK-NonO pathway. Arterioscler Thromb Vasc Biol, 27**,** 1760-7.

ZHANG, F., HU, E. C., TOPP, S., LEI, M., CHEN, W. & LINEAWEAVER, W. C. 2006. Proinflammatory cytokines gene expression in skin flaps with arterial and venous ischemia in rats. J Reconstr Microsurg, 22**,** 641-7.

ZHANG, J., PING, W., CHUNRONG, W., SHOU, C. X. & KEYOU, G. 1997. Nonhypercholesterolemic effects of a palm oil diet in Chinese adults. J Nutr, 127**,** 509S-513S.

ZHANG, J., WANG, C. R., XUE, A. N. & GE, K. Y. 2003. Effects of red palm oil on serum lipids and plasma carotenoids level in Chinese male adults. Biomed Environ Sci, 16**,** 348-54.

ZHANG, P. & OMAYE, S. T. 2001. Antioxidant and prooxidant roles for beta-carotene, alpha-tocopherol and ascorbic acid in human lung cells. Toxicol In Vitro, 15**,** 13-24.

ZHAO, L. & BRINTON, R. D. 2004. Suppression of proinflammatory cytokines interleukin-1beta and tumor necrosis factor-alpha in astrocytes by a V1 vasopressin receptor agonist: a cAMP response element-binding protein-dependent mechanism. J Neurosci, 24**,** 2226-35.

ZILVERSMIT, D. B. 1995. Atherogenic nature of triglycerides, postprandial lipidemia, and triglyceride-rich remnant lipoproteins. Clin Chem, 41**,** 153-8.

ZIMMERMAN, G. A., PRESCOTT, S. M. & MCINTYRE, T. M. 1992. Endothelial cell interactions with granulocytes: tethering and signaling molecules. Immunol Today, 13**,** 93-100.

ZOCK, P. L., DE VRIES, J. H. & KATAN, M. B. 1994. Impact of myristic acid versus palmitic acid on serum lipid and lipoprotein levels in healthy women and men. Arterioscler Thromb, 14**,** 567-75.