Cape Peninsula University of Technology

MODULATION OF POSTPRANDIAL OXIDATIVE STRESS BY ROOIBOS (*ASPALATHUS LINEARIS*) IN NORMOLIPIDAEMIC INDIVIDUALS

By

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DECLARATION

I, Ngiambudulu Mbandu Francisco, declare that the contents of this thesis represent my own work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

______________________________ ____________________

Signed **Date**

ABSTRACT

Consumption of sucrose with a meal containing oxidised and oxidisable lipids cause an increase in oxidative stress which is referred to as postprandial oxidative stress. The modulating effect on postprandial oxidative stress by an antioxidant-rich beverage, fermented rooibos (*Aspalathus linearis*) was compared to that of a commercial soft drink (soda). Both study beverages contained sucrose and were consumed with a standardised fat meal.

The study consisted of two parts, a pilot study (Phase One) where participants consumed either a standardised fat meal with water (control group n = 5) or a standardised fat meal with a sucrose-containing commercial soda (treatment group n = 8) using a parallel design, and the experimental study (Phase Two) where participants (n = 14) consumed the standardised fat meal with the commercial soda (control group) or the rooibos beverage (treatment group) using a crossover design. Specific analytical techniques and methods for determination of plasma glucose, serum insulin, lipid profile, an inflammatory indicator (high sensitive C-reactive protein), plasma antioxidant capacity, whole blood redox status and plasma lipid oxidation biomarkers were used. Results from the pilot study indicated significantly ($P<0.05$) higher postprandial levels of glucose in the control group at 4 hr and 6hr postprandially. The inflammatory biomarker and triglyceride levels were significantly ($P<0.05$) elevated in both groups when compared to their respective baselines. Results also showed the total antioxidant capacity and total glutathione levels in the plasma of both groups to be significantly ($P<0.05$) lowered when compared to the baseline values. The level of lipid oxidation biomarkers in the plasma was significantly ($P<0.05$) higher at 2 hr, 4 hr and 6 hr post time intervals for thiobarbituric acid reactive substances and 4 hr post time interval for conjugated dienes in the participants consuming the standardised fat meal with soda when compared to the baseline value, while this was reflected only at 2 hr post time interval for thiobarbituric acid reactive substances, with the conjugated dienes levels being significantly ($P<0.05$) lowered at 6 hr post time interval in the control group. No differences were shown on inter group level for the pilot study. On inter group level, results from Phase Two showed significant ($P<0.05$) lower levels of plasma glucose at 6 hr post time interval in the treatment group when compared to the control group, with insulin levels being significantly ($P<0.05$) higher in the control group at 4 hr post time interval. When considering the lipid profile, total cholesterol, low-density lipoprotein and triglyceride serum levels were significantly ($P<0.05$) lower in the treatment group at various post time intervals. The high-density lipoprotein levels were also shown to be lower in this group, but as the baseline values (0 hr) of the two groups

differed significantly, this lowered effect cannot be seen as a true reflection of the results. The inflammatory biomarker level was also significantly ($P<0.05$) lower in the treatment group at 6 hr post interval time when compared to the control group. The plasma antioxidant capacity (trolox equivalent antioxidant capacity at 4 hr) and redox status (total glutathione at 2, 4 and 6 hr) of the treatment group were significantly ($P<0.05$) elevated when compared to the control group. In line with these results, significant ($P<0.05$) lower levels of the lipid oxidation biomarkers (conjugated dienes and thiobarbituric acid reactive substances) were also shown in the treatment group at 4 hr, and 2 hr and 4 hr post time intervals, respectively, when compared to the control group.

These results (Phase Two) suggest that the rooibos beverage containing sucrose, when consumed with a standardised fat meal, modulated postprandial oxidative stress as shown by the significantly ($P<0.05$) elevated redox status, lowered inflammation and lipid oxidative damage, with important changes to the lipid profile.

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DEDICATION

I would like to dedicate this thesis to my dearest son, Jibril Bandu Francisco, for his patience and love on these days when we were physically away from each other.

TABLE OF CONTENTS

DECLARATION ..... **ii**
ABSTRACT ..... **iii**
ACKNOWLEDGEMENTS ..... **v**
DEDICATION ..... **vii**
CHAPTER ONE: GENERAL INTRODUCTION **1**
1.1 Aims of the study 2
CHAPTER TWO: LITERATURE REVIEW **3**
2.1 Oxidative stress and postprandial oxidative stress 3
2.1.1 Postprandial hyperglycaemia 8
2.1.1.1 Sucrose as dietary carbohydrate source 8
2.1.1.2 Ingestion, absorption and controversial health aspect of sugar 9
2.1.1.3 Carbohydrate and postprandial oxidative stress 11
2.1.2 Postprandial hyperlipidaemia 12
2.1.2.1 Kinetics of lipids after fat meal 12
2.1.2.2 Lipids, fats and lipid rich meal 13
2.1.2.3 Cholesterol and hypertriglyceridaemia 14
2.1.2.4 Cholesterol and low-density lipoprotein 15
2.1.2.5 Lipid oxidation 15
2.1.2.6 Fat absorption 18
2.1.3 Interactions of carbohydrates in the diet with postprandial lipid metabolism 19
2.2 Cellular injury and diseases implicated as a result of postprandial oxidative stress 20
2.2.1 Cellular injury and chemistry of postprandial oxidative stress 20
2.2.2 Postprandial oxidative stress and its health implications 21
2.2.2.1 Atherosclerosis 21
2.2.2.2 Obesity 23
2.2.2.3 Hypertension 24
2.2.2.4 Diabetes 24
2.2.2.5 Aging 24
2.2.3 Biomarkers of oxidative stress 24
2.3 Dietary intervention strategies to alleviate postprandial oxidative stress 25
2.3.1 Alpha linolenic acid and linoleic acid 26
2.3.2 Red wine (proanthocyanidins) 27
2.3.3 Cysteine 27

2.3.4 Supplements such as vitamins ... 28
2.3.5 Other plant polyphenols ... 28
2.3.5.1 *Camellia sinensis* (tea) ... 29
2.3.5.2 *Aspalathus linearis* (rooibos) ... 30
2.3.5.2.1 Background ... 30
2.3.5.2.2 Processing and chemical composition ... 32
2.3.5.2.2.1 Quercetin and luteolin ... 34
2.3.5.2.2.2 Aspalathin and nothofagin ... 35
2.3.5.2.2.3 Orientin and rutin ... 36
2.3.5.2.3 Health properties of rooibos ... 37
2.3.5.2.3.1 Anti-mutagenic and anti-cancer properties ... 38
2.3.5.2.3.2 Anti-inflammatory and aging properties ... 38
2.3.5.2.3.3 Antispamodic effects ... 38
2.3.5.2.3.4 Immune system modulation ... 38
2.3.5.2.3.5 Vasodilation, antihemolytic, dermatological and anti-allergic effects ... 39
2.3.5.2.3.6 Antimicrobial and viral effects ... 39
CHAPTER THREE: METHODOLOGY ... 41
3.1 Introduction ... 41
3.2 Materials and methods ... 41
3.2.1 Experimental design ... 41
3.2.2 Ethical approval ... 42
3.2.3 Anthropometric measurements ... 43
3.2.4 Dietary intake ... 43
3.2.5 Blood sample collection and processing ... 44
3.3 Analytical biochemical methods ... 46
3.3.1 Herbal tea quantification and activity ... 46
3.3.1.1 Preparation of aqueous fermented rooibos beverage ... 46
3.3.1.2 Soluble solid measurement ... 46
3.3.1.3 Total polyphenol content ... 46
3.3.1.4 Flavonol content ... 47
3.3.1.5 Flavanol content ... 47
3.3.2 Aqueous fermented rooibos and plasma total antioxidant capacity measurement ... 48
3.3.2.1 Oxygen radical absorbance capacity ... 48
3.3.2.2 Ferric reducing antioxidant power ... 49
3.3.2.3 Trolox equivalent antioxidant capacity ... 49
3.3.3 Clinical chemistry ... 50

3.3.3.1 Plasma glucose 50
3.3.3.2 Insulin analysis 51
3.3.3.3 High sensitive C-reactive protein 51
3.3.3.4 Lipid profile 52
3.3.3.4.1 Total cholesterol........ 52
3.3.3.4.2 High-density lipoprotein cholesterol........ 53
3.3.3.4.3 Low-density lipoprotein cholesterol 53
3.3.3.4.4 Triglycerides 54
3.3.4. Oxidative stress measurement 54
3.3.4.1 Glutathione analysis........ 54
3.3.4.2 Lipid oxidation determination 55
3.3.4.2.1 Conjugated dienes 55
3.3.4.2.2 Thiobarbituric acid-reactive substance 56
3.4 Statistical analysis 57
CHAPTER FOUR: RESULTS 59
4.1 Phase One (Pilot study)........ 59
4.1.1 Anthropometry measurement 59
4.1.2 Glucose and insulin levels 59
4.1.2.1 Plasma glucose 59
4.1.2.2 Serum insulin 60
4.1.3 Lipid profile and C-reactive protein analysis........ 62
4.1.3.1 Lipid profile 62
4.1.3.2 High sensitive C-reactive protein 64
4.1.4 Plasma total antioxidant capacity measurement 64
4.1.4.1 Plasma oxygen radical absorbance capacity levels 64
4.1.4.2 Plasma ferric reducing antioxidant power levels........ 67
4.1.4.3 Plasma trolox equivalent antioxidant capacity levels........ 67
4.1.5 Oxidative stress indices and lipid oxidation status 68
4.1.5.1 Total Glutathione levels 68
4.1.5.2 Lipid oxidation status 70
4.1.5.2.1 Conjugated dienes 70
4.1.5.2.2 Thiobarbituric acid reactive substances........ 70
4.2 Phase Two (Experimental study)........ 71
4.2.1 Anthropometry........ 71
4.2.2 Antioxidant content and total antioxidant capacity measurement in rooibos........ 71
4.2.2.1 Phenolic content and soluble solid of the aqueous fermented rooibos beverage 71

4.2.2.2 Total antioxidant capacity of the fermented rooibos beverage 72
4.2.3 Glucose and insulin assay 72
4.2.3.1 Plasma glucose 72
4.2.3.2 Serum insulin 73
4.2.4 Lipid profile and C-reactive protein analysis 75
4.2.4.1 Lipid profile 75
4.2.4.1.1 Total cholesterol 75
4.2.4.1.2 High-density lipoprotein cholesterol 77
4.2.4.1.3 Low-density lipoprotein cholesterol 77
4.2.4.1.4 Triglycerides 78
4.2.4.2 High sensitive C-reactive protein 78
4.2.5 Plasma total antioxidant capacity measurement 79
4.2.5.1 Plasma oxygen radical absorbance capacity levels 79
4.2.5.2 Plasma ferric reducing antioxidant power levels 82
4.2.5.3 Plasma trolox equivalent antioxidant capacity levels 82
4.2.6 Total glutathione level and lipid oxidation status 83
4.2.6.1 Total glutathione 83
4.2.6.2 Conjugated dienes 85
4.2.6.3 Thiobarbituric acid reactive substances 85
CHAPTER FIVE: DISCUSSION AND CONCLUSION 87
CHAPTER SIX: RECOMMENDATIONS 95
REFERENCES 97
APPENDICES 127

LIST OF FIGURES

Figure 2.1: Endogenic and exogenic sources of reactive oxygen species 6
Figure 2.2: The illustration of the regulation of plasma glucose 10
Figure 2.3: Lipid classification 13
Figure 2.4: Basic reaction process of lipid oxidation 17
Figure 2.5: Diagram of postprandial oxidative stress 23
Figure 2.6: Rooibos field 32
Figure 2.7: Rooibos (fermented left and unfermented or "green" right) 32
Figure 2.8: The chemical structure of (A) quercetin and (B) luteolin 35
Figure 2.9: Chemical structure of (A) aspalathin and (B) nothofagin 36
Figure 2.10: Chemical structure of (A) orientin and (B) rutin 37
Figure 3.1: Standardised fat meal with water 44
Figure 3.2: Blood collection procedure 44
Figure 4.1: Serum triglycerides changes 62
Figure 4.2: High sensitive C-reactive protein changes 64
Figure 4.3: Plasma oxygen radical absorbance capacity changes 65
Figure 4.4: Plasma trolox equivalent antioxidant capacity changes 67
Figure 4.5: Total glutathione changes 68
Figure 4.6: Conjugated dienes changes 70
Figure 4.7: Plasma glucose changes 72
Figure 4.8: Insulin changes 73
Figure 4.9: Serum total cholesterol changes 75
Figure 4.10: Low-density lipoprotein cholesterol changes 77
Figure 4.11: Triglycerides changes 78
Figure 4.12: High sensitive C-reactive protein changes 79
Figure 4.13: Plasma oxygen radical absorbance capacity changes 80
Figure 4.14: Plasma ferric reducing antioxidant power changes 82
Figure 4.15: Plasma trolox equivalent antioxidant capacity changes 83
Figure 4.16: Plasma conjugated dienes changes 85
Figure 4.17: Plasma thiobarbituric acid reactive substances changes 86

LIST OF TABLES

Table 2.1: Oxidative stress and involvement in development of various chronic diseases........... 4
Table 2.2: Classes of rooibos phenolic acids.. 33
Table 2.3: Breakdown of natural antioxidant components in rooibos 34
Table 4.1: Anthropometry characteristics of control and treatment groups in Phase One......... 59
Table 4.2: Postprandial changes in blood glucose and insulin levels in participants of Phase One 61
Table 4.3: Postprandial changes in the lipid profile and inflammatory indicator in participants of Phase One 63
Table 4.4: Postprandial changes in plasma antioxidant capacity parameters in participants of Phase One 66
Table 4.5: Postprandial changes in biomarkers of oxidative stress in participants of Phase One 69
Table 4.6: Anthropometric characteristics of the Phase Two study group................................. 71
Table 4.7: Polyphenolic quantification and soluble solid content 71
Table 4.8: Postprandial changes in blood glucose and insulin levels in participants of Phase Two 74
Table 4.9: Postprandial changes in the lipid profile and inflammatory indicator in participants of Phase Two 76
Table 4.10: Postprandial changes in plasma antioxidant capacity parameters in participants of Phase Two 81
Table 4.11: Postprandial changes in biomarkers of oxidative stress in participants of Phase Two 84

LIST OF EQUATIONS

Equation 2.1: Superoxide anion disproportional reaction 4
Equation 2.2: Fenton reaction 5
Equation 2.3: Radical nitric oxide reaction 5
Equation 3.1: The enzymatic reaction method of hexokinase/glucose-6-phosphate dehydrogenase 51
Equation 3.2: Enzymatic Trinder end-point reaction 53
Equation 3.3: Triglycerides sequential enzymatic reactions 54
Equation 3.4: Calculation formula of conjugated dienes 56
Equation 3.5: Calculation formula of thiobarbituric acid-reactive substance 57

LIST OF ABBREVIATIONS

AAPH: 2,2'-Azobis (2-methylpropionamidine) dihydrochloride

ABTS: 2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid)

ATP: Adenosine-5'-triphosphate

BC: Before Christ

BHT: Butylated hydroxy toluene

BMI: Body mass index

BP: Blood pressure

cAMP: Cyclic adenomonophosphate

CD: Conjugated dienes

CHO: Carbohydrates

CPUT: Cape Peninsula University of Technology

Hs CRP: High sensitive C-reactive protein

CV: Coefficient of variation

CVD: Cardiovascular disease

DMACA: 4-(dimethylamino)-cinnamaldehyde

DMACA: 4-dimethylaminocinnamaldehyde

DNA: Deoxyribonucleic acid

DNPH: 2,4-dinitrophenyl hydrazine

DPPH: 1,1-diphenyl-2-picrylhydrazyl

DTNB: 5,5'-Dithiobis 2-nitrobenzoic acid

EC: (-)-Epicatechin

ECG: (-)-Epicatechin gallate

ECQ: (-)-Epicatechin quinones

EDTA: Ethylenediaminetetra acetic acid

EGC:	(-)-Epigallocatechin
EGCG:	(-)-Epigallocatechin gallate
EGCQ:	(-)-Epigallocatechin quinones
FADH:	Flavin-adenine dinucleotine (reduced form)
FAO:	Food and Agriculture Organisation
FRAP:	Ferric reducing ability/power
GR:	Glutathione reductase
GSH:	Glutathione reduced
GSSG:	Glutathione oxidised
H_2O_2:	Hydrogen peroxide
HCl:	Hydrochloric acid
HDL-C:	High-density lipoprotein cholesterol
$HO^{\bullet-}$:	Hydroxyl radical
HNE:	Radical hydroxyl-2-nonenal
IL:	Interleukin
LDL-C:	Low-density lipoprotein cholesterol
LOOH:	Lipid hydroperoxides
LO:	Lipid oxidation
M2VP:	1-methyl-2-vinyl-pyridinium trifluoromethanesulfonate
MDA:	Malondialdehyde
MPA:	Metaphosphoric acid
N:	Number
NADPH:	β- Nicotinamide adenine dinucleotide phosphate
NO:	Nitric oxide
$O_2^{\bullet-}$:	Superoxide anion radical
OPA:	Orthophosphoric acid

ORAC:	Oxygen radical absorbance capacity
OS:	Oxidative stress
PO:	Peroxidase
PUFA:	Poly unsaturated fatty acid
RNS:	Reactive nitrogen species
ROS:	Reactive oxygen species
SASA:	South African Sugar Association
SD:	Standard deviation
SOD:	Superoxide dismutase
TAC:	Total antioxidant capacity
TB:	Theabrownins
TF:	Theaflavins
TBARS:	Thiobarbituric acid-reactive substances
TC:	Total cholesterol
TEAC:	Trolox-equivalent antioxidant capacity
TG:	Triglycerides
TNB:	5-thio-2-nitrobenzoic acid
TPTZ:	2,4,6-tris(2-pyridyl)-s-triazine
TR:	Thearubigin
TRL:	Triglyceride-rich lipoproteins
USA:	United States of America
VLDL:	Very low-density lipoprotein
VS:	Versus
WHO:	World Health Organisation

UNITS OF MEASUREMENT

g:	Gram
hr:	Hour
Kcal:	Kilocalorie
Kg:	Kilogram
Kj:	Kilojoule
mg/dl:	Milligram per decilitre
mg/l:	Milligram per litre
ml U/l:	Millilitre unit per litre
mmol/l:	Millimoles per litre
mM:	Millimolar
nmol CD/ml:	Nanomoles conjugated dienes per millilitre
nmol MDA/l:	Nanomoles MDA per litre
pH:	Negative logarithm of hydrogen ion concentration
µM:	Micro molar
µmol TE/l:	Micromole trolox equivalent per litre
%:	Percentage
°C:	Degrees celsius

CHAPTER ONE: GENERAL INTRODUCTION

Of the 50 million deaths that occur per year worldwide, 40 million occur in developing countries (Yusuf *et al.,* 2002:3). A substantial proportion of these deaths are due to cardiovascular diseases (CVD). The major contributor to the growing burden of cardiovascular disease is atherosclerosis, which accounts for about one third of cardiovascular deaths. Vascular disease is a major cause of morbidity and mortality throughout the world (Thorogood *et al.,* 2007:1). The World Health Organisation (WHO) currently attributes one-third of all global deaths (15.3 million) to CVD and it is projected that by the year 2025 between 80 to 90 % of all CVDs in the world will occur in low income and middle income countries (Joint WHO/FAO, 2003:81). The increase in cardiovascular disease in these countries is due to several causes such as nutritional disorders (Yusuf *et al.,* 2002:3). This is a challenge that is not only medically and epidemiologically, but also socially and politically important. To combat this projected global epidemic of cardiovascular disease, policies that combine sound knowledge of prevention and good clinical care are needed, as well as policies that deal with strategies that promote physical activities and appropriate nutrition. In Africa in general, the lag-time effect of risk factors for CVD mortality rates are the consequence of previous exposure to behavioural risk factors such as insufficient physical activity, increased consumption of tobacco and inappropriate nutrition (Yusuf *et al.,* 2001:2859). In South Africa in particular, vascular diseases are already common in adults. Unhealthy dietary practices include the high consumption of fats and refined carbohydrates and low consumption of fruits and vegetables (WHO/FAO, 2003:81). These diets have reported to induce a phenomenon called postprandial oxidative stress (Bowen & Borthakur, 2004:483).

Postprandial oxidative stress results from sustained postprandial hyperglycaemia and/or hyperlipidaemia (Sies *et al.,* 2005:969). In Western societies and developed countries, a significant part of the day is spent in the postprandial state. Epidemiological and mechanistic studies suggest that perturbations of the postprandial period are involved in the pathogenesis of macro vascular complications (Carroll *et al*., 2003:108). Oxidative stress is involved in many other diseases and many studies have suggested that it may be alleviated by the intake of dietary antioxidants originated from beverages and plant foods (Borek, 2004:333; Lau *et al.,* 2005:S128; Scalbert *et al.,* 2005:215S). The inhibitory effect of diets on oxidative stress may be attributed to many components such as the polyphenols, which is often abundant in beverages derived from plant origin, such as herbal teas.

In order to assess the effect and mechanisms of protection, various biochemical assays determining glucose and insulin concentrations, lipid profile, total antioxidant capacity and oxidative stress biomarkers were used to determine whether dietary rooibos supplementation is associated with changes that can modulate postprandial oxidative stress in healthy individuals. To our knowledge, no previous studies have investigated the effect of rooibos herbal tea on postprandial oxidative stress in healthy individuals.

1.1 Aims of the study

The aims of this study were:

i. to establish oxidative stress levels after a combined sucrose load and standardised fat meal intake in healthy individuals.

ii. to assess the modulating effect of rooibos on postprandial oxidative stress induced by the intake of a standardised fat meal and sucrose.

CHAPTER TWO: LITERATURE REVIEW

2.1 Oxidative stress and postprandial oxidative stress

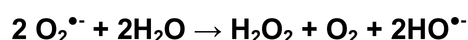
Oxidative stress is a term commonly used to describe the status of oxidative damage within a biological organism (Kuhnt *et al*., 2006:981). This condition occurs during normal metabolic activities when there is an imbalance in oxidants/free radicals and antioxidants in favour of the oxidants (Sies, 1997:291; Atsunor *et al.,* 2003:1675; Blumberg, 2004:3188S; Cigaril *et al.,* 2005:309; Faraci, 2005:186). It has been hypothesised as contributing, directly or indirectly, to the development of several human diseases such as cancer, atherosclerosis, malaria, rheumatoid arthritis, Alzheimer's disease, Parkinson's disease, amyotropic lateral sclerosis, neurodegenerative, chronic-inflammatory, vascular and neoplasic disease (Aruoma, 1994:671; Halliwell, 1994:37; Kathib *et al.,* 2007:3661). A common factor in the development of degenerative diseases is oxidative damage to important cellular components such as lipids, proteins and deoxyribonucleic acid (DNA) (Shelgikar *et al.,* 2005:73). Table 2.1 summarises diseases implicated in oxidative stress with references.

The oxidised or nitrosylated products produced in the development of these human diseases may exhibit decreased biological activity leading to cellular loss of energy metabolism, cell signalling, cell transport, cell injury and death through necrotic or apoptotic mechanisms and other major cellular functions (Vincent *et al.,* 2004:613). These products are also targeted for proteosome degradation associated with further decreasing cellular function. Lipid oxidation is implicated in vascular plaque formation while DNA base oxidation can initiate carcinogenesis (Smolková *et al*., 2004:136). Human body is engaged in physiological metabolism for the maintenance of a state of equilibrium, in which the levels of oxidants and antioxidants should also be balanced. Oxidants such as the free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are products of normal cellular metabolism (Buonocore & Groenendaal, 2007:1). They play a dual role as a deleterious as well as beneficial species, being either harmful or beneficial to living systems (Buonocore & Groenendaal, 2007:1). Reactive oxygen or nitrogen species are molecules and free radicals (chemical species with at least one unpaired electron) derived from molecular oxygen (Freeman & Crapo, 1982:412; Turrens, 2003:335).

Table 2.1: Oxidative stress and involvement in development of various chronic diseases

Oxidative stress implications	Source of information
Aging	Finkel *et al.,* (2000:246)
Apoptosis	Barnerjee *et al*., (2007:10)
Cancer	Okamoto *et al*., (1996:441)
Cardiovascular disease	Dhalla *et al*., (2000:673); Griendling & Fitzgerald (2003:1915)
Endothelial dysfunction	Heitzer *et al.,* (2001:2677); Landmesser *et al.,* (2002:3078)
Hypertension	De Champlain *et al.,* (2004:601)
Infertility	Sharma *et al.,* (1999:2807); Pasqualotto *et al*., (2000:464); Pasqualotto *et al.,* (2001:321)
Inflammation	Gottschling *et al.,* (2001:39)
Neurological disorders	Behl *et al*., (1997:535); Floyd *et al*., (1999:236)

In the context of oxidative stress, free radicals are small molecules or ions that are reactive with small activation energies and have short lifetimes. The small size enables many of the free radicals to penetrate cell membranes (Jensen, 2003:387). Free radicals can be considered as a subgroup of ROS or RNS. Reactive oxygen species are continuously generated via metabolic pathways in the body and exert physiological actions (Beretta *et al.,* 2006:290). A major part of ROS originates as by-products of aerobic metabolism in the mitochondria. The superoxide anion ($O_2^{\bullet-}$) is produced in the inner membrane of the mitochondria as part of the mechanism which reduces oxygen to water. Jensen (2003:388) suggested that $O_2^{\bullet-}$ may enter disproportionation reactions, resulting in the production of hydrogen peroxide, as shown in Equation 2.1.



$$2\,O_2^{\bullet-} + 2H_2O \rightarrow H_2O_2 + O_2 + 2HO^{\bullet-}$$

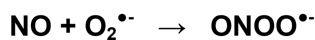
Equation 2.1: Superoxide anion disproportional reaction (Jensen, 2003:388)

In addition to this reaction, a major source of hydrogen peroxide (H_2O_2) is the oxidative deamination of biogenic amines, catalysed by the mitochondrial outer membrane monoamine oxidase (Jensen, 2003:388). Hydrogen peroxide is considered as an oxidant, though it is not a radical. It is relatively long-lived and can diffuse long distances prior to enter into reactions. It may produce the highly reactive hydroxyl radical ($HO^{\bullet-}$) through the Fenton reaction, if a transition metal-like iron is available, as shown in Equation 2.2.

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + HO^{\bullet-} + HO^{\bullet-} \text{ and } O_2^{\bullet-} + Fe^{3+} \rightarrow Fe^{2+} + O_2$$

Equation 2.2: Fenton reaction (Jensen, 2003:388)

Jensen (2003:388) states that RNS such as nitric oxide (NO) is produced in biological tissue such as vascular endothelial cells by nitric oxide synthase. In the context of oxidative stress the most noted reaction of the radical NO is with the radical $O_2^{\bullet-}$. Equation 2.3 illustrates the radical nitric oxide in reaction with superoxide to produce the nitric oxide peroxyl radical.



$$NO + O_2^{\bullet-} \rightarrow ONOO^{\bullet-}$$

Equation 2.3: Radical nitric oxide reaction (Jensen, 2003:388)

There are many different sources from which ROS/RNS are generated (endogenic and exogenic). Most ROS/RNS come from endogenous sources as by-products of physiological and essential metabolic reactions, such as energy generation from mitochondria or the detoxification reactions involving the liver cytochrome P-450 enzyme system (Mussali-Galante *et al.,* 2007:9). Exogenous sources include cigarette smoke, environmental pollutants such as emission from automobiles and industries, consumption of alcohol in excess, asbestos, exposure to ionising radiation and bacterial, protozoal, fungal or viral infections (Joint Working Group AECB's *et al.*, 1998:16-19). Figure 2.1 illustrates the different sources of ROS. Aerobic organisms are using oxygen as an electron acceptor.

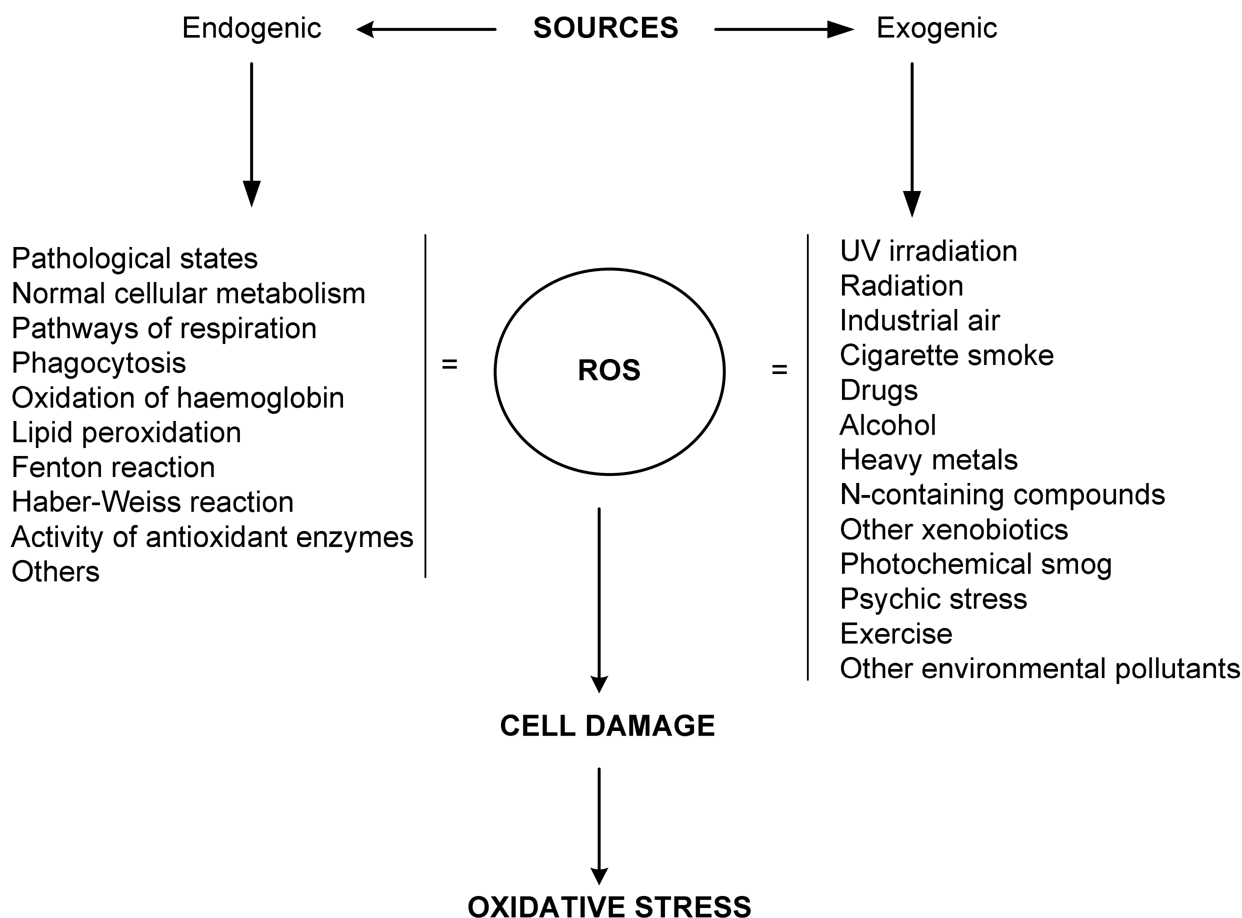


Figure 2.1: Endogenic and exogenic sources of reactive oxygen species (modified from Georgieva, 2003:2)

However, during respiration, free radicals can be partially reduced, forming ROS such as $O_2^{\bullet-}$, H_2O_2 and $HO^{\bullet-}$. Evidence suggests that mitochondria convert 1-2 % of the oxygen consumed into $O_2^{\bullet-}$ (Franca *et al.,* 2006:621). There is increasing evidence (based mostly on experiments derived from isolated mitochondria) suggesting that mitochondrial dysfunction and interrelated intramitochondrial generation of superoxide anion and other ROS and RNS are also implicated in the pathophysiological processes associated with aging, cancer, neurodegenerative and inflammatory disorders, diabetes, and diabetic complications (Shigenaga *et al.,* 1994:10771; Green *et al.,* 2004:S110; Csiszar *et al.,* 2005:285; Mukhopadhyay *et al*., 2007:203; Pacher *et al.,* 2007:315). The free radical-generating system of xanthine and xanthine oxidase, which often produces the superoxide anion, could also generate a combination of superoxide and H_2O_2 or $HO^{\bullet-}$, has been employed in the experimental induction of oxidative stress (Fatokun *et al.,* 2007:34).

Numerous free radical species are normally produced in the body to carry out specific functions. Superoxide, H_2O_2 and NO are free radical ROS that are essential for normal physiology. They prevent disease by assisting the immune system, mediating cell signalling and playing an essential role in apoptosis (Seifried *et al.,* 2007:568). On the other hand, these agents produce highly active singlet oxygen, hydroxyl radicals and peroxynitrite that can attack important molecules (Vincent *et al.,* 2004:616 and Seifried *et al.,* 2007:568). Nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) enzymes generate ROS ($O_2^{\bullet-}$ and its downstream metabolite H_2O_2) outside the cell or within intracellular organelles (Karl-Heinz, 2007:257). The NADPH-dependent oxidase carried by white blood cells generates superoxide which helps cells to kill bacteria, yeast and tumour cells (Babior & Peters, 1981:2321; Floyd, 1990:2589). Elevated levels of oxygen radicals have detrimental effects including DNA and protein damage and lipid oxidation, which primarily affect membrane structure and function leading to cellular injury (Sugino, 2007:S133). These species are produced in pathological and normal lipidaemic individuals. Normal lipidaemic individuals are defined as individuals with normal serum levels of total cholesterol (TC), high-density lipoproteins (HDL), low-density lipoproteins (LDL) and triglycerides (TG). There are also other parameters associated with normolipidaemic healthy individuals in relation to some well known determinants of lipid metabolism such as age, body mass index, dietary intake, body composition and parameters of insulin sensitivity (Castro Cabezas *et al*., 2001:220). All these parameters may be influenced while in the postprandial state.

The postprandial state is the period that comprises and follows a meal, and has received a great deal of attention in the oral glucose tolerance test (Lefebvre & Scheen, 1998:S63). Depending on the composition of the diet, during the postprandial state, three lipoprotein species can be affected: chylomicrons, chylomicron remnant and very low-density lipoproteins (VLDL) (Westphal *et al*., 2000:914). During the postprandial phase chylomicrons and their remnants can penetrate the intact endothelium and cause foam-cell formation; these particles are highly atherogenic after modification (Alipour *et al.,* 2007:466). People in the Western world are non-fasting for most of the day, which consequently leads to a continuous challenge of the endothelium by atherogenic lipoproteins and their remnants (Alipour *et al.,* 2007:466). The accumulation of potentially atherogenic particles is controlled by the balance between formation and clearance (Karpe, 1997:S60). Chylomicrons are rich in triglycerides and they are secreted by the intestine postprandially. High fasting plasma concentrations of triglyceride-rich lipoproteins are associated with an increased risk of coronary artery disease (Karpe, 1997:S61). Postprandial oxidative stress is an independent predictor of future cardiovascular events even in non-diabetic individuals. During the postprandial phase, there is

impairment of the redox status (postprandial oxidative stress) that could be affected by (i) the intake of oxidised/prooxidant species which, when absorbed, can directly modify the redox balance or (ii) the specific intake of macronutrients, whose catabolism can lead to the production of oxygen-radical species (Natella *et al*., 2008:33). The mechanisms, through which increased postprandial glucose (hyperglycaemia) levels and lipid (hyperlidipaemia) concentrations may damage endothelial cells on blood vessel walls, appear to be complex (Lefebvre & Scheen, 2004:S63; Süheyl Ezgü *et al*., 2005:1388).

2.1.1 Postprandial hyperglycaemia

Postprandial hyperglycaemia is an increase in blood glucose, which is induced by the ingestion of a carbohydrate such as sucrose, which is a normal physiological response (Slama *et al.,* 2006:187). This increase may result in vascular damage. There are at least 4 major pathways involved in hyperglycaemia-induced vascular damage: (i) enhanced polyol activity, causing sorbitol and fructose accumulation; (ii) increased formation of advanced glycation end products; (iii) activation of protein kinase C and nuclear factor kappa B; and (iv) increased hexosamine pathway flux (Brownlee, 2001:813).

2.1.1.1 Sucrose as dietary carbohydrate source

Sucrose is an oligosaccharide composed of α-D-glucopyranosyl and β-D-fructofuranoside. It is also known as saccharose, cane sugar or beet sugar. As sucrose does not contain a hemiacetalic linkage, it is classified a “non-reducing” sugar (Queneau *et al.,* 2004:178). Sucrose is water soluble and crystallises into the anhydrous form when hydrolysed in the intestine and yield glucose and fructose. Sugar occurs universally right through out the plant kingdom in flowers, seeds, fruits and roots of plants. Its chemistry is typical of the difficulty to derivatise simple carbohydrates (Jenner, 1980:91).

Epidemiological studies have shown that when impaired, oral glucose tolerance is associated with an increased risk of cardiovascular disease (Karpe, 1997:S61). Many studies have shown that excessive postprandial glucose excursions are accompanied by oxidative stress and, less well known, activation of blood coagulation (Karpe, 1997:S60). The mechanisms responsible for postprandial hyperglycaemia are still poorly understood although excessive postprandial release of glucose into the circulation has been uniformly found in patients with impaired glucose tolerance and type 2 diabetes mellitus (Bell *et al*., 1989:45; McMahon *et al*., 1989:291; Mitrakou *et al*., 1990:1381). The type of dietary carbohydrate (such

as starch, lactose, glucose and sucrose) has a profound influence on insulin action (Rajasekar & Anuradha, 2007:1176).

The average amount of consumed sugar reported for South Africans are generally lower than those reported in the United States of America, where populations consume the equivalent of 82 g added sugar per day (Morgan & Zabik, 1981:405). However, generally it is inappropriate to compare sugar intake in South Africa with that reported in studies from most European countries, because such studies have not all used the same classification system for sugar. Denmark, France and Germany reported the average sugar intake in terms of sucrose, whereas the Netherlands also include lactose (Ruxton *et al*., 1999:504). Some scientists believe that a diet high in added sugars is suggested to dilute micronutrients and displace nutrient-dense foods (Charlton *et al.,* 1998:331; Skinner *et al.,* 1999:58). Evidence of micronutrient dilution in diets high in added sugar comes from numerous publications (Charlton *et al.,* 1998:331). A study reported that specific micronutrients decreased in South African men and women over the age of 65, while the proportion of energy from sugar in the diet increased (Charlton *et al.,* 1998:332).

However, reasons given in support of people limiting the intake of sugar added to food, is based on the evidence that a high intake of sugar increases the risk of certain chronic diseases (Steyn *et al*., 2002:599). The term 'added sugar' is used throughout, referring to all monosaccharides and disaccharides that are added to foods and drinks during preparation (Krebs-Smith, 2001:527S). Due to its effects, the World Health Organisation (WHO) recommends a sugar intake that contributes to < 10% of the total energy intake per day (Young *et al.,* 2007:74).

2.1.1.2 Ingestion, absorption and controversial health aspect of sugar

Sun & Empie (2007:2) reported that during ingestion and absorption, sucrose is hydrolysed into fructose and glucose by stomach acid and cleaving enzymes in the digestive tract. As this sucrose does not appear in the blood stream, sucrose metabolism requires the cleavage of the glycosidic bond between glucose and fructose, which is catalysed by two enzymes, namely sucrose synthase and invertases (Gonzalez & Cejudo, 2007:839).

Sucrose synthase activity is associated with anabolic processes, requiring uridine diphosphate-glucose as substrate, whereas cleavage by invertases which produces glucose and fructose is associated with active growth and cell expansion (Gonzalez & Cejudo, 2007:839). Both sugars are then carried by specific transporters: glucose by the sodium-

dependent glucose co transporters (SGLT1) and fructose by the fructose transporters (GLUT5) (Palmer, 2006:1). Glucose in the blood stimulates the pancreas to release insulin into the blood. Insulin helps the cells to take glucose from the blood so that cells can use the glucose for energy production. Figure 2.2 demonstrates the regulation of plasma glucose in: (a) a normal fasting state and (b) in a normal fed state. It is also interesting to note that even when sucrose is used in soft drinks, the sucrose is often more than 50% hydrolysed into fructose and glucose (Sun & Empie, 2007:2). Once absorbed, the body cannot distinguish between the sugars from fruit, fruit juice, cane or starch. They are all used in the same way as a source of energy for the body. As a result, eating is followed by an increase in the levels of glucose and insulin in the blood. After an oral sugar loading in normal subjects, the background antioxidant defences has been reported to decrease and oxidative stress to increase (Ma *et al.,* 2005:250).

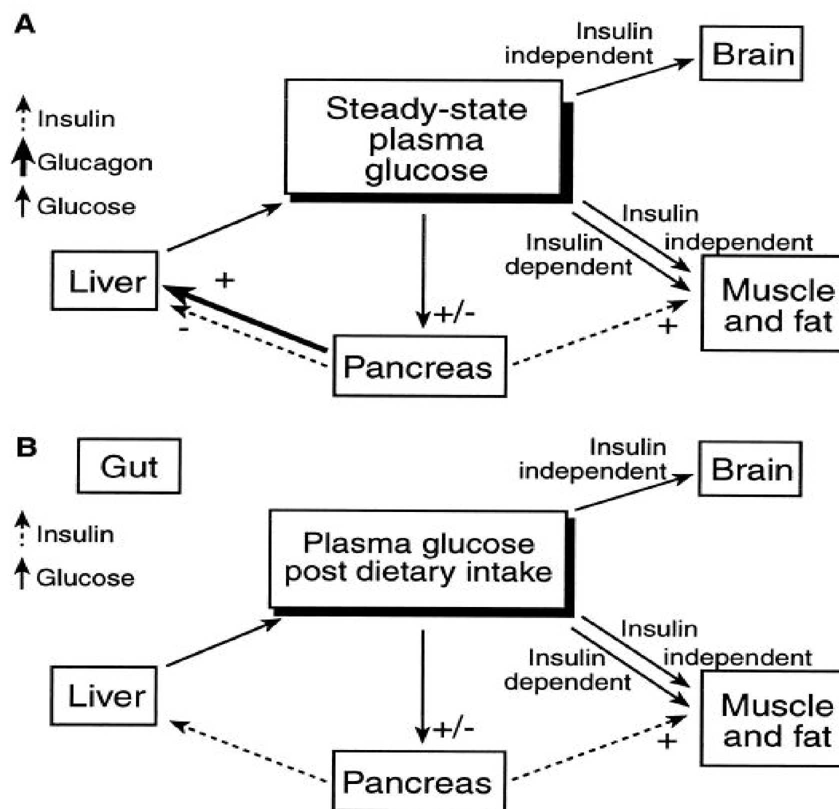


Figure 2.2: The illustration of the regulation of plasma glucose in: (A) the normal fasting state and (B) the normal fed state (modified from Gavin III, 2001:5H)

There is significant evidence that high sucrose diets may alter intracellular metabolism, which in turn facilitates accelerated aging through oxidative damage (Sandra, 2003:1). However, a reduction in sugar intake is almost invariably associated with an increase in fat intake, and a corresponding increase in energy intake (Ruxton *et al.,* 1999:505). Sugar can also cause premature aging (Lee & Cerami, 1992:63), increased free radical oxidative stress (Ceriello, 2000a:27), higher levels of TC, TG, LDL and HDL (Scanto & Yudking, 1969:1).

However, eminent bodies such as the WHO and Food and Agricultural Organisation (FAO) of the United Nations agree that sugar, like other carbohydrate-containing foods, has a place in a balanced diet. WHO/FAO (2003) and Mardis (2001:87) reported that there is no evidence that sucrose, other sugars and starches cause life-style related diseases such as heart disease, obesity and diabetes. Sugar is a healthy part of our meals. Carbohydrates, including sugar, are the preferred sources of the body's fuel for brainpower, muscle energy and functioning of cells. Over-consumption of sucrose has been linked to some adverse health effects. Nilsson & Hultman (1974:5) reported that due to its fructose content, sucrose may preferentially restore liver glucogen rather than muscle glycogen after exercise.

2.1.1.3 Carbohydrate and postprandial oxidative stress

As humans find themselves in a postprandial state for most of the day, prolonged exposure to dietary factors, which may impair endothelial function or delay postprandial recovery of the endothelium could negatively affect the cardiovascular risk profile (de Koning & Rabelink, 2002:12). It is known that a glucose challenge impairs endothelium-dependent vasorelaxation which is believed to be caused by the generation of oxidative stress. Ceriello *et al.,* (1998a:329) and Kawano *et al.,* (1999:146) reported that markers of oxidative stress are increased after a glucose challenge. In addition, meals and oral glucose challenges also lead to a postprandial reduction in antioxidant defence systems (Ceriello *et al.,* 1998b:1530). Glucose, independent from the effect of insulin, impairs endothelium-dependent vasorelaxation in a hyperglycaemic forearm model assessed by venous occlusion strain-gauge plethysmography. Beckman *et al.,* (2001:1618) and Van Etten *et al.,* (2001:58) hypothesised that this effect can be restored by an antioxidant supplement, such as vitamin C in combination with vitamin E. Such restoration has also been shown after an oral glucose challenge (Title *et al.,* 2000:2186). Evidence exist that hyperglycaemia increases free radical production by protein glycation (including lipoprotein), glucose autoxidation and activation of the polyol pathway (Carroll & Schade, 2003:25). The mechanisms, through which increased postprandial glucose level concentrations may damage endothelial cells on blood vessel walls, appear to be

complex (Lefèbvre & Scheen, 2004:S63; Süheyl Ezgü *et al.*, 2005:1388). These mechanisms include the activation of protein kinase C, increased expression of adhesion molecules, increased adhesion and uptake of leucocytes, increased production of proliferative substances such as endothelins, increased proliferation of endothelial cells, increased synthesis of collagen IV and fibronectin, and decreased production of nitric oxide (Lefèbvre & Scheen, 2004:S63). Grant and co workers (1994:853) also showed that sucrose in a rich fat meal amplifies the postprandial excursion of serum, lipoprotein triglyceride and cholesterol concentrations by decreasing TG clearance, thus delaying the clearance of postprandial hypertriglyceridaemia. In addition, the insulin-resistant states accentuate the postprandial hyperlipidaemia concentration (Karpe, 1997:S60).

2.1.2 Postprandial hyperlipidaemia

Postprandial hyperlipidaemia refers to elevated levels of lipids such as TG, chylomicrons and remnant lipoproteins in blood following a meal containing lipids (O'Keefe *et al.,* 2008:250). Thus, it could be a risk factor for cardiovascular disease (Natella *et al.,* 2002:7720).

2.1.2.1 Kinetics of lipids after fat meal

The postprandial lipidaemia phenomenon is well known to cause endothelial dysfunction, but its fundamental mechanism is still under debate (Tsai *et al.,* 2004:315). Many studies have demonstrated that individuals in this phenomenon may show elevated levels of lipid parameters. Consumption of a typical fat-containing meal (30-60 g of fat) cause a pronounced increase in postprandial lipidaemia such as circulating TGs, after 1 hr and can remain high for 5-8 hr (Dubois *et al.,* 1998:31). Tsai *et al.,* (2004:315) has found that, after a high fat meal intake, the levels of TGs remained significantly increased after 2 hr, 4 hr and 6 hr and resulted in endothelial dysfunction in healthy participants. Another study conducted in healthy participants comparing a high fat meal with a low fat meal, also showed a significant increase in TG levels after consumption of the high fat meal (3 hr, 4 hr and 5 hr postprandially) when compared to the low fat meal (Blendea *et al.,* 2005:1341). Consuming a fat meal containing 15 g of fat, did not affect the TG levels in normolipidaemic adults after 3 hr (Dubois *et al.,* 1998:35). A typical fat-containing meal, as mentioned above, could also affect the level of total cholesterol, HDL-C and LDLC as research has shown the total cholesterol to increase after 4 hr of consuming a high fat load/meal (Wilmink *et al.,* 2001:579). However, low-fat diets have been shown to lower plasma LDL-C as well HDL-C levels (Eastwood, 2003:551). The

HDL-C and LDL-C levels were lower at 3 hr after the consumption of a meal containing only 15 g fat, while after the consumption of a 50 g fat meal, levels were higher (Dubois *et al.,* 1998:35). It is important to know that the total cholesterol level gradually increases from youth to middle age, with associated increases in LDL and decreases in HDL levels, but these changes could also be dependent on the amount of fat consumed (Eastwood, 2003:550).

2.1.2.2 Lipids, fats and lipid rich meal

Lipids are substances that are insoluble in water but soluble in organic solvents such as alcohol, chloroform, ether, acetone, hexane and benzene and comprise of a wide spectrum of organic compounds that differ greatly in their chemical and physical properties as well as their physiological roles (Kaplan & Pesce, 1984:918). They include a spectrum of substances such as fatty acids, sterols, triacylglycerols (more commonly called triglycerides), phosphorous-containing compounds (phospholipids), fat-soluble vitamins, bile acids, waxes and other complex fats. Figure 2.3 shows the classification of lipids.

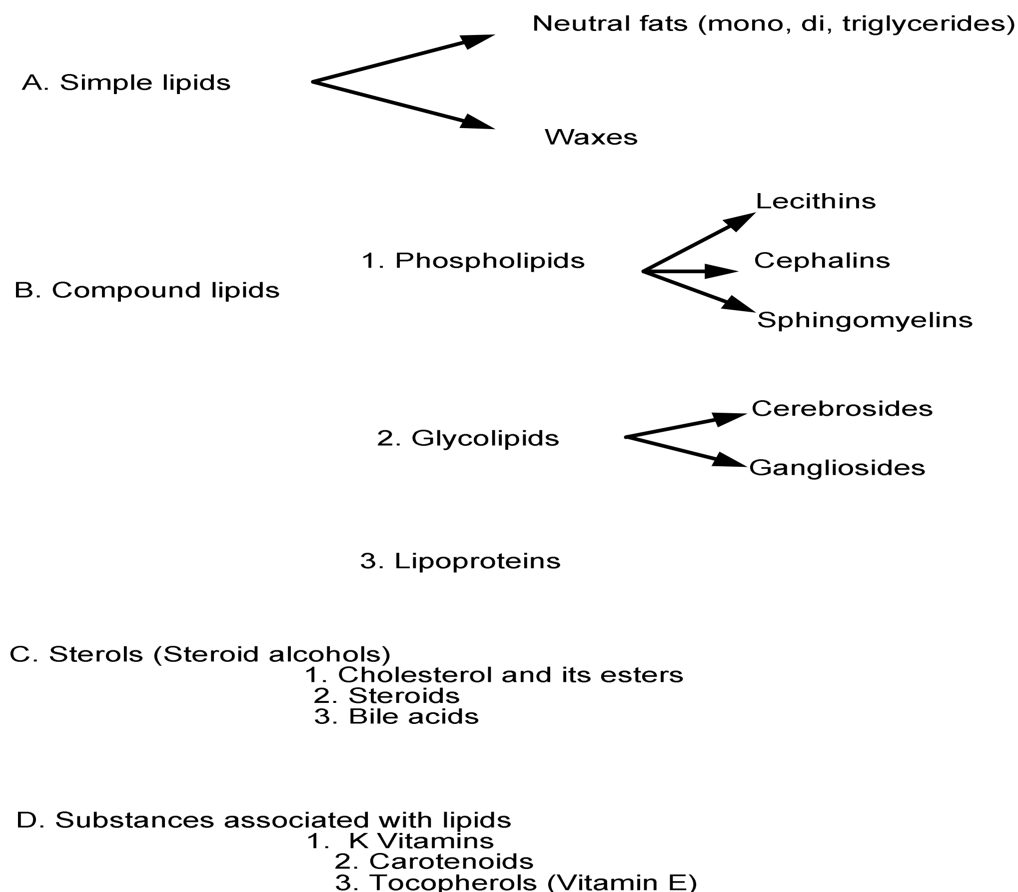


Figure 2.3: Lipid classification (as compiled from Fahy, 2005:846-858)

After lipid digestion, micelles and fatty acids are taken up by passive diffusion, facilitated diffusion and active transport through the enterocyte membrane (Hinsberger & Sandhu, 2004:610). Within the cytosol they bind to a fatty-acid binding protein which carries them from the cell membrane to the smooth endoplasmatic reticulum (ER). The concentration gradient is maintained and therefore the uptake of fatty acids into the cell is facilitated. In the smooth ER fatty acids and monoglycerides are transformed into TG and phospholipids (PL), whereas fat-soluble vitamins are bound to apolipoproteins produced in the rough ER and form chylomicrons (Hinsberger & Sandhu, 2004:610). Research has shown that cholesterol is an essential lipid for mammalian life, but a high serum cholesterol level can almost guarantee the eventual onset of vascular diseases and in some cases can lead to death (Rozner & Garti, 2006:435; Lancelot & Grauby-Heywang 2007:81).

2.1.2.3 Cholesterol and hypertriglyceridaemia

Cholesterol is an amphiphilic molecule consisting of a planar tetracyclic skeleton, a hydrophobic tail, and a polar hydroxyl group. Before absorption into enterocytes, cholesterol traverses the unstirred water layer and reaches the brush border (Westergaard & Dietschy, 1976:97). This process is therefore carried out by cholesterol carriers, mainly micelles. The formation of micelles and cholesterol incorporation into micelles are therefore considered important for cholesterol absorption, although micellar solubilisation may not be the rate limiting step (Wang *et al.,* 2006:38). Cholesterol is a multifunctional sterol molecule with a role in the regulation of protein activity, including signalling protein in the brain, oncogenic G protein, membrane proteins, and enzymes such as proteases of amyloid protein. It is also a component of all cellular membranes. It undergoes oxidation in the presence of heat, air, and light (Rozner & Garti, 2006:435). In the intestine the absorption of dietary cholesterol and fatty acids is followed by the assembly of chylomicrons in, and release from, the intestinal cells into the lymph system. Therefore, these triglyceride-rich particles become progressively smaller by the action of lipoprotein lipase generating remnant-like particles (RLPs) (De Koning & Rabelink, 2002:13). Many features of postprandial lipid metabolism such as an increase in the concentrations of RLPs, TG and oxidised low-density lipoproteins, are proatherogenic and the repetitive nature of this process during the day may contribute greatly to the development of atherosclerosis. Amongst the features mentioned above, the most characteristic feature of lipid metabolism after a lipid rich meal intake is postprandial hypertriglyceridaemia (De Koning & Rabelink, 2002:13). Postprandial hypertriglyceridaemia leads to decreased low-density lipoprotein (LDL-C) size which is more prone to oxidation (Mckeone *et al.,* 1993:1926).

2.1.2.4 Cholesterol and low-density lipoprotein

Cholesterol is required in the membranes of mammalian cells for normal cellular function and is either synthesised in the endoplasmic reticulum or derived from the diet. When derived from the diet, it is transported by the bloodstream as lipoprotein cholesterol. Therefore LDL-C are produced in the normal metabolic function and are taken into the cell by LDL-C receptor-mediated endocytosis in clathrin-coated pits, and then hydrolysed in lysosomes. The LDL-C particles of which the central core contains 1600 molecules of cholesterol ester and 170 molecules of TG, is surrounded by a monolayer of 700 phospholipid molecules, consisting primarily of lecithin, small amounts of sphingomyelin and lysolecithin, and 600 molecules of cholesterol (Gotto *et al*., 1986:126). Almost half of the fatty acids in LDL-C are polyunsaturated fatty acids (PUFAs) and the amount of PUFAs and antioxidants varies significantly within individuals and all depends on the diet. This relation between PUFA and antioxidants may result in a great variation in LDL oxidation susceptibility (Ramos *et al*., 1995:2113). Low-density lipoprotein oxidation is the type of LDL-C that has undergone deleterious alterations. Oxidised LDL, either in animals or humans, has been reported to be a better predictor of atherosclerosis and cardiovascular disease than regular LDL-C (CoLO, 2005:84). The circulating oxidised LDL is additive to the global risk assessment score for cardiovascular risk prediction based on age, gender, total cholesterol and high-density lipoprotein cholesterol (HDL-C), diabetes, hypertension and smoking (Mertens *et al*., 2001:2073). Reactive nitrogen species generated by the myeloperoxidase- H_2O_2 – NO_2 system of monocytes convert LDL-C into an atherogenic form that is avidly taken up and degraded by macrophages, leading to foam-cell formation (Podrez *et al*., 1999:1547). The circulating oxidised LDL does not only originate from extensive metal ion-induced oxidation in the blood, but also from mild oxidation in the arterial wall by cell-associated lipoxygenase and/or myeloperoxidase (Mertens *et al*., 2001:2073).

2.1.2.5 Lipid oxidation

Increased generation of ROS and oxidative stress may be of crucial importance in the pathogenesis of endothelial damage. Furthermore, there is a relationship between endothelial damage, glycemic control, and disorders of lipid metabolism (Neri *et al.,* 2005:1764). Bartsch and Nair (2004:28) believe that current attention is directed to persistent oxidative and nitrosative stress and excess lipid oxidation (LO) which is induced by inflammatory processes, impaired metal transport or dietary imbalance causing accumulation of massive DNA damage, together with deregulation of cell homeostasis. Lipids, which are prone to free radical attack,

may be altered by these free radicals, resulting in LO, which has been implicated in various diseases (Devasagayam *et al.,* 2004:794).

The mechanisms of lipid oxidation may be divided into three classes (Halliwell & Gutteridge, 1999:45): (i) Auto oxidation of lipids that is a process where electrons are removed from lipids by free radicals, resulting in the increased production of more free radicals. This involves a catalytic chain reaction where hydroperoxides are formed via loss of hydrogen radicals in the presence of trace metals, light or heat (Tejero *et al*., 2004:336). (ii) In the second class, photo-oxidation of lipids occurs when the oxidation reactions are induced by light, resulting in the loss of one or more electrons as a result of photo-excitation. Several natural photosensitisers such as tetrapyrroles (bilirubin), flavins, chlorophyll, hemoproteins and reduced pyridine nucleotides (NADH) are known to generate singlet oxygen (Girotti, 1989:497). (iii) The third class is the enzymatic oxidation, where enzymes such as those of the cytochrome P450 family participate in oxidation process of cholesterol to give rise to a specific hydroxycholesterol molecule (Tejero *et al*., 2004:336; Niki *et al.,* 2005:670).

Lipid oxidation is defined as a process whereby unstable species such as free radicals “steal” electrons from the lipids in cell membranes. Oxidation of the unsaturated fatty acids in lipids is one of the most fundamental chemical reactions, because they contain multiple double bonds with methylene -CH_2- groups that possess especially reactive hydrogens and they generate a very complex set of volatile oxidation products that can cause spoiling or rancidity (Frankel, 2005:9). Lipid oxidation process is divided into three stages: Initiation, propagation and termination (Frankel, 2005:15).

The initiation stage starts with abstraction of hydrogen from a -CH2- group of unsaturated fatty acids where the carbon radical is usually stabilised by a molecular rearrangement forming conjugated dienes (CDs), which are alkene compounds containing two double bonds separated by a single bond (Facundo *et al*., 2004:958). The propagation stage starts when the unstable fatty acid radical molecule reacts readily with molecular oxygen, creating a peroxyl-fatty acid radical, which is also an unstable species. This radical reacts with another free fatty acid producing different fatty acid radical CDs (Poirier *et al.,* 2001:1598). The cycle continues as the newly formed fatty acid radical reacts in the same way. The CDs react with O_2, forming peroxyl radicals that react with hydrogen atoms from other lipids, and producing lipid hydroperoxides (LOOH) or forming cyclic peroxides. In the presence of iron and copper, LOOH breaks down to produce several varieties of aldehydes such as hydroxyl-2-nonenal (HNE) and malondialdehyde (MDA) (Udilova *et al*., 2003:1481; Facundo *et al.,* 2004:958). Malondialdehyde is one of the most important thiobarbituric acid reactive

substances (TBARS) in LO; it allows not only the reaction with cellular nucleophiles but leads to self-condensation to form MDA oligomers (D'Abrosca *et al*., 2007:4135). The oxidative decomposition products such as LOOH and TBARS may also cause cellular damage in the body as in atherosclerosis, cancer, various inflammatory diseases and aging. It is more accurate to use the term TBARS instead of MDA for the products of the reaction with thiobarbituric acid (Goçmen *et al*., 2004:429). The final or termination stage of lipid oxidation involves the interaction of peroxyl radical with not only an antioxidant but also sometimes with another peroxyl radical. The basic reaction process of LO is described in Figure 2.4.

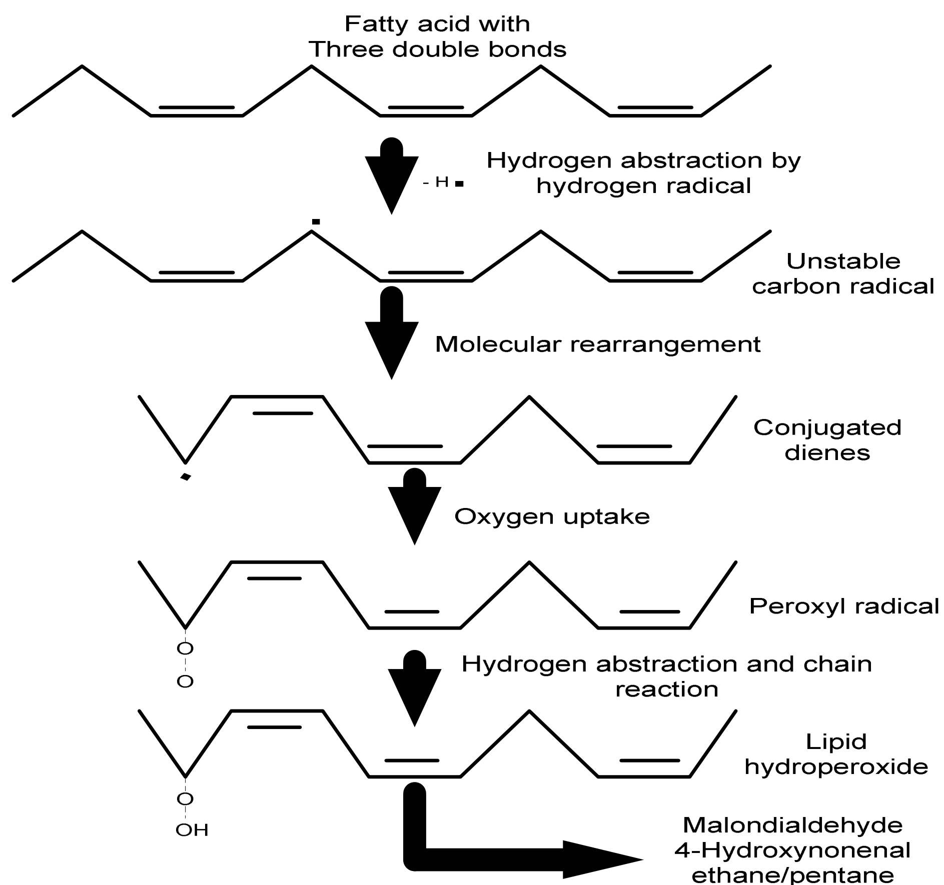


Figure 2.4: Basic reaction process of lipid oxidation (Young & McEneny, 2001:360)

Lipid oxidation occurs in isolated mitochondria, lysosomes and microsomes (D'Abrosca *et al.,* 2007:4135). The destruction of unsaturated fatty acids, which occurs in lipid oxidation, has been linked with altered membrane structure and enzyme inactivation. In addition to lipid hydroperoxides and lipid radicals, LO generates activated oxygen species such as $HO^{\bullet -}$ and

$O_2^{\bullet-}$ radicals. In addition, oxidative degradation of polyunsaturated membrane lipids leads to a variety of toxic carbonyl products such as hydroxyalkenals and other products (D'Abrosca *et al.,* 2007:4135). Lipid oxidation products are also considered as compounds harmful to human health (Sasaki *et al*., 2001:407). Oxidative stress generates ROS that can interact with phospholipids and unsaturated fatty acids to generate other products (Feng *et al.,* 2006:125). Although LO occurs under normal physiological conditions, it is increased during viral or bacterial infection, xenobiotic and radiation exposure, and inflammation (Feng *et al*., 2006:126). Lipid oxidation and HNE formation are triggered by the attack of ROS on PUFAs.

Reactive oxygen species are generated inter alia via redox-cycling of polycyclic aromatic hydrocarbon-quinone (PAH-quinone) intermediates and by respiratory burst after stimulation of phagocytes in inflamed tissues. Thus, HNE is converted to 2, 3-epoxy-4-hydroxynonanal by fatty acid hydroperoxides, which subsequently reacts covalently with DNA to form etheno-bridged base modifications (Nair *et al.,* 2007:97). *In vitro* studies have shown that oxidative stress is associated with impaired endothelium-dependent arterial dilation (Rubanyi & Vanhoutte, 1986:H822). Diets containing high levels of lipid oxidation products accelerate the development of atherosclerosis in animals (Staprans *et al*., 1996:533). Furthermore, Gittelsohn *et al*., (1998:541) reported that the method of preparation of food is also associated with increased risk for diabetes and impaired glucose tolerance. The choice of cooking method and the addition of fat during the preparation of foods may be associated with chronic diseases such as glucose intolerance. Thus, non-enzymatic LO and formation of lipid-peroxides can be initiated by adding ascorbate in the presence of oxygen and Fe^{2+} or Fe^{3+} ions to various tissue preparations such as homogenates, mitochondria, microsomes and nuclei obtained from various tissues and species (Gavazza & Catalá, 2006:247). Among cellular macromolecules, PUFAs exhibit the highest sensitivity to oxidative damage; this highest sensitivity could be explained by a power function of the number of double bonds found in fatty acid molecule (Catalá, 2006:1484).

2.1.2.6 Fat absorption

The primary function of the gastrointestinal tract (GIT) is to transform ingested food consisting of carbohydrates, proteins, fat and macronutrients, into smaller components and to absorb these nutrients (Hinsberger & Sandhu, 2004:605). The first step of this process is the mechanical act of chewing which reduces the size of the food and mixes the food. As a consequence of eating, several enzyme-containing fluids are secreted into the GIT and this aids hydrolysis of proteins, fats and carbohydrates (Hinsberger & Sandhu, 2004:605).

A normal Western adult diet contains a daily amount of approximately 60 to 80 g of fat. Of this, about 95% are long-chain TG, which are insoluble in water and the remaining 5% are cell membrane phospholipids, cholesterol, other sterols and fat-soluble vitamins (Hinsberger & Sandhu, 2004:610). Fat digestion starts in the mouth and stomach, where an emulsion is made from the ingested food. By this mechanical process, the dietary lipids are released from interacting proteins to form an emulsion of smaller particles. In the stomach a small amount of the TG undergo hydrolysis induced by a gastric lipase able to function in an acidic environment. In the intestine, bile acids emulsify fats and fat-soluble vitamins. Following a high-fat meal, triglyceride-rich lipoproteins are assembled in the gut and absorbed via the lymph into the blood circulation, producing a temporary hyperlipidaemia (Gershkovich & Hoffman, 2007:24). Triglycerides absorbed from the intestine are transported into circulation by chylomicrons, and are cleared from circulation by lipoprotein lipase (LPL) (Sasase *et al.,* 2007:9). Isolation and characterisation of proteins involved in transport and metabolic fate of long-chain fatty acids have provided new insights into the molecular basis of fat absorption (Petit *et al.,* 2007:38). The last step in fat digestion is the formation of mixed micelles by the interaction of fatty acids and monoglycerides with bile salts (Carrey, 1983:652). In the absorption of a fat-containing meal, bile acids must be present in the upper small intestine in concentrations sufficient to allow the formation of micelles (Smith *et al*., 1998:128). There are three distinct stages during the digestion of a fat load at which the presence of cephalic phase stimuli could potentially exert an effect; (i) the fasting state, before ingestion, (ii) during the first 1-2 hr after ingestion when TG is still present within the GIT and has just started to appear in the plasma and (iii) 5-6 hr after ingestion when there is no longer TG present within the lumen of the GIT but lipids are still present within the lacteals and the enterocytes. This is when a second meal would normally be eaten and so has caused a second meal effect (Robertson, 2006:6). Following transport across the sarcolemma, long-chain fatty acids are activated by fatty acyl-CoA synthase and then the resulting long-chain fatty acyl-CoA is converted to fatty acyl carnitine by carnitine palmitoyl transferase I and afterwards oxidised in the mitochondria, otherwise esterified to TG by glycerolphosphate acyl-transferase (Stowe *et al*., 2006:4282).

2.1.3 Interactions of carbohydrates in the diet with postprandial lipid metabolism

Chance and co-workers (1971:232) were the first to describe the generation of ROS by mitochondria. Excessive ingestion of caloric dense and easy digestible foods cause abnormal surges in blood glucose and TG levels (Ceriello *et al*., 2005:2523; Jakulj *et al.,* 2007:938). This high intake of energy increases the metabolic capacities of the mitochondria in the over-

nourished muscle and adipose tissues. Sucrose and free fatty acids flood the Krebs cycle, stimulating an excess of the reduced form of nicotinamide adenine dinucleotide production, which outstrips the capacity of oxidative phosphorylation and drives the transfer of single electrons to oxygen, creating free radicals (Monier *et al.,* 2006:1681; O'Keefe *et al.*, 2008:250). Long-chain non esterified free fatty acids can affect the mitochondrial generation of ROS in two ways: (i) by depolarisation of the inner membrane due to the uncoupling effect and (ii) by partly blocking the respiratory chain (Schönfeld & Wojtczak, 2007:1032). Postprandial oxidative stress is characterised by an increased susceptibility towards oxidative damage after consumption of a meal rich in lipids and/or carbohydrates. This has been associated with an increased risk for atherosclerosis and related disorders (Tsai *et al.,* 2004:315). Postprandial oxidative stress with impairment of endothelial function occurs after consumption of fat-rich meals (Blackhurst & Marais, 2006:551). Grant and co workers, (1994:853) also showed that carbonated beverages containing sucrose with a lipid-rich meal amplifies the postprandial excursion of serum and lipoprotein triglyceride and cholesterol concentrations by decreasing triglyceride clearance. Postprandial oxidative stress is an independent predictor of future cardiovascular events leading to cellular injury even in non diabetic individuals (O'Keefe *et al.*, 2008:249).

2.2 Cellular injury and diseases implicated as a result of postprandial oxidative stress

2.2.1 Cellular injury and chemistry of postprandial oxidative stress

Reactive oxygen species, whether produced endogenously as a consequence of normal cell functions or derived from external sources, pose a constant threat to cells living in an aerobic environment (Martindale & Holbook, 2002:1). Current evidence suggests that $O_2^{\bullet -}$ and H_2O_2 cause injury to cells as a result of the generation of a more potent oxidising species. In addition to $O_2^{\bullet -}$ and H_2O_2, the third essential component of the complex that mediates lethal cell injury is a cellular source of ferric iron (Farber, 1994:17). Oxidative stress has traditionally been viewed as a stochastic process of cell damage resulting from aerobic metabolism while antioxidants have been viewed simply as free radical scavengers. Reactive oxygen species are widely used as second messengers to propagate pro-inflammatory or growth-stimulatory signals (Hensley *et al.*, 2000:1456). Cells contain a number of antioxidant defences to minimise fluctuations in ROS, but ROS generation often exceeds the cell's antioxidant capacity resulting in oxidative stress (Martindale & Holbook, 2002:1). Cells exposed to ROS at high concentration, or for extended periods undergo cellular DNA damage which is widely known to induce cell death via either apoptosis and/or necrosis (Song *et al.,* 2006:1).

There are three important components to ROS balance: (i) ROS (free radical) production, (ii) antioxidant defences and (iii) repair mechanisms (Cohen, 2007:111). Oxygen-derived free radicals such as $O_2^{\bullet-}$ and $HO^{\bullet-}$ are thought to be linked to the onset of various pathological conditions (Grokas *et al.,* 2007:60). Under oxidative stress, carbohydrates, lipids, and proteins as well as DNA are the major targets of free radicals (Kalousová *et al*., 2005:40).

Lipid oxidation has been suggested to be involved in the aetiology of several chronic diseases (Mayne, 2003:933S). Lipid oxidation is considered to be a main mechanism of cell injury in aerobic organisms that are subjected to oxidative stress. It is triggered and promoted by different radical and non radical members of the ROS family, or by the catalytic decomposition of preformed lipid hydroperoxides, in tissues by several agents including most notably the transition metals and microsomal cytochromes (Sen *et al.,* 2006:20). The formation of lipid peroxides results in the destruction of the original lipid, leading to the loss of membrane integrity, which contributes to the deterioration of biological systems during aging and disease (Brock *et al.,* 2007:171). Lipid oxidation products are formed by non enzymatic reactions of unsaturated lipids with ROS, such as $O_2^{\bullet-}$ and $HO^{\bullet-}$ in the circulation or extra vascular space (Sakata *et al.,* 2002:22). Thus, LO is one of the most important expressions of oxidative stress induced by ROS (Cigaril *et al.,* 2005:309). Oxidative stress may represent: (i) adduction of products of glycoxidation and/or LO or (ii) direct oxidation of protein side-chains. These conditions may result in protein damage (Sayre *et al.,* 2001:722).

2.2.2 Postprandial oxidative stress and its health implications

2.2.2.1 Atherosclerosis

Atherosclerosis disease and its major complications such as myocardial infarction and ischemic stroke is still a major health problem in the world (Ludewig *et al*., 2002:154). It is regarded as a chronic inflammatory response affecting the intima of arteries, due to the accumulation of cholesterol deposits in macrophages in arteries. This cholesterol deposition leads to a proliferation of certain types of cells within the arterial walls that impinge gradually on the vessel lumen and impede blood flow (Amann, 2008:1599). The development observed may be quite insidious, therefore could last for decades until the formation of an atherosclerotic lesion, by means of physical forces from blood flow. In addition, the atherosclerotic lesion becomes disrupted in this regards and all profound arterial wall components are exposed to flowing blood, which in turn may lead to thrombosis and compromising the supply of oxygen to rich various organs such as the heart and brain (Weitz *et al.,* 2004:265S). The loss of brain

and heart function because of reduced blood flow is termed stroke and heart attack, respectively. These two clinical manifestations of atherosclerosis are often referred to as coronary artery disease (CAD) and cerebrovascular disease, and are commonly referred to as cardiovascular disease (Stocker & Keaney, 2004:1382; Hansson, 2005:1685). The origin of the inflammation is still under discussion (Stocker & Keaney, 2004:1381; Hansson, 2005:1685). According to many investigators, some hypotheses point to the fact that sustained postprandial hyperglycaemia and hyperlipidaemia as shown in Figure 2.5 are the origin of atherosclerosis disease (Tsai *et al.,* 2004:315). Investigations showed that excess dietary fat is associated with the development of atherosclerosis in humans (Tell *et al.,* 1994:979). The postprandial dysmetabolism induces atherosclerosis when there are excessive intakes of carbohydrates and/or lipids. Quickly digestible food results in abnormal rises in the levels of blood glucose, triglycerides and free fatty acids. This excess intake of energy-containing substrates could essentially swamp the metabolic capabilities of the mitochondria in the over nourished muscle and adipose tissues (Ceriello *et al.,* 2004:816). This build-up of nicotinamide adenine dinucleotide increases the mitochondrial proton gradient and this drives the transfer of single electrons to oxygen following by the production of free radicals such as superoxide anion. Thus, this proportionate increase induces postprandial oxidative stress that could lead to atherosclerosis (O'Keefe & Bell, 2007:901). In respect to the underlying pathology of cardiovascular disease, there are a number of cardiovascular risk factors that need considerable attention, such as obesity, hypertension and diabetes (Stocker & Keaney, 2004:1382 and 1383).

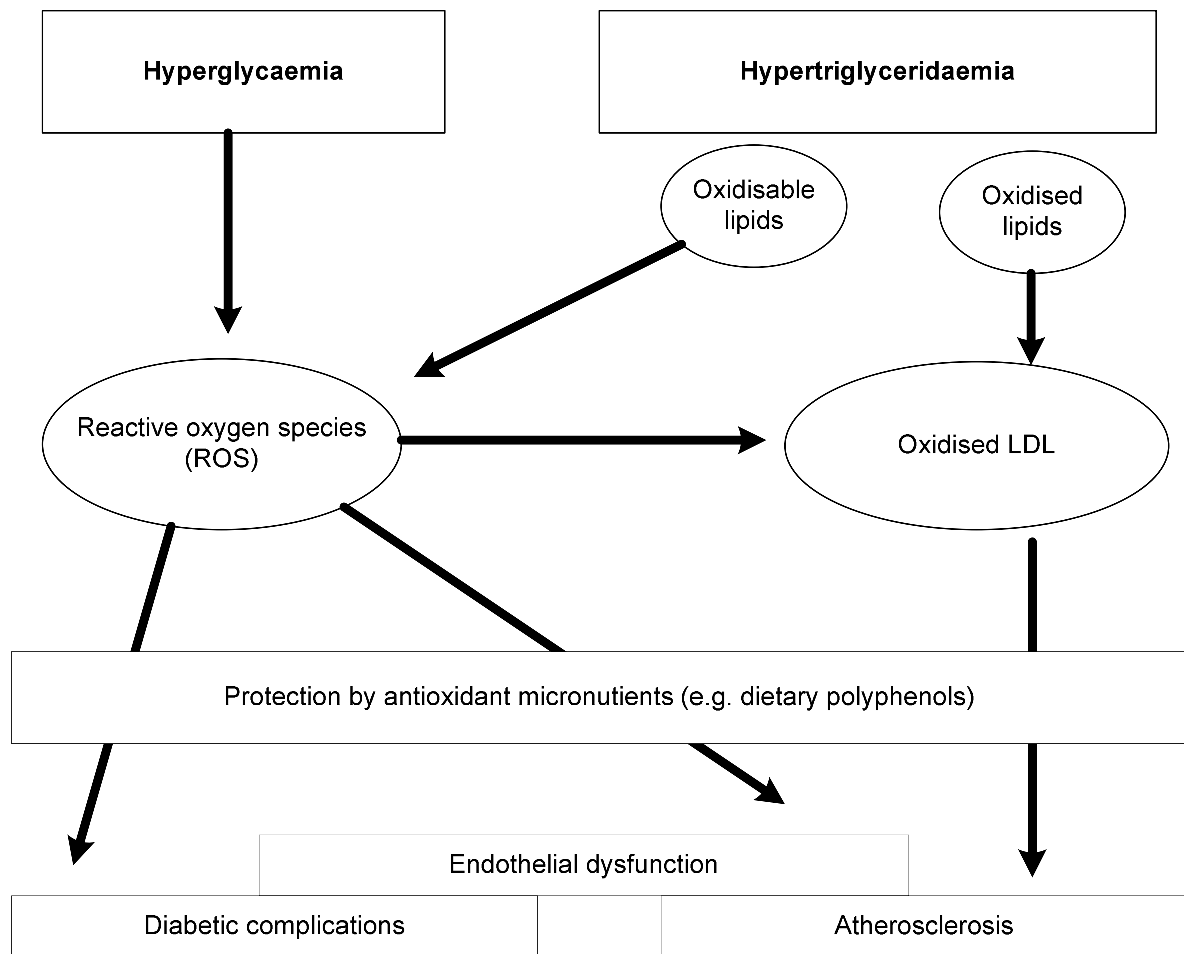


Figure 2.5: Diagram of postprandial oxidative stress and its relation to atherosclerosis and diabetes (adapted from Sies et al., 2005:970)

2.2.2.2 Obesity

Obesity defined as an excess body weight with an abnormal high preponderance of body fat, is considered as a condition that increases the incident risk of cardiovascular disease (Stocker & Keaney, 2004:1382). Although, exact mechanisms to explain this phenomenon is under investigatigation, there are a number of other risk factors for cardiovascular disease such as hypertension, low HDL cholesterol, and diabetes mellitus (Wilson *et al.,* 1999:1105). It has been suggested that postprandial oxidative stress induced from sustained hyperglycaemia or hyperlipidaemia is associated with metabolic syndrome diseases such as obesity (Sies *et al.*, 2005:969).

2.2.2.3 Hypertension

Hypertension, also called high blood pressure, may be defined as a systolic blood pressure in excess of 140 mmHg or a diastolic blood pressure above 90 mmHg (Devereux *et al*., 1983:471). It has been reported that there appears to be linear relation throughout the world between blood pressure elevation and the increased incidence of atherosclerotic vascular disease (MacMahon *et al.,* 1990:765).

2.2.2.4 Diabetes

Diabetes has been of growing concern, due to the adoption of a Western diet with a high fat content and few fruits and vegetables, often leading to obesity. This increases the risk of type 2 diabetes mellitus, which can be fatal. In South Africa in particular, death rates from diabetes and obesity increased between 1997 and 2004 with the female death rates higher than male death rates (Lehohla, 2006). Postprandial hyperglycaemia and hyperlipidaemia which lead to postprandial oxidative stress increase the risk of diabetes and it is also the common pathway through which the majority of diabetic complications occur (Ceriello, 2000b:131). Also, in patients with diabetes, it increases the risk of coronary atherosclerosis, which is increased three to five fold when compared with non diabetics despite controlling for other risk factors (Bierman, 1992:647; Pyorala *et al*., 1987:463). A number of other known risk factors for coronary diseases such as hypertension and abnormal lipids are also more common in diabetics than the normal population (Bierman, 1992:647; Nishigaki *et al.,* 1981:373). Thus, diabetes represents a major contributing factor to atherosclerosis (Stocker & Keaney, 2004:1382).

2.2.2.5 Aging

Aging is characterised by a progressive loss of muscle mass, leading by a decrease of muscle protein synthesis stimulation in the postprandial state (Dardevet *et al.,* 2002:95). It has been reported to be another confounding factor associated with delayed and impaired clearance of postprandial lipidaemia (Krasinski *et al.,* 1990:883).

2.2.3 Biomarkers of oxidative stress

Oxidative stress biomarkers are specific biochemicals in the body, which have particular molecular features that makes it useful for measuring the progress of disease or the effects of treatment. A biomarker of oxidative stress enables the identification of a modified

biological molecule and provides an understanding of the nature of the denaturing radical along with the location of oxidative damage (Young *et al.,* 2007:73). Biomarkers of oxidative stress can be classified into several groups: reactive oxygen species, by- or end-products of oxidative damage, antioxidant enzymes and redox status of endogenous antioxidants (Orhan *et al.,* 2006:1006). As highly sensitive markers of oxidative damage in mammalian systems, biomarkers may provide information on three progressive levels of disease outcome: (i) as measurable endpoints of oxidative damage to proteins, amino acids, oxidised lipids, oxidised DNA bases, (ii) as functional markers of blood flow and platelet aggregation and (iii) as endpoints related to a specific disease (Young *et al*., 2007:74). There are several biomarkers that may be assayed in order to determine the oxidation/redox status in different biological samples and they include reduced glutathione, oxidised glutathione, glutathione reductase, glutathione peroxidase, glutathione-S-transferase, superoxide dismutase, catalase, conjugated dienes, lipid hydroperoxides and thiobarbituric acid reactive substances (Linares *et al*., 2009:152). It is advisable to combine the results of two or more different oxidative stress markers in order to draw an acceptable conclusion. In recent years, many biomarkers for measuring components of oxidative damage to lipids, proteins and DNA have been developed (Lee *et al.,* 2006:344). The increase or decrease of oxidative stress biomarker level can be associated with dietary response (Dietrich *et al.,* 2002:12; Sánchez-Moreno *et al.,* 2004:657).

2.3 Dietary intervention strategies to alleviate postprandial oxidative stress

In the last few decades, a substantial increase in research pertaining to natural antioxidants has been observed, due to their non-toxicity and interaction with various endogenous and exogenous free radicals in living cells (Joshi *et al.,* 2007:125). Antioxidants are believed to slow down the formation of free radicals and protect the body from ROS or RNS damage. The antioxidant defence process includes: scavenging free radicals to prevent their propagation, enzymatic hydrolysis of ester bonds to remove peroxidised fatty acids from lipids, sequestration of transition metal ions and enzyme-catalysed reduction of peroxides (Thomas, 2000:716). Antioxidants are classified into 2 groups according to the mechanism by which they prevent or retard oxidation: primary (chain-breaking) antioxidants like α-tocopherols which interrupt oxidation by converting free radicals into more stable species, while the secondary (preventive) antioxidants such as ascorbic acid and phenolic compounds react with oxygen before the start of oxidation (Neri *et al.,* 2005:1765). The antioxidant defence system of the body consists of several mechanisms: (i) non enzymatic antioxidants such as polyphenols, glutathione (GSH), uric acid, albumin, bilirubin and ceruloplasmin and (ii) antioxidant enzymes such as superoxide dismutase, catalase, GSH-peroxidase, GSH-reductase, GSH tranferases

(Vasankari *et al.,* 1997:396). To combat the potentially deleterious effects of free radicals and oxidants, aerobic cells should break down superoxides using superoxide dismutases and peroxides using glutathione peroxidases to catalyse the reactions (Thomas, 2000:716). Therefore nutrients, both water soluble and lipid soluble, comprise an important aspect of the antioxidant defence system in humans (Mayne, 2003:933S). All antioxidants such as polyphenols have a chemical element referred to as a redox potential, which is the measurement of their ability to be oxidised. They are by this action also classified as reducing agents, which are needed to quickly block the chain reaction caused by free radicals before cell damage occurs. Phenolic compounds or polyphenols are reducing agents, which constitutes one of the most numerous and widely distributed groups of substances in the plant kingdom. More than 8000 phenolic structures are currently known of these secondary metabolism plant products (Droebner *et al.,* 2007:2). Thus, intake of supplements or dietary polyphenols present in nuts, dark chocolate, pomegranates, berries, pigmented plant-based foods and drinks such as red wine and herbal teas may help to protect the vascular endothelium from postprandial oxidative stress and inflammation independently from their effects on postprandial hyperglycaemia and hyperlipidaemia (Bayard *et al.,* 2007:53).

2.3.1 Alpha linolenic acid and linoleic acid

Alpha linolenic acid (ALA) and linoleic acid (LA) belong to the n-3 (omega-3) and n-6 (omega-6) series of polyunsaturated fatty acids, respectively. They are defined ‘‘essential’’ fatty acids since they are not synthesised in the human body and are completely obtained from the diet (Russo, 2009:937).

Omega-3 fatty acids (ω-3 FAs) are oils found in fish and plants. It has been advised to couple PUFA ingestion with concurrent consumption of antioxidants in order to counteract the oxidative damage of tissue lipids (Song *et al.,* 2000:3032). Many lipid studies have indicated that increased consumption of long chain ω-3 FAs may be of therapeutic value in a variety of inflammatory conditions (Calder, 2006:197). Several benefits of long chain ω-3 FAs have been reported in humans following treatment with lipid extracts, with clinical functional and inflammatory markers being significantly improved (Emelyanov *et al.,* 2002:596). The long chain ω-3 FAs also impair inflammatory gene expression and promote anti-inflammatory resolvins and protectins (Calder, 2006:197). The ω-3 fatty acids have been reported to lower postprandial hyperlipidaemia levels by 16-40% in a dose-dependent fashion in part by up regulating lipoprotein lipase activity and accelerating the clearance of chylomicrons (Park & Harris, 2003:461). Also the ω-3 FA supplementations help to prevent lipid oxidation by safeguarding erythrocytes from oxidative injury (Iraz *et al.,* 2005:169).

A healthy diet should consist of around 2 - 4 times more omega-6 fatty acids than omega-3 fatty acids, the Western diet is very high in omega-6 fatty acids relative to n-3 fatty acids and they are generally found in plants (Enser *et al.,* 2000:201). Omega-6 fatty acids have been reported to reduce human postprandial oxidative stress (Berry *et al.,* 2008:922). But research has found that an excess intake causes an imbalance, which is a significant factor in the rising rate of CVD and inflammatory disorders in the Western countries (Simopoulos, 2002:495).

2.3.2 Red wine (proanthocyanidins)

Proanthocyanidin is a common component of foods and beverages that originate from plants (Santos-Buelga *et al*., 2000:1094). They are a group of polyphenolic bioflavonoids diverse in chemical structure, characteristics and pharmacology. Studies have reported proanthocyanidins to manifest a wide range of biological effects such as anti-inflammatory, antiallergic and vasodilatory actions (Buening *et al*., 1981:71; Kolodziej *et al*., 1995:415). Proanthocyanidins are polyphenolic compounds are present in cereals, legume seeds and particularly abundant in some fruits and fruit juices such as red grape juice, thus also present in red wine. Proanthocyanidins have a strong antioxidant activity and scavenge ROS and RNS (Arteel & Sies, 1999:167). Due to their potential health effects, proanthocyanidins have attracted increasing interest in medicine (Natella *et al*., 2002:7720). Evidence shows that proanthocyanidins taken with food prevent the postprandial oxidative stress by inhibiting lipid oxidation, platelet aggregation and capillary permeability and fragility and also modulate the activity of enzyme systems including cyclooxygenase and lipooxygenase (Bors & Saran, 1987:289; Kolodziej *et al*., 1995:415; Natella *et al*., 2002:7724; Ursini & Seranian, 2002:599).

2.3.3 Cysteine

Glutathione is a tripeptide synthesised from a combination of the amino acids, L-glutamic acid, L-cysteine and glycine, and it is reported to be involved in a variety of physiological functions (Blouet *et al.,* 2007:1089). Glutathione is a major determinant of the intracellular redox balance and is involved in protection against free radical induced oxidative stress (Schafer & Buettner, 2001:1191). However, cysteine is a non-essential amino acid, belonging to the glucogeneic group that is formed from methionine (an essential amino acid) and from serine (a non-essential amino acid) in the organism (Scorolli *et al.,* 2008:381). Endowed with a group of sulfhydryls, cysteine when participating in glutathione synthesis is often used in elevated concentrations whereby, it constitutes one of the principal intracellular

antioxidant systems in humans, acting as a reserve of equivalent reductions. Thus, these equivalent reductions may be used for removing toxic peroxides and oxidised radicals which are formed during cellular metabolism under aerobic conditions and in great quantities during inflammation (Scorolli *et al.,* 2008:381). Therefore scientists have proven that an increased intake of dietary cysteine may be beneficial to maintain glutathione redox status and reduce oxidative stress injuries (Blouet *et al*., 2007:1089).

2.3.4 Supplements such as vitamins

Antioxidant vitamins have been used as supplements, thus diets containing vitamins such as vitamins E (α-tocopherol) and C (ascorbic acid) have been reported to inhibit oxidation of LDL, lower the level of C-reactive protein and interleukin-6 (IL-6), inhibit the plasminogen activator and restore arterial flow (Plotnick *et al.,* 1997:1682; Devaraj & Jialal, 2000a:195; Devaraj & Jialal, 2000b:792; Devaraj *et al*., 2002:528). Vitamin C has many different functions in humans. Beside its well-known role as an antioxidant, vitamin C serves as a cofactor in several important enzyme reactions, including those involved in the synthesis of cholesterol, amino acids, catecholamines, carnitine, and certain peptide hormones (Chatterjee *et al.,* 1975:24). Vitamin E consists of eight different forms that are produced by plants alone and have similar chromanol structures: monomethyl (δ-) tocopherol, dimethyl (β- or γ -) and trimethyl (α-) and the corresponding tocotrienols (Singh *et al.,* 2005:154). Vitamin E has been reported to be a powerful antioxidant with anti-inflammatory properties. Research has shown that vitamin E also has potential beneficial effects with regard to CVD (Singh *et al.,* 2005:151). Studies in human subjects and animal models have demonstrated that *α*-tocopherol supplementation decreased lipid oxidation and superoxide production by impairing the assembly of nicotinamide adenine dinucleotide phosphate (reduced form) oxidase (Singh *et al.,* 2005:151). Vitamin C and E consumed synergistically have been shown to prevent the adverse changes in postprandial oxidative stress markers and endothelial function induced by a high-fat meal (Plotnick *et al.,* 1997:1686 and Ceriello *et al*., 1998b:1529).

2.3.5 Other plant polyphenols

Growing interest in nutrition and preventive health care research for the evaluation and development of natural bioactive and antioxidant active products from plant materials due to their polyphenol contents, has been demonstrated in recent times (Naithani *et al.,* 2006:176). Polyphenols are substances widely distributed in plants such as teas and herbals (Ullah *et al.,* 2008:187). These antioxidants found in herbal tea play an important role as part of a healthy

diet (Halliwell, 1999:70). Many studies have reported the beneficial effect of tea and herbal tea polyphenols in human subjects and experimental animals (Okamura & Tamura, 2004:189; Matsuda *et al.,* 2007:214). Tea and herbal tea consumption could be helpful in maintaining and promoting health particularly in respect of CVD prevention (Sumpio *et al.,* 2006:818-819).

2.3.5.1 *Camellia sinensis* (tea)

Tea is a widely consumed beverage around the world (Nishitani & Magesaka, 2004:675) and ranks second only to water consumption (Muthumani & Senthil, 2006:103; Galati *et al*., 2006:570). The tea plant (*Camellia sinensis*) is believed to have originated in the landmass encompassing Tibet, Western China, and Northern India (http://www.whfoods.com/). According to ancient Chinese legend, the origin of tea is attributed to a Chinese scholar and herbalist, Emperor Shen Nung, who lived around 2737 B.C. One day Emperor Nung was boiling water for an evening meal while resting under a wild tea tree, when leaves from the wild tea bush accidentally fell into the pot of water he was boiling. Thus, tea was discovered and it spreaded worldwide (Zee *et al.,* 2003:3).

Tea plants are widely cultivated in South-East Asia, including China, India, Japan, Taiwan, Sri Lanka, Indonesia and many African countries including South Africa (Luximo-Ramma, 2005:357). There are four types of tea produced: green, oolong, black and white tea. All derive from the same plant *Camellia sinensis*. Fresh leaves and buds are pan-fried, rolled and dried and used to prepare green tea. Oolong is made from sun wilted fresh leaves that has been slightly bruised and partially fermented. Black tea is made by fully fermenting the slightly wilted leaves (Ferrara *et al.,* 2001:397), while white tea is made from immature tea leaves that has undergone little processing (Santana-Rios *et al.,* 2001:62). This classification depends on the differences in the manufacturing processes and the fermentation times (Galati *et al.,* 2006:570). The leaves of the tea plant are used both as a medicinal and social beverage. Since 3000 B.C., traditional Chinese medicine has recommended green tea for headaches, body aches and pains, digestion, enhancement of immune defences, detoxification, as an energiser and to prolong life (Ferrara *et al*., 2001:397).

During the past decade, numerous studies have suggested possible effects of green and black teas and their polyphenolic constituents, as antioxidants in *in vitro* and *in vivo* models of cancer and cardiovascular disease (Luczaj & Skrzydlewska, 2005:910). Teas are reported to contain natural antioxidants such as vitamins and polyphenols (flavonoids such as flavanols, flavonols and isoflavones), co-enzyme Q10, carotenoids, selenium, zinc and other phytochemicals (Naithani *et al.,* 2006:176). Polyphenols can act as free radical-scavengers,

quenching $HO^{\bullet -}$ or $O_2^{\bullet -}$ (*al.,* 2007:617). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Atoui *et al.,* 2005:28). In addition, they also have metal chelation potential. The antioxidant activity of plant phenolics has been studied in relation to the prevention of coronary diseases and cancer, as well as age-related degenerative brain disorders (Parr & Bolwell, 2000:985). Research on the putative health effects of tea has demonstrated the contribution of phytochemicals, particularly phenolic acids, flavonoids, catechins and other flavanol derivatives to the above-mentioned benefits (Luximo-Ramma *et al.,* 2005:359). Phenolic compounds constitute 50–70% of a tea water extract and are the main quality parameters for teas (Yao *et al.,* 2006:614). Polyphenol activities are mediated partly by their free radical scavenging, antioxidant and metal complexing actions (Bahorun *et al.,* 2004:1554). The main proportion (approximately 90%) of total phenolic compounds in tea is flavonoids. In green tea, catechins (flavanols and flavanol gallates) are the major polyphenols while their complex oxidation products, theaflavins (TF), thearubigins (TR) and theabrownins (TB) serve as quality markers in black tea (Yao *et al*., 2006:614).

The antioxidant activity of *Camelia sinensis* tea has been assessed in *in vitro* and *in vivo* model systems (Hodgson *et al.,* 2000:1103). The antioxidant activity of this tea is associated with a number of its constituents, which have the ene-diol functionality, often in an electron rich B-ring system. Ingestion of tea polyphenols improve endothelial dysfunction and lowers the susceptibility of LDL lipids to oxidation postprandially (Sies *et al.,* 2005:969). Short- and long-term black tea beverage consumption was also shown to reverse endothelial vasomotor dysfunction in patients with coronary artery disease (Duffy *et al.,* 2001:154). Also to a lesser extend, *in vitro* studies have shown tea catechins as potent inhibitors of carcinogenesis at the three stages of cancer development (Fujiki, 1999:589; Suganuma *et al.,* 1999:339). However in hypercholesterolemic rats, evidence showed that green tea considerably reduced serum and liver cholesterol, the atherogenic index, liver weight and lowering the deposition of lipids (Yang & Koo, 1997:505). It is also important to know that the type of tea consumed varies in different countries (Brannon, 2007:2).

2.3.5.2 *Aspalathus linearis* (rooibos)

2.3.5.2.1 Background

Aspalathus linearis plant is from the family of *Fabaceae* and tribe of *Crotalarieae,* well known as rooibos in Afrikaans and “Rotbush” in German (Joubert *et al.,* 2008:377). The stems

are slender and the leaves are linear and needle like, 2-6 cm long. Rooibos herbal tea is also referred to as bush tea or Masai tea and is an indigenous South African product of the redbush plant, *Aspalathus linearis* (Joubert *et al*., 2008:376). *Aspalathus linearis* is a leguminous shrub indigenous to the Cedarberg Mountains around Clanwilliam and its surrounding area near Cape Town, South Africa (Rabe *et al.,* 1994:1559; van der Bank *et al.,* 1995:257; Joubert *et al.,* 2004:133). The genus *Aspalathus* comprises about 278 species and is endemic to South Africa (van Heerden *et al.,* 2003:886). The species are concentrated in the Cape Province, with a few spreading to southern KwaZulu-Natal. Rooibos herbal tea production only occurs in the Clanwilliam area, a region 200 to 300 km north of Cape Town, within and adjacent to the Cedarberg and Olifantsrivier mountain ranges, and on the Bokkeveld plateau (Nel *et al.,* 2007:117). Rooibos has been used by indigenous people, the Khoikhoi, since 1772 (Marais *et al.,* 2000:43). Its leaves and stems are used for the manufacturing of rooibos herbal tea (Bramati *et al.,* 2002:5513). Rooibos is increasingly recognised as one of the relatively few economic plants, which has made a remarkable transition from a local wild resource to a cultivated crop in the 20th century (Bramati *et al.,* 2002:5513). The demand for this indigenous South African herbal beverage by the international market has significantly increased to such an extent that the tonnage currently exceeds domestic use (Van der Merwe *et al.,* 2006:43). Although, traces of alkaloid spateine were found in rooibos, it is a unique beverage rich in phenolic components; rooibos is naturally caffeine-free and have low tannin content (Blommaert & Steenkamp, 1978:49; Rabe *et al.,* 1994:1559; Reynecke *et al.,* 1949:397; Van Wyk & Verdoorn, 1989:520). The beneficial properties are partly ascribed to the phenolic constituents in the plant, which are enzymatically modified during processing fermentation. Several phenolic compounds are known to occur in *Aspalathus linearis* (van Heerden *et al.,* 2003:886). Its predominant contents are dihydrochalcones, flavonols and flavones (Rabe *et al.,* 1994:1559). Figure 2.6 shows a rooibos field.

Figure 2.6: Rooibos field (image kindly provided by Rooibos Limited, Clanwilliam)

2.3.5.2.2 Processing and chemical composition

Two types of rooibos are manufactured namely fermented and unfermented “green” as shown in Figure 2.7. Fermented rooibos is produced after the rooibos plant is harvested during the hot summer months and early autumn between January and April by topping the whole bush to approximately. 45 cm (Joubert *et al.,* 2008:387).

Figure 2.7: Rooibos (fermented left and unfermented or “green” right)

It is advisable that the active growth should not be exceeding 50 cm, otherwise the plant material will produce a weak tea. No flowers should be present, as they are unfavourable to quality. The leaves become redbrown when the shredded plant material is fermented in heaps at ambient temperature for about 12-14 hours, before being sun-dried (Joubert *et al.,* 2008:387). Unfermented rooibos or “green” rooibos is also obtained by retaining the green leaf and by keeping the oxidative changes to a minimum. Therefore, quality “green” rooibos is

produced either by drying of shredded plant material without delay under vacuum, or drying of whole shoots to a critical moisture content before shredding or steaming of the fresh shoots to inactivate enzymes before shredding (Joubert *et al.,* 2008:388). Rooibos has been reported to also contain phenolic acids that have shown antioxidant activity (Rabe *et al*., 1994:1564). Phenolic acids are polyphenolic substances that are present in all plants and have been studied extensively in cereals, legumes, nuts, olive oil, vegetables, fruits, tea, and red wine (Kris-Etherton *et al.,* 2003:71). The phenolic acids identified in rooibos may be classified into two classes namely hydroxybenzoic acids and hydroxycinnamic acids as shown in Table 2.2.

Table 2.2: Classes of rooibos phenolic acids

Hydroxybenzoic acids	**Hydroxycinnamic acids**
Syringic acid *	Caffeic acid *
Protocatechuic acid *	Ferulic acids *
p-Hydroxybenzoic acid *	p-Coumaric acid *
Ferulic acids *	Vanillic acid *

* Rabe *et al*., 1994:1559

The polyphenol antioxidants identified in rooibos include the monomeric flavonoids, dihydrochalcones (aspalathin and nothofagin), quercetin, rutin, isoquercitrin, orientin, isoorientin, luteolin, vitexin, isovitexin, and chrysoeriol (Joubert, 1996:409). Table 2.3 shows the breakdown of natural antioxidant components in rooibos.

Table 2.3: Breakdown of natural antioxidant components in rooibos

Dihydrochalcone glycosides	Flavone glycosides	Flavonol glycosides
Aspalathin (Koeppen & Roux,1966)	Iso-orientin (Koeppen *et al.,*1962; Koeppen & Roux, 1965b)	Rutin (Koeppen *et al.,* 1962)
Nothofagin (Joubert, 1996)	Orientin (Koeppen *et al.,*1962; Koeppen & Roux, 1965a)	Isoquercitrin (Koeppen *et al.,* 1962)
	Vitexin (Rabe *et al.,* 1994)	Quercetin (Snyckers & Salemi, 1974)
	Iso-vitexin (Rabe *et al.,* 1994)	

Between 35 - 68 % of the total polyphenol content in the unfermented rooibos samples comprise of aspalathin (Schulz *et al.,* 2003:543). The flavonoids are classified into five categories: monomeric flavanols, flavanones, anthocyanidins, flavones and flavonols (Huang *et al.,* 1999:999). The dihydrochalcone aspalathin is unique to rooibos (Joubert *et al*., 2003:270).

2.3.5.2.2.1 Quercetin and luteolin

Two of the flavonoids in rooibos, quercetin and luteolin, are potent antioxidants also found in many fruits and vegetables. Quercetin is one of the most frequently found and studied flavonoids and has many known biological activities, including antioxidant action (Dok-Go *et al.,* 2003:135). Quercetin has been shown in many cell-free experimental systems to scavenge reactive oxygen radicals and to reduce oxidative DNA damage and lipid oxidation (Dok-Go *et al.,* 2003:135). Luteolin, the flavone subclass of flavonoids, usually occurs as glycosylated forms in tea and as an aglycone in perilla seeds (Shimoi *et al.,* 1998:220). It has been reported to be non-mutagenic, antimutagen, antitumorigenic; anti-inflammatory and anti-allergic and has been recognised as a hydroxyl radical scavenger (Choi, 2007:870; Shimoi *et al.,* 1998:220). Figure 2.8 shows the chemical structure of quercetin and luteolin.

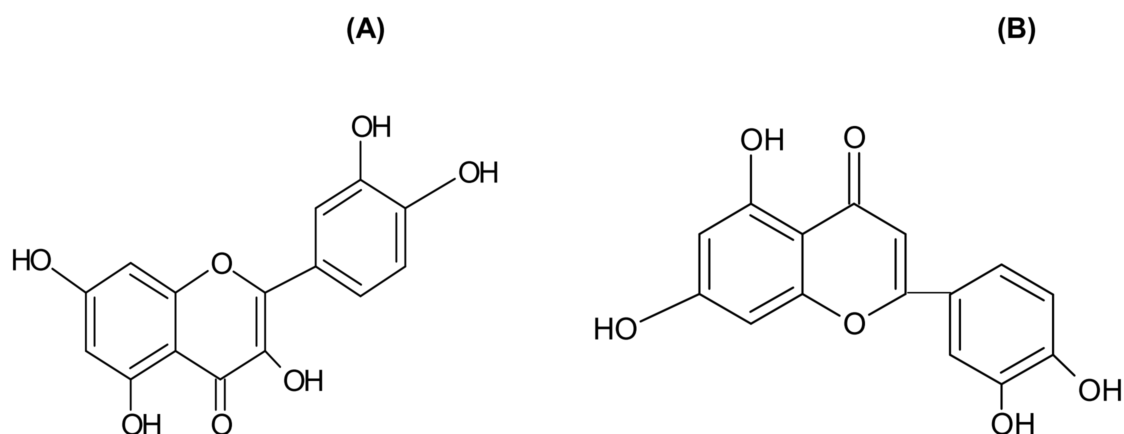


Figure 2.8: The chemical structure of (A) quercetin and (B) luteolin (Huang *et al.,* 1999:1002)

2.3.5.2.2.2 Aspalathin and nothofagin

Aspalathin (2',3,4,4',6'-pentahydroxy-3-C-β-D-glucopyranosyldihydrochalcone) is a unique polyphenol only found in rooibos and is one of the most abundant monomeric flavonoids in rooibos (Jaganyi & Wheeler, 2003:121). Aspalathin is one of the substances in rooibos that mimics superoxide dismutase (SOD) for its antioxidant activity (Yoshikawa *et al.,* 1990:173). Aspalathin is present in both fermented as well as unfermented "green" rooibos contributing to its antioxidant activity (Jaganyi & Wheeler, 2003:121; Marnewick *et al.,* 2005:194). Crude aspalathin and ethyl acetate fractions are the most potent radical scavengers with the highest total polyphenol concentrations and would be the obvious choice for exploitation as natural antioxidants (Joubert *et al.,* 2004:137). Aspalathin is not so well studied as quercetin and luteolin.

Nothofagin (2'.4,4',6'-tetrahydroxy-3-C-β-D-glucopyrasyldihydrochalcone) is similar in structure to aspalathin except for the hydroxylation pattern of the B-ring (Joubert, 1996:403). It has only been identified in one other natural source besides rooibos, namely the heartwood of the red beech tree *Nothofagus fusca*, family of *Fagaceae*, which is native to New Zealand (Hillis & Inoue, 1967:59). A serving of unfermented/"green" rooibos has considerably more aspalathin and nothofagin than an equal serving of fermented rooibos, because a portion of these flavonoids oxidises to other antioxidant compounds during fermentation. Figure 2.9 shows the chemical structure of aspalathin and nothofagin.

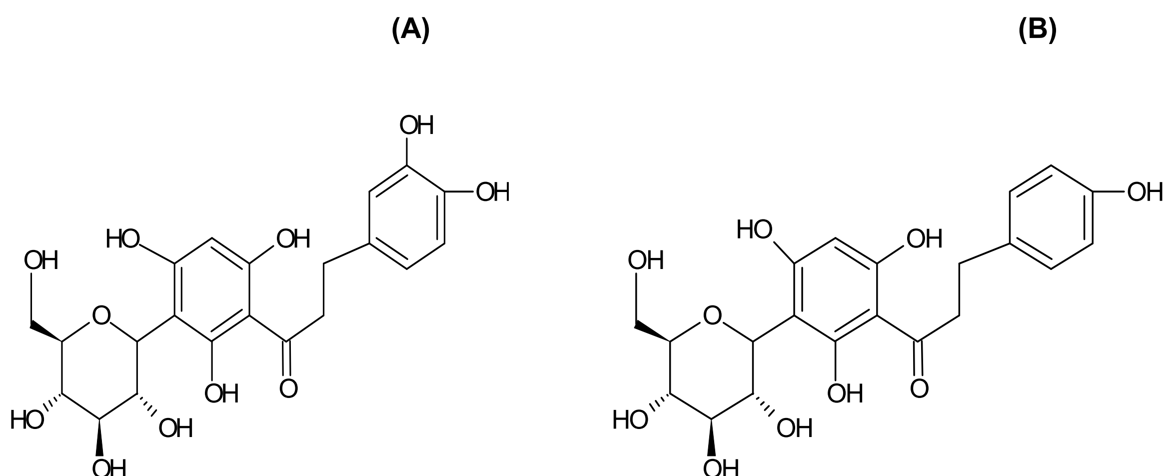


Figure 2.9: Chemical structure of (A) aspalathin and (B) nothofagin (Jaganyi & Wheeler, 2003:121; Joubert, 1996:403)

2.3.5.2.2.3 Orientin and rutin

Orientin is a *C*- glycosyl-luteolin compound, which exists widely in plants of many families including *Aspalathus linearis* and can be identified as 8-C-P-D'glucopyranosyl-luteolin (Koeppen & Roux, 1965:444). It has a wide variety of biological activities such as radioprotection, vasorelaxant, antioxidative property, free-radical-inhibiting and antiviral activity (Li *et al.,* 2007:221). As orientin is a potent free radical scavenger with the growing significance of a potential beneficial role in human health, there is an increasing demand for analysing its *in vivo* pharmacokinetics (Li *et al*., 2008:429). Figure 2.10 illustrates the chemical structure of orientin and rutin.

Rutin is a flavonol glycoside consisting of the flavonol quercetin and the disaccharide rutinose (Pastukhow *et al.,* 2007:60). Rutin (quercetin-3-rhamnosyl glucoside), a natural flavone derivative, has been associated with health benefits and was first discovered in buckwheat in the 19th century (Yang *et al.,* 2008:1061).

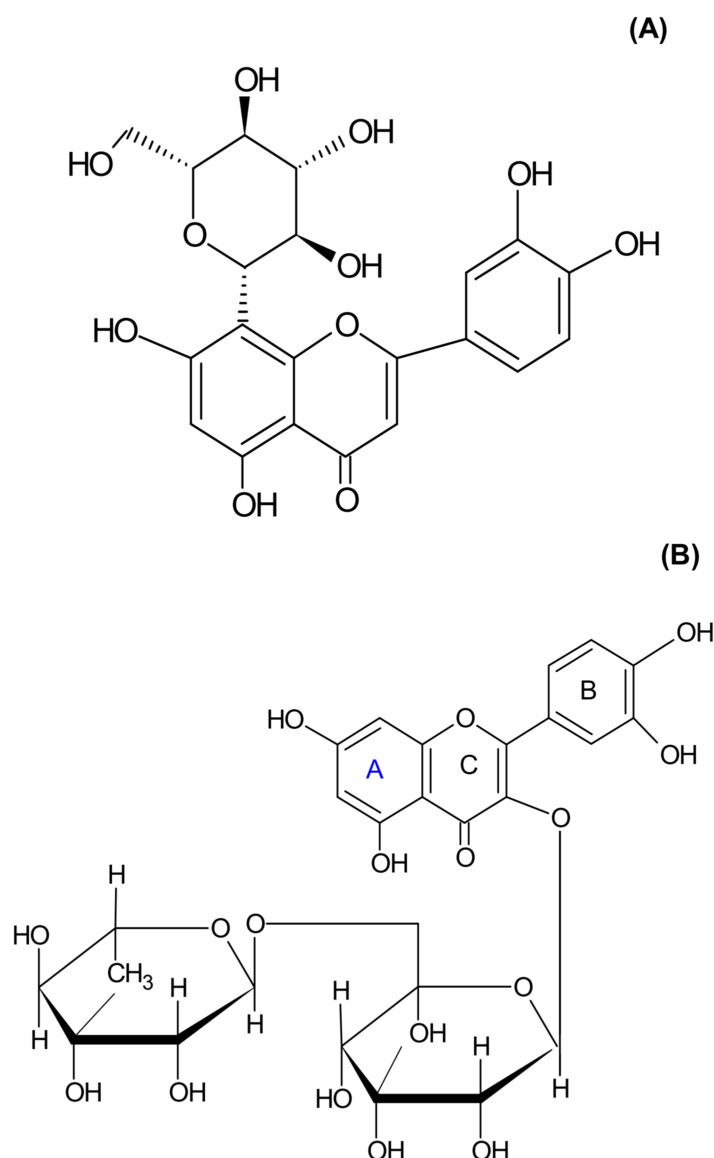


Figure 2.10: Chemical structure of (A) orientin and (B) rutin (Adapted from Li *et al.,* 2008:430; Pastukhov *et al.,* 2007:61)

2.3.5.2.3 Health properties of rooibos

There is a great interest in the role of herbal teas for maintenance of health and in the prevention of disease (Vaidyanathan & Walle, 2002:897). Rooibos has been used as an herbal beverage and to a lesser extent as an herbal medicine in South Africa before the 1800's (Marnewick *et al.,* 2004:146). A growing body of evidence suggests that moderate consumption of herbal teas, including rooibos, may have protective effects against several forms of disease where oxidative stress plays a role (Breet *et al.,* 2005:984).

2.3.5.2.3.1 Anti-mutagenic and anti-cancer properties

Many studies have reported that rooibos contains anti-mutagenic properties (Marnewick *et al.,* 2000:164 and Lamošová *et al.,* 1997:39). Contribution of the flavonoids to the anti-mutagenic potency of rooibos as determined *in vitro* depends on their relative concentrations in the aqueous extracts, which may vary depending on different environmental factors (Marnewick *et al.,* 2000:164; Van der Merwe *et al.,* 2006:50; Snijman *et al.,* 2007:121). Also, in rat model study, green tea fraction with the highest flavanol/proanthocyanidin content, showed the highest protective activity (99%) against hepatic microsomal lipid oxidation and completely inhibited the formation of skin tumour (Marnewick *et al.,* 2005:193).

2.3.5.2.3.2 Anti-inflammatory and aging properties

Rooibos is known to have anti-inflammatory properties. Khan & Gilani, (2006:468) suggested, the medicinal use of rooibos in hyperactive gastrointestinal, respiratory and cardiovascular diseases has the potential to be developed as a remedy for congestive airway disorders. Rooibos was reported to protect several regions of the rat brain (frontal cortex, occipital cortex, hippocampus and cerebellum) against lipid oxidation accompanying aging (Inanami *et al.,* 1995:86).

2.3.5.2.3.3 Antispamodic effects

Based on studies in the jejunum by Gilani *et al.,* (2006:365) that the potential therapeutic use of rooibos in K^+ channel activation and smooth muscle relaxation for the treatment of asthma has been recognised. In animal experimental, involving rabbits, guinea-pigs and rats, concentrated rooibos extract, containing approximately 120 and 199 mg quercetin equivalents/g extract of total polyphenols and flavonoids, respectively, exhibited bronchodilatory, antispasmodic and blood pressure lowering effects (Khan & Gilani, 2006:465).

2.3.5.2.3.4 Immune system modulation

A rooibos water extract stimulated antibody production in murine splenocytes when using anti-ovalbumin and sheep red blood cells, while it lacked any effects in lipopolysaccharide-stimulated splenic B-cells (Kunishiro *et al.,* 2001:2137). With Wistar rat model, oral administration of a rooibos water extract significantly restored the anti-ovalbumin-induced antibody production after cyclosporin A-treatment. Rooibos also stimulated IL-2 generation in murine splenocytes *in vivo* (Kunishiro *et al.,* 2001:2137). An aqueous fraction,

which was obtained after column fractionation of a hot water extract of rooibos, increased the immunoglobulin M (IgM) production in anti-OVA-stimulated murine splenocytes, which was also associated with the production of IL-10 (Ichiyama *et al.,* 2007:589).

2.3.5.2.3.5 Vasodilation, antihemolytic, dermatological and anti-allergic effects

A study by Persson *et al.,* (2006:1139) investigated the effect of rooibos on the angiotensin-converting enzyme (ACE), which also catalyses the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor. The effect of rooibos on the production of the vasodilator, nitric oxide (NO), in cultured endothelial cells from human umbilical veins as model system, was also included in the study. No significant inhibition of ACE was found, but a significant dose dependent increase in NO production resulted after an incubation of the cells with rooibos for 24 h.

In another study, Japanese quails were fed rooibos, either by supplementing their drinking fluid with a rooibos extract or the feed with milled plant material for 180 days. Results from this study did not show any changes in the erythrocytes fragility when using peroxide- or hypotonia-induced hemolysis (Simon *et al.,* 2000:365).

Rooibos intake also decreased the incidence of viral *herpes simplex* within 2–3 days in patients who received a dilute infusion of rooibos at least once a week, while patients with atopic dermatitis were relieved of the itchy sensation in their skin (Shindo & Kato, 1991:388). Another study conducted by Hesseling & Joubert (1982:1037) showed no modulating effect by rooibos in humans on a type I skin response, after a skin prick test using 16 common allergens.

2.3.5.2.3.6 Antimicrobial and viral effects

A dose-dependent growth inhibitory effect against *Escherichia coli* were shown by hot water extracts and their ethyl acetate extracts, prepared from fermented and unfermented rooibos (Scheepers, 2001). The hot water extracts of fermented and unfermented rooibos also inhibited the growth of *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus mutans, Listeria monocytogenes* and *Saccharomyces cerevisiae*, while the hot water extract of fermented rooibos also stimulated the growth of Zygosaccharomyces *rouxii*. Another study also showed a rooibos alkaline water extract, prepared from plant material that was first subjected to hot

water extraction, suppressed HIV-induced cytopathicity of HIV- (HTLV-III) infected MT-4 cells *in vitro* (Nakano *et al.,* 1997a:128 and Nakano *et al.,* 1997b:267).

It is important to note that most of the abovementioned studies were done in experimental animals and very little information is available on the effect rooibos has in humans. To date, no studies have reported on the modulating effect rooibos might have using an animal lipidaemic model. Therefore, the present study investigated the effect of endogenous ROS-induced by a lipid-rich meal and/or consumption of sucrose on human postprandial oxidative stress. In order to prevent/modulate oxidative stress, the approach used in this study included the intake of rooibos, a South African indigenous herbal tisane with known polyphenolic antioxidant properties. The choice of rooibos as study beverage was due to its good antioxidant ability to scavenge free radicals and also it's up-regulation of certain metal chelation reactions (Lee & Jang, 2008:285).

CHAPTER THREE: METHODOLOGY

3.1 Introduction

The purpose of this study was to investigate the effect of sucrose in combination with a standardised fat meal and antioxidant intervention on markers of oxidative stress in healthy volunteers. A previous study showed that a rich fat meal (1 g lipid / kg body weight) was well tolerated by volunteers and could demonstrate the effects that a sucrose load (1.5 g / kg body weight) have on lipid clearance without seriously compromising the comfort of the volunteers (Grant *et al.,* 1994:854). In hyperlipidaemic and hyperglycaemic subjects, endothelium-dependent vasodilatation was impaired in the postprandial state, making postprandial oxidative stress an important potential factor inducing risk for cardiovascular disease (Sies *et al*., 2005:969). There is convincing evidence that postprandial changes of both lipids and glucose can potentially predict cardiovascular risk, better than fasting values or even long-term integration parameters, such as glycated haemoglobin (Manuel-y-Keenoy *et al*., 2005:475). Postprandial oxidative stress is attenuated when dietary antioxidants are supplied together with a meal rich in oxidised or oxidisable lipids (Sies *et al.,* 2005:969). The validity of the quantities was based on the approximate equivalence of a typical fast-food meal, e.g. hamburger meal when consumed with a commercial soda.

3.2 Materials and methods

3.2.1 Experimental design

Research participants for this study were registered students at the Cape Peninsula University of Technology (CPUT). After advertising on campus via internal e-mail and posters, those who were interested to participate in the study received information describing the nature and objectives of the study. The study was conducted in two phases: Phase One (n = 13) was a pilot study using a parallel design and required of participants to consume either a standardised fat meal and water (designated control group, n = 5) or a standardised fat meal and a commercial sucrose beverage (designated treatment group n = 8), with Phase Two (n = 14) being the experimental study with a crossover design, that consisted of participants that consumed the standardised fat meal and commercial sucrose beverage on one occasion (designated control group) and the standardised fat meal and fermented rooibos beverage containing sucrose on another occasion two weeks later (designated treatment group).

Research participants in Phase One were screened (see Appendix B.2) with respect to the criteria as described by the American Heart Association (2002) for apparent healthy individuals. The inclusion criteria included apparent health (blood pressure ≤ 120/80 mmHg), non-diabetic (fasting glucose < 7.0 mmol/l), normolipidaemic (fasting triglyceride concentration < 1.7 mmol/l and fasting cholesterol concentration < 5.818 mmol/l), aged between 18 - 35 years, relative stable body mass index of 20-25 kg/m^2. While participants in Phase Two were selected according to their age, blood pressure and BMI (the reason being that they were their own controls in the crossover design). Exclusion criteria for both phases included a history of documented cardio-artery disease, a history of or treatment with medications for diabetes mellitus, use of lipid-altering drugs more recently than 4 weeks before the study, taking any vitamin or antioxidant supplements, having any serious medical illnesses (such as liver, kidney or thyroid disease), smoking and females under menstruation, pregnancy, lactation or using contraceptives.

Participants from both study phases were requested to abstain from alcohol and vigorous exercise for 24 hours and fast for 12 hours before they reported to the experimental study venue (Science building, Cape Town Campus, CPUT) for each 8 hour study day. Written informed consent (see Appendix B. 5) for the study was obtained from the volunteers after the study procedures were explained to them. In Phase Two, twenty two participants were initially recruited, but during the crossover period, 8 opted out because of personal reasons, leaving 14 participants that completed Phase Two. Apart from the participants not being allowed to take any vitamin and antioxidant supplements, they were required to maintain their normal diet throughout the study period.

No run-in period or the requirement of the participants to consume a standardised evening meal before each 8 hour study day were included in this study design when considering previously published studies (Grant *et al.,*1994:854; Ceriello *et al.,* 1999:1504; Devaraj *et al.,* 2008:868).

3.2.2 Ethical approval

Ethical approval for this study was obtained from the Health and Wellness Sciences Faculty Research Ethics Committee of the Cape Peninsula University of Technology before commencement of the study.

3.2.3 Anthropometric measurements

Anthropometric measurements, which consisted of the inclusion criteria, were used in both study phases and were made before the experimental day using standard procedures (Lohman *et al*., 1988:35). The participants height was measured using a stadiometer (Seca, Kraaifontein, South Africa) and their weight recorded using a digital scale (Adam Medical Scale, Kraaifontein, South Africa). Body mass index (BMI) was calculated using the standard formula (weight [kg] / height [m^2]), while the systolic and diastolic blood pressure were measured using a blood pressure monitor (Rossimax Medical®, Kraaifontein, South Africa). Anthropometric data were recorded on the anthropometry and blood pressure forms (see Appendix B. 1), on which the age and sex were also recorded.

3.2.4 Dietary intake

The standardised fat meal consumed by the research participants contained (50.1 g) fat and consisted of 150 g cooked sausage (beef and pork), 95 g cooked beef patty and chicken viennas. The fat content of the meal was chosen to mimic what would be typically consumed in a Western diet. This was then combined with either water (566 ml = control group) or 59.5 g of sucrose (566 ml commercial soda drink = treatment group) in Phase One. The same standardised fat meal was consumed in Phase Two with either the commercial soda drink (500 ml) containing 52.5 g (10.5 g per 100 ml) of sucrose (control group) or with 500 ml fermented rooibos herbal tea (2% w/v) containing 52.5 g of sucrose (treatment group). Candy sweets containing 16.5 g of sucrose were also included on both occasions, totalling the amount of sucrose consumed by both groups in Phase Two to 69 g. Based on the results obtained from the pilot study, the candy was included to increase the amount of sucrose consumed with the standardised fat meal. The beverages were consumed within 15 minutes after consuming the fat meal. The commercial soda drinks, candy sweet and ingredients of the standardised fat meal were purchased from a local supermarket, while the rooibos tea bags were supplied by Rooibos Ltd, Clanwilliam. The standardised fat meal as shown in Figure 3.1 was prepared by qualified dieticians of the Consumer Sciences department of the Cape Peninsula University of Technology.

Figure 3.1: Standardised fat meal with water

3.2.5 Blood sample collection and processing

Blood samples were taken from the median cubital vein on the anterior forearm by a registered nurse or phlebotomist (Figure 3.2) and drawn from each participant at various time intervals, first before consuming the fat meal and beverage, designated as time 0 hour (also referred to as baseline), as well as at 2, 4 and 6 hours post ingestion with minimal stasis. Participants were only allowed to visit the computer room situated in close proximity to the experimental study venue during the intervals between blood collections.

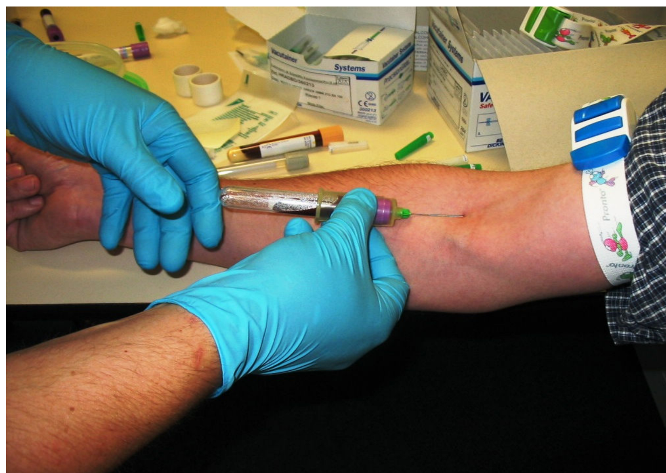


Figure 3.2: Blood collection procedure

Participants also had access to videos (set up in the same experimental study venue) that they could watch during these intervals in order to minimise physical activity as it could affect some of the blood parameters.

For plasma samples, two FX (5.0 ml) ethylene-diaminetetraacetic acids (EDTA)-containing vacutainer/siliconised test tubes were used at each designated time. These test tubes were purchased from LASEC (South Africa). Blood sample tubes were gently inverted for sufficient mixing and kept on ice until arrival at the laboratory and promptly centrifuged the same day. Centrifugation of blood was done at 4000 rpm for 8 minutes at 4°C in an Eppendorf centrifuge 5810R, within an hour after collection. Plasma was transferred to 2 ml micro tubes (4 aliquots) and stored at -80°C for the analysis of plasma antioxidant capacity and lipid oxidation.

For serum samples two FX (5.0 ml) vacutainer/siliconised test tubes containing a separating gel and no additives or antioxidant were used at each designated time interval. The test tubes were purchased from LASEC (South Africa). Blood sample tubes were kept on ice until arrival at the Laboratory where they were processed. Blood tubes were centrifugated at 4000 rpm for 8 minutes at 4°C in an Eppendorf centrifuge 5810R within an hour after collection. Supernatant (serum) was transferred to 2 ml micro tubes (4 aliquots) and stored at -80°C until the assays were done. Serum samples were used for lipid profile, insulin and CRP analysis.

For blood glucose samples, one FX (2.5 ml) vacutainer test tube containing heparin as anticoagulant was used to collect blood at each designated study time. Test tubes were also purchased from LASEC (South Africa). Blood samples were processed after being kept on ice before the arrival at the laboratory. Separation of blood by centrifugation was done as described in previous paragraphs. The supernatants were transferred to 1.5 ml micro tubes (2 aliquots) and stored at -80°C until the assay was done.

Thus, for the erythrocyte total glutathione samples, 50 µl whole EDTA blood was aliquoted into 1.5 ml micro tubes and stored at -80°C on the day the blood sample was drawn. The following day samples were thawed and immediately mixed with 350 µl of cold 5% monophosphoric acid (MPA) solution (1:8 dilution of original sample). Samples were vortexed for 15-20 seconds and centrifuged at 10 000 rpm for 10 minutes, whereafter supernatants (MPA extract) were stored at -80°C for spectrophotometric determination.

3.3 Analytical biochemical methods

3.3.1 Herbal tea quantification and activity

3.3.1.1 Preparation of aqueous fermented rooibos beverage

Fermented rooibos was prepared by pouring freshly boiled tap water onto herbal tea leaves at a concentration of 2% (w/v). The mixtures were stirred manually with a plastic spatula for 5 minutes to allow for extraction of polyphenols and other water soluble components. These aliquots of the extracts were cooled to room temperature and used for analytical testing.

3.3.1.2 Soluble solid measurement

The soluble solid content of the fermented rooibos extract was determined by first weighing empty conical tubes in quadruplicate. Thereafter 1 ml aliquots of rooibos water extract (as prepared in 3.3.1.1) was added into conical tubes and dried at 55°C for 72 hours. The weight of each tube was recorded again after drying. The soluble solid was calculated by subtracting the weight of the empty tubes from the weight of the rooibos water extract dried tubes. Values were expressed as mg soluble solid per litre water extract.

3.3.1.3 Total polyphenol content

The total polyphenol in the fermented rooibos extract was determined using the Folin Ciocalteu method with gallic acid as the standard. Total polyphenols were measured according to the method described by Waterhouse (2005:1). The freshly prepared rooibos sample (section 3.3.1.1) was diluted ten times, with 100 µl of herbal tea extracts being diluted in 900 µl of distilled water. Using a clear 96 micro well plate, 25 µL of standards (gallic acid) and herbal tea samples were added to each dedicated well (standards were prepared from a gallic acid stock solution). Standards and samples were done in triplicate. To all standard and sample wells, 125 µl Folin Ciocalteu reagent was added using a multichannel pipette. After incubating for 5 minutes at room temperature, 100 µl Na_2CO_3 was added to each well, whereafter the plate was incubated for 2 hours at room temperature before reading on a Multiskan plate reader (Multiskan spectrum, the Thermo Electron Corporation - Vantaa, Finland) at a wavelength of 765 nm. Reagent preparation is further described in appendix A (1.1).

Calculations were performed using a Microsoft Excel® spreadsheet 2007. Results were expressed as mg/l. An intra assay coefficient of variation of ≤ 5% was accepted.

3.3.1.4 Flavonol content

The flavonol content of the rooibos extract was determined using quercetin as the standard. The freshly prepared rooibos was diluted ten times, where 100 μl of rooibos extracts were diluted with 900 μl of distilled water in a micro tube. In the designated wells of a clear 96 micro well plate, 12.5 μl of standards (quercetin) and rooibos extract samples were added to each well in triplicate. This was followed by 12.5 μl of 0.1% hydrochloric acid (HCl) in 95% ethanol. Thereafter 225 μl of 2% HCl was added to this solution. The plate was incubated for 30 minutes at room temperature before taking a reading. The absorbance was measured at 360 nm on Multiskan plate reader (Multiskan spectrum, Thermo Electron Corporation - Vantaa, Finland). Calculations were performed using a Microsoft Excel® spreadsheet 2007 and the results were expressed in mg/l and reagents preparation is further detailed in appendix A (1.1). An intra assay coefficient of variation of ≤ 5% was accepted.

3.3.1.5 Flavanol content

The flavanol content of the rooibos extract was determined using the 4-dimethylaminocinnam-aldehyde (DMACA) reagent which reacts with flavanols to form a characteristic light blue colour that is measured at 640 nm (Delcour *et al.,* 1985:38; Treutter, 1989:187). Catechin was used as standard. Rooibos extracts were prepared as mentioned in section 3.3.1.1. The freshly prepared rooibos was diluted ten times whereafter 50 μl of catechin standards and samples were added in triplicate to the designated well of a clear 96 micro well plate in triplicate. The reaction was initiated by the addition of 250 μl of DMACA reagent into each well. The micro well plate was incubated for 30 minutes at room temperature before the absorbance was measured at 640 nm on a Multiskan plate reader (Multiskan spectrum, Thermo Electron Corporation - Vantaa, Finland) after ensuring that the temperature was at 25°C. Calculations were performed using a Microsoft Excel® spreadsheet 2007. The results were expressed in mg/l and reagents preparation is further detailed in appendix A (1.1). An intra assay coefficient of variation of ≤ 5% was accepted.

3.3.2 Aqueous fermented rooibos and plasma total antioxidant capacity measurement

Three assays were used to determine the total antioxidant capacity (TAC) of the various rooibos samples as well as plasma of samples study participants. Methods have been developed to assess the total antioxidant capacity of serum or plasma because of some difficulties in measuring each antioxidant component separately and interactions among antioxidants. The following assays are commonly used and have been extensively evaluated: the oxygen radical absorbance capacity (ORAC), the ferric reducing ability of plasma (FRAP) and the 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox)-equivalent antioxidant capacity (TEAC). In all the total antioxidant capacity assays, the intra assays coefficient of variation of ≤ 10% was accepted.

3.3.2.1 Oxygen radical absorbance capacity

The total antioxidant capacity of rooibos was determined by the oxygen radical absorbance capacity (ORAC) method as described by Ou and co-workers (2001:4620). The loss of fluorescence in the presence of the peroxyl radical generator, 2,2'-azobis-2-amidinopropane hydrochloride (AAPH), reflects the antioxidant activity and can be compared with a standard (Trolox) using a fluorometer (Fluoroskan ascent) at an excitation wavelength of 485 nm and an emission wavelength of 530 nm at 37°C. The rooibos extract was diluted 1:100 using distilled water. Twelve microlitres of each standards, controls and rooibos samples were added to respective wells of the black 96 micro well plate in triplicate. From the fluoroscein stock solution 10 µl was added to 2 ml phosphate buffer (in Eppendorf tube) and then further diluted by adding 240 µl of this solution to 15 ml phosphate buffer. Using a multichannel pipette, 138 µl of this solution was added into each well of a black 96 well micro plate. The reaction was initiated with the addition of AAPH (50 µl) and read on a Fluorescence plate reader (Fluoroskan ascent, Thermo Electron Corporation - Vantaa, Finland) every 5 minutes for 2 hours. The final volume of the assay was 200 µl and the sample readings were compared with a standard series using trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid).

Plasma samples were deproteinated using a 1:1 dilution with perchloric acid (PCA) by adding 100 µl of 0.5 M PCA to 100 µl of plasma sample in a microfuge tube. The solution was thoroughly mixed using vortex mixes for 1 minute and centrifuged at 4000 rpm for 5 minutes; whereafter the supernatant fluid was transferred to microfuge tubes. These supernatants were stored at -40º C and thawed at 4ºC on the day of the analysis. For analysis the supernatants were diluted 1:5 using the ORAC buffer (see appendix A 1.4) before determining the ORAC as described above. All plasma samples were done in triplicate. Calculations were performed

using a Microsoft Excel® spreadsheet 2007. Values were expressed as µmol Trolox Equivalent/l. Reagents preparation is further detailed in appendix A (1.2).

3.3.2.2 Ferric reducing antioxidant power

Ferric reducing antioxidant power (FRAP) in the rooibos solution and plasma was determined using a spectrophotometric method as described by Benzie & Strain (1996:71). The rooibos extract (preparation described in 3.3.1.1) was diluted 1:10 with distilled water. At a low pH, when the ferric-tripyridyltriazine (Fe^{3+} –TPTZ) complex is reduced to the ferrous (Fe^{2+}) form, an intense blue colour with an absorbance maximum at 593 nm develops. L-ascorbic acid was used as the standard and all samples were analysed in triplicate. Ten microlitres of each of the standards, controls and samples (rooibos extract diluted or plasma) were added to designated wells of a clear 96 micro well plate. This was followed by the addition of 300 µl of the FRAP reagent (see appendix 2.1.4) to each well using a multichannel pipette. The final volume of the assay was 310 µl. The plate was incubated for 30 minutes at 37°C, whereafter the absorbance was measured at 593 nm, using a Multiskan plate reader (Multiskan spectrum, Thermo Electron Corporation - Vantaa, Finland). All calculations were performed using a Microsoft Excel® spreadsheet 2007 and results were expressed in µM vitamin C equivalents/l and reagents preparation is further detailed in appendix A (1.2).

3.3.2.3 Trolox equivalent antioxidant capacity

In the present study the trolox equivalent antioxidant capacities of the rooibos and plasma samples were determined using the method of Rice-Evans & Miller (1994:28). All rooibos extracts were diluted 1:10 with distilled water. Shortly the method is based when antioxidants are added to the trolox equivalent antioxidant capacity (TEAC) test system, they can either scavenge the 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) radical formed or interfere with the radical generating process. The radical cation ($ABTS^{\bullet+}$) formed can be monitored by measurement of its characteristic absorbance maxima at 734 nm. Measurement of the absorbance at a specified wavelength enables the calculations of a percentage scavenging. The method was done by adding 25 µl of standards (see appendix A 1.8 for trolox standard preparation), control and samples (rooibos extracts or supernatant of plasma) in triplicate to the appropriate wells of clear 96 micro well plate. The ABTS reagent was diluted with absolute ethanol to read an absorbance of approximately 2 ± 0.1 nm (≈ 1ml ABTS reagent mixed with 20 ml ethanol absolute) and 300 µl of this ABTS reagent was added to each well using a multichannel pipette. Reagents preparation is further detailed in appendix

A (1.2). The plate was incubated for 30 minutes at room temperature before the absorbance was measured at 734 nm and the Multiskan plate reader (Multiskan spectrum, Thermo Electron Corporation - Vantaa, Finland) temperature was settled at 25°C.

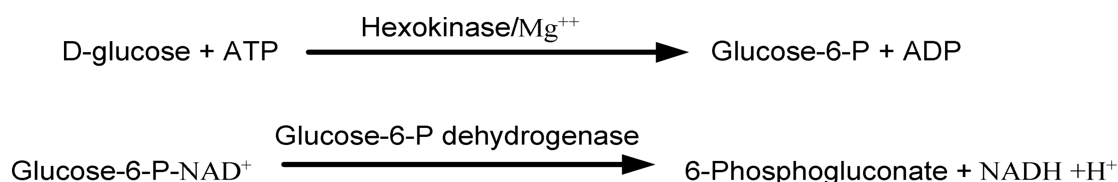
It is important to note that plasma samples were deproteinated (1:1 with 0.5 M of PCA) before analysis (see ORAC sample analysis 3.3.2.1). On the day of assay the stored deproteinated plasma supernatants were thawed and analysed with no further dilution required. All calculations were performed using a Microsoft Excel® spreadsheet 2007. Values were expressed as µmol Trolox Equivalent/l.

3.3.3 Clinical chemistry

Biochemical parameters (except serum insulin and C-reactive protein) were measured using a chemistry analyser, Medica Easy Random Access (EasyRA^{TM}, Bedford, Massachusetts, USA). Reagents for the biochemical analysis (except serum insulin and C-reactive protein) were supplied by the same company (glucose, total cholesterol, high-density lipoprotein and triglycerides kit test) and stored at 2-8°C. This chemistry analyser has a maximum throughput of 150 tests/ hour. The analyser utilises a new wireless radio frequency identification technology to manage the utilisation and protocols for up to 24 on-board reagents. This analyzer uses absorbance photometry for the chemistry tests with all reagents packaged in liquid form in single or dual reagent wedges ready to use. Plasma samples were thawed after being stored at -80°C before analysis was done in triplicate. Sample volume of 100 µL was used to analyse plasma glucose and lipid profile tests. The use of a chemistry analyser calibrator was employed before the test. Assays were automatically analysed at their respective absorbances. Plasma glucose and lipid profile assays were expressed in mmol/l. The principle of each assay method used with this analyser is described below. In all clinical chemistry assays, the intra assays coefficient of variation of ≤ 3% was accepted.

3.3.3.1 Plasma glucose

Glucose is the major carbohydrate present in blood. Glucose derived from dietary intake is converted to glycogen for storage in the liver and muscles or transformed to fatty acids for storage in adipose tissue. The absorbance is measured at 340 nm with 700 nm as a blanking wavelength. The concentration of glucose in human plasma was determined using enzymatic reaction method hexokinase/glucose-6-phosphate dehydrogenase (Neeley, 1972:509). Equation 3.1 describes the glucose enzymatic method:



Equation 3.1: The enzymatic reaction method of hexokinase/glucose-6-phosphate dehydrogenase

3.3.3.2 Insulin analysis

Plasma insulin was determined by a two-site immunoenzymometric assay using a ST AIA-PACK IRI kit on TOSOH AIA System analyser (TOSOH Bioscience, B-3980 Tessenderlom, Belgium). Insulin present in the test sample bound with monoclonal antibody immobilized on a magnetic solid phase and enzyme-labelled monoclonal antibody in the AIA-PACK. The magnetic beads are washed to remove unbound enzyme-labelled monoclonal antibody and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labelled monoclonal antibody that binds to the beads is directly proportional to the IRI concentration in the test sample.

The immunoenzymometric method using serum as described below was used for this assay. Serum stored at -80°C were gradually thawed at room temperature (18-25°C) and mixed gently. Lyophilised substrate was completely dissolved. Serum samples were diluted 1:10 in order to obtain a reading between 10 and 300 mlU/L. The AIA Systems perform all reagent handling operations automatically. The AIA system can store two different calibration curves for each analyte at one time. The diluted factor was entered in the software, a standard curve constructed and the unknown sample concentrations were calculated using the standard curve. The results were expressed in mlU/L and all materials and reagents were obtained from TOSOH (TOSOH Bioscience N.V., B-3980 Tessenderlo-Belgium).

3.3.3.3 High sensitive C-reactive protein

The High sensitive C-reactive protein (hs-CRP) is a plasma protein which is produced and released by the liver under the stimulation of cytokines such as tumour necrosis factor-α and interleukins (IL) 1 and 6 (Wang & Hoy, 2007:37). Its measurement has been emerged as an acute risk marker for cardiovascular disease (Ridker *et al*., 1997:978; Ridker, 2003:368;

Singh *et al.*, 2007:780), inflammation (Stuveling *et al.,* 2004:107; Chen *et al.,* 2007:1) and oxidative stress (Dohi *et al.,* 2007:65). Human serum high sensitive CRP concentration is determined using an ultra sensitive turbidimetric immunoassay measuring antigen-antibody reactions by the end-point method, with a detection limit of 0 - 1.0 mg/dl and a high measurement range (0 – 1.5 mg/dl CRP) on a Hitachi 917 automated chemistry analyzer (Roche Diagnostics, Australia). The method is based on the reaction of anti-human CRP antibody, with human CRP in the sample to form insoluble antibody-antigen complexes. Thawed serum samples, standards and control (8 µl) were mixed with 1000 µl of buffer and the optical density (OD1) was read at 600 nm. Hereafter, 240 µl of CPR latex was added, mixed and incubated for 5 minutes at room temperature, before reading the optical density (OD2) again at 600 nm. The results were expressed in mg/dl and the use of general chemistry calibrator was employed, using Chem Trol Plus reagent as a commercial quality control calibrator. Calibration values are described in appendix B 2.

3.3.3.4 Lipid profile

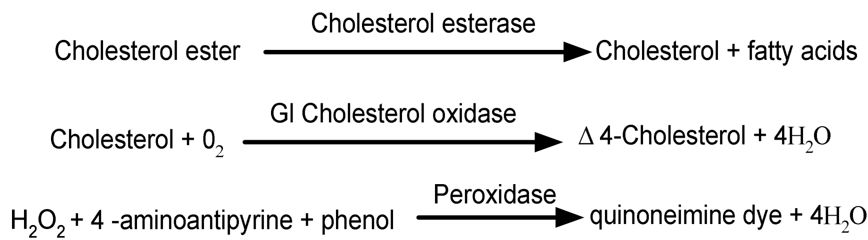
Serum total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C, low-density lipoprotein-cholesterol (LDL-C) and triglycerides (TG) were among the parameters investigated for the lipid profile.

3.3.3.4.1 Total cholesterol

Cholesterol is an essential lipid that acts as a major structural constituent of plasma membranes, and is a precursor of steroid hormones and bile acids (Hojo *et al.,* 2007:135).

The total cholesterol (TC) concentration in serum is the result of lipid metabolism and is affected by heredity, organ functions (e.g. liver, kidney, thyroid and endocrine) and diet. In this study, the total serum cholesterol was determined using enzymatic Trinder end-point reaction, based on the work of Allain *et al.,* (1974:470) see Equation 3.2.

A sample volume of 100 µl serum was used to measure TC. Samples were anlysed in triplicate. The indicator, quinoneimine, is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase which turns red, the intensity of which is read at 520 nm with 600 nm as a blanking wavelength and is directly proportional to the concentration of cholesterol in the serum sample.



Equation 3.2: Enzymatic Trinder end-point reaction

3.3.3.4.2 High-density lipoprotein cholesterol

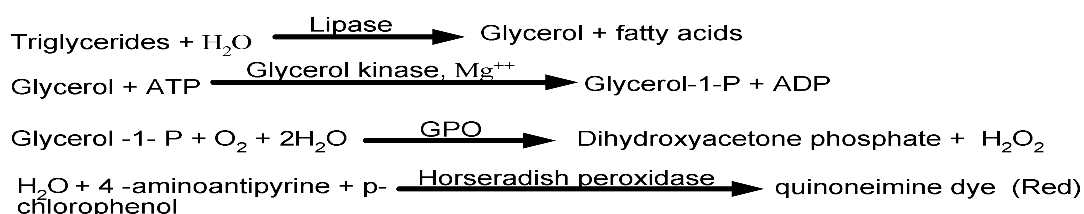
Reagent kits from Medica (Bedford, Massachusetts, USA) were used to measure the participant's high-density lipoprotein cholesterol (HDL-C). A sample volume of 100 µl was used to determine HDL-C in triplicate. No sample treatment was required. This direct assay method of measuring HDL-C involves the removal of other non-HDL lipoproteins via a selective precipitation reaction. In the second step, the detergent solubilises the HDL-C specifically, which then reacts with a chromagen to develop a colour, which can be read optically at 600 nm. The intensity of the colour at the maximum absorbance of 600 nm is proportional to the concentration of HDL-C in the sample. After completion of the assay, the Medica EasyRA Chemistry Analyzer calculates the HDL concentration from the ratio of the corrected unknown sample's absorbance to the corrected absorbance of the calibrator multiplied by the calibrator value.

3.3.3.4.3 Low-density lipoprotein cholesterol

The study used an indirect calculation for low-density lipoprotein cholesterol (LDL-C) using the modified Friedewald formula (Puavilai & Laoragpongse, 2004:589). The modified Friedewald formula is as follow: [modified Friedewald LDL-C] = ((Total cholesterol − HDL-C) − (1/6 TG). Studies have found that the standard Friedewald formula could overestimate LDL-C concentration, therefore it should be either directly assayed or be calculated by a modified Friedewald formula (Seyed-Ali *et al.,* 2008:318). The standard Friedewald formula incorporates fasting triglyceride levels. This modified Friedewald formula is used when the TG level is < 300 mg/dl (see addendum in Appendix B. 4). It has been reported to have an accuracy of 83.8% when compared to directly measured LDL ± 10 mg. This formula is more accurate than the standard Friedewald formula (Puavilai, 2009:182). Calculations were performed using a Microsoft Excel® spreadsheet 2007 and results obtained were expressed in mmol/l.

3.3.3.4.4 Triglycerides

The assay method for measuring triglycerides (TG) is via several sequential enzymatic reactions as described by Fossati & Prencipe (1982:2077) and involved a Trinder-type reaction mechanism as seen in Equation 3.3 (Trinder, 1969:159). Triglycerides were determined after enzymatic hydrolysis with lipoprotein lipases. A serum sample volume of 100 µl was used to determine TG in triplicate. When the reaction takes place, the indicator is a red quinone formed from hydrogen peroxide, 4-aminoantipyrine and 4-chlorophenol under the catalytic influence of peroxidase. The intensity of the red colour at the maximum absorbance at 520 nm is proportional to the triglycerides concentration in the sample.



Equation 3.3: Triglycerides sequential enzymatic reactions

3.3.4. Oxidative stress measurement

3.3.4.1 Glutathione analysis

Glutathione (GSH) is synthesised from L-glutamate, L-cysteine and glycine in two consecutive steps, catalysed by γ-glutamyl-cysteine synthase and glutathione synthase (Griffith, 1999:923). Glutathione is an important endogenous antioxidant that functions directly in elimination of toxic peroxides and aldehydes (Samiec *et al.*, 1998:699*).* Plasma glutathione in humans has been reported to be altered in several pathophysiological states. Because the different analytical methods for GSH have been validated and are calibrated against GSH standards, the variability that might occur could be due to differences in sampling, processing and/or storage of samples (Jones *et al.,* 1998:176). Glutathione is easily oxidised to the disulfide dimer (GSSG). Glutathione disulfide (GSSG) is produced during the reduction of hydroperoxides by glutathione peroxidase. Glutathione disulfide is reduced to GSH by glutathione reductase and it is the reduced form that exists mainly in biological systems.

The method as described by Asensi *et al*., (1999:269) was used. The sulfhydryl group reacts with 5,5'-dithiobis-2-nitrobenzic acid (DTNB) and produces a yellow coloured 5-thio-2-nitrobenzoic acid (TNB). The mixed disulfide, GSTNB (GSH and TNB) that is concomitantly produced, is reduced by glutathione reductase to recycle the GSH and produce more TNB. The rate of TNB production is directly proportional to the concentration of GSH in sample. Because glutathione reductase is used in the assay, both GSH and GSSG are measured and the assay reflects total glutathione. The concentration was determined measured at 412 nm after addition of NADPH on a Multiskan spectrum plate reader (Thermo Electron Corporation - Vantaa, Finland). For total GSH, 5µl of the MPA-treated whole blood sample was added to 300 µl GSH/GSSG Buffer A (appendix A 1.1) in microfuge tubes (1:61 dilution). This was followed by 50 µl standards, blank or samples to the respective wells of a 96 micro well plate. To these wells 50 µl of the DTNB solution using multichannel pipette were added, whereafter 50 µl of the enzyme solution, glutathione reductase (GR), using a multichannel pipette, were added. The microplate was shaken and incubated for 5 minutes at 25°C. To start the reaction, 50 µl of NADPH were added to each well and the absorbance was measured at 412 nm for 5 minutes at 30 second intervals. The final sample dilutions of total GSH was 1:488. Reagent preparation is further detailed in appendix A (1.3). Total glutathione values were expressed in µM and all calculations were performed using a Microsoft Excel® spreadsheet 2007. An intra assay coefficient of variation of ≤ 10% was accepted.

3.3.4.2 Lipid oxidation determination

While one single assay of lipid oxidation is probably not sufficient to serve as a marker for cardiovascular risk, there is a need for the measuring of several markers (Goçmen *et al.,* 2004:429). In this study, conjugated dienes (CD) and thiobarbituric acid reactive substances (TBARS) were measured as indicators of lipid oxidation biomarkers of oxidative stress. All the lipid oxidation assays had the intra assays coefficient of variation of ≤ 10%.

3.3.4.2.1 Conjugated dienes

Conjugated dienes have been reported to be an important indicator of lipid oxidation and are the initial markers of oxidative stress (Gümüslü *et al.,* 1997:214). Molecules that contain CDs absorb maximally at 215-250 nm, depending on the presence of substituent groups nearby. Conjugated dienes are characterised by a peak absorbance at approximately 234 nm, with a lesser peak at 260-280 nm. A potential problem with the measurement of CDs

in biological samples is the interfering background absorption from PUFAs, haem proteins, purines and pyrimidines.

The method, as described by Recknagel & Glende (1984:333), was used to determine CD concentrations in human plasma samples. Thawed plasma samples (100 µl) were mixed with 405 µl of a chloroform/methanol mixture (2:1) in a 1.5 ml micro tube and kept on ice. Solutions were then vortexed for 1 minute, centrifuged at 8000 rpm for 15 minutes at 4°C. The top aqueous layer was carefully removed and the bottom organic chloroform layer was transferred into clean microcentrifuge tubes. The organic chloroform layers were dried under nitogen (N_2) gas in a Techne sample concentrator (Labotec, South Africa). To each of the dried residues 1 ml cyclohexane was added and vortexed for 1 minute. Thereafter, 300 µl of the solution was transferred to 96 micro well plate and the absorbance was determined at 234 nm spectrophotometrically (Multiskan spectrum, Thermo Electron Corporation - Vantaa, Finland). The concentration of CDs is determined by the extinction coefficient of 0.265×10^4 $M^{-1}cm$ using 96 micro well plate. Cyclohexane was used as blank. Reagents preparation is further detailed in appendix A (1.3). All CD calculations were performed according to the Equation 3.4 using a Microsoft Excel® spreadsheet 2007. The results expressed as nmol CD/ml per plasma.

$$\frac{A_{234}(\text{sample}) - A_{234}(\text{blank})}{0.265} \quad X\ 10$$

Equation 3.4: Calculation formula of conjugated dienes

3.3.4.2.2 Thiobarbituric acid-reactive substance

The TBARS spectrophotometric method, although sensitive, is also non-specific because compounds such as bilirubin, amino acids, oxidised proteins, aldehydes, ketones and esters may also react with thiobarbituric acid (TBA). Another possible problem is that significant amounts of malondialdehyde (MDA) might be lost via volatilisation or cross-linking with proteins. The heat required for the reaction can promote the formation of new TBARS during the assay. The MDA or its enol form, 3-hydroxy-2-propenal, is another particular product of lipid oxidation and it is an important biomarker of lipid oxidation and is an endogenous by-product of prostaglandin and leukotriene biosynthesis (Gümüslü *et al.,* 1997:214).

In this study, the plasma degradation products (thiobarbituric acid-reactive substance) of lipid oxidation were photometrically determined after reaction with thiobarbituric acid. The method as described by Drapper *et al*., (1993:355) with slight modifications, was used to measure the thiobarbituric acid-reactive substance (TBARS). In a 96 micro well plate, 6.25 µl of 4 mM cold mix of butylated hydroxytoluene (BHT) and ethanol and also 50 µl of 0.2 M ortho-phosphoric acid were added to 50 µl plasma using a microcentrifuge tube. This mixture was then vortexed for 10 seconds wher after 6.25 µl TBA reagent was added. The resulting solutions were again vortexed and heated to 90°C for 45 minutes in water bath. Cooling the samples on ice for 2 minutes followed. Whereafter the samples were left at room temperature for 5 minutes. Hereafter, 500 µl *n*-butanol and 50 µl saturated NaCl were added, vortexed for 10 seconds and centrifuged at 12000 rpm for 2 minutes at 4°C. From this mixure, 300 µl from the top phase were added to 96 well micro plate and the absorbance was determined at 532 and 572 nm in a Multiskan plate reader (Multiskan spectrum, Thermo Electron Corporation - Vantaa, Finland). Results were expressed as nmol MDA/l plasma and reagents preparation is further detailed in appendix A (1.3). The TBARS was calculated in a Microsoft Excel® spreadsheet 2007, with the extinction coefficient of 0.138 as seen in Equation 3.5.

$$\frac{A_{532} - A_{732}}{0.138} \text{ X } 33.4$$

Equation 3.5: Calculation formula of thiobarbituric acid-reactive substance

3.4 Statistical analysis

In both study phases, results are given as means with their standard deviation (SD) and percentage changes. In Phase One, data were analysed for normality and responses to the two test meals were compared by one-way analysis of variance (ANOVA) for repeated, paired measurement with time, diet and subjects as factors. Where data were not equal and also where the interactions or the factors were statistically significant (P<0.05), Tukey-Kramer Multiple-Comparison Test was used to compare all pairwise differences between the means. Data were statistically analysed using NCSS V 07.1.14 Version (software released in January 2009 - Kaysville, Utah). While in Phase Two, data were analysed for normality and responses to the two test meals were compared by one-way analysis of variance for repeated, paired measurement with time, diet and subjects as factors. If the data was found not to have a normal distribution, a log transformation was performed. Where the interactions or the factors were statistically significant (P<0.05) Student-Newman-Keuls test for all pairwise comparisons test

was used to determine whether the value at a given time and dietary treatment differed from baseline (a significant postprandial response). Moreover, a paired sample *t*-test was used to determine significant differences between dietary treatments at a given time point. Data were statistically analysed using MedCalc Version 11.1.1.0 software.

CHAPTER FOUR: RESULTS

4.1 Phase One (Pilot study)

4.1.1 Anthropometry measurement

The anthropometry data of control and treatment groups of the pilot study are shown in Table 4.1. No significant (P>0.05) differences existed between both groups with regards to age, BMI and blood pressure.

Table 4.1: Anthropometry characteristics of control and treatment groups in Phase One

	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Systolic BP (mm Hg)	Diastolic BP (mm Hg)
Control Group (n=5)	25.2 ± 2.9	64.7 ± 4.8	174.2 ± 8.3	21.8 ± 1.4	128.6 ± 10.1	80.9 ± 8.9
Treatment Group (n=8)	23.3 ± 3.1	69.7 ± 12.5	173.7 ± 10.8	22.6 ± 2.9	135.0 ± 12.3	84.6 ± 10.6

Data in columns are presented as mean ± SD, n = number of subjects, BMI = body mass Index, BP = blood pressure; all subjects were males.

4.1.2 Glucose and insulin levels

4.1.2.1 Plasma glucose

We tested whether the intake of a standardised fat meal consumed with water (control group) or sucrose (treatment group) affected the plasma glucose levels of the participants differently. As shown in Table 4.2, the control group exhibited significant (P<0.05) higher serum glucose levels after 4 and 6 hr, (15% and 13%, respectively); with no significant differences (P>0.05) in the treatment group. When considering the inter group variations it is important to note that the baseline values (0 hr) of plasma glucose levels in the control group differed significantly (P<0.05) from that of the treatment group.

4.1.2.2 Serum insulin

Both control and treatment groups showed higher levels of serum insulin at 2 hr and 4 hr (39% and 39% for control group and 49% and 62% for treatment group) respectively, when compared to the baseline (0 hr) values, although not significant. In both groups, these levels returned to baseline after 6 hr as shown in Table 4.2. When comparing the inter group variations, no significant differences were shown between the serum insulin levels in either groups (Table 4.2).

Table 4.2: Postprandial changes in blood glucose and insulin levels in participants of Phase One

	Control group (n = 5)				Treatment group (n = 8)			
	0hr	2hr (%)	4hr (%)	6hr (%)	0hr	2hr (%)	4hr (%)	6hr (%)
Glucose	4.6 ± 0.74a*	4.4 ± 0.49a (-4%)	5.3 ± 0.28b (15%)	5.2 ± 0.13b (13%)	5.1 ± 0.57a*	4.4 ± 0.43a (-14%)	5.1 ± 0.29a (0%)	5.2 ± 0.54a (2%)
Insulin	6.9 ± 1.99a	9.6 ± 5.89a (39%)	9.6 ± 5.56a (39%)	6.0 ± 2.00a (-13%)	3.7 ± 0.97a	5.5 ± 1.98a (49%)	6.0 ± 1.38a (62%)	3.7 ± 1.60a (0%)

Values in columns are means ± SD; means followed by the same letter do not differ significantly (P>0.05). If letters differ, then P<0.05 when compared to the baseline value; values in parenthesis are percentage (%) changes compared to baselines, n = number of participants per group; time 0 hour is designated as baseline; glucose are expressed in mmol/l and insulin expressed in mIU/L. The underlined values represent significant (*=P<0.05) differences when comparing the control group with the treatment group.

4.1.3 Lipid profile and C-reactive protein analysis

4.1.3.1 Lipid profile

When comparing the serum variables of total cholesterol (TC), HDL-C, LDL-C and TG, no significant differences were shown when comparing the control and treatment groups (Table 4.3). On intra group level, the TGs were significantly ($P<0.05$) higher in the treatment group at 2 hr and 4 hr (44% and 56%, respectively) when compared with the baseline (0 hr). At 6 hr, the TG levels returned to baseline (0 hr) as shown in Table 4.3 and Figure 4.1. While in the control group, the TGs level was significantly ($P<0.05$) higher only at 4 hr (20%) postprandially when compared to the baseline.

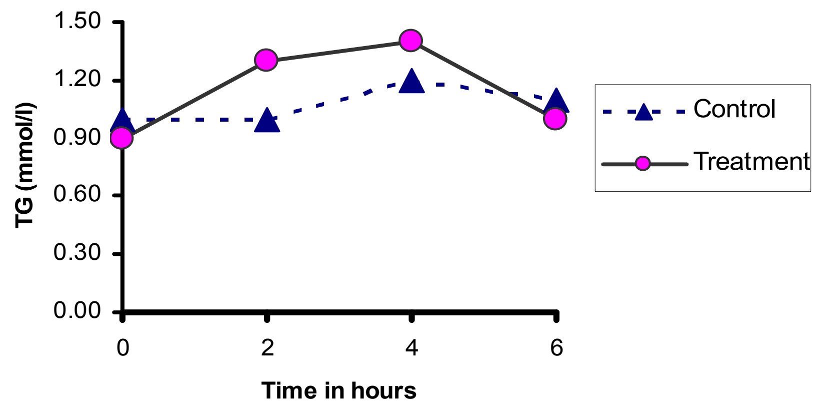


Figure 4.1: Serum triglycerides changes after the intake of the standardised fat meal (dashed line) and standardised fat meal with sucrose (continuous line)

Table 4.3: Postprandial changes in the lipid profile and inflammatory indicator in participants of Phase One

	Control group (n = 5)				Treatment group (n = 8)			
	0hr	2hr (%)	4hr (%)	6hr (%)	0hr	2hr (%)	4hr (%)	6hr (%)
T C	4.2 ± 0.80a	4.6 ± 0.27a	4.5 ± 0.58a	4.3 ± 0.61a	4.4 ± 0.94a	4.5 ± 1.10a	4.4 ± 0.91a	4.4 ± 0.73a
		(10%)	(7%)	(2%)		(2%)	(0%)	(0%)
HDL-C	1.1 ± 0.21a	1.2 ± 0.11a	1.3 ± 0.21a	1.2 ± 0.13a	1.1 ± 0.11a	1.2 ± 0.22a	1.2 ± 0.32a	1.3 ± 0.21a
		(9%)	(18%)	(9%)		(9%)	(9%)	(18%)
LDL-C	2.9 ± 0.56a	3.2 ± 0.31a	3.0 ± 0.50a	2.9 ± 1.06a	3.2 ± 0.86a	3.1 ± 0.89a	3.0 ± 0.76a	2.9 ± 0.55a
		(10%)	(3%)	(0%)		(-3%)	(-6%)	(-9%)
TG	1.0 ± 0.37a	1.0 ± 0.45a	1.2 ± 0.31b	1.1 ± 0.38a	0.9 ± 0.17a	1.3 ± 0.38b	1.4 ± 0.47b	1.0 ± 0.23a
		(0%)	(20%)	(10%)		(44%)	(56%)	(11%)
hs-CRP	0.3 ± 0.12a	0.3 ± 0.13a	0.6 ± 0.09b	0.5 ± 0.05b	0.4 ± 0.12a	0.3 ± 0.15b	0.5 ± 0.08c	0.5 ± 0.10c
		(0%)	(100%)	(67%)		(-25%)	(25%)	(25%)

Values in columns are means ± SD; means followed by the same letter do not differ significantly (P>0.05). If letters differ then P<0.05 when compared to the baseline value; values in parenthesis are percentage (%) changes compared to baselines; n = number of participants per group; time 0 hour is designated as baseline; TC = total cholesterol, HDL-C = high-density lipoprotein cholesterol, LDL = low-density lipoprotein cholesterol, TG = triglycerides are expressed in mmol/l and hs-CRP = high sensitive C-reactive protein expressed in mg/dl.

4.1.3.2 High sensitive C-reactive protein

Both groups (control and treatment) showed a significantly (P<0.05) higher level of hs-CRP at 4 hr (100% and 25%) and at 6 hr (67% and 25%), respectively, post ingestion time intervals when compared to their baseline values (Table 4.3 and Figure 4.2). The hs-CRP levels in the treatment group was significantly (P<0.05) lower at 2 hr (-25%) when compared to the baseline value. When comparing the two groups, the mean hs-CRP concentrations did not differ significantly (Table 4.3).

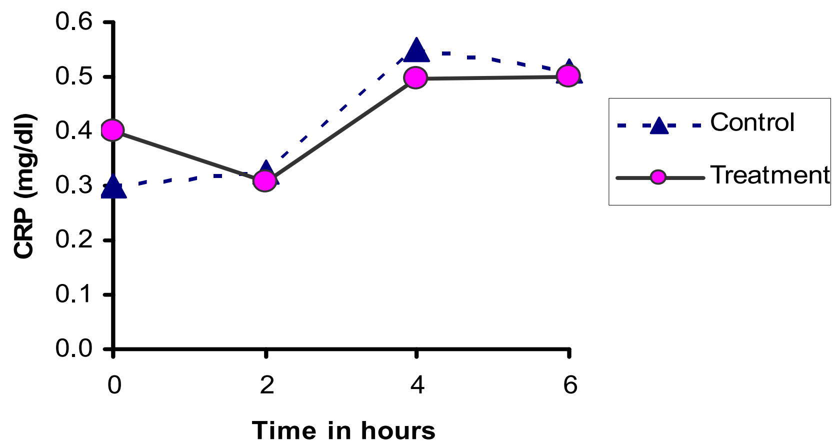


Figure 4.2: High sensitive C-reactive protein changes after the intake of the standardised fat meal (dashed line) and standardised fat meal with sucrose (continuous line)

4.1.4 Plasma total antioxidant capacity measurement

4.1.4.1 Plasma oxygen radical absorbance capacity levels

No significant differences were shown when comparing oxygen radical absorbance capacity (ORAC) values of the two groups (Table 4.4 and Figure 4.3). When comparing intra group variations, differences in the ORAC values (Table 4.4 and Figure 4.3) were significantly (P<0.05) lowered in both groups at 2 hr when compared with their baselines (-25% and -21%, respectively).

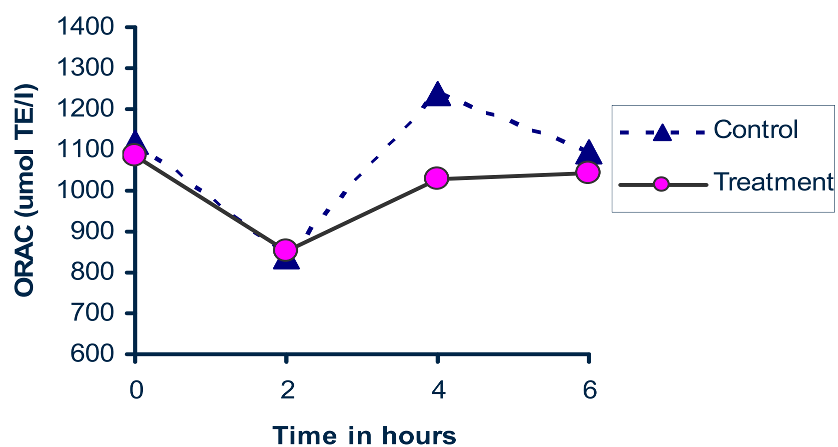


Figure 4.3: Plasma oxygen radical absorbance capacity changes after the intake of the standardised fat meal (dashed line) and standardised fat meal with sucrose (continuous line)

Table 4.4: Postprandial changes in plasma antioxidant capacity parameters in participants of Phase One

	Control group (n = 5)				Treatment group (n = 8)			
	0hr	**2hr (%)**	**4hr (%)**	**6hr (%)**	**0hr**	**2hr (%)**	**4hr (%)**	**6hr (%)**
ORAC	1119 ±138a	837 ± 195b	1238 ±147a	1093 ± 102a	1084 ± 132a	852 ± 104b	1031 ±144a	1043 ± 136a
		(-25%)	(11%)	(-2%)		(-21%)	(-5%)	(-4%)
FRAP	672 ± 55a	655 ± 171a	719 ± 29a	616 ± 127a	619 ± 88a	616 ± 101a	620 ± 34a	600 ± 51a
		(-3%)	(7%)	(-8%)		(-0.5%)	(0.2%)	(-3%)
TEAC	1171 ± 277a	702 ± 199b	685 ± 204b	599 ± 231b	956 ±189a	651 ± 134b	621 ± 163b	721 ±127b
		(-40%)	(-42%)	(-49%)		(-32%)	(-35%)	(-25%)

Values in columns are means ± SD; means followed by the same letter do not differ significantly ($P>0.05$). If letters differ then $P<0.05$ when compared to the baseline value; values in parenthesis are percentage (%) changes compared to baselines; n = number of participants per group; time 0 hour is designated as baseline; ORAC = oxygen radical absorbance capacity and TEAC = trolox equivalent antioxidant capacity are expressed in µmol TE/l and FRAP = ferric reducing antioxidant power, expressed in µM vitamin C/l.

4.1.4.2 Plasma ferric reducing antioxidant power levels

Both control and treatment groups showed an overall decrease in the antioxidant activity at 2 and 6 hr when compared to their respective baseline (0 hr) values, although not significant. When comparing inter group variations, no significant differences were found in the FRAP values (Table 4.4).

4.1.4.3 Plasma trolox equivalent antioxidant capacity levels

Comparing inter group differences, no significant differences were shown between groups (Table 4.4). However, both groups after the consumption of a standardised fat meal with/without the sucrose beverage showed significantly ($P<0.05$) lower levels at 2 hr (-40% and -32%); 4 hr (-42% and -35%) and 6 hr (-49% and -25%), respectively when compared with their baselines (Table 4.4 and Figure 4.4).

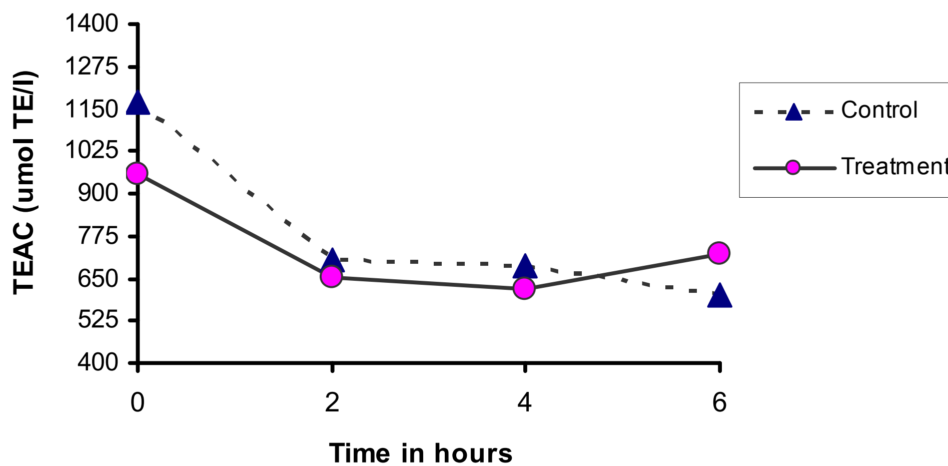


Figure 4.4: Plasma trolox equivalent antioxidant capacity changes after the intake of the standardised fat meal (dashed line) and standardised fat meal with sucrose (continuous line)

4.1.5 Oxidative stress indices and lipid oxidation status

The oxidative stress indices were determined by measuring the total glutathione, CD and TBARS levels in blood and plasma samples.

4.1.5.1 Total Glutathione levels

Total glutathione (tGSH), the primary endogenous antioxidant, was measured in the blood of all Phase One study participants. No significant differences were found in the total glutathione levels when comparing both groups (Table 4.5). When comparing intra group differences, study participants from both groups showed significantly ($P<0.05$) lower levels at 2 hr (-32% and -25%); 4 hr (-33% and -51%) and 6 hr (-46% and -65%), respectively, when compared to their baselines (0 hr) (Table 4.5 and Figure 4.5).

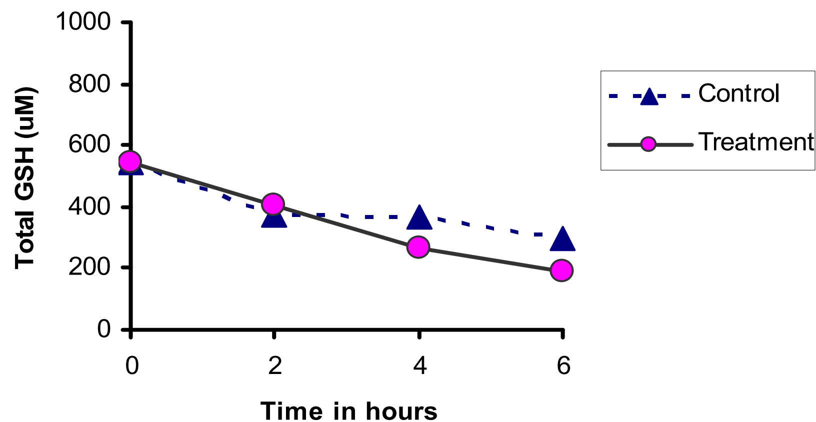


Figure 4.5: Total glutathione changes after the intake of the Standardised fat meal (dashed line) and standardised fat meal with sucrose (continuous line)

Table 4.5: Postprandial changes in biomarkers of oxidative stress in participants of Phase One

	Control group (n = 5)				Treatment group (n = 8)			
	0hr	**2hr (%)**	**4hr (%)**	**6hr (%)**	**0hr**	**2hr (%)**	**4hr (%)**	**6hr (%)**
tGSH	543 ± 225a	371 ± 274b	365 ± 145b	295 ± 146b	542 ± 192a	404 ± 173b	264 ± 79b	192 ± 88b
		(-32%)	(-33%)	(-46%)		(-25%)	(-51%)	(-65%)
CD	12 ± 2a	12 ± 1a	11 ± 1a	9 ± 1b	10 ± 2a	10 ± 1a	12 ± 1b	11 ± 2a
		(0%)	(-8%)	(-25%)		(0%)	(20%)	(10%)
TBARS	2.4 ± 1a	3.1 ± 1b	2.1 ± 1a	2.2 ± 0a	2.7 ±1a	3.4 ±1b	3.2 ± 1b	3.6 ± 1b
		(29%)	(-13%)	(-8%)		(26%)	(19%)	(33%)

Values in columns are means ± SD; means followed by the same letter do not differ significantly (P>0.05). If letters differ then P<0.05 when compared to the baseline value; values in parenthesis are percentage (%) changes compared to baselines; n = number of participants per group; time 0 hour is designated as baseline; total glutathione (tGSH) is expressed in μM, conjugated dienes (CD) is expressed in nmol CD/ml and thiobarbituric acid reactive substances (TBARS) is expressed in nmol MDA/l plasma.

4.1.5.2 Lipid oxidation status

4.1.5.2.1 Conjugated dienes

Conjugated diene levels in the treatment group (Table 4.5 and Figure 4.6) were significantly ($P<0.05$) higher at 4 hr (20%) when compared to the baseline (0 hr), while returning to the baseline level at 6 hr. While the levels in the control group did not show this significant increase, but were rather significantly ($P<0.05$) less (-25%) at 6 hr when compared to the baseline value. No significant differences were shown between the plasma CD levels in the treatment group when compared with the control group (Table 4.5).

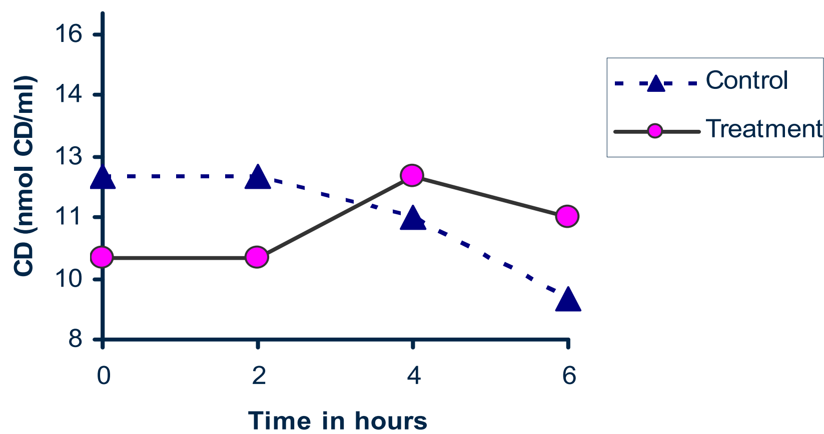


Figure 4.6: Conjugated dienes changes after the intake of the standardised fat meal (dashed line) and standardised fat meal with sucrose (continuous line)

4.1.5.2.2 Thiobarbituric acid reactive substances

The mean TBARS levels are shown in Table 4.5 with both groups showing significantly ($P<0.05$) higher levels at 2 hr time interval (29% and 26%, respectively) when compared to the baseline. The treatment group also show significantly higher levels at 4 hr and 6 hr (19% and 33%, respectively) time intervals. The TBARS levels of the control group at 6hr did not differ from that of the baseline, while the TBARS levels of the treatment group remained significantly ($P<0.05$) increased at 6 hr when compared to the baseline value. When comparing the two groups, no significant differences were shown.

4.2 Phase Two (Experimental study)

4.2.1 Anthropometry

The anthropometric measurements were taken as in the pilot study (Phase One). Table 4.6 below shows the anthropometric data for the study participants in Phase Two.

Table 4.6: Anthropometric characteristics of the Phase Two study group

	N (M/F)*	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Systolic BP (mmHg)	Diastolic BP (mm Hg)
Study group	14 (7/7)*	24.1 ± 4.4	62.8 ± 10	165 ± 10	22.5 ± 2.0	120.4 ± 9	79.6 ± 8.5

Data in columns are presented as mean ± SD, N = number of participants, BMI = body mass index, BP = blood pressure and * number of males (M) and females (F).

4.2.2 Antioxidant content and total antioxidant capacity measurement in rooibos

4.2.2.1 Phenolic content and soluble solid of the aqueous fermented rooibos beverage

The rooibos water extract used for measurement of the antioxidant content was prepared as described in section 3.3.1.1. Participants consuming the 500 ml of rooibos beverage consumed 271.25 mg total polyphenols, with the flavonols contributing 78.75 mg and the flavanols contributing 11.7 mg (Table 4.7). The soluble solid content of the fermented rooibos beverage was determined as 904 mg per serving of 500 ml.

Table 4.7: Polyphenolic quantification and soluble solid content of the rooibos beverage

	Phenolic content of rooibos beverage	
	mg/l	mg/serving (500 ml)
Total polyphenols	542.5 ± 6.3	271.25
Flavonols	157.5 ± 11.7	78.75
Flavanols	23.3 ± 7.2	11.7
Soluble solids	1808 ± 96.5	904

Data in columns are presented as mean ± SD or only means.

4.2.2.2 Total antioxidant capacity of the fermented rooibos beverage

The rooibos water extract used for measurement of the antioxidant capacity was also prepared as described in section 3.3.1.1. The ORAC assay showed activity levels of 10393 ± 501 µmol TE/l and 5196.5 ± 250.5 per serving of 500 ml, with the FRAP assay showing values of 1489 ± 188 µmol vitamin C equivalents/L and 744.5 ± 94 when expressed per serving size of 500 ml. The TEAC activity was determined as 2189 ± 472 µmol TE/l and 1094.5 ± 236 when expressed per serving of 500ml.

4.2.3 Glucose and insulin assay

4.2.3.1 Plasma glucose

The plasma glucose levels of the group that consumed the standardised fat meal with sucrose beverage (control group) and those who consumed the standardised fat meal and rooibos beverage (treatment group) are shown in Table 4.8. When comparing the control and treatment groups, significantly ($P<0.05$) lower glucose levels were observed in the treatment group 6 hr post ingestion. When comparing intra group variations, significantly ($P<0.05$) lower plasma glucose levels were observed in the treatment group at 2 hr and 6 hr (-22% and -18%, respectively) post ingestion time intervals when compared to the baseline (0 hr) value. The glucose levels in the control group were also significantly ($P<0.05$) lowered at the 2 hr (-16%) time interval while returning to baseline level after 4 hr and 6 hr (Table 4.8 and Figure 4.7).

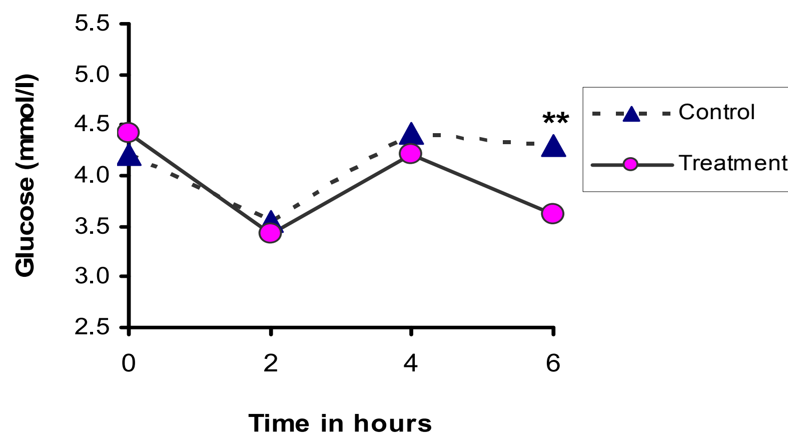


Figure 4.7: Plasma glucose changes after the intake of the standardised fat meal with sucrose (dashed line) and the standardised fat meal with rooibos (continuous line) **= P<0.001 when comparing the two groups

4.2.3.2 Serum insulin

When comparing the control and treatment groups, a significantly ($P<0.05$) higher insulin level was seen at 4 hr post ingestion time in the control group. Serum insulin levels in the control group at 2, 4 and 6 hr post ingestine times did not differ significantly when compared to the baseline (0 hr), but in the treatment group serum insulin levels were significantly higher ($P<0.05$) after 2 hr when compared to 0 hr (Table 4.8 and Figure 4.8).

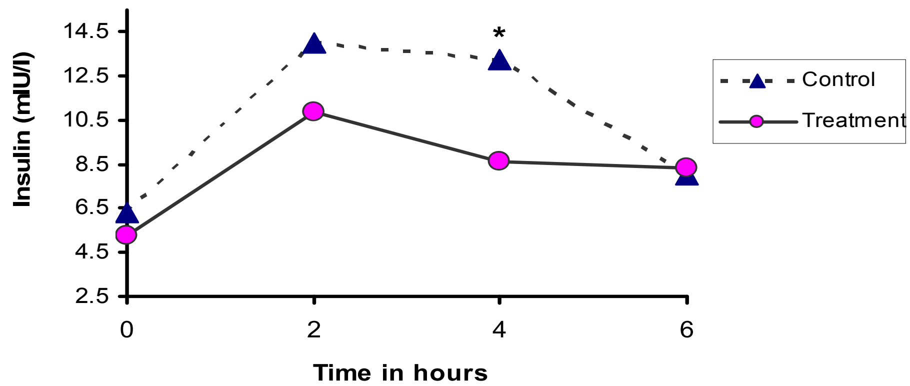


Figure 4.8: Insulin changes after the intake of the standardised fat meal with sucrose (dashed line) and the standardised fat meal with rooibos (continuous line), *= P<0.05 when comparing the two groups

Table 4.8: Postprandial changes in blood glucose and insulin levels in participants of Phase Two

	Control group (n = 14)				Treatment group (n = 14)			
	0hr	**2hr (%)**	**4hr (%)**	**6hr (%)**	**0hr**	**2hr (%)**	**4hr (%)**	**6hr (%)**
Glucose	4.2 ± 0.56a	3.6 ± 0.52b	4.4 ± 0.42a	4.3 ± 0.41a**	4.4 ± 0.36a	3.4 ± 0.76b	4.2 ± 0.74a	3.6 ± 0.67b**
		(-16%)	(5%)	(2%)		(-22%)	(-5%)	(-18%)
Insulin	6.2 ± 3.74a	14.0 ± 10.20a	13.3 ± 7.31a*	8.0 ± 4.56a	5.3 ±1.86a	10.8 ± 3.35b	8.6 ± 5.39a*	8.3 ± 5.51a
		(120%)	(109%)	(26%)		(105%)	(63%)	(58%)

Values in columns are means ± SD, means followed by the same letter do not differ significantly (P>0.05), if letters differ then P<0.05 when compared to the baseline value; values in parenthesis are percentage (%) changes compared to baseline (0 hr); n = number of participant per group; glucose are expressed in mmol/l and insulin expressed in mIU/l. The underlined values represent significant (* = P<0.05; ** = P<0.001) differences when comparing the control group with the treatment group.

4.2.4 Lipid profile and C-reactive protein analysis

4.2.4.1 Lipid profile

The postprandial characteristics of the serum TC, HDL-C, LDL-C and TG levels of both groups are shown in Table 4.9.

4.2.4.1.1 Total cholesterol

When comparing the control group with the treatment group, the serum total cholesterol levels were significantly (P<0.0001; P<0.001; P<0.0001, respectively) lowered in the treatment group at 2 hr, 4 hr and 6 hr post ingestion time intervals as shown in Table 4.9, but not in the control group. On intra group level, the total cholesterol levels in the treatment group were significantly (P<0.05) lower at 6 hr (-28%) when compared to baseline, while no significant differences were shown in the control group (Figure 4.9).

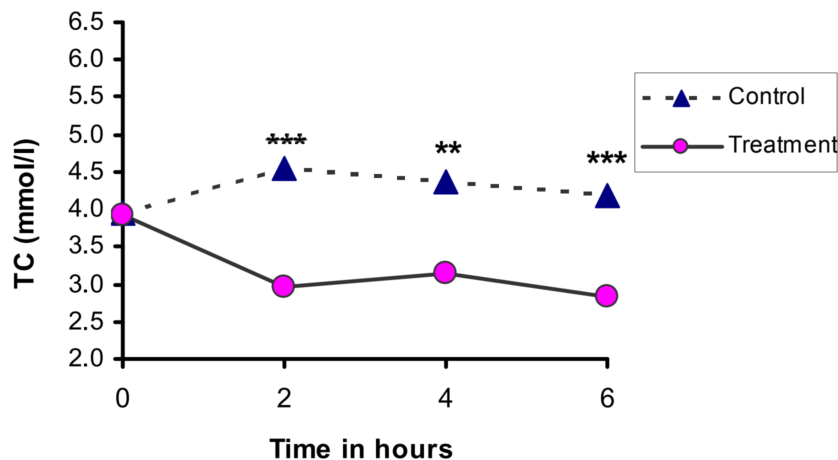


Figure 4.9: Serum total cholesterol changes after the intake of the standardised fat meal with sucrose (dashed line) and the standardised fat meal with rooibos beverage (continuous line), **= P<0.001; ***= P<0.0001 when comparing the two groups

Table 4.9: Postprandial changes in the lipid profile and inflammatory indicator in participants of Phase Two

	Control group (n = 14)				Treatment group (n = 14)			
	0hr	**2hr (%)**	**4hr (%)**	**6hr (%)**	**0hr**	**2hr (%)**	**4hr (%)**	**6hr (%)**
T C	4.0 ± 0.94a	4.5 ± 0.79a***	4.4 ± 0.94a**	4.2 ± 0.77a***	3.9 ± 1.07a	3.0 ± 0.84a***	3.2 ± 1.19a**	2.8 ± 0.66b***
		(15%)	(10%)	(6%)		(-25%)	(-20%)	(-28%)
HDL-C	1.09 ± 0.18a*	1.12 ± 0.14a***	1.11 ± 0.17a***	1.08 ± 0.17a***	0.92 ± 0.19a*	0.83 ± 0.15a***	0.87 ± 0.19a***	0.86 ± 0.17a***
		(4%)	(2%)	(-0.3%)		(-10%)	(-5%)	(-7%)
LDL-C	2.8 ± 0.87a	3.3 ± 0.75a***	3.1 ± 0.88a*	3.0 ± 0.76a***	2.9 ± 0.93a	2.1 ± 0.71a***	2.2 ± 1.14b*	1.9 ± 0.54b***
		(18%)	(11%)	(7%)		(-28%)	(-24%)	(-34%)
TG	0.5 ± 0.15a	0.7 ± 0.26a	1.0 ± 0.32b*	0.9 ± 0.24b**	0.5 ± 0.28a	0.6 ± 0.32a	0.8 ± 0.39a*	0.5 ± 0.21a**
		(36%)	(97%)	(78%)		(24%)	(52%)	(6%)
hs-CRP	0.30 ± 0.04a	0.37 ± 0.06a	0.34 ± 0.05a	0.28 ± 0.03a*	0.20 ± 0.06a	0.18 ± 0.04a	0.12 ± 0.03a	0.07 ± 0.02a*
		(22%)	(13%)	(8%)		(-13%)	(-42%)	(-64%)

Values in columns are means ± SD; means followed by the same letter do not differ significantly (P > 0.05), if letters differ then P < 0.05 when compared to the baseline value; values in parenthesis are percentage (%) changes compared to baselines (0 hr); n = number of participants per group; TC= total cholesterol, HDL-C= high-density lipoprotein cholesterol, LDL-C= low-density lipoprotein cholesterol, TG= triglycerides are all expressed in mmol/l and hs-CRP= high sensitive C-reactive protein expressed in mg/dl. The underlined values represent significant (*= P<0.05, **= P<0.001, ***= P<0.0001) differences when comparing the control group with the treatment group.

4.2.4.1.2 High-density lipoprotein cholesterol

When comparing inter group differences, the treatment group showed a significantly (P<0.05; P<0.0001) lower HDL-C level at baseline (0hr), 2 hr, 4 hr and 6hr post ingestion time intervals when compared to the control group (Table 4.9). When considering these inter group variations it is important to note that the baseline values (0 hr) of serum HDL-C in the control group differed significantly (P<0.05) from that of the treatment group and could account for the decrease shown at all other time intervals when comparing inter group differences, which cannot be seen as a true result. The intra group variations showed no significant (P>0.05) differences in both groups, when compared to the baseline values.

4.2.4.1.3 Low-density lipoprotein cholesterol

The low-density lipoprotein cholesterol (LDL-C) levels were significantly (P<0.05; P<0.0001) lowered in the treatment group at all postprandial intervals when compared with that of the control group (Table 4.9; Figure 4.11). When comparing the intra group variations, significantly (P<0.05) lower levels (-24% at 4 hr and -34% at 6 hr, respectively) were also evident in the treatment group but not in the control group.

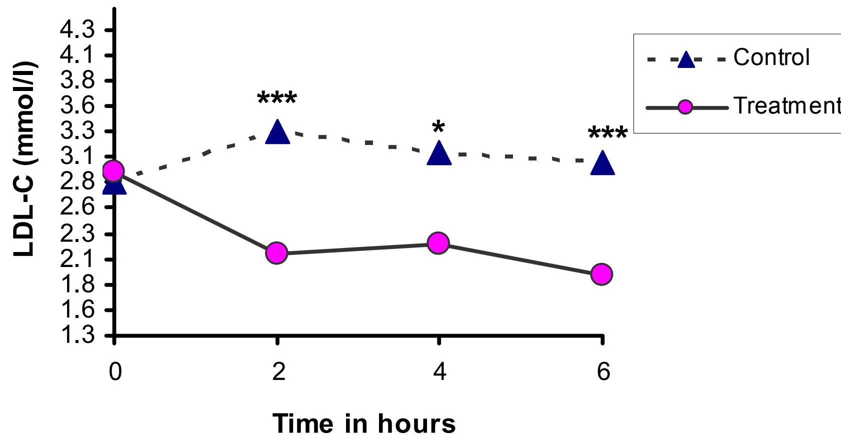


Figure 4.10: Low-density lipoprotein cholesterol changes after the intake of the standardised fat meal with sucrose (dashed line) and the standardised fat meal with rooibos (continuous line), *= P<0.05; ***= P<0.0001 when comparing the two groups

4.2.4.1.4 Triglycerides

The triglyceride (TG) levels were significantly (P<0.05, P<0.001) lower in the treatment group at 4 hr and 6 hr post ingestion time interval, when compared to that of the control group (Table 4.9; Figure 4.11). When comparing the intra group variations, consumption of the standardised fat meal with the sucrose beverage caused a significant (P<0.05) higher level of TGs in the control group at 4 and 6 hr when compared to the baseline value, while no significant differences were shown in the treatment group.

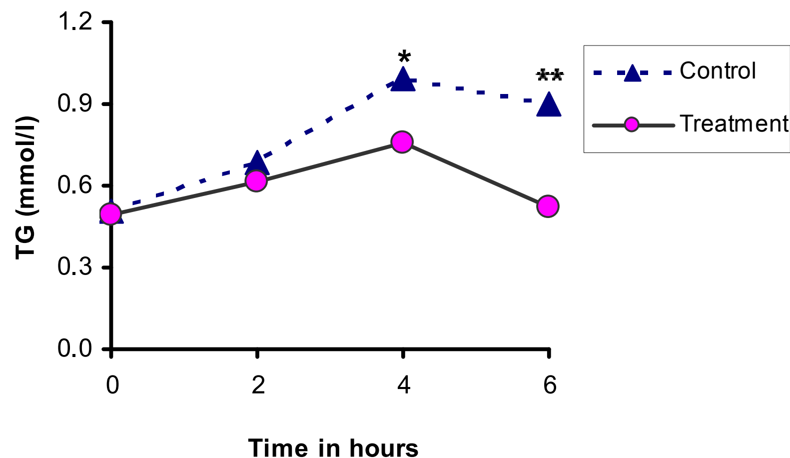


Figure 4.11: Triglycerides changes after the intake of the standardised fat meal with sucrose(dashed line) and the standardised fat meal with rooibos (continuous line), *= P<0.05; **= P<0.001 when comparing the two groups

4.2.4.2 High sensitive C-reactive protein

The levels of high sensitive C-reactive protein (hs-CRP) of the two groups consuming the standardised fat meal either with the sucrose or rooibos beverages are shown in Table 4.9. When comparing the control group with the treatment group, hs-CRP levels were significantly (P<0.05) lower in the treatment group at 6 hr post ingestion (Figure 4.12). The intra group differences showed no significant variations in either of the two groups at the time intervals when compared with their respective baseline values.

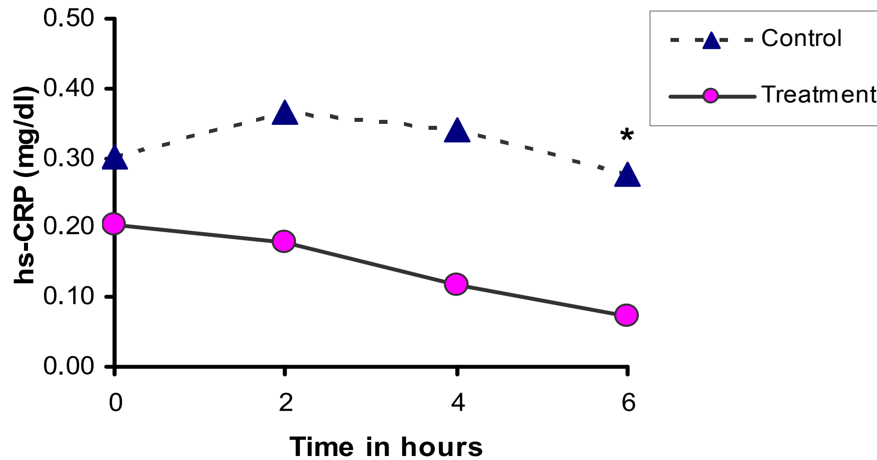


Figure 4.12: High sensitive C-reactive protein changes in participants after consumption of the standardised fat meal with sucrose (dashed line) and the standardised fat meal with rooibos (continuous line); *= P<0.05 when comparing the treatment group with the control group

4.2.5 Plasma total antioxidant capacity measurement

As mentioned in the pilot study, three methods were used to determine the total antioxidant capacity in the plasma samples of Phase Two study participants.

4.2.5.1 Plasma oxygen radical absorbance capacity levels

When comparing both intra and inter group differences, no significant differences in the levels of oxygen radical absorbance capacity (ORAC) were shown at all post ingestion time intervals in both groups (Table 4.10 and Figure 4.13).

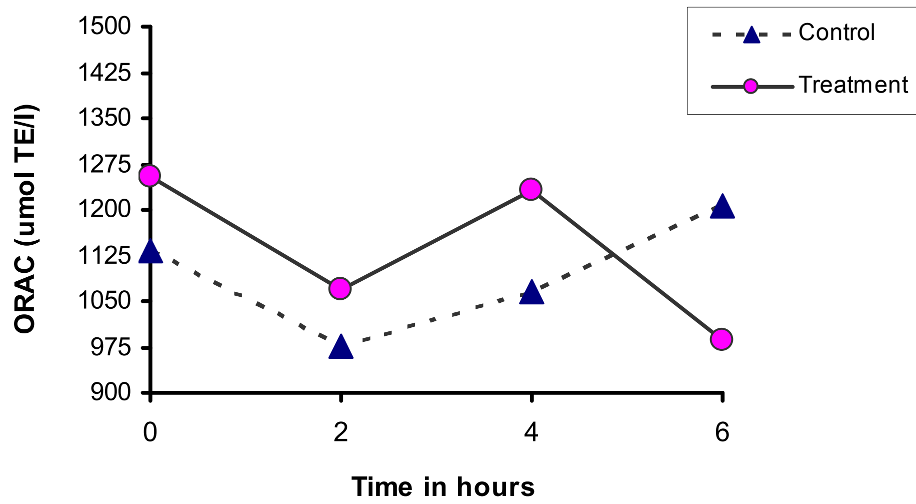


Figure 4.13: Plasma oxygen radical absorbance capacity changes after the intake of the standardised fat meal with sucrose (dashed line) and the standardised fat meal with rooibos (continuous line) when comparing control and treatment groups

Table 4.10: Postprandial changes in plasma antioxidant capacity parameters in participants of Phase Two

	Control group (n = 14)				Treatment group (n = 14)			
	0hr	2hr (%)	4hr (%)	6hr (%)	0hr	2hr (%)	4hr (%)	6hr (%)
ORAC	1132 ± 245a	976 ± 303a	1065 ± 251a	1205 ± 246a	1256 ± 329a	1070 ± 213a	1232 ± 226a	987 ± 209a
		(-14%)	(-6%)	(6%)		(-15%)	(-2%)	(-21%)
FRAP	198 ± 29a*	238 ± 33a**	248 ± 50b	255 ± 43b*	151 ± 34a*	176 ± 28a**	320 ± 83b	207 ± 45b*
		(20%)	(25%)	(29%)		(17%)	(113%)	(38%)
TEAC	388 ± 98a	388 ± 114a	391 ± 263a*	448 ± 145a	455 ± 125a	455 ± 145a	775 ± 127b*	370 ±139a
		(0%)	(1%)	(15%)		(0%)	(70%)	(-19%)

Values in columns are means ± SD; means followed by the same letter do not differ significantly (P>0.05), if letters differ then P<0.05 when compared to the baseline value; values in parenthesis are percentage (%) changes compared to baselines (0 hr); n = number of participants per group; ORAC= oxygen radical absorbance capacity and TEAC= trolox equivalent antioxidant capacity, are expressed in µmol TE/l and FRAP= ferric reducing antioxidant power, expressed in µM vitamin C/l; the underlined values represent significant (*= P<0.05 and **= P<0.001) differences when comparing the control group with the treatment group.

4.2.5.2 Plasma ferric reducing antioxidant power levels

When considering the inter group variations it is important to note that the baseline values (0 hr) of serum FRAP levels in the control group differed significantly (P<0.05) from that of the treatment group and could account for the decreases shown at 2 hr and 6 hr time intervals, which cannot be seen as a true result (Table 4.10). When comparing intra group variations, the FRAP values in the control group were significantly (P<0.05) higher at 4 hr and 6 hr (25% and 29%, respectively) when compared to the baseline value, while in the treatment group they were significantly (P<0.05) higher at 4 hr and 6 hr (113% and 38%) post ingestion time intervals when compared to the baseline value (Table 4.10 and Figure 4.14).

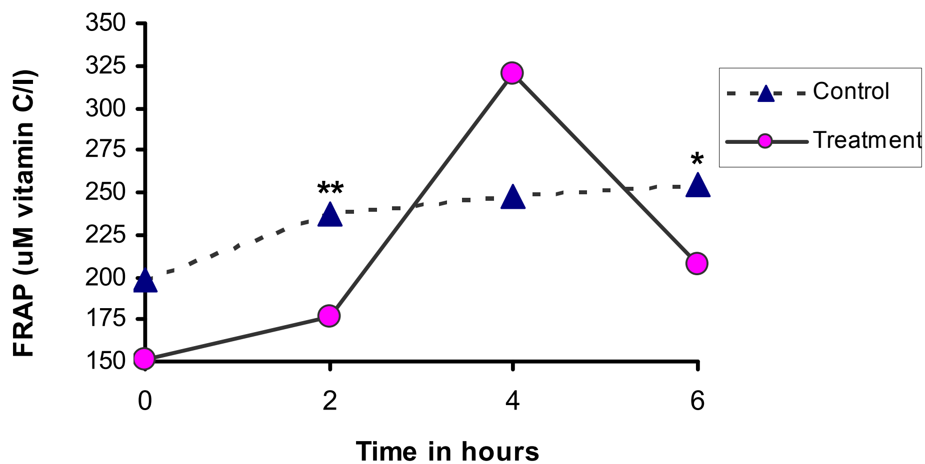


Figure 4.14: Plasma ferric reducing antioxidant power changes in participants after consuming the standardised fat meal with sucrose (dashed line) or the standardised fat meal with rooibos (continuous line), *= P<0.05, **= P<0.001

4.2.5.3 Plasma trolox equivalent antioxidant capacity levels

The treatment group showed a significantly (P<0.05) higher level of plasma TEAC capacity at 4 hr post ingestion when compared to the control group (Figure 4.15). When comparing the intra group variations, plasma TEAC activity were observed to be significantly (P<0.05) higher in the treatment group at 4 hr (70%) when compared to the baseline (0 hr) value (Table 4.10). While the control group did not show any, significant differences at all post ingestion time intervals, when compared to baseline value.

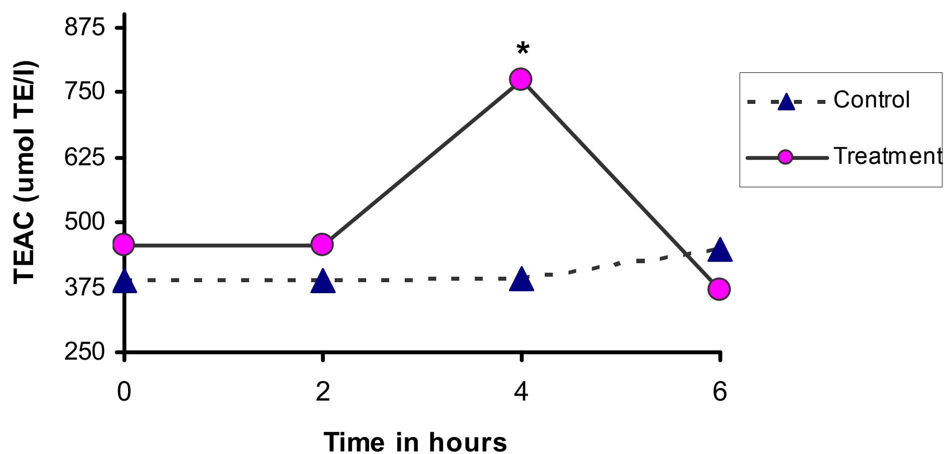


Figure 4.15: Plasma trolox equivalent antioxidant capacity changes after consumption of the standardised fat meal with sucrose (dashed line) and the standardised fat meal with rooibos (continuous line)
*= P<0.05 when comparing the two groups

4.2.6 Total glutathione level and lipid oxidation status

The total glutathione levels were determined in whole blood samples, while the lipid oxidation status was evaluated by measuring the CD and TBARS levels in plasma samples.

4.2.6.1 Total glutathione

When comparing inter group variations, a significantly (P<0.05) higher level of tGSH was observed in the treatment group at 2 hr, 4 hr and 6 hr intervals when compared to the control group at the same time intervals. However, the intra group variations were not significantly (P>0.05) different in both groups (Table 4.11).

Table 4.11: Postprandial changes in biomarkers of oxidative stress in participants of Phase Two

	Control group (n = 14)				Treatment group (n = 14)			
	0hr	**2hr (%)**	**4hr (%)**	**6hr (%)**	**0hr**	**2hr (%)**	**4hr (%)**	**6hr (%)**
tGSH	847 ± 113a	745 ± 134a*	800 ± 93a	835 ± 93a	1015 ± 192a	959 ± 413a*	1005 ± 232a	1052 ± 249a
		(-12%)	(-5%)	(-1%)		(-5%)	(-1%)	(4%)
CD	14 ± 2a	16 ± 1a**	16 ± 3b***	14 ± 2a	13 ± 2a	13 ± 2a**	12 ± 2a***	13 ± 2a
		(14%)	(14%)	(0%)		(0%)	(-8%)	(0%)
TBARS	2.2 ± 1a	2.8 ± 1a	3.5 ± 1a**	2.7 ± 1a	2.4 ±1a	1.8 ±1a	1.5 ± 0a**	2.1 ± 1a
		(27%)	(59%)	(51%)		(-23%)	(-35%)	(-11%)

Values in columns are means ± SD; n = number of participant per group; means followed by the same letter do not differ significantly (P>0.05), if letters differ then P<0.05 when compared to the baseline value; values in parenthesis are percentage (%) changes compared to baselines (0 hr); tGSH = total glutathione expressed in μM; CDs = conjugated dienes expressed as nmol CD/ml and TBARS = thiobarbituric acid reactive substances expressed in nmol MDA/l plasma. The underlined values represent significant (*= P<0.05, **= P<0.001 and ***= P<0.0001) differences when comparing the control group with the treatment group.

4.2.6.2 Conjugated dienes

The conjugated dienes (CDs) level in the treatment group was significantly ($P<0.001$; $P<0.0001$) lower at 2 hr and 4 hr post ingestion time when compared to the control group (Table 4.11). When comparing the intra group CD variations, CD levels in the control group at 4 hr interval were significantly ($P<0.05$) higher when compared with the baseline value, while no significant differences were shown in the treatment group at all the post ingestion time intervals when compared with the baseline (Figure 4.16).

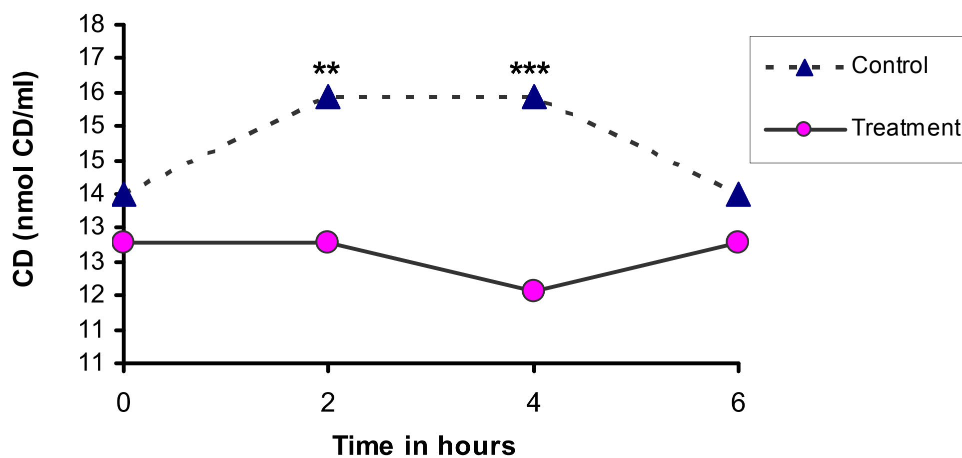


Figure 4.16: Plasma conjugated dienes changes after the intake of the Standardised fat meal with sucrose (dashed line) and the standardised fat meal with rooibos (continuous line), **= P<0.001; ***= P<0.0001 when comparing the treatment and the control groups

4.2.6.3 Thiobarbituric acid reactive substances

The postprandial thiobarbituric acid reactive substances (TBARS) levels in the treatment group as seen in Table 4.11 and Figure 4.17 was significantly ($P<0.001$) lower at 4 hr post ingestion time interval when compared to that of the control group. When comparing intra group variations, no significant differences were shown for either of the treatment group or control groups at all time intervals when compared with the respective baseline values.

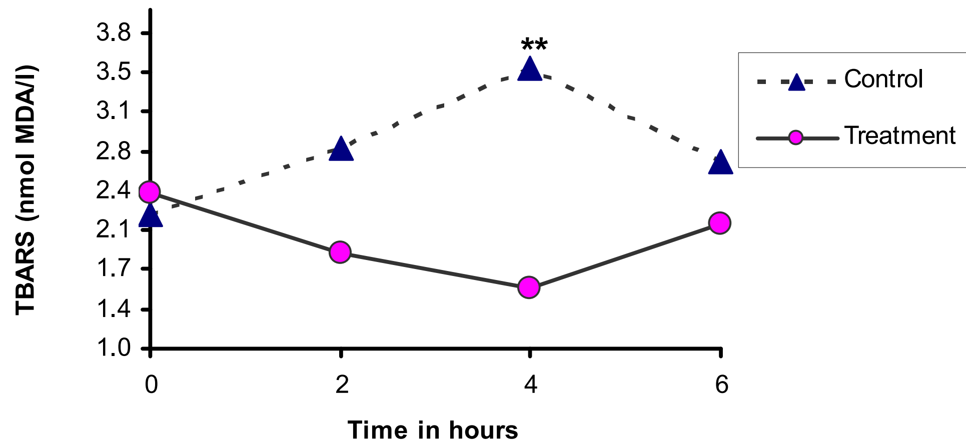


Figure 4.17: Plasma thiobarbituric acid reactive substances changes after the intake of the standardised fat meal with sucrose (dashed line) and the standardised fat meal with rooibos (continuous line), **= P<0.001 when comparing the treatment group with the control group

CHAPTER FIVE: DISCUSSION AND CONCLUSION

The pilot study was designed to assess the effect of sucrose ingestion on postprandial oxidative stress after consumption with a standardised fat meal. This scenario was specifically chosen as one of many that mimics a Western style, fast food meal. In order to assess the combined effects of a standardised fat meal consumed with a commercial sucrose beverage (carbonated soft drink), the study measured various blood biochemical markers. The pilot study consisted of a small number of normolipidaemic subjects randomly divided into two groups: a control group (n = 5) consuming the standardised fat meal and a treatment group (n = 8) consuming the standardised fat meal with a commercial sucrose-containing soft drink to assess the effect on postprandial oxidative stress.

When considering the effect on blood glucose and insulin levels, it is important to note that the baseline values of glucose in both participant groups in the pilot study were not similar, thus rendering the absolute values difficult to compare. In this instance, the percentage change was used to compare the two groups and no significant differences were shown in blood glucose and insulin levels when comparing the two groups. The blood glucose levels were significantly higher in the control group at 4 hr and 6 hr postprandially when compared to the baseline (0 hr), but not in the treatment group who consumed the commercial sucrose beverage. This may in part be related to the first time point chosen for postprandial sampling, and could represent the terminal phase of insulin action in the case of the treatment group (Cozma *et al.,* 2002:1274). In addition, the no significant difference found in insulin level at all post postprandial could also be influenced by the high inter-subjects group variance (Grant *et al.,* 1994:857). Thirty and sixty minutes after a meal had been suggested in previous studies to detect elevated blood glucose levels after a meal containing sucrose (1.5 g/kg body weight) and fat (1 g lipid/kg body weight) (Grant *et al.,* 1994:856). Plasma glucose levels increasing after a meal may be due to both the ingestion of glucose molecules as well as other macromolecules (Gavin III, 2001:4H). In addition, glucose affects the trans epithelial transport of nutrients permeating the cell barrier by paracellular transcellular passive diffusion and facilitated transport (D'Souza *et al.,* 2003:6). The mechanism involved in this glucose-induced regulation of uptake process is not yet fully identified. An alteration of the physical properties of the enterocyte brush border, which in turn regulates the activity of membrane transporters, could be involved but other regulation pathways should also be considered (Lairon *et al*., 2007:221).

During an increase of plasma glucose, the presence of an intracellular imbalance of NADH/NAD$^+$ ratio and the labile non-enzymatic glycation, processes that may occur very rapidly, may provoke oxidative stress. It has been shown that both of these phenomena are accompanied by the production of free radicals (Ceriello *et al*., 1999:1506). Postprandial hyperinsulinaemia has been shown to cause a late postprandial accumulation of intestinally derived apoB48-containing chylomicrons in healthy humans (Harbis *et al.,* 2001:467). There is an indirect regulation of intestinal lipid uptake by dietary glucose (Lairon *et al.,* 2007:220). This hypothesis has recently been confirmed by several *in vivo* and *in vitro* studies (Lairon *et al*., 2007:221).

The serum total cholesterol, HDL-C, LDL-C and triglycerides levels were not significantly different for all the post time intervals when comparing both groups. However, significantly higher levels of triglycerides were shown in both groups when compared to their baseline levels. Plasma triglycerides in the post absorptive state are partly derived from absorbed lipids and partly from glucose-induced hepatic synthesis (Ceriello *et al*., 1999:1507). Results from the pilot study indicates that, when sucrose was consumed with the standardised fat meal, significantly increased triglyceride levels were already evident at 2 hr post ingestion time, when compared with elevated levels only at 4 hr when no sucrose was consumed with the standardised fat meal. The postprandial clearance of serum triglycerides is influenced, indicating that sucrose amplifies the postprandial triglyceridaemia by significantly increasing the triglycerides levels in the serum; which could be mediated by a decreased triglyceride clearance after the ingestion of fat rich meal consumed with sucrose (Grant *et al.,* 1994:859). Postprandial lipidaemia is a useful tool to evaluate the role of triglycerides in coronary artery disease as a more informative means of characterising triglycerides metabolism. In multivariate analyses, hypertriglycerides are independent predictors of coronary artery disease (Patsch *et al.,* 1992:1336; Lefebvre & Scheen, 1998:S63).

In this pilot study, both test meals also showed higher in hs-CRP levels at 4 and 6 hr post ingestion interval times. Inflammation has been shown to play a key role in the development of atherosclerosis and circulating markers, such as C - reactive protein, correlate with the tendency to develop ischemic events (Libby *et al.,* 2002:1135). Therefore reducing the incidence of this inflammatory disease with diet may be possible.

When considering the results of postprandial antioxidant capacity, the standardised fat meal consumed with/without sucrose caused significantly lower blood ORAC levels after 2 hr time interval, with no significant differences between the two groups. The standardised fat meals consumed with/without sucrose did not affect the FRAP activities, with TEAC activities

significantly decreased at all time intervals postprandially. In the case of total antioxidant capacity measurements, it is important to note that different antioxidants respond differently in different measurement methods. For example, uric acid in serum contributes 19% to TEAC activity but only 7% to ORAC activity, whereas α-tocopherol, ascorbic acid, and bilirubin contribute more to TEAC activity than to the ORAC activity (Cao & Prior, 1998:1314). The ORAC methodology is arguably the most accepted and accurate indicator of antioxidant status, mainly because it is based on measurements of fluorescence rather than absorbance (Cao & Prior, 1998:1315). No significant changes in the total glutathione levels of participants when comparing the two groups were shown, but the total glutathione levels were significantly lower in both groups when compared to their respective baseline values. Glutathione, an endogenous antioxidant, contributes in the modulation of oxidative stress. In this pilot study, the observed decrease in tGSH was concurrent with the decreased activity of ORAC at 2 hr and TEAC at 2 hr, 4 hr and 6 hr. In the case of the control group, this could be due to the increase found in blood glucose levels at the same time points, whereas with the treatment group a concurrent increase in triglyceride levels with decreasing antioxidant activity and tGSH levels were shown.

In terms of oxidative lipid damage, consumption of the standardised fat meal with/without the sucrose beverage did not show any changes in CDs and TBARS when comparing the two groups. However, when comparing time intervals of both groups with their respective baseline values, participants consuming the standardised fat meal with sucrose exhibited higher lipid oxidation levels as shown by the significantly increased levels of CDs after 4 hr and TBARS after 2 hr, 4 hr and 6 hr when compared with the baseline level. The level of CDs in the control group was not significantly higher, but rather lower at 6 hr, while the level of TBARS increased only at 2 hr postprandially when compared to the baseline. Thus, results from this pilot study suggested that the intake of the sucrose-containing commercial soda with the standardised fat meal resulted in an elevated level of lipid oxidation in the blood, as shown by the higher levels of CDs and increased time period of TBARS levels. Together with the increased levels of triglycerides, these results are suggestive of an increased postprandial oxidative stress in the participants. These findings contributed to the decision to increase the sucrose intake (59.5g in the pilot study) to 69g and continue with the second phase of the study to determine the possible modulation of postprandial oxidative stress with a dietary antioxidant, such as rooibos. It is hypothesized that the postprandial reduction of antioxidant defences may significantly contribute to the increased level of lipid oxidation. Thus, the endogenous antioxidant mechanisms failed to protect or prevent the dietary induced-oxidative stress. This could be due to decreased activities of scavenging enzymes or

overproduction of free radicals or both, leading to lipid oxidation (Shirpoor *et al*., 2008:119). With regards to postprandial oxidative stress, Ursini *et al*., (1998:251) previously demonstrated that at 2 hr postprandial, a typical English/American breakfast with the nutrient composition as being 11% protein, 34% carbohydrate and 55% fat, comprising approximately 1200 kcal (5021 kj), in nine healthy males subjects aged 30 - 60 years, resulted in significant (123%) postprandial increases in plasma biomarkers of oxidative stress such as lipid hydroperoxides.

During the second phase of this study, 14 participants consumed the standardised fat meal with a commercial sucrose beverage mimicking a fast food meal (control group) and two weeks later were crossed over to consume the rooibos beverage with the standardised fat meal (the treatment group). Dietary patterns high in saturated fats, trans-fatty acids, refined sugar and lacking sufficient antioxidants and omega-3 fatty acids, may cause an activation of metabolic diseases and the innate immune system via an excessive production of pro-inflammatory cytokines associated with a reduced production of anti-inflammatory cytokines (Giugliano *et al.,* 2006:677). There are many dietary strategies associated with a decreased cardiovascular risk and generation of inflammation. One such a strategy was used in the second phase of our investigation. The fermented/traditional rooibos beverage for this study was quantified in terms of antioxidant content as well as activity and was found to be in line with previously published results (Von Gadow *et al.,* 1997b:75; Marnewick *et al.,* 2000:160).

When considering the blood glucose levels of the study participants in Phase Two, both groups showed significantly lowered levels (2 hr for the control group; 2, and 6 hr for the treatment group) when compared to their respective baseline values. When comparing the two groups/occasions consumption of the rooibos beverage with the standardised fat meal caused significantly lower levels of blood glucose at 6 hr postprandially. This effect was not shown for the control group. Previously, it has been reported that hyperglycaemia induces overproduction of superoxide by the mitochondrial electron transport chain, which accounts for the molecular mechanisms implicated in glucose-mediated vascular damage (Brownlee, 2001:813). The oxidation of glucose leads to an increase production of free radicals and other reactive oxygen species, which are believed to cause, together with glycation, a wide magnitude of diabetic complications and also cardiovascular diseases (Bucala & Cerami, 1992:1; Bayraktutan, 2002:2). Postprandial plasma glucose level is an important determinant of this onset (Schchiri *et al.,* 2000:B21). Participants that consumed the study diet with the rooibos beverage, also showed significantly higher insulin levels at 2 hr time interval when compared to the baseline value, while no differences were detected when the standardised fat meal and commercial soda was consumed. However, when comparing both groups, participants consuming the

study diet with the commercial soda, showed significantly higher insulin levels at 4 hr. Research showed that the ingestion of sucrose causes a significant increase in insulin and blood glucose, however during rest, glucose is oxidised immediately when entering the cell (Benadé *et al.,* 1973:207; Daly, 2003:865S). It is therefore, inappropriate to speculate that elevated insulin levels predict CVD as this relationship is controversial, mainly because it is often not clear exactly what is being measured by insulin assays (Lautt, 2007:763). For e.g when using a specific assay for proinsulin and insulin, research has found that proinsulin levels rather than insulin levels were a better predictor of development of coronary heart disease in a study of older Swedish males (Zethelius *et al.,* 2005:862). Also, literature supports proinsulin levels as a better marker than insulin, but the slow metabolic degradation of proinsulin may lead to greater fasting levels of proinsulin and thus reflect postprandial hyperinsulinaemia (Kronborg *et al.,* 2007:1607; Lautt, 2007:763). It is important to note that, the increase found in insulin in this study, was not only caused by these phenomena (blood glucose and proinsulin) but could also be due to other influencing factors such as amino acids.

When comparing the lipid profile and inflammatory biomarker (hs-CRP) of the two groups, study participants consuming rooibos with the standardised fat meal showed significantly lower levels of total cholesterol, HDL-C and LDL-C at 2 hr, 4 hr and 6 hr and TGs levels at 4 hr and 6 hr, while hs-CRP level at 6 hr was also significantly lowered in this group. It is important to notice that the HDL-C baseline levels (0 hr) of the two groups differed significantly from each other, thus rendering the values difficult to compare, and could account for the decrease in HDL-C levels shown. It can also be speculated that this significant lowering of the HDL-C levels is probably not a direct effect of rooibos but could be explained by the lipid structural perturbation as observed in total cholesterol and TGs (Figure 4.9 and Figure 4.11). After a high fat meal the apolipoproteins B48-containing chylomicron remnants accumulate and increase, therefore reported to be correlated with an elevation in triglycerides (Mamo *et al.,* 1997:288; Smith *et al.,* 1999:204). However, a recently completed 6 weeks rooibos intervention study did not show any decrease in HDL-C levels of the participants at risk for developing CVD (Macharia, 2009:57). This study showed that consuming 6 cups of rooibos for 6 weeks the total cholesterol, LDL-C and TG levels were decreased. As the results of both the present study and that by Macharia (2009:57) are suggestive of a lipid profile modulatory role by rooibos, further investigations are justified. The TG levels in the control group were significant higher at 4 hr and 6 hr time intervals, while in the treatment group no significant differences were noted when compared to the baseline values. Other studies conducted in normolipidaemic or hyperlipidaemic adults where conventional lipid-lowering drugs (lipid-lowering drugs that have the potency of reducing postprandial hyperlipidaemia) such as

fibrates and/or statins were used for a certain period ranging between 4 and 18 weeks, have shown decreases of between -28 and -42% of TC, LDL and TGs (Gylling *et al.,* 1995:17; O'Keefe *et al.,* 1995:480; Genest *et al.,* 2000:164; Parhofe *et al.,* 2000:4224). More specific, the effect of atorvastatin in normolipidaemic adults on postprandial lipoprotein metabolism showed a significant decrease in total cholesterol (-28%), trigycerides (-30%) and LDL-C (-41%) following an oral fat load. In the present study, levels of total cholesterol and LDL-C in the treatment group were significantly lowered by -28%, and -24 and -34%, respectively. Previous studies have reported that an increased consumption of green tea (*Camellia sinensis*) has been associated with decreased total cholesterol, LDL-C and TG levels in human blood, suggested it to be inversely related to the risk of cardiovascular disease (Sies *et al.,* 2005:971; Wang *et al.,* 2007:4). Another study reporting on the consumption of a plant extract to modulate the blood lipid profile is that of Durak and co-workers (2004:373) where a garlic extract was consumed by normotensive and hypertensive subjects with high blood cholesterol levels, showed the extract to lower the level of total cholesterol and to improve the blood lipid profile to a significant extent.

Considering the plasma antioxidant capacity, consuming the standardised fat meal with a commercial soda or rooibos had varying effects. No effects were shown on the ORAC activity when comparing the two groups, while the TEAC activities were significantly higher after the rooibos consumption at 4 hr time interval and the FRAP activities being significantly higher at 2 hr and 6 hr in this group as well. With respect to the FRAP levels, the increased activities are probably not a true reflection, as the baseline levels of both groups differed significantly and influenced the final comparative outcome. A previous study has found that the ORAC of subjects with metabolic syndrome, consuming either a high-fat fast-food-style or the American Heart Association-recommended heart-healthy meal, was shown to be not significantly different at all post ingestion times (Devaraj *et al.,* 2008:867). Many factors can influence the antioxidant capacity, including endogenous antioxidants such as glutathione and vitamin C and the level of free radicals such as ROS/RNS present in the human body. In addition, dietary antioxidants may be effective in scavenging only one of these types of free radicals, but may also be relatively ineffective towards another type of free radical or chelating metal. The level of antioxidants ingested could also play an important role (Moore & Yu, 2008:118). Polyphenols that are absorbed are conjugated and may appear as glucuronoids and sulphates in the intestines and later in the liver. These metabolites are present in the plasma within an hour at a level of >0.01 µmol/l and can be detected in a 24 hr urine sample after a single oral dose (Lee *et al.*, 1995:396; Seeram *et al.,* 2004:67). Many factors exist that could influence the absorption of these polyphenolic compounds and their metabolites, including the amount of polyphenols

consumed and the route of administration and duration of intervention as well as the measurement used to determine the total antioxidant capacity *in vivo* (Kampa *et al.,* 2002:2; Manach *et al.,* 2004:727). In this study, some of these parameters could have contributed to the varying effects in the total antioxidant capacities as shown by the postprandial results.

When considering the redox status of the participants consuming the study diet with the commercial soda or rooibos beverage, a significantly higher level of tGSH at all post ingestion times were shown in participants consuming the rooibos beverage. When the two lipid oxidation biomarkers were measured, the consumption of the study diet with rooibos also significantly lowered the CDs levels at 2 hr and 4 hr, and the TBARS levels at 4 hr, when compared to the participants that consumed the study diet with the commercial soda. It appears that this lowering effect is not sustainable for longer than 4 hr and can potentially provide short-term protection against postprandial oxidative stress. The significantly higher levels of the endogenous antioxidant, tGSH, as well as the increased antioxidant activity (TEAC) could account for the significantly lower levels of lipid oxidation in the participants who consumed the rooibos beverage with the standardised fat meal, as this combined effect was not seen in the participants who consumed the standardised fat meal and commercial soda beverage. In this regard, a previous study has reported that green tea (*Camellia sinensis*) polyphenols to be excellent antioxidants that directly scavenges free radicals and inhibits lipid peroxide formation (Wang *et al.,* 2007:4). Green tea in particular, has shown to protect against cardiovascular and renal diseases in several studies, human and rat models (Stangl *et al.*, 2006:218). Despite being a powerful antioxidant *in vitro*, black tea did not increase the resistance of human plasma to lipid oxidation ex *vivo* (Cherubini *et al.,* 1999:386), with other studies reporting green tea to exhibit more health benefits than black tea in terms of antioxidant capacity, as each tea is different in terms of composition and concentration of antioxidant compounds (Lee *et al.*, 2002:785). Rooibos also contains several phenolic compounds, such as aspalathin, which is the main polyphenolic constituent of this herbal tea, that has been shown to act as an active scavenger of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (von Gadow *et al*., 1997a:636). Rooibos, due to its antioxidant and antimutagenic properties has been reported to be involved in the prevention of some diseases in rat model, where oxidative stress plays an important role, including cancer (Marnewick *et al*., 2005:200; Marnewick *et al.,* 2009:220). Other endogenous factors/mechanisms, such as the levels of glutathione, bilirubin and uric acid have also been shown to be involved (Ames *et al.,* 1981:6858; Stocker *et al.,* 1987:1043; Alptekin *et al.,* 1996:167).

In conclusion, the results from both study phases indicate that the consumption of a standardised fat meal with or without sucrose intake, increased postprandial oxidative stress. The results from phase Two suggested that the ingestion of the sucrose-containing rooibos beverage with the standardised fat meal, did not further promote the postprandial oxidative stress, but to the contrary, decreased the short-term-induced postprandial oxidative stress by modulating the lipid profile, antioxidant activity and endogenous antioxidant level, and reducing lipid oxidation. The consequences of oxidative reactions occurring in the course of these time points may represent some risk for metabolic and non-metabolic diseases and fatal/non-fatal heart diseases. Therefore it has been suggested that antioxidant supplementation of the diet may prevent or reduce many of these risks (Kay & Holub, 2003:452). Postprandial oxidative stress is characterised by a susceptible increase of the organism toward oxidative damage after consumption of a fat meal and/or carbohydrates (Ursini & Sevanian, 2002:599; Bowen & Borthakur, 2004:477). As many non-fasting reactions may represent major risk factors for diseases, postprandial risk analysis should become an important diagnostic tool in the prevalence of diseases in the future (Kay & Holub, 2003:452). From the study results, it is suggested that the fermented rooibos beverage (containing sucrose) may have a place as a dietary supplement in alleviating short-term induced postprandial oxidative stress.

Several limitations to this study should be considered when interpreting the results presented here, i) a relative small sample size of study participants (n = 14) in Phase Two; ii) omission of a third control group where the participants only consumed rooibos without the inclusion of sucrose; iii) omission of a run-in period and/or standardised evening meal before commencement of the study the following day; iv) Phase Two (crossover design) did not include a randomisation of the participants; v) shorter postprandial time intervals (e.g. 30 or 60 minutes) for blood collection were not included and vi) full analysis of the study diet is not included.

CHAPTER SIX: RECOMMENDATIONS

The work conducted in this study has focused on the possible modulating role of rooibos as a dietary antioxidant component on postprandial oxidative stress, induced by the intake of sucrose and a standardised fat meal. Though rooibos may function well in projects designed as such, there are still large gaps in our knowledge concerning the quantity and type of antioxidants to be used in order to alleviate oxidative stress and specifically the burden of disease in humans. Although there is more information available concerning this herbal tea, which is increasingly being used in oxidative stress studies, postprandial clinical nutritional baseline information could also be extended to this herbal tea.

Many human studies have addressed changes in postprandial hyperglycaemia and hyperlipidaemia using dietary interventions, but this study is amongst the few and the results presented in this thesis confirms that the consumption of a standardised fat meals and/or sucrose-containing beverage contributes to postprandial oxidative stress. The intake of dietary antioxidants, such as rooibos, may modulate this effect, but studies using greater sample numbers and measuring more oxidative stress parameters and shorter time intervals are necessary to substantiate and further elucidate the results from this study. However, results from this study recommend the intake of rooibos as part of a balanced diet for the overall well being and reduction of oxidative damage to the body.

Several questions for future research have been raised by the results presented here. The following studies may further determine the modulatory effects of rooibos on postprandial oxidative stress:

I. Evaluating the effect of rooibos on postprandial oxidative damage of proteins and DNA in normal individuals.

The intake of both meals (fat meal and sucrose) increased lipid oxidation and the data suggests that this could be alleviated by using rooibos as a dietary supplement. Due to the time constraint, oxidative damage to other important cellular components such as proteins and DNA could not be executed. Many cardiovascular and cerebrovascular diseases and cancers are associated with Western lifestyle, especially related to diet (Kolonel *et al.,* 2004:519). The accumulation of ROS/NRS leading to oxidative stress has been associated with intracellular events leading to protein and DNA damage (Martindale & Holbrook, 2002:1). It is therefore

important to also evaluate the effect of rooibos on postprandial-induced oxidative damage to proteins and DNA.

II. Investigating the effect of both types of rooibos (fermented and unfermented) on postprandial oxidative stress in normal individuals.

The data presented in our study demonstrate, the fermented rooibos beverage to modulate postprandial oxidative stress in normal lipidaemic individuals, after an intake of a standardised fat meal and commercial sucrose beverage. Investigating whether unfermented/"green" rooibos could also improve the postprandial oxidative stress in these same individuals would help us, to develop better strategies for prevention of postprandial oxidative stress where this unique herbal tea is involved.

III. Studies should also be designed to address *in vivo* effects of postprandial variations, time of ingestion and digestion, nutritional status and bioavailability of rooibos components/metabolites or a comparison study with conventional lipid-lowering drugs.

Our research demonstrated the short-term modulatory effect of a fermented rooibos beverage taken with a standardised fat meal and sucrose diet. As antioxidant activities have been shown to vary among individuals and are directly related to the total polyphenols found in the herbal teas (Mousavinejad *et al.,* 2009:1274), it is important to have a baseline understanding whether this antioxidant beverage should be taken before or after a fat meal or rather be taken throughout the day. Further investigation can also identify the life span of antioxidants found in both types of rooibos (fermented and unfermented) at the specific quantities (percentages) when consumed.

REFERENCES

Alipour, A., Elte, J.W.F., Van Zaanen, H.C.T., Rietveld, A.P. and Castro cabezas, M. 2007. Postprandial inflammation and endothelial dysfunction. *Biochemical Society Transactions*, **35(3):** 466-469.

Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W. and Fu, P.C. 1974. Enzymatic determination of total serum cholesterol. *Clinical Chemistry,* **20(4):** 470-475.

Alptekin, N., SeçKin, S., Doğru-Abbasoğlu, S., Yelkenci, Koçk-toker, N., Toker, G. and Uysal, M. 1996. Lipid peroxides, glutathione, γ-glutamylcysteine syntetase and γ-glutamyltranspeptidase activities in several tissues of rats following water-immersion stress. *Pharmacological Research,* **34(3/4):** 167-169.

Amann, K. 2008. Media calcification and intima calcification are distinct entities in chronic kidney disease. *Clinical Journal of the American Society of Nephrology,* **3:** 1599-1605.

American Heart Association. 2002. Third report of the aational cholesterol education panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult treatment panel II) Final report. *Circulation,* **106:** 3134-3191.

Ames, B.N., Cathcart, R., Schwiers, E. and Hochstein, P.1981. Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. *Proceedings of the National Academy of Sciences,* **78:** 6858-6862.

Aoshima, H., Hirata, S. and Ayabe, S. 2007. Antioxidant and anti-hydrogen peroxide activities of various herbal teas. *Food Chemistry,* **103:** 617-622.

Asensi, M., Sastre, J., Pallardo, F.V., Lloret, A., Lehner, M., Garcia-de-la Asuncion, J. and Viňa, J. 1999. Ratio of reduced to oxidized glutathione as indicator of oxidative stress status and DNA damage. *Methods in Enzymology,* **299:** 267-276.

Arteel, G. E. and Sies, H. 1999. Protection against peroxynitrite by cocoa polyphenol oligomers. *Federation of European Biochemical Societies Letters,* **462:** 167-170.

Atoui, A.K., Mansouri, A., Boskou, G. and Kefalas, P. 2005. Tea and herbal influsion: Their antioxidant activity and phenolic profile. *Food Chemistry,* **89:** 27-36.

Atsunor, N., Noriaki, T. and Eiji, K. 2003. Oxidative stress and billuribin. *Japanese Journal of Clinical Experimental Medicine,* **80(9):** 1675-1677.

Babior, M and Peter, W.A. 1981. The O_2^- - producing enzyme of human neutrophils. *Journal of Biology Chemistry,* **256:** 2321-2323.

Bahorun, T., Luximon-Ramma, A., Crozier, A. and Aruoma, O.I. 2004. Total phenol, flavonoids, proanthocyanidins and vitamin C levels and antioxidant activities of Mauritian vegetables. *Journal of Agriculture and Food Science,* **84:** 1553-1561.

Barnerjee, S., Maity, P., Mukherjee, S., Sil, A.K., Panda, K., Chattopadhyay, D. and Chatterjee, I.B. 2007. Black tea prevents cigarette smoke induced apoptosis and lung damage. *Journal of Inflammation,* **4(3):** 1-12.

Bartsch, H. and Nair, J. 2004. Oxidative stress and lipid oxidation-derived DNA-lesion in inflammation driven carcinogenesis. *Cancer Detection and Prevention,* **28:** 385-391.

Bayard, V., Chamorro, F., Motta, J. and Hollenberg , N. 2007. Does flavonol intake influence mortality from nitric oxide-dependent processes? Ischemic heart disease, stroke, diabetes mellitus, and cancer in Panama. *International Journal of Medical Sciences,* **4(1):** 53-58.

Bayraktutan, U. 2002. Free radicals, diabetes and endothelial dysfunction. *Diabetes, Obesity and Metabolism,* **4:** 1-33.

Beckman, J.A., Goldfine, A.B., Gordon, M.B. and Creager, M.A. 2001. Ascorbate restores endothelium-dependent vasodilation impaired by acute hyperglycemia in humans. *Circulation,* **103:** 1618-1623.

Bell, P.M., Firth, R.G. and Rizza, R.A. 1989. Assessment of the postprandial pattern of glucose metabolism in non-diabetic subjects and patients with non-insulin dependent diabetes mellitus using a simultaneous infusion of [2(3)H] and [3(3)H] glucose. *Metabolism,* **38(1):** 38-45.

Behl, C., Skutella, T., Lezoualc'h, F., Post, A., Widmann, M., Newton, C.J. and Holsboer, F. 1997. Neuroprotection against oxidative stress by estrogen: structure-activity relationship. *Molecular Pharmacology,* **51:** 535-541.

Benadé, A. J. S., Wyndham, C. H., Jansen, C. R., Rogers, G. G. and de Bruin, E. J. P. 1973. Plasma insulin and carbohydrate metabolism after sucrose ingestion during rest and prolonged aerobic exercise. *Pflügers Archives,* **342:** 207-218.

Benzie, I.F.F. and Strain, J.J. 1996. The Ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power". The FRAP assay. *Annal Biochemistry,* **239:** 70-76.

Beretta, G., Aldini, G., Facino, R.M., Russell, R.M., Krinsky, N.I. and Yeum, K-J. 2006. Total antioxidant performance: A validated fluorescence assay for the measurement of plasma oxidizability. *Analytical Biochemistry,* **354:** 290-298.

Berry, S.E, Tydeman, E.A, Lewis, H.B, Phalora, R, Rosborough, J, Picout, D.R. and Ellis, P.R. 2008. Manipulation of lipid bioaccessibility of almond seeds influences postprandial lipemia in healthy human subjects. *American Journal of Clinical Nutrition,* **88(4):** 922-929.

Bierman, E.L. 1992. George Lyman Duff Memorial Lecture. Atherogenesis in diabetes. *Arteriosclerosis and Thrombosis,* **12:** 647-656.

Blackhurst, D.M. and Marais, A.D. 2006. Concomitant consumption of red wine and polyunsaturatedbfatty acids in edible oil does not influence the oxidation status of chylomicron lipids despite increasing plasma catechin concentration. *Nutrition, Metabolism and Cardiovascular Diseases,* **16:** 550-558.

Blendea, M.C., Bard, M., Sowers, J.R. and Winer, N. 2005. High-fat meal impairs vascular compliance in a subgroup of young healthy subjects. *Metabolism Clinical and Experimental,* **54:** 1337-1344.

Blommaert, K.L.J. and Steenkamp, J., 1978. Tannien- en moontlike kafeïeninhoud van rooibostee, *Aspalathus* (Subgen. Nortiera) *linearis* (Burm. Fil) R. Dahlgr. *Agroplantae*, **10:** 49.

Blouet, C., Mariotti, F., Azzout-Marniche, D., Mathe, V., Mikogami, T., Tome, D. and Huneau, J-F. 2007. Dietary cysteine alleviates sucroses-induced oxidative stress and insulin resistance. *Free Radical Biology & Medicine,* **42:** 1089-1097.

Blumberg, J. 2004. Use of biomarkers of oxidative stress in research studies. *Journal of Nutrition*, **134:** 3188S-3189S.

Borek, C. 2004. Dietary antioxidants and human cancer. *Integrative Cancer Therapies,* **3(4):** 333-341.

Bors, W. and Saran, M. 1987. Radical scavenging by flavonoid antioxidants. *Free Radical Research Communication,* **2:** 289-294.

Bowen, P. E. and Borthakur, G. 2004. Postprandial lipid oxidation and cardiovascular disease risk. *Current Atherosclerosis Reports,* **6:** 477-484.

Bramati, L., Minoggio, M., Gardana, C., Simonetti, P., Mauri, P. and Pietta, P. 2002. Quantitative characteriztion of flavonoid compounds in rooibos tea (*Aspalathus linearis*) by LC-UV/DAD. *Journal of Agriculture and Food Chemistry*, **50:** 5513-5519.

Brannon, C. 2007. Green tea: New benefits from an old favourite? *Nutrition Dimention*, 1-26.

Breet, P. Kruger, H. S., Jerling, J. C. and Oosthuizen, W. 2005. Actions of black tea and rooibos on iron status of primary school children. *Nutrition Research*, **25:** 983-994.

Brock, J.W.C., Ames, J.M., Thorpe, S.R. and Baynes, J.W., 2007. Formation of methionine sulfoxide during glycoxidation and lipoxidation of ribonuclease A. *Archives of Biochemistry and Biophysics,* **457:** 170-176.

Brownlee, M. 2001. Biochemistry and molecular cell biology of diabetic complications. *Nature*, **414:** 813-820.

Bucala, R. and Cerami, A. 1992. Advanced glycosylation: chemistry, biology and implications for diabetes and aging. *Advanced Pharmacology,* **23:** 1-34.

Buening, M. K., Chang, R. L., Huang, M. T., Fortner, J. G., Wood, A. W. and Conney, A. H. 1981. Activation and inhibition of benzo(a)pyrene and aflatoxin B1 metabolism in human liver microsomes by naturally occurring flavonoids. *Cancer Research,* **41:** 67-72.

Buonocore, G. and Groenendaal, F. 2007. Anti-oxidant strategies. *Seminars in Fetal and Neonatal Medicine,* **12(4)**: 287-295.

Calder, P.C., 2006. Polyunsaturated fatty acids and inflammation. *Prostaglandins Leukotrienes and Essential Fatty Acids,* **75:** 197-202.

Cao, G. and Pior, R.L., 1998. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clinical Chemistry,* **44:** 1309-1315.

Carrey, M.C. 1983. Lipid digestion and absorption. *Annual Revue of Physiology,* **45:** 651-77.

Carroll, M.F. and Schade, D.S. 2003. Timing of antioxidant vitamin ingestion alters postprandial pro-atherogenic serum markers. *Circulation,* **108:** 24-31.

Castro Cabezas, M., Halkes, C.J., Meijssen, S. van Oostrom, A.J. and Erkelens, D.W. 2001. Diurnal triglycerine profiles: a novel approachto study triglyceride. *Artherosclerosis,* **155:** 219-228.

Catalá, A. 2006. An overview of lipid oxidation with emphasis in outer segments of photoreceptors and the chemiluminescence assay. *The International Journal of Biochemistry and Cell Biology,* **38:** 1482-1495.

Ceriello, A. 2000a. Oxidative stress and glycemic regulation. *Metabolism,* **49(2 Suppl 1):** 27-29.

Ceriello, A. 2000b. The post-prandial state and cardiovascular disease: relevance to diabetes mellitus. *Diabetes Metababolic Research Review,* **16:** 125-132.

Ceriello, A., Bortolotti, N. and Crescentini, A.1998a. Antioxidant defences are reduced during the oral glucose tolerance test in normal and non-insulin dependent diabetic subjects. *European Journal Clinical Investigation,* **28:** 329-333.

Ceriello, A., Bortolotti, N. and Motz, E. 1998b. Meal-generated oxidative stress in type 2 diabetic patients. *Diabetes Care,* **21:** 1529-1533.

Ceriello, A., Bortolotti, N., Motz, E., Pieri, C., Marra, M., Tonutti, L., Lizzio, S., Feletto, F., Catone, B. and Taboga, C. 1999. Meal-induced oxidative stress and low-density lipoprotein oxidation in diabetes: The possible role of hyperglycemia. *Metabolism,* **48(12):** 1503-1508.

Ceriello, A., Assaloni, R., Da Ros, R., Maier, A., Piconi, L., Quagliaro, L., Esposito, K. and Giugliano, D. 2005. Effect of atorvastatin and irbesartan, llone and in combination, on postprandial endothelial dysfunction, oxidative stress, and inflammation in type 2 diabetic patients. *Circulation,* **111:** 2518-2524.

Ceriello A. and Motz, E. 2004. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? Arteriosclerosis, *Thrombosis Vascular Biology,* **24:** 816-823.

Chance, B. and Oshino, N. 1971. Kinetics and mecanisms of catalase in peroxisomes of the mitochodrial. *Biochemistry,* **122:** 225-233.

Charlton, K.E., Wolmarans, P. and Lombard, C.J. 1998. Evidence of nutrient dilution with high sugar intakes in older South Africans. *Journal of Human Nutrition and Dietetics,* **11:** 331-343.

Chatterjee, I. B., Majumder, A. K., Nandi, B. K. and Subramanian, N. 1975. Synthesis and some major functions of vitamin C in animals. *Annal of New York Academy of Science,* **258:** 24-47.

Chen, C., Nan, B., Lin, P. and Yao, Q., 2008. C-reactive protein increase plasminogen activator inhibitor-1 expression in human endothelial cells. *Trombosis Research,* **122(1):** 125-133).

Cherubini, A., Beal, M.F. and Frei, B. 1999. Black tea increases the resistance of human plasma to lipid oxidation in vito, but not ex vivo. *Free Radical Biology & Medicine,* **27(3/4):** 381-387.

Choi, E.-M. 2007. Modulatory effects of luteolin on osteoblastic function and inflammatory mediators in osteoblastic MC 3T3-E1 cells. *Cell Biology International,* **31:** 870-877.

Cigaril, P., Kurutas, E.B., Gul, M., Kilinc, M., Aral, M. and Guven, A. 2005. The effects of oxidative stress in urinary tract infection during pregnancy. *Mediators of Inflammations,* **2005(5):** 309-311.

Cohen, A., Klasing, K. and Ricklefs, R. 2007. Measuring circulating antioxidants in wild birds. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology,* **147:** 110-121.

CoLO A. 2005. LDL cholesterol: “bad” cholesterol or bad science? *Journal of American Physicians and Surgeons,* **10(3):** 83-89.

Cozma, L.S., Luzio, S.D., Dunseath, G.J., Langendorg, K.W. and Pieber, T. 2002. Comparison of the effects of three insulinotropic drugs on plasma insulin levels after a standard meal. *Diabetes Care,* **25:** 1271-1276.

Csiszar, A., Pacher, P., Kaley, G. and Ungvari, Z. 2005. Role of oxidative and nitrosative stress, longevity genes and poly(ADP-ribose) polymerase in cardiovascular dysfunction associated with aging. *Current Vascular Pharmacology,* **3**: 285-291.

D’Abrosca, B., Fiorentino, A., Pacifico, S., Cefarelli, G., Uzzo, P., Letizra, M. and Monaco, P. 2007. Natural feruloyl monoglyceride macrocycles as protecting factors against free-radical damage of lipidic membranes. *Bioorganic & Medicinal Chemistry Letters,* **17:** 4135-4139.

Daly, M. 2003. Sugars, insulin sensitivity, and the postprandial state. *The American Journal of Clinical Nutrition,* **78(4):** 865S-872S.

Dardevet, D., Sornet, C., Bayle, G., Prugnaud, J., Pouyet, C. and Grizard, J. 2002. Postprandial stimulation of muscle protein synthesis in old rats can be restored by a leucine-supplemented meal. *Journal of Nutrition,* **132:** 95-100.

De Champlain, J., Wu, R., Girouard, H., Karas, M., El Midaoui, A., Laplante, M.A. and Wu, L. 2004. Oxidative stress in hypertension. *Clinical Experimental Hypertension,* **26(7-8):** 593-601.

De Koning, E.J.P. and Rabelink, T.J. 2002. Endothelial function in the post-prandial state. *Atherosclerosis Supplements,* **3:** 11-16.

Delcour, J.A. and Janssens de Varebeke, D.1985. A new colometric assay for flavonoids in pilsner beer. *Journal of the Institute of Brewing,* **91:** 37-40.

Dermibağ, R., Yilmaz, R., Gür, M., Kunt, A.S., Koçyiğit, A., Celik, H., Güzel, S. and Selek, S. 2006. DNA damage and plasma total antioxidant capacity in patients with slow coronary artery flow. *Türk Kardiyoloji Dermeği Arşivi,* **34:** 89-93.

Devaraj, S. and Jialal, I. 2000_a. Low-density lipoprotein postsecretory modification, monocyte function, and circulating adhesion molecules in type 2 diabetic patients with and without macrovascular complications: the effect of α-tocopherol supplementation. *Circulation,* **102:**191-196.

Devaraj, S. and Jialal, I. 2000_b Alpha tocopherol supplementation decreases serum C-reactive protein and monocyte interleukin-6 levels in normal volunteers and type 2 diabetic patients. *Free Radical Biology & Medicine,* **29:** 790-792.

Devaraj, S., Cabo Chan, A.V. and Jialal, I. 2002. α-Tocopherol supplementation decreases plasminogen activator inhibitor-1 and P-selectin levels in type 2 diabetes patients. *Diabetes Care,* **25:** 524-529.

Devaraj, S., Wang-Polagruto, J., Polagruto, J., Keen, C.L. and Jialal, I. 2008. High-fat, energy-dense, fast-food-style breakfast results in an increase n oxidative stress in metabolic syndrome. *Metabolism Clinical and Experimental,* **57:** 867-870.

Devasagayam, T.P.A., Tilak, J.C., Boloor, K.K., Sane, K.S., Ghaskadbi, S.S. and Lele, R.D. 2004. Free radical and antioxidants in human health: Current status and future prosects. *Journal of Association of Physicians of India,* **52:** 794-804.

Devereux, R.B., Pickering, T.G., Harshfield, G.A., Kleinert, H.D., Denby, L., Clark, L., Pregibon, D., Jason, M. and Kleiner, B. 1983. Left ventricular hypertrophy in patients with hypertension: importance of blood pressure response to regularly recurring stress. *Circulation,* **68:** 470-476.

Dhalla, N.S., Temsah, R.M. and Netticadan, T. 2000. Role of oxidative stress in cardiovascular disease. *Journal of Hypertension,* **18(6):** 655-673.

Dietrich, M., Block, G., Hudes, M., Morrow, D.J., Norkus, E.P., Traber, M.G. Cross, C.E. and Packer, L. 2002. Antioxidant supplementation decreases lipid oxidation biomarker F2-isoprostanes in plasma of smokers. *Cancer Epidemiology, Biomarkers & Prevention,* **11:** 7-13.

Dohi, Y., Takase, H., Sato, K. and Ueda, R. 2007. Association among C - reactive protein, stress and traditional risk factors in healthy Japanese subjects. *International Journal of Cardiology,* **115:** 63-66.

Dok-Go, H., Lee, K. H., Kim, H. J., Lee, E. H., Lee, J., Song, Y. S., Lee, Y-H., Jin, C., Lee, Y. S. and Cho, J. 2003. Neuroprotective effects of antioxidative flavonoids, quercetin, (+)-dihydroquercetin and quercetin 3-methyl ether, isolated from *Opuntia ficus-indica var. Saboten*. *Brain Research,* **965:** 130-136.

Drapper, H.H., Squires, E.J., Mahmood, H., Wu, J., Agarual, S. and Hadley, M.A. 1993. Comparative evaluation of thiobarbituric acid methods for the determination of malondialdhyde in biological materials. *Free Radicals Biology & Medicine,* **15(4):** 353-363.

Droebner, K., Ehrhardt, C., Poetter, A., Ludwig, S. and Planz, O. 2007. CYSTUS052, a polyphenol-rich plant extract, exerts anti-influenza virus activity in mice. *Antiviral Research,* **76:** 1-10.

D'Souza, V.M., Shertzer, H.G., Menon, A.G., Giovanni M. and Pauletti, G.M. 2003. High glucose concentration in isotonic media alters caco-2 cell permeability. *Pharmaceutica Science Journal,* **5(3):** 1-9.

Dubois, C., Beaumier, G., Juhel, C., Armand, M., Portugal, H. and Pauli, A.M. 1998. Effects of graded amounts (0-50 g) of dietary fat on postprandial lipemia and lipoproteins in normolipidemic adults. *American Journal of Clinical Nutrition,* **67:** 31-38.

Duffy, S.J., Keaney, J.F., Holbrook, M., Gokce, N., Swerdloff, P.L., Frei, B. and Vita, J.A. 2001. Short- and long-term black tea consumption reverses endothelial dysfunction in patients with coronary artery disease. *Circulation,* **104:** 151-156.

Durak, I., Kavutcu, M., Aytaç, B., Avci, A., Devrim, E. Ozbek, H. and Oztürk, H.S. 2004. Effect of garlic extract consumption on blood lipid and oxidant/antioxidant parameters in humans with high blood cholesterol. *Journal of Nutritional Biochemistry,* **15:** 373-377.

Eastwood, M. 2003. *Principals of human nutrition*. 2nd ed, Blackwell Science Ltd. p. 1-680.

Emelyanov, A., Fedoseev, G., Krasnoschekova, O., Abulimity, A., Trendeleva, T. and Barnes, P.J., 2002. Treatment of asthma with lipid extract of New Zealand greenlipped mussel: a randomised clinical trial. *European Respiratory Journal,* **20:** 596-600.

Enser, M., Richardson, R., Wood, J.D., Gill, B.P. and Sheard, P.R. 2000. Feeding linseed to increase the n-3 PUFA of pork: fatty acid composition of muscle, adipose tissue, liver, and sausages. *Meat Science,* **55:** 201-212.

Facundo, H.T.F., Brandt, C.T., Owen, J.S. and Lima, V.L.M. 2004. Elevated levels of erythrocyte-conjugated dienes indicate increased lipid oxidation in Schistosomiasis *mansoni* patients. *Brazilian Journal of Medical and Biological Research,* **37:** 957-962.

Fahy, E ., Subramaniam, S., Brown, H.A., Glass, C.K., Merrill Jr., A. H., Murphy, R.C.,Raetz,C.R.H., Russell, D.W., Seyama, Y., Shaw W., Shimizu, T., Spener, F., Van Meer, G.,VanNieuwenhze, M.S., White, S.H., Witztum, J.L. and Dennis., E.A. 2005. A comprehensive classification system for lipids. *Journal of Lipid Research,* **46:** 839-861.

Faraci, F.M. 2005. Oxidative stress: the curse that underlies cerebral vascular dysfunction? *American Stroke Association,* **36:** 186-188.

Farber, J.L. 1994. Mechanisms of cell injury by activated oxygen species. *Environmental Health Perspective,* **104:** 17-24.

Fatokun, A.A., Stone, T.W. and Smith, R.A. 2007. Hydrogen peroxide mediates damages by xanthine and xanthine oxidase in cerebellar granule neuronal cultures. *Neuroscience Letters,* **416:** 34-38.

Feng, Z., Hu, W., Marnett, L.J. and Tang, M-S. 2006. Malondialdehyde, a major endogenous lipid oxidation product, sensitizes human cells to UV-and BPDE-induced killing and mutagenesis through inhibition of nucleotide excision repair. *Mutation Research,* **601:** 125-136.

Ferrara, L., Montesano, D. and Senatore, A. 2001. The distribution of minerals and flavoids in the tea plant (*Camellia sinensis*). *IL Farmaco,* **56:** 397-401.

Floyd, R.A. 1990. Role of oxygen free radicals in carcinogenesis and brain ischemia. *Federation of American Societies for Experimental Biology,* **4(9):** 2587-2597.

Floyd, R.A. 1999. Antioxidant, oxidative stress and degenerative neurological disorders. *Society for Experimental Biology and Medicine. Proceedings,* **222(3):** 236-245.

Fossati, P. and Prencipe, L. 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry,* **28(10):** 2077-2080.

Franca, M.B., Panek, A.D. and Eleutherio, E.C.A. 2007. Oxidative stress and its effets during dehydratation. *Comparative Biochemistry and Physiology Part A,* **146:** 621-631.

Frankel, E.N. 2005. *Lipid oxidation.* Published by the Oily Press. 2nd ed. Bridgwater TA7 0YZ, England. 1-410.

Freeman, B.A. and Crapo, J.D. 1982. Biology of disease: Free radicals and tissue injury. *Laboratory Investigation,* **45(5):** 412-26.

Fujiki, H. 1999. Two stages of cancer prevention with green tea. *Journal of Cancer Research and Clinical Oncology,* **125:** 589-597.

Gavazza, M.B. and Catala′, A. 2006. The effect of α-tocopherol on lipid oxidation of microsomes and mitochondria from rat testis. *Postaglandins, Leukotrienes and Essential Fatty Acids,* **74:** 247-254.

Gavin III, J.R. 2001. Pathophysiologic mechanisms of postprandial hyperglycemia. *American Journal of Cardiology,* **88(6A):** 4H-8H.

Genest, J., Nguyen, N.H., Theroux, P., Davignon, J. and Cohn, J.S. 2000. Effect of micronized fenofibrate on plasma lipoprotein levels and hemostatic parameters of hypertriglyceridemic patients with low levels of high-density lipoprotein cholesterol in the fed and fasted state. *Journal of Cardiovascular Pharmacology,* **35**: 164-172.

Georgieva, N.V. 2005. Oxidative stress as a factor of disrupted ecological oxidative balance in biological system. *Bulgarian Journal of Veterinary Medicine,* **8(1):** 1-11.

Gershkovich, P. and Hoffman, A. 2007. Effect of high-fat meal on absorption and disposition of lipophilic compounds: The importance of degree of association with triglyceride-rich lipoproteins. *European Journal of Pharmaceutical Sciences,* **32:** 24-32.

Gilani, A.H., Khan, A., Ghayur, M.N., Ali, S.F. and Herzig, J.W. 2006. Antispasmodic effect of rooibos tea (*Aspalathus linearis*) is mediated through K^+ channel activation. *Basic Clinical Pharmacology and Toxicology,* **99:** 365-373.

Giokas, D.L., Vlessidis, G.A. and Evmiridis, N.P. 2007. On line selection detection of antioxidants free-radical scavenging activity based on Co(II)/EDTA-induced luminal chemiluminescence by flow injestion analysis. *Analytica Chimica Acta,* **589:** 59-69.

Girotti, A.W. 1989. Photodynamic lipid in biology systems. *Photochemistry and Photobiology,* **51(4):** 497-509.

Gittelsohn, J., Wolever, T.M.S., Harris, S.B., Giraldo, R, H., Hanley, A.J.G. and Zinman, B. 1998. Specific patterns of food consumption and preparation are associated with diabetes and obesity in a native Canadian community. *Journal of Nutrition,* **128:** 541-547.

Giugliano, D., Ceriello, A. and Esposito, K. 2006. The effects of diet on inflammation: emphasis on the metabolic syndrome. *Journal of the American College of Cardiology,* **48(4):** 677-685.

Goçmen, A.Y., Gümüslü, S. and Semiz, E. 2004. Association between paraoxonase -1 activity and lipid oxidation indicator levels in people living in the Antalya region with angiographically document coronary artery disease. *Clinical Cardiology,* **27:** 426-430.

Gonzalez, M.-C. and Cejudo, F.J. 2007. Gibberellin-regulated expression of neutral and vacuolar invertase genes in petioles of sugar beet plants. *Plant Science,* **172:** 839-846.

Gotto, A. M., Jr., Pownall, H. J. and Havel, R. J. 1986. Introduction to the plasma lipoproteins. *Methods Enzymology,* **128:** 3-41.

Gottschling, B.C., Maronpot, R.R., Hailey, J.R., Peddade, S., Moomaw., C.R., Klaunig, J.E. and Nyska, A. 2001. The role of oxidative stress in indium phosphate induced lung carcinogenesis in rats. *Toxicological Sciences,* **64:** 28-40.

Grant, K.I., Marais, M.P. and Dhansay, M.A. 1994. Sucrose in a lipid-rich meal amplifies the postprandial excursion of serum and lipoprotein triglyceride and cholesterol concentrations by decreasing triglyceride clearance. *The American Journal of Clinical Nutrition,* **59:** 853-860.

Green, K., Brand, M.D. and Murphy, M.P. 2004. Prevention of mitochondrial oxidative damage as a therapeutic strategy in diabetes. *Diabetes,* **53(Suppl 1):** S110-S118.

Griendling, K.K. and FitzGerald, G.A. 2003. Oxidative stress and cardiovascular injury. Part: basic mechanism and in vitro monitoring of ROS. *Circulation,* **108:** 1912-1915.

Griffith, O.W. 1999. Biological and pharmacological regulation of mammalian glutathione synthesis. *Free Radical Biology & Medicine,* **27:** 922-935.

Gümüslü, S., Serterser, M., Özben, T., Balkan, S. and Balkan, E. 1997. Inhibitory role of N^{ω}-nitro-L-arginine methyl ester (L-NAME), a potent nitric oxide synthase inhibitor, on brain malondialdehyde and conjugated diene levels during focal cerebral ischemia in rats. *Clinica Chimica Acta,* **267:** 213-223.

Gylling, H., Relas, H. and Miettinen, T.A. 1995. Postprandial vitamin A and squalene clearances and cholesterol synthesis of and on treatment in type III hyperlipoproteinemia. *Atherosclerosis,* **115**: 17-26.

Halliwell, B. 1994. Free radicals, antioxidants and disease: curiosity, cause or consequence? *Lancet,* **344:** 721-724.

Halliwell, B. 1999a. Food-derived antioxidants. Evaluating their importance in food and *in vivo. Food Science and Agricultural Chemistry,* **1:** 67-109.

Halliwell, B. and Gutteridge, J.M.C. 1999b. *Free radicals in biology and medicine*. 3rd ed. pp 1-88. Oxford University Press.

Hillis, W.E. and Inoue, T. 1967. The polyphenols of nothogagus species- II. *Phytochemistry,* **6:** 59-67.

Hansson, G.K. 2005. Inflammation, atherosclerosis, and coronary artery disease. *New England Journal of Medicine,* **352:** 1685-695.

Harbis, A., Defoort, C., Narbonne, H., Juhel, C., Senft, M., Latgé, C., Delenne, B., Portugal, H., Atlan-Gepner, C., Vialettes, B. and Lairon, D. 2001. Acute hyperinsulinism modulates plasma apolipoprotein B-48 triglyceride–rich lipoproteins in healthy subjects during the postprandial period. *Diabetes,* **50:** 462-469.

Heitzer, T., Schlinnzig, T., Krohn, T., Meinertz, T. and Münzel, T. 2001. Endothelial dysfunction, oxidative stress, and risk of cardiovascular event in patient with coronary artery disease. *Circulation,* **104:** 2673-2678.

Hensley, K., Robinson, K.A., Gabbita, S. P., Salsman, S. and Floyd, R.A. 2000. Reactive oxygen species, cell signaling, and cell injury. *Free Radical Biology & Medicine,* 10:1456-62.

Hesseling, P.B. and Joubert, J.R. 1982. The effect of rooibos tea on the type I allergic reaction. *South African Medical Journal,* **62:** 1037-1038.

Hinsberger, A. and Sandhu, B.K. 2004. Digestion and absorption. *Current Paediatrics,* **14(7):** 605-611.

Hodgson, J. M., Puddey, I. B., Croft, K. D., Burke, V., Mori, T. A., Caccetta, R. A. A. and Beilin, L. J. 2000. Acute effects of ingestion of black and green tea on lipoproprotein oxidation. *American Journal of Clinical Nutrition,* **71:** 1103-1107.

Hojo, K., Hakamata, H., Ito, A., Kotani, A., Furukawa, C., Hosokawa, Y.Y. and Kusu, F. 2007. Determination of total cholesterol in serum by high-performance liquid chromatography with electrochemical detection. *Journal of Chromatography A,* **1166:** 135-141.

Holbrook, N.J. and Ikeyama, S. 2002. Age-related decline in cellular response to oxidative stress: links to growth factor signaling pathways with common defects. *Biochemical Pharmacology,* **64:** 999-1005.

http://www.whfoods.com/genpage.php?tnamefoodspice&dbud=146, the tea plant.

Huang, Q. L., Jin, Y., Zhang, L. N., Cheung, P. C. K. and Kennedy, J. F. 2007. Structure, molecular size and antitumor activities of polysaccharides from *Poria cocos mycelia* produced in fermenter. *Carbohydrate Polymers,* **70:** 324-333.

Huang, Y.-T., Hwang, J.-J., Lee, P -P., Ke, F.-C.., Huang, J.- H., Huang, C.- J., Kandaswami, C., Middleton, Jr. and Lee, M. - T. 1999. Effects of luteolin and querctin, inhibitors of tyrosine kinase, on cell growth and metastasis - associated properties in A431 cells overexpressing epidermal growth factor receptor. *British Journal of Pharmacology,* **128:** 999-1010.

Ichiyama, K., Tai, A. and Yamamoto, I. 2007. Augmentation of antigen-specific antibody production and IL-120 generation with a fraction from rooibos (*Aspalathus linearis*) tea. *Bioscience, Biotechnology and Biochemistry,* **71:** 589-602.

Inanami, O., Asanuma, T., Inukai, N., Jin, T., Shimokawa, S., Kasai, N., Nakano, M., Sato, F. and Kuwabara, M. 1995. The suppression of age-related accumulation of lipid peroxides in rat brain by administration of rooibos tea (*Aspalathus linearis*). *Neuroscience Letters,* **196:** 85-88.

Iraz, M., Erdogan, H., Ozyurt, B., Ozugurlu, F., Ozgocmen, S. and Fadillioglu, E. 2005. Omega-3 essential fatty acid supplementation and erythrocyte oxidant/antioxidant status in rats. *Annals of Clinical & Laboratory Science,* **35:** 169-173.

Jaganyi, D. and Wheeler, P. J. 2003. Rooibos tea: Equilibrium and extraction kinetics of aspalathin. *Food Chemistry,* **83:** 121-126.

Jakulj, F., Zernicke, K., Bacon S.L., van Wielingen, L.E., Key, B.L., West, S.G. and Campbell, T.S. 2007. A high-fat meal increases cardiovascular reactivity to psychological stress in healthy young adults. *Journal of Nutrition,* **137:** 935-939.

Jenner, M.R. 1980. *Development in food carbohydrates-2.* in C.K. ed. Applied Science Publishers: p. 91.

Jensen, S.J.K. 2003. Oxidative stress and free radicals. *Journal of Molecular Structural,* **667:** 387-392.

Joint working group assembled from the AECB's Advisory Committees, AECB Staff, health Canada staff and Ontario ministry of environment and energy staff. 1998. Assessment and management of cancer risks from radiological and chemical hazards. *Health Canada Atomic Energy Control Board,* 16-19.

Jones, D.P., Carlson, J.L., Samiec, P.S., Sternberg Jr, P., Mody Jr, V.C., Reed, R.L. and Brown, L, A.S. 1998. Glutathione measurement in human plasma evaluation of sample collection, storage and derivatization condition for analysis of dansyl derivatives by HPLC. *Clinica Chimica acta,* **275:** 175-184.

Joshi, R., Kamat, J.P. and Mukherjee, T. 2007. Free radical scavenging reaction and antioxidant activity of embelin: Biochemical and pulse radiolytic studies. *Chemical Biology Interaction,* **167:** 125-134.

Joubert, E., Gelberblom, W.C.A., Louw, A. and de Beer, D. 2008. South African herbal teas: *Aspalathus linearis*, *Cyclopia spp*, and Athrixia phylicoides - A review. *Journal of Ethnopharmacology,* **119:** 376-412.

Joubert, E. 1996. HPLC quantification of the dihydrochalcones, aspalathin and nothofagin in rooibos tea (*Aspalathus linearis*) as affected by processing. *Food Chemistry,* **55(4):** 403-411.

Joubert, E., Otto, F., Grüner, S. and Weinreich, B. 2003. Reserved – phased HPLC determination of mangiferin, isomangiferin and hesperidin in cyclopia and the effect of composition of *C. genistoides*. *European Food and Research Technology,* **216:** 270-273.

Joubert, E., Winterton, P., Britsz, T.J. and Ferreira, D. 2004. Superoxide anion and α,α-diphenyl-β-picrylhydrazyl radical scavenging capacity of roibos (*Aspalathus linearis*) aqueous extracts, crude phenol fractions, tannin and flavonoids. *Food Reseach International,* **37:** 133-138.

Kalousová, M., Zima, T., Tessar, V., Dusilova-Sulkava, S. and Skrha, J. 2005. Advanced glycoxidation end products in chronic diseases - clinical chemistry and genetic background. *Mutation Research,* **579:** 37-46.

Kampa, M., Nistikaki, A., Tsaousis, V., Maliaraki, N., Notas, G. and Castanas. E. 2002. A new automated method for the determination of the total antioxidant capacity (TAC) of human plasma, based on the crocin bleaching assay. *BMC Clinical Pathology,* **2:** 1-16.

Kaplan, L.A. and Pesce, A.J.1984. Theory analysis and correlation. *Clinical Chemistry.* The C.V. Mosby Company, p. 918.

Karl-Heinz, K. 2007. Aging: A revisited theory based on free radicals generated by NOX family NADPH oxidases. *Experimental Gerontology,* **(42)4:** 256-262.

Karpe, F. 1997. Mechanisms of postprandial hyperlipidaermia-remnants and coronary artery disease. *Diabete Medicine,* **3:** S60-S66.

Kay, C.D. and Holub, B.J. 2003. The postprandial effect of dietary antioxidants in humans. *Current Artherosclerosis Reports,* **5(6):** 452-458.

Kathib, S., Musa, R. and Vaya, J. 2007. An exogenous marker: A Novel approach for the characterization of oxidative stress. *Bioorganic and Medical Chemistry,* **15:** 3661-3666.

Kawano, H., Motoyama, T., Hirashima, O., Hirai, N., Miyao, Y., Sakamoto, T., Kugiyama, K., Ogawa, H. and Yasue, H.1999. Hyperglycemia rapidly suppresses flow-mediated endothelium-dependent vasodilation of brachial artery. *Journal of American College of Cardiology,* **34:** 146-154.

Khan, A. and Gilani, A.H., 2006. Selective broncholdilatory effect of rooibos tea (*Aspalathus linearis*) and its flavonoid, chrysoeriol. *European Journal of Nutrition,* **45:** 463-469.

Koeppen, B. H., Smit, C. J. B. and Roux, D. G. 1962. The flavone C-glycosides and the flavononol *O*-glycosides of Aspalathus acuminatus (Rooibos tea). *Journal of Biochemistry*, **83:** 507-511.

Koeppen, B. H. and Roux, D. G. 1965a. *C*-glycosulflavonoids. *Biochemistry Journal,* **95:** 444-448.

Koeppen, B. H. and Roux, D. G. 1965b. *C*-glycosulflavonoids. The chemistry of orientin and iso-orientin. *Journal of Biochemistry,* **97:** 444-448.

Koeppen, B. H. and Roux, D. G. 1966 C-glycosulflavonoids. The chemistry of aspalathin. *Journal of Biochemistry,* **99:** 604-609.

Kolodziej, H., Haberland, C., Woerdenbag, H. J. and Konings, A. W. T. 1995. Moderate cytotoxicity of proanthocyanidins to human tumor cell lines. *Phytotherapy Research,* **9:** 410-415.

Kolonel, L.N., Altshuler, D. and Henderson, B.E., 2004. The multiethnic cohort study: exploring genes, lifestyle and cancer risk. *Nature Reviews Cancer***, 4(7):** 519-527.

Krasinski, S.D., Cohn J.S., Schaefer E.J. and Russell R.M. 1990. Postprandial plasma retinyl ester response is greater in older subjects compared with younger subjects. Evidence for delayed plasma clearance of intestinal lipoproteins. *Journal of Clinical Investigation,* **85:** 883-892.

Krebs-Smith, S.M. 2001. Choose beverages and foods to moderate your intake of sugar: Measurement requires quantification. *Journal of Nutrition,* **131:** 527S-535S.

Kris-Etherton, P.M., Heker, K.D., Bonanone, A., Coval, S.M., Binkoski, A.E., Hilpert, H.F., Griel, A.E. and Ethrton, T.D. 2003. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *The American Journal of Medicine,* **113(9):** 71-88.

Kronborg, J., Johnsen, S.H., Njølstad, I., Toft, I., Eriksen, B.O. and Jenssen, T. 2007. Proinsulin: insulin and insulin:glucose ratios as predictors of carotid plaque growth: a population-based 7 year follow-up of the Tromsø Study. *Diabetologia,* **50:** 1607-1614.

Kuhnt, K., Wagner, A., Kraft, J., Basir, S and Jahreis, G. 2006. Dietary supplementation with 11 trans- and 12 trans-18:1 and oxidative stress in humans. *The American Journal Clinical Nutrition,* **84:** 981-988.

Kunishiro, K., Tai, A. and Yamamoto, I. 2001. Effects of rooibos tea extract on antigenspecific antibody production and cytokine generation *in vitro* and *in vivo*. *Bioscience, Biotechnology and Biochemistry,* **65:** 2137-2145.

Lairon, D., Play, B. and Jourdheuil-Rahmani, D. 2007. Digestible and indigestible carbohydrates: interactions with postprandial lipid metabolism. *The Journal of Nutritional Biochemistry,* **18(4):** 217-227.

Lamošová, D., Juráni, M., Greksák, M., Nakano, M. and Vaneková, M. 1997. Effect of rooibos tea (*Aspalathus linearis*) on chick skeletal cell growth in culture. *Comparative Biochemistry and Physiology,* **116(1):** 39-45.

Lancelot, E. and Grauby-Heywang, C. 2007. Comparison of the interaction of dihydrocholesterol and cholesterol with sphingolipid or phospholipids Langmuir monolayers. Colloids and Surfaces: *Biointerfaces,* **59:** 81-86.

Landmesser, U., Spiekermann, S., Dikalov, S., Tatge, H., Wilke, R., Kohler, C., Harrison, D.G., Hornig, B. and Drexler, H. 2002. Vascular oxidative stress and endothelial dysfunction in patients with chronic heart failure: Role of xanthine oxidase and extracellular superoxide dismutase. *Circulation,* **106:** 3073-3078.

Lau, F.C., Shukitt-Hale, B. and Joseph, J.A. 2005. The beneficial effects of fruit polyphenols on brain aging. *Neurobiology of Aging,* **26S:** S128-S132.

Lautt, W.W. 2007. Postprandial insulin resistance as an early predictor of cardiovascular risk. *Therapeutics and Clinical Risk Management,* **3(5):** 761-770.

Lee, A.T. and Cerami, A. 1992. The role of glycation in aging. *Annals of the New York Academy of Science,* **663:** 63-70.

Lee, C-Y. J., Issac, H.B., Wang, H., Huang, S.H., Long, L-H., Jenner, A.M., Kelly, R.P. and Halliwell, B. 2006. Cautions in the use of biomarkers of oxidative damage; the vascular and antioxidant effects of dark soy sauce in humans. *Biochemistry and Biophysic Research Communications,* **344:** 906-911.

Lee, E-J. and Jang, H-D. 2004. Antioxidant activity and protective effect on DNA strand scission of rooibos tea (Aspalathus linearis). *BioFactors,* **21(1-4):** 285-292.

Lee, K.W., Lee, H.J. and Lee, C.Y. 2002. Antioxidant activity of black tea vs. green tea. *Journal of Nutrition,* **132:**785-786.

Lee, M.J., Wang, Z.Y., Li, H., Chen, L., Sun, Y., Gabbo, S., Balentine, D.A. and Yang, C.S. 1995. Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemiology, Biomarkers and Preventors,* **4(4):** 393-399.

Lefèbvre, P.J. and Scheen, A.J. 1998. The postprandrial state and risk of cardiovascular disease. *Diabetic Medicine,* **15(S4):** S63-S68.

Lehohla, P. 2006. Knowing causes of death is crucial for planning. http://www.statssa.gov.za/ news_archive/14 September 2006.

Li, D., Wang, Q., Yuan, Z.F., Zhang, L., Xu, L., Cui, Y. and Duan, K. 2008. Pharmacokenetics and tissue distribution study of orientin in rat by liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis,* **47:** 429-434.

Li, X., Huo, T., Qin, F., Lu, X. and Li, F. 2007. Determination and pharmacokinetics of orientin in rabbit plasma by liquid chromatography after intravenous administration of orientin and *Trollius chinensis* Bunge extract. *Journal of Chromatography B,* **853:** 221-226.

Libby, P., Ridker, P.M. and Attilio Maseri, A. 2002. Inflammation and atherosclerosis. *Circulation,* **105:** 1135-1143.

Linares, V., Alonso, V., Albina, M.L., Bell´es, M., Sirvent, J.J., Domingo, J.L. and S´anchez, D.J. 2009. Lipid oxidation and antioxidant status in kidney and liver of rats treated with sulfasalazine. *Toxicology,* **256:** 152-156.

Lohman, T., Roche, A. and Martorell, R. (eds). 1988. *Anthropometric standardization reference Manual*. Campaingn IL: Human kinetics Books, p. 1-112.

Luczaj, W. and Skrzdlewska, E. 2005. Antioxidative properties of black tea. *Preventive Medicine,* **40:** 910-918.

Ludewig, B., Zinkernagel, R.M. and Hengartner, H. 2002. Arterial inflammation and atherosclerosis. *Trends in Cardiovascular Medicine,* **12(4):** 154-159.

Luximo-Ramma, A., Bahorun, T., Crozier, A., Zbarsky, V., Datla, K.P., Dexter, D.T. and Aruoma, O.I. 2005. Characterization of the antioxidant functions of flavonoids and proanthocyanidins in Mauritian balck teas. *Food Research International,* **38:** 357-367.

Ma, S. W., Tomlinson., B. and Benzie, I.F.F. 2005. A study of the effect of oral glucose loading on plasma oxidant: antioxidation balance in normal subjects. *European Journal of Nutrition,* **44:** 250-254.

Macharia, M. 2009. Modulation of oxidative stress biomarkers by rooibos in adults at risk for developing cardiovascular disease. 1-89.

MacMahon, S., Peto, R., Cutler, J., Collins, R., Sorlie, P., Neaton, J., Abbott, R., Godwin, J., Dyer, A. and Stamler, J. 1990. Blood pressure, stroke, and coronary heart disease. Part 1: Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet,* **335:** 765-774.

Mamo, J. C. L., Yu, K. C. W., Elsegood, C. L. Smith, D., Vine, D., Gennat, H.C., Voevodin, M. and Proctor, S.D. 1997. Is atherosclerosis exclusively a postprandial phenomenon*? Clinical and Experimental Pharmacology and Physiology*, **24:** 288-293.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., and Jiménez, L. 2004. Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition,* **79:** 727-747.

Manuel-y-Keenoy, B., van Campenhout, A., Aerts, P., Vertommen, J., Abrams, P., van Gaal, L.F., van Gils, C. and De Leeuw, I.H. 2005. Time course of oxidative stress status in the postprandrial and postabsorptive state in type 1 diabetes mellitus: Relationship to glucose and lipi changes. *Journal of the American College of Nutrition,* **24(6):** 474-485.

Marais, C., van Rensburg, W.J., Ferrera, D. and Steenkamp, J.A., 2000. (S)- and (R)-Erodictyol-6-C-β-D-glucopyranoiside, novel keys to the fermentation of rooibos (*Aspalathus linearis*). *Phytochemistry,* **55:** 43-49.

Mardis, A.L.2001. Current knowledge of the health effects of sugar intake. *Center for Nutrition Policy and Promotion,* **13(1):** 87.

Marnewick, J.L., Gelderblom, W.C.A. and Joubert, E. 2000. An investigation on the antimutagenic properties of South African herbal teas. *Mutation Research,* **471:** 157-166.

Marnewick, J.L., Batenburg, W., Swart, P., Joubert, E., Swanevelder, S. and Gelderblom, W.C.A. 2004. Ex vivo modulation of chemical-induced mutagenesis by subcellular liver fractions of rats treated with rooibos (*Aspalathus linearis*) tea, honeybush (*Cyclopia intermedia*) tea, as well as green and black (*Camellia sinensis*) teas. *Mutation Research,* **558:** 145-154.

Marnewick, J., Joubert, E., Joseph, S., Swanevelder, S., Swart, P. and Gelderblom, W. 2005. Inhibition of tumor promotion in mouse skin by extract of rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermerdia*), unique South African herbal tea. *Cancer Letters,* **224:** 193-202.

Marnewick, J.L. and Gelderblom, W.C.A. 2006. A comporative study on the antimutagenic properties of aqueous extracts of *Asparlathus linearis* (rooibos), different *Cyclopia spp.* (honeybush) and *Camellia sinensis* teas. *Mutation Research,* **611:** 42-53.

Marnewick, J.L., van der Westhuizen, F.H., Joubert, E. Swanevelder, S., Swart, P. and Gelderblom, W.C.A. 2009. Chemoprotective properties of rooibos *(Aspalathus linearis),* honeybush (Cyclopia intermedia) herbal and green and black *(Camellia sinensis)* teas against cancer promotion induced by fumonisin B1 in rat liver. *Food and Chemical Toxicology,* **47:** 220-229.

Martindale, J.L. and Holbrook, N.J., 2002. Cellular response to oxidative stress: Signaling for suicide and survival. *Journal of Cellular Physiology,* **192:** 1-15.

Matsuda, K., Nishimura, Y., Kurata, N., Iwase, M. and Yasuhara, H. 2007. Effects of continuous ingestion of herbal teas on intestinal CYP3A in the rat. *Journal of Pharmacological Sciences,* **103(2):** 214-221.

Mayne, S.T. 2003. Antioxidant nutrients use of biomarkers of exposure and oxidative stress in epidemiologic research. *Journal of Nutrition,* **133:** 933S-940S.

McKeone, B.J., Patsch, J.R. and Pownall, H.J. 1993. Plasma triglycerides determine low-density lipoprotein composition, physical properties, and cell-specific binding in cultured cells. *Journal of Clinical Investigation,* **91(5):** 1926-1933.

McMahon, M., Marsh, H.M. and Riza, R.A. 1989. Effects of basal insulin supplementation on disposition of mixed meal in obese patient with NIDDM. *Diabetes,* **38(3):** 291-303.

Mertens, A. and Holvoeri, P. 2001. Oxidized LDL and HDL: antagonists in atherothrombosis. *Federation of American Societies for Experimental Biology,* **15:** 2073-2084.

Mitrakou, A., Kelly, D., Veneman, T., Jenssen, T., Pangburn, T., Reilly, J. and Gerich, J. 1990. Contribution of abnormal muscle and liver metabolism to postprandial hyperglycemia in NIDDM. *Diabetes,* **39:** 1381-1390.

Monier, L., Mas, E., Ginet, C., Michel, F., Villon, L., Cristol, J-P. and Colette, C. 2006. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *Journal of the American Medical Association,* **295:** 1681-1687.

Moore, J. and Yu, L. 2008. *Methods for antioxidant capacity estimation of wheat and wheat-based food products.* In Yu, L. Wheat antioxidants, Wiley, 118-119.

Morgan, K.J. and Zabik, M.E. 1981. Amount and food sources of total sugar intake by children ages 5 to 12 years. *The American Journal of Clinical Nutrition,* **34(3):** 404-413.

Mousavinejad, G., Emam-Djomeh, Z., Rezaei, K. and Khodaparast, M.H.H. 2009. Identification and quantification of phenolic compounds and their effects on antioxidant activity in pomegranate juices of eight Iranian cultivars. **115(4):** 1274-1278.

Mukhopadhyay, P., Rajesh, M., Yoshihiro, K., Harko, G. and Pacher, P. 2007. Simple quantitative detection of mitochondrial superoxide productive in lives cells. *Biochemical and Biophysical Research Communications,* **358(1):** 203-208.

Mussali-Galante, P., Rodriguez-Lara, V., Avila-Costa, M.R. and Fortoul, T.I. 2007. *Mechanisms of vanadium toxicity in vadadium, Its impact on health*, eds: Fortoul, T.I. and Avila-Costa, M.R. Nova Science Publishers, Inc., 1-79.

Nair, J., De Flora, S., Izzotti, A. and Bartsch, H. 2007. Lipid oxidation-derived etheno-DNA adducts in human atherosclerotic lesions. *Mutation Research,* **621:** 95-105.

Naithani, V., Nair, S. and Kakkar, P. 2006. Decline in antioxidant capacity of Indian herbal teas during storage and its relation to phenolic content. *Food Research International,* **39:** 176-181.

Nakano, M., Itoh, Y., Mizuno, T. and Nakashima, H. 1997b. Polysaccharide from *Aspalathus linearis* with strong anti-HIV activity. *Bioscience, Biotechnology and Biochemistry,* **61:** 267-271.

Nakano, M., Nakashima, H. and Itoh, Y. 1997a. Anti-human immunodeficiency virus activity of oligosaccharides from rooibos tea (*Aspalathus linearis*) extracts in vitro. *Leukemia,* **11(supplement 3):** 128-130.

Natella, F., Belelli, F., Gentili, V., Ursini, F. and Scaccini, C. 2002 Grape seed proanthocyanidins prevent plasma postprandial oxidative stress in humans. *Journal of Agricultural and Food chemistry,* **50(26):** 7720-7725.

Natella, F., Ramberti, A. and Scaccini, C. 2008. A role of BRCA1-associated protein BARD1 in oxidative stress response and signaling and proliferation control. *In oxidants in biology*. Springer Netherlands, p. 10-35.

Neeley, W.E. 1972. Simple automated determination of serum or plasma glucose by a hexokinase/glucose-6 phosphate dehydrogenase method. *Clinical Chemistry,* **18(6):** 509-515.

Neri, S., Signorelli, S.S., Torrisi, B., Pulvirenti, D., Mauceri, B., Abate, G., Ignaccolo, L., Bordonaro, F., Cilio, D., Calvagno, S. and Leotta, C. 2005. Effects of antioxidant supplementation on postprandial oxidative stress and endothelial dysfunction: A single - blind, 15 - Day clinical trial in patients with untreated type 2 diabetes, subjects with impaired glucose tolerance, and healthy controls. *Clinical Therapeutics,* **27(11):** 1764-1773.

Nel, E., Binns, T. and Bek, D. 2007. Alternative foods and community based development: rooibos tea production in South Africa’s West Coast mountains. *Applied Geography,* **27:** 112-129.

Nilsson, L.H., and Hutman, E. 1974. Liver and muscle glycogen in man after glucose and fructose infusion. *Scandinavian Journal of Clinical and Laboratory Investigation,* **33:** 5-10.

Niki, E., Yoshida, Y., Saito, Y. and Noguchi, N. 2005. Lipid oxidation: Mechanisms, inhibition, and biological effects. *Biochemical and Biophysical Research Communications,* **338:** 668-676.

Nishigaki, I., Hagihara, M., Tsunekawa, H., Maseki, M. and Yagi, K. 1981. Lipid peroxide levels of serum lipoprotein fractions of diabetic patients. *Biochemica Medicine,* **25:** 373-378.

Nishitani, E. and Magesaka, Y.M. 2004. Simultaneous determination of catechins, caffeine and other phenolic compounds in tea using new HPLC method. *Journal of Food Composition and Analysis,* **17:** 675-685.

Okamoto, k., Toyokumi, S., Kim, W.J., Oqawa, O., Kakehi, Y., Arao, S., Hiai, H. and Yoshida, O. 1996. Overexpression of human mut T homologue gene messager RNA in renal- cell carcinoma: evidence of persistent oxidative stress in cancer. *International Journal of Cancer,* **65(4):** 437-441.

Okamura, S. and Tamura, H-O. 2004. Effect of herbal teas on conjugation reactions in a human colon carcinoma cell line, caco-2. *Journal of Health Science,* **50(2):** 189-192.

O'Keefe, J.H. and Bell, D.S.H. 2007. Postprandial hyperglycemia/ hyperlipidemia (postprandial dysmetabolism) is a cardiovascular risk factor. *The American Journal of Cardiology,* **100(5):** 899-904.

O'Keefe, J.H., Gheewala, N.M. and O'Keefe J.O. 2008. Dietary strategies for improving post-Prandial glucose, lipids, inflammation, and cardiovascular health. *Journal of the American College of Cardiology,* **51(3):** 249-255.

O'Keefe, J.H., Harris, W.S., Nelson, J. and Windsor, S.L. 1995. Effects of pravastatin with niacin or magnesium on lipid levels and postprandial lipemia. *The American Journal of Cardiology,* **76(7):** 480-484.

Orhan, H., Guerer-Orhan, H., Vriese, E., Vermeulen, N.P.E. and Meerman, J.H.N. 2006. Application of lipid oxidation and protein oxidation biomarkers for oxidative damage in mammalian cells. A comparison with two fluorescent probes. *Toxicology in Vitro,* **20:** 1005-1013.

Ou, B., Hampsch-Woodill, M. and Prior, R.L. 2001. Development and validation of an improved oxygen radical absorbance capacity using fluorescein as fluorescent probe. *Journal of Agriculture Food and chemistry,* **49:** 4619-4626.

Pacher, P., Beckman, J.S. and Liaudet, L. 2007. Nitric oxide and peroxynitrite in health and disease. *Physiological Reviews,* **87**: 315-424.

Palmer, M. 2006. Metabolism course notes, University of Waterloo.

Parhofer, K.G., Barrett, P.H. and Schwandt, P. 2000. Atorvastatin improves postprandial lipoprotein metabolism in normolipidemlic subjects. *Journal of Clinical Endocrinology and Metabolism,* **85:** 4224-4230.

Park, Y. and Harris W. 2003. Omega-3 fatty acid supplementation accelerates chylomicron triglyceride clearance. *Journal of Lipid Research,* **244:** 455-463.

Parr, A., and Bolwell, G.P. 2000. Phenols in man: The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of the Science of Food and Agriculture,* **80:** 985-1012.

Pastukhow, A.V., Levchenko, L. A. and Sadkow, A.P. 2007. Spectroscopic study on binding of rutin to human serum albumin. *Journal of Molecular Structure*, **842:** 60-66.

Pasqualotto, F.F., Sharma, R.K., Nelson, D.R., Thomas Jr, A.J. and Agarwal, A. 2000. Relationship between oxidative stress, semen characteristics and clinical diagnostics in men undergoing infertility investigation. *Fertility and Sterility,* **73(3):** 458-464.

Pasqualotto, F.F., Sharma, R.K., Kobayashi, H., Nelson, D.R., Thomas Jr, A.J. and Agarwal, A. 2001. Oxidative stress in normospermic men undergoing infertility evaluation. *Journal of Andrology,* **22(2):** 316-322.

Pastukhov, A.V., Levchenko, L.A. and Sadkov, A.P. 2007. Spectroscopy study on binding of rutin to human serum albumin. *Journal of Molecular Structure,* **847:** 60-66.

Patsch, J.R., Miesenbock, G., Hopferwieser,T. Muhlberger, V., Knapp, E., Dunn, J.K., Gotto Jr, A.M. and Patsch, W. 1992. Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. *Arteriosclerosis and Thrombosis,* **12:** 1336-1345.

Persson, I.A-L., Josefsson, M., Persson, K. and Andersson, R.G.G. 2006. Tea flavanols inhibit antiotensin-converting enzyme activity and increase nitric oxide production in human endothelial cells. *Journal of Pharmacy and Pharmacology,* **58:** 1139-1144.

Petit, V., Niot, I., Poirier, H. and Besnard, P. 2007. Absoption intestinale des acides gras: Faits et incertitutudes fatty acids intestinal absorption: Facts and uncertainties. *Nutrition Clinique et Metabolisme,* **21:** 38-45.

Plotnick, G.D., Corretti, M.C. and Vogel, R.A. 1997. Effect of antioxidant vitamins on the transient impairment of endothelium-dependent brachial artery vasoactivity following a single high-fat meal. *Journal of the American Medical Association,* **278:** 1682-1686.

Podrez, E. A., Schmitt, D., Hoff, H. F. and Hazen, S. L. 1999. Myeloperoxidase-generated reactive nitrogen species convert LDL into an atherogenic form *in vitro*. *Journal of Clinical Investigation,* **103:** 1547-1560.

Poirier, B., Michel, O., Bazin, R., Bariéty, J., Chevalier, J., Myara, I. and Gaston, A-T. 2001. Conjugated dienes: A critical trait of lipoprotein oxidizability in renal fibrosis. *Nephrol Dial Transplant,* **16:** 1598-1606.

Puavilai, W. and Laoragpongse, D. 2004. Is calculated LDL-C by using the new modified Friedewald equation better than the standard Friedewald equation? *Journal of Medical Association of Thailand,* **87:** 589-593.

Puavilai, W., Laorugpongse, D., Deerochanawong, C., Muthapongthavorn, N. and Srilert, P. 2009. The accuracy in using modified Friedewald equation to calculate LDL from non-fast triglyceride: A pilot study. *Journal of Medical Association of Thailand,* **92(2):** 182-187.

Pyorala, K., Laakso, M. and Uusitupa, M. 1987. Diabetes and atherosclerosis: an epidemiologic view. *Diabetes/Metabolism Research and Reviews,* **3:** 463-524.

Queneau, Y., Fitremann, J. and Trombotto, S. 2004. The chemistry of unprotected sucrose: the selectivity issue. *Comptes Rendus Chimie,* **7(2):** 177-188.

Rabe, C., Steenkamp, J.A., Joubert, E., Burger, J.F.W. and Ferreira, D. 1994. Phenolic metabolites from rooibos tea (*Aspalathus linearis*). *Phytochemistry,* **35(6):** 1559-1565.

Rajasekar, P. and Anuradha, C.V. 2007. Fructose-induced hepatic gluconeogenesis: Effect of L-carnitine. *Life Sciences,* **80:** 1176-1183.

Ramos, P., Gieseg, S. P., Schuster, B. and Esterbauer, H. 1995. Effect of temperature and phase transition on oxidationresistance of low-density lipoprotein. *Journal of Lipid Research,* **36:** 2113-2128.

Recknagel, R.O. and Glende Jr, E.A. 1984. Specrophotometric detection of lipid conjugated dienes. *Methods in Enzymology,* **105:** 331-337.

Reynecke, J., Coetzee, W.H.K. and Bester, J.J.A. 1949. Rooibos tea. A preliminary report on the composition. *Farming in South Africa,* **24:** 397-412.

Rice-Evans, C. and Miller, N.J. 1994. Total antioxidant status in plasma and body fluids. *Methods Enzymology,* **234:** 279-293.

Ridker, P.M., Cushman, M., Stampter, M.J., Tracy, R.P. and Hennekens, C.H. 1997. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *England Journal Medicine,* **336:** 973-979.

Ridker, P.M. 2003. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation,* **107:** 363-369.

Robertson, M.D. 2006. Food perception and postprandial lipid metabolism. *Physiology & Behavior,* **89:** 4-9.

Rozner, S. and Garti, N. 2006. The activity and absorption relationship of cholesterol and phytosterols. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* **282-283:** 435-456.

Rubanyi, G.M. and Vanhoutte, P.M. 1986. Superoxide anions and hyperoxia inactivate endothelium derived relaxing factor. *American Journal of Physiology,* **250:** H822-H827.

Russo, L.G. 2009. Dietary n S 6 and n S 3 polyunsaturated fatty acids: From biochemistry to clinical implications in cardiovascular Prevention. *Biochemical Pharmacology,* **77:** 937-946.

Ruxton, C.H., Garceau, F.J. and Corttrell, R.C. 1999. Guidelines for sugar consumption in Europe: Is a quantitative approach justified? *European Journal of Clinical Nutrition,* **53(7):** 503-519.

Sakamoto, K., Tominaga, Y., Yamauchi, K., Nakatsu, Y., Sakumi, K., Yoshiyama, K., Egashira, A., Kura, S., Yao, T., Tsuneyoshi, M., Maki, H., Nakabeppu, Y. and Tsuzuki, T. 2007. MUTYH-Null mice are susceptible to spontaneous and oxidative stress–induced intestinal tumorigenesis. *Cancer Research,* **67:** 6599-6604.

Sakata, N., Moh, A. and Takebayashi, S. 2002. Contribution of superoxide to reduced antioxidant activity of glucoxidative serum albumin. *Heart Vessels,* **17:** 22-29.

Samiec, P.S., Drews-Botsch, C., Flagg, E.W., Kurtz, J.C., Sternberg Jr, P. Reed, R.L. and Jones, D.P. 1998. Glutathione in human plasma: Decline in association with aging, age-related mascular degeneration and diabetes. *Free Radical Bioloy & Medicine,* **24(5):** 699-704.

Sánchez-Moreno, C., Dorfman, E.S., Lichtenstein, H.A. and Martín, A. 2004. Dietary fat type affects vitamins C and E and biomarkers of oxidative status in peripheral and brain tissues of golden Syrian hamsters. *Journal of Nutrition,* **134:** 655-660.

Sandra, B. 2003. The double danger of high fructose corn syrop. http://www.westonaprice.org/ modernfood/highfructose.html.

Santana-Rios, G., Orner, G.A., Amantana, A., Provost, C., Wu, S.Y. and Dashwood, R.H. 2001. Potent antimutagenic activity of white tea in comparison with green tea in the Salmonella assay. *Mutation Research,* **495(1-2):** 61-74.

Santos-Buelga, C. and Scalbert, A. 2000. Proanthocyanidins and tanninlike compounds in human nutrition. *Journal of Food Science and Agriculture,* **80:** 1094-1117.

Sasase, T., Morinaga, H., Yamamoto, H., Ogawa, N., Matsui, K., Miyajima, K., Kawai, T., Mera, Y., Masuyama, T., Shimohara, M., Ohta, T. and Matsushita, M. 2007. Increased fat absorption and impaired fat clearance cause postprandrial hypertriglyceridemia in spontaneously diabetic torui rat. *Diabetes Research and Clinical Pratice,* **78(1):** 8-15.

Sasaki, K., Mitsumoto, M. and Kawabata, K. 2001. Relationship between lipid oxidation and fat content in Japanese Black beef longissimus muscle during storage. *Meat Science,* **59**: 407-410.

Sayre, L. M., Smith, M.A. and Perry, G. 2001. Chemistry and biochemistry of oxidative stress in neurodegerative disease.*Journal of Medicinal Chemistry,* **8:** 721-738.

Scalbert, A., Johnso, I.T. and Saltmarsh, M. 2005. Polyphenols: antioxidants and beyond. *The American Journal of Clinical Nutrition,* **81(suppl):** 215S-217S.

Scanto, S. and Yudkin, J. 1969. The effect of dietary sucrose on blood lipids, serum insulin, platelet adhesiveness and body weight in human volunteers. *Postgraduate Medicine Journal,* **45:** 602-607.

Schafer, F.Q. and Buettner, G.R. 2001. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radical Biology & Medicine*, **30(11):** 1119-1212.

Schchiri, M., Kishikawa, H., Ohkubo, Y. and Wake, N. 2000. Long-term results of the Kumamoto Study on optimal diabetes control in type 2 diabetic patients. *Diabetes Care,* **23(Suppl 2):** B21-B29.

Scheepers, S. 2001. Anti-microbial activity of rooibos tea (*Aspalathus linearis*) on food spoilage organisms and potential pathogens. M.Sc. (Food Science) Thesis. Stellenbosch University, Stellenbosch, South Africa.

Schönfeld, P. and Wojtczak, L. 2007. Fatty acids decrease mitochondrial generation of reactive oxgen species at the reserve electron transport but increase it at the forward transport. *Biochimica et Biophysica Acta,* **1767:** 1032-1040.

Schulz, H., Jourbet, E. and Schütze, W. 2003. Quantification of quality parameters for reliable evaluation of green rooibos (*Aspalathus linearis*). *European Food research and Technology,* **216:** 539-543.

Scorolli, L., Meduri, A., Morara, M., Scalinci, S.Z., Greco, P., Meduri, R.A. and Colombati, S. 2008. Effect of cysteine in transgenic mice on healing of corneal epithelium after excimer laser photoablation. *Ophthalmologica,* **222:** 380-385.

Seeram, N.P., Lee, R. and Heber, D. 2004. Bioavailability of ellagic acid in human plasma after consumption of ellagitannins from pomegranate (Punica granatum L.) juice. *Clinica Chimica Acta,* **348:** 63-68.

Seifried, H.E., Anderson, D.E., Fisher, E.I. and Milner, J.A. 2007. A review of the interaction among dietary antioxidants and reactive oxygen species. *Journal of Nutrition Biochemistry,* **18:** 567-579.

Sen, T., Sen, N., Tripathi, G., Chatterjee, U. and Chakrabarti, S. 2006. Lipid oxidation associated cardiolipin loss and membrane depolarization in rat brain mitochondria. *Neurochemistry International,* **46:** 20-27.

Seyed-Ali, A., Mohammad-Ali, B., Katayoun, G-M., Parvin, T. and Seyed-Mohammad, D. 2008. The impact of low serum triglyceride on LDL-cholesterol estimation. *Archives of Iranian Medicine,* **11(3):** 318-321.

Sharma, R.K., Pasqualotto, F.F., Nelson, D.R., Thomas Jr, A.J. and Agarwal, A. 1999. The reactive oxygen species-total antioxidant capacity score is a new measurement of oxidative stress to predict male infertility. *Human Reproduction,* **14(11):** 2801-2807.

Shelgikar, P.J., Deshpande, K.H., Sardeshmukh, A.S., Katkam, R.V. and Suryakar, A.N. 2005. Role of oxidants and antioxidants in ARF patients undergoing hemodialysis. *Indian Journal of Nephrology,* **15:** 73-76.

Shigenaga, M.K., Hagen, T.M. and Ames, B.N. 1994. Oxidative damage and mitochondrial decay in aging. *Proceedings of the National Academy of Sciences,* **91:** 10771-10778.

Shimoi, K., Okanda, H., Furugori, M., Goda, T., Takasa, S., Suzuki, M., Hara, Y., Yamamoto, H. and Kinae, N. 1998. Intestinal absorption of luteolin and luteolin 7-O-β-glucoside in rats and humans. *Federation of American Societies for Experimental Biology,* **438:** 220-224.

Shindo, Y. and Kato, K., 1991. Effect of rooibos tea on some dermatological diseases. In: *Proceedings of the International Symposium on tea Science*, the organizing committee of ISTS, Shizuoka, Japan, 385-389.

Sies, H. Oxidative stress: 1997. Oxidants and antioxidants. *Experimental Physiology,* **82(2):** 291-295.

Sies, H. 1999. Glutathione and its role in cellular functions. *Free Radical Biology & Medicine,* **27(9/10):** 916-921.

Sies, H., Stahl, W. and Sevanian, A. 2005. Nutritional, dietary and postprandial oxidative stress. *Journal of Nutrition,* **135:** 969-972.

Simon, M., Horovska, L., Greksak, M., Dusinsky, R. and Nakano, M. 2000. Antihemolytic effect of rooibos tea (*Aspalathus linearis*) on red blood cells of Japanese quails. *General Physiology and Biophysics,* **19:** 365-371.

Simopoulos, A.P. 2002. Omega-3 Fatty acids in inflammation and autoimmune diseases. *Journal of the American College of Nutrition,* **21(6):** 495-505.

Singh, U., Devaraj, S. and Jialal, I. 2005. Vitamin E, oxidative stress, and inflammation. *Annual Reviews of Nutrition,* **25:** 151-174.

Singh, U., Devaray, S., Vasquez-Vivar, J. and Jialal, I. 2007. C-reactive protein decreases endothelial nitric oxide sythase activity via uncoupling. *Journal of Molecular and Cellular Cardiology,* **43:** 780-791.

Skinner, J.D, Canuth, B.R., Moran, J. 3rd, Houck, K. and Coleta, F. 1999. Fruit juice intake is not related to children's growth, **103:** 58-64.

Slama, G., Elgrably, F., Sola, A., Mbemba, J. and Larger, E. 2006. Postprandial glycaemia: a plea for the frequent use of delta postprandial glycaemia in the treatment of diabetic patients. *Diabetes & metabolism,* **32(2):** 187-192.

Smit, A.B., van Kesteren, R.E., van Minner, L.J., Spijker, S., van Heerikhuizen, H. and Geraerts, W.P.M. 1998. Towards understanding the role of insulin in the brain: lesson from insulin-related signalling systems in the invertebrate brain. *Progress in Neurobiology,* **54:** 35-54.

Smith, A.F., Beckett, G.J., Walker, S.W. and Rae, P.W.H. 1998. 6th ed. *Clinical Biochemistry,* Blackwell Science, p. 1-259.

Smith, D., Watts, G. F., Dane-Steward, C. and Mamo, J. C. L. 1999. Post-prandial chylomicron response may be predicted by a single measurement of plasma apolipoprotein B48 in the fasting state. *European Journal of Clinical Investigation,* **29(3):** 204-209.

Smolková, B., Dušinská, M., Rašlová, K., Geraldine McNeill, G., Spustová, V., Blažíček, P., Horská, A. and Collins, A. 2004. Seasonal changes in markers of oxidative damage to lipids and DNA; correlations with seasonal variation in diet. *Mutation Research,* **551:** 135-144.

Snijman, P.W., Swanevelder, S., Joubert, E., Green, I.R. and Gelderblom, W. 2007. The antimutagenic activity of the major flavonoids of rooibos (*Aspalathus linearis*): Some dose-response effects on mutagen activation-flavonoid interactions. *Mutation Research,* **631:** 111-123.

Snyckers, F. O. and Salemi, G. 1974. Studies of South African medicinal plants. Part 1: Quercetin as the major in vitro active component of rooibos tea. *Journal of South African Chemistry Institute,* **27:** 5-7.

Song, J.H., Fujimoto, K. and Miyazawa, T., 2000. Polyunsaturated (n-3) fatty acids susceptible to oxidation are increased in plasma and tissue lipids of rats fed docosahexaenoic acid-containing oils. *Journal of Nutrition,* **130:** 3028-3033.

Song, J., Li, J., Lulla, A., Mark Evers, B. and Chung, D.H. 2006. Protein kinas D protects against oxidative stress – induced intestinal epithetial cell injury via Rho/ROK/PKC-δ pathway activation. *American Journal of Physiology Cell Physiology,* **290:** C1469-C1476.

Stangl, V., Lorenz, M. and Stangl, K. 2006. The role of tea and tea flavonoids in cardiovascular health. *Molecular Nutrition and Food Research,* **50:** 218-228.

Staprans, I., Rapp, J.H., Pan, X.M., Hardman, D.A. Feingold, K.R. 1996. Oxidized lipids in the diet accelerate the development of fatty steaks in cholesterol-fed rabbits. *Arteriosclerosis, Thrombosis and Vascular Biology,* **16:** 533-538.

Steyn, N.P., Myburgh, N.G. and Nel, J.H. 2003. Evidence to support a food-based dietary guideline on sugar consumption in South Africa. *Bulletin of the World Health Organization,* **81(8):** 599-608.

Stocker, R. and Keaney Jr J.F. 2004. Role of oxidative modifications in atherosclerosis. *Physiological Reviews,* **84:** 1381-478.

Stocker, R., Yamamoto, Y., McDonagh, A.F., Glazer, A.N. and Ames, B.N. 1987. Bilirubin is an antioxidant of possible physiologic importance. *Science,* **235**: 1043-1046

Stowe, K.A., Burgess, S.C., Merritt, M., Sherry, A.D. and Malloy, C.R. 2006. Storage and oxidation of long-chain fatty acids in the C57/BL6 mouse heart as measured by NMR spectroscopy. *Federation of European Biochemical Societies Letters,* **580:** 4282-4287.

Stuveling, E.M., Hillege, H.L., Bakker, S.J.L., Asselbergs, F.W., de Jong, P.E., Gans, R.O.B. and De Zeeuw, D. 2004. C-reactive protein and microalbuminura differ in their association with various domains of vascular disease. *Atherosclerosis,* **172:** 107-114.

Suganuma, M., Okabe, S., Sueoka, N., Sueoka, E., Matsuyama, S., Imai, K., Nakachi, K. and Fujiki, H. 1999. Green tea and cancer chemoprevention. *Mutation Research,* **428:** 339-344.

Sugino, N. 2007. The role of oxygen radical radical mediated signaling pathways in endometrial function. *Placenta,* **21:** S133-S136.

Süheyl Ezgü, F., Hasanoğlu, A., Tümer, L., Özbay, T., Aybay, C. and Gündüz, M. 2005. Endothelial activation and inflammation in prepurbertal obese turkish children. *Metabolism Clinical and Experimental,* **54:** 1384-1389.

Sumpio, B.E., Cordova, A.C., Berke-Schlessel, D.W., Qin, F. and Chen, Q.H. 2006. Green tea, the "Asian paradox," and cardiovascular disease. *Journal of the American College of Surgeon,* **202(5):** 813-825.

Sun, S.Z. and Empie, M.W. 2007. Lack of findings for the association between obesity risk and usual sugar-sweetened beverage consumption in adults – A primary analysis of databases of CSFII-19994-1998, NHANESIII, and combined NHANES 1999-2002. *Food and Chemical Toxicology,* **45:** 1523-1536.

Tejero, I., González-Lafont A., Lluch, J.M. and Erikson, L.A. 2004. Photo-oxidation of lipids by singlet oxygen: a theoretical study. *Chemical Physics Letters,* **398:** 336-342.

Tell, G.S., Evans, G.W., Folsom, A.R., Shimakawa, T., Carpenter, M.A. and Heiss, G. 1994. Dietary fat intake and carotid artery wall thickness: the atherosclerosis risk in communities (ARIC) study, **139(10):** 979-989.

Thomas, M.J. 2000. The role of free radical and antioxidants. *Nutrition,* **16(7-8):** 716-718.

Thorogood, M., Connor, M., Tollman, S., Hundt, G.L., Fowkes, G. and Marsh, J. 2007. A cross-sectional study of vascular risk factors in a rural South African population: data from the Southern African stroke prevention initiative (SASPI). *BMC Public Health,* **7(326):** 1-10.

Title, L.M., Cummings, P.M., Giddens, K. and Nassar, B.A. 2000. Oral glucose loading acutely attenuates endothelium-dependent vasodilation in health adults without diabetes: an effect prevented by vitamins C and E. *Journal of the American College of Cardiology,* **36:** 2185-2191.

Treutter, D. 1989. Chemical reaction detection of catechins and proanthocyanidins with 4-dimethylaminocinnamaldehyde. *Journal of Chromatography,* **467:** 185-193.

Trinder, P. 1969. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *Journal of Clinical Pathology,* **22:** 158-16.

Tsai, W-C., Li, Y-H., Lin, C.C., Chao, T-H. and Chen, J-H. 2004. Effects of oxidative stress on endothelial function after a high-fat meal. *Clinical Science,* **106:** 315-319.

Tsai-Yueh, L., Ming-Jai, S., Yi-Fan, Y., Yen-Bin, L., Hsui-Chuan, L., Chau-Chung, W. and Yuan-Teh, L. 2004. Effect of hypercholesterolemia on myocardial function in New Zealand white rabbits. *Journal of Biomedical Science,* **11(6):** 829-837.

Turrens, J.F. 2003. Mitochondrial formation of reactive oxygen species. *Journal of Physiology,* **552(2):** 335-344.

Udilova, N., Jurek, D., Marian, B., Gilles, L., Schulte-Hermann, R. and Nohl, H. 2003. Induction of lipid oxidation in biomembranes by dietary oil component. *Food and Chemical Toxicology,* **41:** 1481-1489.

Ullah, M.F and Khan, M.W. 2008. Food as medicine: Potential therapeutic tendencies of plant derived polyphenolic compounds. *Asian Pacific Journal of Cancer Prevention,* **9:** 187-196.

Ursini, F. and Sevanian, A. 2002. Postprandial oxidative stress. *Biological Chemistry,* **383(3-4):** 599-605.

Ursini, F., Zamburlini, A., Cazzolato, G., Maiorino,M., Bittolo Bon, G. and Sevanian, A. 1998. Postprandial plasma lipid hydroperoxides: A possible link between diet and atherosclerosis. *Free Radical Biology & Medicine,* **25(2):** 250-252.

Vaidyanathan, J. B. and Walle, T. 2002. Glucuronidation and sulfation of the tea flavanoid (-) – epigallatecatechin by the human and rat enzymes. *Drug Metabolism and Disposition,* **30:** 897-903.

Van der Bank, M., van Wyk, B.E. and Van den Bank, H. 1995. Biochemical genetic variation in four wild population of *Aspalathus linearis* (Rooibos tea). *Biochemical Systematics and Ecology,* **23(2):** 257-262.

Van der Merwe, J.D., E. Joubert, E., E.S. Richards, E.S., Manley, M., Snijman, P.W., Marnewick, J.L. and Gelderblom, W.C.A. 2006. A comparative study on the antimutagenic properties of aqueous extracts of *Aspalathus linearis* (rooibos), different *Cyclopia spp.* (honeybush) and *Camellia sinensis* teas. *Mutation Research,* **(611):** 42-53.

Van Heerden, F.R., van Wyk, B.-E., Viljoen, A.M. and Steenkamp, P.A. 2003. Phenolic variationin wild population of *Aspalathus linearis* (rooibos Tea). *Biochemical Systematics and Ecology,* **31:** 885-895.

Van Wyk, B.-E. and Verdoorn, G.H., 1989. Alkaloids of the genera *Aspalathus*, *Rafnia* and *Wiborgia* (Fabaceae–Crotalarieae). *South African Journal of Botany,* **55:** 520-522.

Vasankari, T.J., Kujala, U.M., Rusko, K., Sarna. S. and Ahotupa, M. 1997. The effect of endurance exercise at moderate altitude on serum lipid oxidation and antioxidative function in humans. *European Journal of Applied Physiology,* **75:** 396-399.

Vincent, M. A., Russel, W. J., Low, P. and Feldman, E.L. 2004. Oxidative stress in the pathogenesis of diabetic neuropathy. *Endocrine Reviews,* **25(4):** 612-628.

Von Gadow, A., Joubert, E. and Hansmann, C. F. 1997a. Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*), r-tocopherol, BHT, and BHA. *Journal of Agriculture Food Chemistry,* **45(1):** 632-638.

Von Gadow, A., Joubert, E. and Hansmann, C.F. 1997b. Comparison of the antioxidant of rooibos tea (*Aspathus linearis*) with green, oolong and black tea. *Food Chemistry,* **60(1):** 73-77.

Wang, B-S., Yu, H.M., Chang, L.W., Yen, W.J. and Duh, P-D. 2008. Protective effects of pu-erh tea on LDL oxidation and nitric oxide generation in macrophages cells. *LWT,* **41:** 1122-1132.

Wang, C-Z., Mehendale, S.R. and Yuan, C-S. 2007. Commonly used antioxidant botanicals: active constituents and their potential role in cardiovascular Illness. *The American Journal of Chinese Medicine,* **35(4):** 543-558.

Wang, Y., Jones, P.J.H., Woollett, L.A., Buckley, D.D., Yao, L., Granholm, N.A., Tolley, E.A. and Heubi, J.E. 2006. Effects of chenodeoxycholic acid and deoxycholic acid on cholesterol absorption and metabolism in humans. *Translation Research,* **148:** 37-45.

Wang, C-Z., Mehendale, S.R. and Yuan, C-S. 2007. Commonly Used Antioxidant Botanicals: Active Constituents and their Potential Role in Cardiovascular Illness. *The American Journal of Chinese Medicine,* **35(4):** 543-558.

Wang, Z. and Hoy, W.E. 2007. C-reactive protein and the risk of developing type 2 diabetes in Aboriginal Australians. *Diabetes Research and Clinical Pratice,* **76:** 37-43.

Waterhouse, A. 2005. Folin-Ciocalteau method for total phenol in wine. Department of Viticulture and Enology, University of California, Davis. http://waterhouse.ucdavis.edu/phenol/folinmicro.htm/http://www.whfoods.com/genpage.php?tnamefoodspice&dbud=146, the tea plant.

Weitz, J.I., Hirsh, J. and Samama, M.M. 2004. New anticoagulant drugs: The seventh ACCP conference on antithrombotic and thrombolytic therapy. *CHEST,* **126:** 265S-286S.

Westphal, S., Orth, M., Ambrosch, A., Osmundsen, K. and Luley, C. 2000. Postprandial chylomicrons and VLDLs in severe hypertriacylglucerolemia are chylomicron remnants after treatment with n-3 fatty acids. *The American Journal of Clinical Nutrition,* **71:** 914-20.

Westergaard, H. and Dietschy, J.M. 1976. The mechanism whereby bile acid micelles increase the rate of fatty acid and cholesterol uptake into the intestinal mucosal cell. *The Journal of Clinical Investigation,* **58:** 97-108.

Wilmink, H.W., Twickler, M.B., Banga, J.D., Dallinga-Thie, M., G., Eeltink, H., Erkelens, D.W., Rabelink, T.J. and Stroes, E.S. 2001. Cardiovascular Research, Effect of statin versus fibrate on postprandial endothelial dysfunction: role of remnant-like particles. *Cardiovascular Research,* **50: 577-582.**

Wilson, P.W., Kannel, W.B., Silbershatz, H. and D'Agostino, R.B. 1999. Clustering of metabolic factors and coronary heart disease. *Archives of Internal Medicine***,** **159:** 1104-1109.

World Health Organization (WHO) and Food and Agriculture Organization (FAO) expert consultation, 2003. Diet, nutrition and the prevention of chronic diseases. *WHO Technical Report Series,* **916**: 1-149.

Yang, J., Guo, J. and Yuan, J. 2008. In vitro antioxidant properties of rutin. *Swiss Society of Food Science and Technology,* **41(6):** 1060-1066.

Yang, T.T.C. and Koo, M.W.L. 1997. Hypocholesterolemic effects of Chinese tea. *Pharmacological Research,* **35:** 505-512.

Yao, L.H., Jiang, Y.M., Caffin, N., D'Arcy, B., Datta, N., Liu, X., Singanusong, R. and Xu, Y. 2006. Phenolic compounds in tea from Australian Supermarkets. *Food Chemistry,* **96:** 614-620.

Yoshikawa, T., Naito, Y., Oyamada, H., Ueda, S., Tanigawa, S., Takemura, T., Sugina, S. and Kondo, M. 1990. *Scavenging effect of Aspalathus linearis (Rooibos tea) on active oxygen species*. In I. Emerit, L. packer and C. Auclair (Eds); Antioxidants in therapy and preventive medicine, New York: Plenum Press, 171-174.

Young, I.S. and McEneny, J. 2001. Lipoprotein oxidation and atherosclerosis. *Biochemical Society of Transactions,* **29(2):** 358-362.

Young, J., Mc Kinney, S.B., Ross, B.M., Wahle, K.W.J. and Boyle, S.P. 2007. Biomarkers of oxidative stress in schizophrenic and control subjects. *Postglandins Leukemia and Essential Fatty acids,* **76:** 73-85.

Yusuf, S., Ôunpuu, S. and Anand, S. 2002. The global epidemic of atherosclerotic cardiovascular disease. *Medical Principles Practices,* **11(suppl 2):** 3-8.

Yusuf, S., Reddy, S., Ôunpuu S. and Anand, S. 2001. Global burden of cardiovascular diseases: Part II: Variations in cardiovascular disease by specific ethnic groups and geographic regions and prevention strategies. *Journal of the American Heart Association,* **104:** 2855-2864.

Zee, F., Sato, D., Keith, L., Follett, P., Randall, T. and Hamasaki, R.T. 2003. Small-scale tea growing and processing in Hawaii. Cooperative extentension service. *New Plants for Hawaii,* 1-16.

Zethelius, B., Lithell, H. and Hales, C.N. 2005. Insulin sensitivity, proinsulin and insulin as predictors of coronary heart disease. A population-based 10-year, follow-up study in 70-year-old men using the euglycaemic insulin clamp. *Diabetologia,* **48:** 862-867.

APPENDICES

APPENDIX A

1. Reagents, Buffers standards and solutions used

1.1 Reagents of total antioxidant content assays

Folin reagent

Folin-Ciocalteus phenol reagent	1 ml
Distilled H_2O	9 ml

Mix well and prepare fresh on day of assay

Sodium carbonate

Sodium Carbonate	7.50 g
Distilled H_2O	100 ml

HCl-Methanol

HCl (32 %)	250 ml
Methanol	750 ml

Store at room temperature

Dimethylamino-cinnamaldehyde (DMACA)

DMACA	0.25 g
HCL-Methanol mixture	500 ml

Prepare fresh on day of analysis.

Ethanol (10 %)

Ethanol	50 ml

Distilled H_2O	450 ml

Store at room temperature

Ethanol (95 %)

Ethanol	25 ml
Distilled H_2O	475 ml

Store at room temperature

HCl (0.1%) in Ethanol (95 %)

HCL (30 %) (In 500 ml volumetric flask)	1.667 ml

Fill up to the mark with 95 % Ethanol

Store at room temperature

HCl (2 %)

HC (in 500 ml volumetric flask)	33.33 ml

Fill up to the mark with distilled H_2O

Store at room temperature

Gallic acid standard (800 mg/L stock solution) (Total Polyphenol)

Gallic acid	0.040 g
Ethanol (10%)	50 ml

Solution should give an absorbance of 0.695 ± 0.017 at 280 nm.

Gallic acid Control (200 mg/L) (Total Polyphenol)

Gallic acid	0.010 g
Ethanol (10%)	50 ml

Solution should give an absorbance of 0.695 ± 0.017 at 280 nm.

Standard Catechin hydrate (1 mM)

Catechin hydrate	0.0145 g
Methanol	50 ml

Solution should give an absorbance of 0.36 ± 0.009 at 310 nm.

Control Catechin hydrate (200 µM)

Catechin hydrate	0.0029 g
Methanol	50 ml

Solution should give an absorbance of 0.72 ± 0.018 at 310 nm.

Standard Quercetin (80 mg/L stock solution)

Quercetin	0.0040 g
Ethanol (95 %)	50 ml

Solution should give an absorbance of 0.575 ± 0.014 at 280 nm

Control Quercetin (30 mg/L)

Quercetin	0.0015 g
Ethanol (95%)	50 ml

1.2 Reagents of total antioxidant capacity assays

Dilute HCL (40mM)

Conncetrated HCL (32%)	1.46 ml
Make up with distilled H_2O to	1 L

TPTZ (10 mM)

TPTZ	0.031 g

HCL (40mM)	10 ml

Dissolve in water bath at 50º C and make fresh on day of assay

Ferric chloride (20mM)

$FeCl_3.6H_2O$	0.054 g
Distilled H_2O	10 ml

Make fresh on day of assay

FRAP reagent

Acetate buffer	10 ml
TPTZ solution	1 ml
$FeCl_3$ solution	1 ml
Distilled H_2O	1.2 ml

FRAP Acetate buffer (300mM, pH 3.6)

Sodium acetate	1.627 g
Glacial acetic acid	16 mL
Make up with distilled H_2O to	1000 ml

ORAC Phosphate buffer (75 mM, pH 7.4)

First solution

Sodium di-hydrogen orthophosphate-1-hydrate	1.035 g
Distilled H_2O	1000 ml

Second solution

Di-sodium hydrogen orthophosphate dehydrate	1.335 g
Distilled H_2O	1000 ml

Mix 18ml of first soulution with 82 ml second solution. Check the pH and adjust with either phosphate buffer.

Fluorescein sodium salt (Stock solution)

$C_{20}H_{10}Na_2O$	0.0225 g
ORAC Phosphate buffer	50 ml

Store at 4ºC in dark container

AAPH (25 mg/ml)

AAPH	0.150 g
ORAC Buffer	6 ml

Perchloric acid (0.5 M)

Distilled H_2O	195 ml
PCA (70%)	15 ml

Store at room temperature

Standard trolox (ORAC) (500 uM stock solution)

6-hydroxy-2,5,7,8-tetra-methylchroman 2-carboxylic acid	0.00625g
ORAC phosphate buffer	50 ml

Solution should give an absorbance of 1.290 ± 0.032 at 289 nm.

Control trolox (ORAC) (250 uM stock solution)

6-hydroxy-2,5,7,8-tetra-methylchroman 2-carboxylic acid	0.00312 g
ORAC phosphate buffer	50 ml

Solution should give an absorbance of 0.0645 ± 0.0016 at 289 nm

Standard trolox (TEAC) (1.0 mM stock solution)

Trolox	0.0125 g
Ethal absolute	50 ml

Solution should give an absorbance of 1.42 ± 0.035 at 289 nm

Control trolox (TEAC) (200 μM)

Trolox	0.0025 g
Ethanol absolute	50 ml

2, 2'-azino-bis (3-ethylbezothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) (7 mM)

$ABTS^{\bullet+}$	0. 0192 g
Distilled H_2O	5 ml

Potassium-peroxodisulphate ($K_2S_2O_8$) (140 mM)

Potassium - peroxodisulphate	0.1892 g
Distilled H_2O	5 ml

ABTS mix

ABTS	5 ml
Potassium-peroxodisulphate	88 μl

This must be done 24 hours before starting the assay

1.3 Reagents for redox and lipid oxidation assays

GSH/GSSG Buffer A (500 mM)

Buffer A

Monobasic sodium phosphate (0.2 M)	69 g

Distilled H_2O	1000 ml
Buffer B	
Dibasic sodium phosphate (0.2 M)	89 g
Distilled H_2O	1000 ml
Buffer A + Buffer B	
Buffer A	160 ml
Buffer B	840 ml of
Add EDTA to this (A+B) solution	0.186 g
(pH to 7.5)	
GSH Standard solution (3.0 uM)	
Glutathione reduced (GSH)	0.0922 g
GSH/GSSG Buffer A	100 ml
GSSG Standard solution (1.5 uM)	
Glutathione oxidised (GSSG)	0.0918 g
GSH/GSSG Buffer A	100 ml
MPA (5%)	
MPA	5 g
Distilled H_2O	100 ml
NADPH (1 mM)	
NADPH	0.83 g
GSH Buffer	1 ml

Prepare fresh and keep on ice

M2VP (30 mM)

M2VP	0.00808 g
HCl (0.1 N)	1 ml

DTNB

DTNB	0.00012 g
GSH Buffer	1 ml

Prepare fresh and keep on ice

Glutathione reductase (GR)

Glutathione	16.00 µl
GSH Buffer	984 µl

Chloroform-Methanol (2:1)

$CHCl_3$	60 ml
Methanol	30 ml

Butylated hydroxy toluene (BHT) (4 mM)

Butylated hydroxy toluene	0.00881 g
Ethanol	10 ml

Ortho-phoshoric acid (0.2 M)

Concetrated ortho-phoshoric acid	625 µl
Add distilled H_2O	49 375 µl

Sodium hydroxide (0.1 M)

NaOH	0.2 g
Distilled H_2O	50 ml

Thiobarbituric acid (0.11 M)

TBA	0.158 g
NaOH (0.1 M)	10 ml

2. Materials and apparatus

Chemicals and apparatus used were obtained from the following suppliers:

ADAM®, Kraaifontein South Africa	Medical scale
Becton, Dickson and Company, United Kingdom	BD vacuntainer SST™, BD vacuntainer FX
Boeco, Germany	M-240 Centrifuge
Calicom Trading 267 (PTY) Ltd, Gauteng, South Africa	C-RP kit
Greiner Bio-one GmbH, Austria	Vacuntainer K_3E K_3 EDTA tubes
Greiner bio-one, Germany	Microtitre plates, 96-well flat-bottomed, Transparent
Labotec, Gauteng, South Africa	Techne sample concentrator
LASEC (PTY) Ltd, Gauteng, South Africa	XA110 RADWAG Balance
Merck Chemicals (PTY) Ltd, Gauteng, South Africa	Butan-1-ol, ethanol, glacial acetic acid 5810R Centrifuge, chloroform, HCl (32%), Methanol, Orthophosphoric acid, sodium di-hygrogen orthophospahete-1-Hydrate,sodium di-hydrogen-Orthophosphate-1-dehydrate, Sodium hydroxide

Supplier	Product
Medica Easy Random Access (EasyRA™) Bedford, Massachusetts, USA	Random access chemistry analyser (EasyRA™)
Merck Schuchardi OHG, Germany	DMACA
Nunclon™ Delta surface, Danmark	NUNC microtitre plates, 96-well Flat-bottomed, transparent and black
Precisa, Swizerland	Precisa 2200CS balance
Rossmax Medical®, Kraaifontein, South Africa	Blood pressure monitor
Seca, Kraaifontein, South Africa	Stadiometer
Sigma-Aldrich Chemie GmbH, Germany	AAPH, ABTS, Ascorbic acid, BHT, DTNB, DNPH, 6-hydroxy-2,5,7,8-Tetramethylchroman-2 carboxylic Acid, Iron III chloride hexane, glutathione reductase, NADPH, TPTZ
Sigma Chemical Company, United Kingdom	Cyclohexane
TOSOH Bionscience N.V., B-3980 Tessenderlo (Belgium)	TOSOH AIA system analysers
Thermo Electron Corporation, Vantaa, Finland	Fluoroskan ascent and Multiskan spectrum

APPENDIX B

1. ANTHROPOMETRIC MEASUREMENT FORM

Anthropometry and Blood Pressure Form

Subject number:	**Date:**
Date of birth:	**Age:**

	Date:				**Date:**		
	Baseline:				**Intervention completion:**		
Measurement:	1	2	3		1	2	3
Blood pressure Systolic and diastolic (mm Hg)							
Weight (kg)							
Height (cm)							
Body Mass Index (kg/m^2)							

2. ADDENDUM OF SCREENING INCLUSIONS CRITERIA (FASTING GLUCOSE, TOTAL CHOLESTEROL AND TRIGLYCERIDES) OF PHASE ONE

Number of participants	Glucose	Total cholesterol	Triglycerides
Control group (n = 5)			
01	5.6	3.5	0.7
02	4.1	3.5	0.7
03	4.3	5.3	2.1
04	5.1	4.0	0.6
05	4.7	5.8	0.6
Average	4.8	4.4	0.9
Treatment group (n = 8)			
01	4.7	3.6	0.5
02	5.1	5.4	0.6
03	4.6	3.4	0.4
04	5.4	4.6	1.1
05	5.4	4.3	1.0
06	5.2	3.6	1.2
07	4.8	4.7	0.4
08	4.5	5.0	0.7
Average	5.0	4.3	0.7

Data are presented as mean and expressed as mmol/l

3. CALIBARTION FOR THE AUTOMATED SYSTEM

Component	Manufacturer	Method	SI-Unit	Target value	Control range
hs-CRP	Chem Trol plus, Level 1	Immunoturbidimetry	mg/dl	19.9	14.5 - 25.3

4. ADDENDUM OF TRIGLYCERIDES VALUES (PHASE TWO) EXPRESED IN mg/dl

	Control group				Treatment group			
	TG0	TG2	TG4	TG6	TG0	TG2	TG4	TG6
2.1	61	106	132	86	67	111	112	53
2.2	60	107	129	89	68	103	113	53
2.3								
4.1				73	37	62	66	46
4.2				74	34	62	67	49
4.3								
5.1	43	41	58	66	35		62	33
5.2	43	42	58	66	44		65	34
5.3								
6.1		75	120	136		67	69	55
6.2		78	110	142		66	103	58
6.3								
15.1	40	59	127	66	54	61	86	50
15.2	39	58	130	66	56	66	90	50
15.3								
21.1	41	49	74	90	51	38		
21.2	44	46	67	91	47	35		
21.3								
22.1	53	60	66	71	116	115	144	94
22.2	52	59	67	69	114	117	166	97
22.3								
23.1	34	51	65		30	31	36	27
23.2	34	58	67		31	33	38	27
23.3								
25.1	58	56	92	70	37	48	41	38
25.2	61	57	86	66	33	51	51	38
25.3								
32.1	37	35	51	70	45	46	52	63
32.2	36	35	50	71	45	47	57	62
32.3								
35.1	59	50	73	72	53	34	66	51
35.2	64	49	82	76	46	34	74	48
35.3								
39.1	22	21	68	67	20		41	26
39.2	23	22	79	69	23		45	27
39.3								

Data are in duplicate and presented as mean and expressed as mg/dl; TG= Triglycerides; 0, 2, 4 and 6 designated as hours.

5. INFORMED CONSENT FORM

INFORMED VOLUNTEER CONSENT DOCUMENT

I,___________________________________, agree to participate in the research study entitled "THE EFFECT OF ROOIBOS ON POSTPRANDIAL OXIDATIVE STRESS FOLLOWING A HIGH FAT AND SUCROSE MEAL IN NORMOLIPIDAEMIC INDIVIDUALS". Conducted by Mr. N.M. Francisco, registered Master's degree (Biomedical Technology) student from the Cape Peninsula University of Technology (CPUT) under the supervision of Dr. J. L. Marnewick (Room 3.73, Oxidative Stress Research Centre, Sciences Building, Faculty of Health and Wellness Sciences, CPUT, Tel: 021-4608314, E-mail: marnewickj@cput.ac.za).

I understand that my participation is voluntary. I can refuse to participate or quit at any time without giving any reason, and without penalty. I can ask to have all information obtained about me in the study to be returned to me, removed from the research records, or destroyed.

Purpose and Benefits

This study will investigate the short-term changes in various blood markers of oxidative stress after healthy volunteers have consumed a high fat meal in combination with sucrose (referred to as Phase I of the study). Once this has been completed, the effect of a natural occurring antioxidant, Rooibos tea will then be investigated on these oxidative stress markers after a high fat meal and sucrose has been consumed (referred to as Phase II of the study).

The modulation of oxidative stress markers in the blood could have important health implications such as enhancing the resistance of low-density cholesterol to oxidative damage which has been implicated in the development of heart disease. At the end of the study, all participants in the study will get to know their health status (blood pressure, Body Mass Index, cholesterol level, liver and kidney function and glucose level) at no cost, an otherwise very costly undertaking.

Procedures

If I volunteer to take part in this study, I will be asked to do the following:

1. Answer questions about my health, food, nutrition and physical activity.

2. Take part in a study (Phase I and II) over a 6 month period in which the first day will consist of the ingestion of sucrose and a standardised fat meal (Phase I) and blood collection. The procedure will be repeated after a two week period. Phase II will follow

within the next 6 months and entails ingestion of sucrose, a standardised fat meal as well as Rooibos tea and blood will be collected. This procedure will also be repeated after a two week period.

3. If I am willing, a qualified phlebotomist or nursing sister will take samples of my blood before the sucrose ingestion and standardised fat meal challenge. And at 4 (Four) consecutive occasions after the ingestion. Blood samples will be drawn from me with a minimum stasis at 0, 120, 240 and 360 minutes. Blood samples will be analysed to determine levels of enzymes, blood glucose, cholesterol and lipids as well as antioxidant status.

4. Someone from the study may call me to clarify my information.

I understand that these questions and blood tests are not for diagnostic purposes. My blood will not be tested for HIV-AIDS. If I have questions about my test results, I should see a physician.

Risks, Stress and Discomfort

There are no known risks to participate in this study, but I may experience some discomfort when my blood is drawn or when the researchers ask me questions about health, nutrition, physical activity and smoking habits. The risks of drawing blood from my forearm include the unlikely possibilities of a small bruise or localized infection and bleeding. These risks will be reduced by using a qualified phlebotomist or nursing sister to draw the blood.

No individually-identifiable information about me, or provided by me during the research, will be shared with others without my written permission, except if it is necessary to protect my welfare (for example, if I were injured and need physician care) or if required by law. I will be assigned a unique number and this number will be used on all of the questionnaires I fill out. The investigator will answer any further questions about the research, now or during the course of the project.

I understand that there will be no laboratory or catering costs to me for participation in this study and that I will not be compensated to participate in the study.

I declare that I have read the details of the project or have listened to the oral explanation thereof, and declare that I understand it. I have had the opportunity to discuss relevant aspects with the researcher and declare that I voluntarily participate in the project. I hereby give consent to participate in the project.

I declare that I take part in this study on my own risk and that the Cape Peninsula University of Technology, any of its workers or students are not responsible if anything should happen to me during the course of the experiment. I also declare not to sue the CPUT for any personal losses or damage that might occur during the project due to negligence from the CPUT, its workers, students or other subjects that take part in this study.

________________________ __________________ _________________

Name of Participant **Signature** **Date**

Telephone: ______________

Email: ____________________

_________________ ___________________ _________________

Name of Researcher **Signature** **Date**

Please sign both copies, keep one and return one to the researcher

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