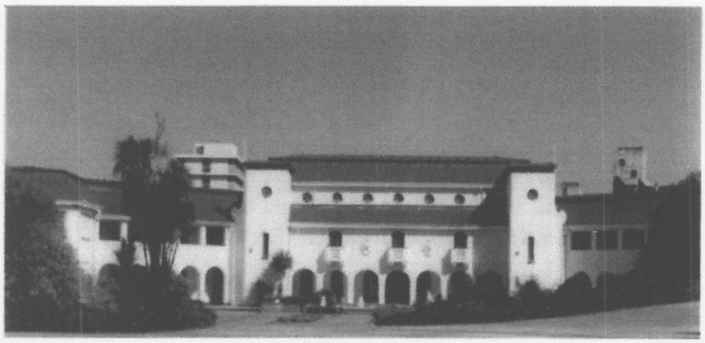
YUNIBESITI YA BOKONE-BOPHIRIMA
NORTH-WEST UNIVERSITY
NOORDWES-UNIVERSITEIT

THE EFFECTS OF A HIGH WALNUT AND UNSALTED CASHEW NUT DIET ON THE ANTIOXIDANT STATUS OF SUBJECTS WITH DIAGNOSED METABOLIC SYNDROME

LISA DAVIS B.Sc (Dietetics), RD



Mini-dissertation submitted in partial fulfilment of the requirements for the degree Magister Scientiae in Dietetics in the School of Physiology, Nutrition and Consumer Sciences of the North-West University, Potchefstroom Campus

Supervisor : Prof W Oosthuizen
North-West University

Co-supervisor : Dr. Du T Loots
North-West University

Wees nie bevrees nie, want Ek is met jou; kyk nie angstig rond nie, want Ek is jou God. Ek versterk jou, ook help Ek jou, ook ondersteun Ek jou met my reddende regterhand."

Jesaja 41:10

ACKNOWLEDGEMENTS

This study would not have been possible without the support and assistance of many people. I would like to thank each and every person involved in this study most sincerely. I wish to express special thanks to:

- God for giving me the ability to pursue my dreams, and making it possible for me to complete my studies successfully.

- My supervisor, Prof. Welma Oosthuizen for her motivation, guidance, patience and insight throughout the course of my studies. Thank you for helping me to appreciate the challenging world of research. I have learned a great deal from you.

- Dr. Du Toit Loots, my assistant supervisor, for all the advice and insight in writing this dissertation. Thank you for being so helpful and patient during the laboratory analysis of the food samples. You have inspired my interest in laboratory analysis.

- Dr. Grieta Hanekom for her involvement in the planning of the menus. Thank you for your kind words and encouragement.

- Dr. Francois van der Westhuizen for his assistance in the laboratory. Thank you for your time, patience and advice.

- Sr. Chrissie Lessing, for the competent and professional manner in which she motivated the participants and handled the blood samples. Thank you for the interest you showed during the study.

- To the Medical Research Council of South Africa. Thank you for giving me the opportunity to complete my studies as a full time student.

- To my mother, father and sisters for all their support, encouragement and love throughout the course of my studies. Thank you for always believing in me. Special thanks to my mother and father for giving me the opportunity to pursue my dreams and ambitions.

- Schalk, for the warmth and beauty you breathe into my life. Thank you for all the love, encouragement, support and understanding.

- To all my family and friends for their support and encouragement.

- To everyone in this department who helped with the execution of the study.

- To the National Research Foundation and the Technology and Human Resources for Industry Programme who provided funding for the study.

- To Tiger brands, Pick 'n Pay, Clover and Unilever-BestFoods-Robertsons, who donated various foods for the controlled feeding program.

- To Ms. E. Uren for the language editing of this dissertation.

OPSOMMING

Motivering

Metaboliese sindroom word gekenmerk deur verskillende risikofaktore insluitend 'n verhoogde risiko vir koronêre hartsiekte (KHS) en word gekenmerk as siektes van die moderne samelewing. Eienskappe van metaboliese sindroom sluit in abdominale obesiteit, verhoogde triasielgliserol (TG) konsentrasies, verhoogde klein, digte lae-digtheidslipoprotein (LDL)-partikels, verlaagde hoë-digtheidslipoprotein cholesterol (HDL-C), insulienweerstandbiedendheid, hipertensie, glukose-intoleransie, inflammasie, en/of tipe 2 diabetes mellitus.

Metaboliese sindroom word dikwels met oksidatiewe stres geassosieer, moontlik as gevolg van 'n langdurige blootstelling aan verhoogde glukose vlakke. Daar is 'n verskeidenheid natuurlike antioksidante (glutatioon, β-karoteen, vitamien C, polifenole) wat moontlik oksidatiewe skade aan biologiese strukture kan voorkom. Neute is ryk aan onversadigde vetsure, proteïene, vesel, mikronutriënte, fitochemikalieë en anti-oksidante. Dit kan dus gespekuleer word dat die hoë anti-oksidantinhoud van neute 'n voordelige effek op die anti-oksidantstatus van individue met metaboliese sindroom kan hê.

Doel

- Om die effek van 'n hoë okkerneut en 'n hoë ongesoute kasjoeneutdieet op die anti-oksidantstatus van persone met metaboliese sindroom, te ondersoek.

Metodes

Agt en sestig vrywilligers met gediagnoseerde metaboliese sindroom (na aanleiding van die ATP III kriteria) is gewerf om aan die parallel, gerandomiseerde, gekontrolleerde voedingstudie deel te neem. Vrywilligers is hoofsaaklik uit die Noordwes-Universiteit, Potchefstroom kampus, en omliggende omgewing gewerf. Na 'n inloopfase van drie weke, waartydens die proefpersone 'n gebalanseerde dieet gevolg het, is die proefpersone ewekansig in drie groepe verdeel waarna die vrywilligers óf okkerneute óf kasjoeneute (60 – 108g/dag) as deel van 'n omsigtige dieet, óf 'n omsigtige dieet sonder neute, gevolg

het. Die intervensie is vir agt weke gevolg. Vastende bloedmonsters is aan die begin van die intervensie (na die inloop fase) en aan die einde van die intervensie geneem. Anti-oksidantveranderlikes insluitend "oxygen radical absorbance capacity (ORAC)", gereduseerde glutatioon (GSH)/geoksideerde glutathioon (GSSG), en "diacron reactive oxygen metabolites (dRom)" is aan die begin van die intervensie gemeet. C-reaktiewe proteïen (CRP), fibrinogeen en plasminogeen activeerder-inhibeerder aktiwiteit ($PAI\text{-}1_a$) as merkers van inflammasie is ook gemeet. Die anti-oksidantkapasiteit en die polifenol inhoud van die drie diëte, die okkerneute en die kasjoeneute is aan die einde van die intervensie bepaal.

Resultate

'n Betekenisvolle verlaging in dRom, en betekenisvolle verhogings in GSSG, die redokstatus van glutatioon (GSH/GSSG), en ORAC is waargeneem in al die groepe van basis na end. GSH het onveranderd gebly van basis na end in al drie groepe. Geen betekenisvolle verskille in die veranderings van basis na end tussen groepe in dRom ($p = 0.92$), GSSG ($p = 0.99$), GSH/GSSG ($p = 0.86$), ORAC ($p = 0.10$), en GSH ($p = 0.34$) is waargeneem nie.

Die totale polifenolinhoud van die okkerneut en die kontrole diëte was eenders en was betekenisvol hoër in vergelyking met die kasjoeneutdieet. Die anti-oksidantkapasiteit van die okkerneut- en kasjoeneutdiëte het die neiging getoon om hoër as die kontrole dieet te wees ($p = 0.07$ en $p = 0.06$ onderskeidelik). CRP, fibrinogeen en $PAI\text{-}1_a$ konsentrasies het nie betekenisvol tussen groepe verskil nie.

Gevolgtrekking

Geen betekenisvolle verskille in GSH, GSSG, GSH/GSSG, dRom, en ORAC is tussen die okkerneut, kasjoeneut, en kontrole diëte opgemerk nie. Daar blyk dus geen voordelige effek van die insluiting van okkerneute en kasjoeneute in die dieet op die anti-oksidantstatus van die proefpersone te wees nie.

Sleutelwoorde

Neute; Anti-oksidantstatus; Metaboliese sindroom; Inflammasie; Insulienweerstand; Obesiteit

ABSTRACT

Motivation

Metabolic syndrome is a constellation of risk factors predisposing to coronary heart disease (CHD) and is classified as a "disease of modern civilization". Characteristics of the metabolic syndrome include abdominal obesity, increased triacylglycerol (TG) concentrations, increased small dense low-density lipoprotein (LDL) particles, decreased high-density lipoprotein cholesterol (HDL-C), hypertension, insulin resistance, inflammation, glucose intolerance and/or type 2 diabetes mellitus.

Subjects with metabolic syndrome may be susceptible to oxidative stress due to their prolonged exposure to elevated glucose levels. A variety of natural antioxidants exists (e.g. glutathione, β-carotene, vitamin C, polyphenols) that may prevent oxidative damage to biological structures. Nuts are rich sources of unsaturated fatty acids, protein, fibre, micronutrients, phytochemicals and antioxidants. Due to their high antioxidant content, it can, therefore, be speculated that nuts may play a role in the prevention of oxidative stress in subjects with the metabolic syndrome.

Objective

- To investigate the effect of a high walnut and a high unsalted cashew nut diet on the antioxidant status of subjects with metabolic syndrome.

Methods

Sixty eight subjects with diagnosed metabolic syndrome (according to the ATP III criteria) were recruited to take part in this parallel, randomized, controlled feeding trial. Subjects were mainly recruited from the North-West University, Potchefstroom Campus and surrounding areas. After a run-in period of three weeks during which the participants followed a prudent diet, subjects were randomly divided into three groups receiving either walnuts or cashew nuts (63 – 108g/day) as part of a prudent diet, or continued with the prudent control diet. The intervention was followed for eight weeks. Fasting blood samples were taken at the beginning (after the three week run-in period) and at the end of

the intervention. Antioxidant variables including oxygen radical absorbance capacity (ORAC), reduced glutathione (GSH)/oxidized glutathione (GSSG), diacron reactive oxygen metabolites (dRom) were measured at the beginning and the end of the intervention. C-reactive protein (CRP), fibrinogen and plasminogen activator-inhibitor activity ($PAI\text{-}1_a$) were also measured as markers of inflammation. The antioxidant capacity and the polyphenol content of the diets and the walnuts and cashew nuts were determined at the end of the intervention.

Results

A significant decrease in dRom and significant increases in GSSG, the redox status of glutathione (GSH/GSSG) and ORAC were observed in all three groups from baseline to end. GSH remained unchanged from baseline to end in all three groups. No significant differences in changes in dRom ($p = 0.92$), GSSG ($p = 0.99$), GSH/GSSG ($p = 0.86$), antioxidant capacity ($p = 0.10$) and GSH ($p = 0.34$) were observed from baseline to end between groups.

The total polyphenol content of the walnut and control diets were similar and significantly higher than the cashew nut diet. The antioxidant capacity of the walnut and cashew nut diets showed a tendency to be higher than the control diet ($p = 0.07$ and $p = 0.06$ respectively). CRP, fibrinogen and $PAI\text{-}1_a$ concentrations did not differ significantly between groups.

Conclusion

No significant differences between the groups receiving walnuts, cashew nuts or no nuts were observed in GSH, GSSG, GSH/GSSG, dRom or ORAC. Therefore, there seems to be no beneficial effect of the inclusion of walnuts and cashew nuts in the diet on the antioxidant status of the participants.

Key words

Nuts; Antioxidant status; Metabolic syndrome; Inflammation; Insulin resistance; Obesity

ABREVIATIONS

A	
ASAP	Antioxidant supplement in atherosclerosis prevention study
ATP III	Adult Treatment Panel III
B	
BMI	Body mass index
C	
CHD	Coronary heart disease
CHO	Carbohydrate
CRP	C-reactive protein
CVD	Cardiovascular disease
D	
DEPPD	N,N-diethyl-para-pherylendiamine
dRom	Diacron reactive oxygen metabolite assay
F	
Fe^{11}	Ferrictripyridyltriazine
Fe^{111}	Ferroustripyridyltriazine
FFA	Free fatty acid
FRAP	Ferric reducing antioxidant power
FSIGT	Frequent sampling and intravenous glucose tolerance test

G

GSH	Reduced glutathione
GSSG	Oxidized glutathione

H

HDL-C	High density lipoprotein cholesterol
HOMA	Homeostatic model assessment
hs	High sensitivity

I

IGT	Impaired glucose tolerance
IL	Interleukin

K

KANWU	Kuopio, Aarhus, Naples, Wollogong and Uppsala (acronym)
KHS	Koronêre hartsiekte

L

LDL	Low density lipoprotein
LGI	Low glyceamic index

M

MDA	Malondialdehyde
MUFA	Mono-unsaturated fatty acids

N

NCEP	National Cholesterol Education Programme
NHANES III	Third National Health and Nutrition Examination Survey

SYMBOLS

α	Alpha
β	Beta
↓	Decrease
↑	Increase
↔	No change

TABLES

TABLES USED IN PREFACE

TABLE 1: Research Team.....p. XX

TABLES USED IN CHAPTER ONE

TABLE 1: Dietary fat and insulin sensitivity in healthy subjects or in individuals with type 2 diabetes or impaired glucose tolerance: high-fat vs. low-fat diets.....p. 8

TABLE 2: Recommendations for the clinical management of the metabolic syndrome.....p. 13

TABLE 3: The relationship between antioxidants and some of the risk factors for the metabolic syndrome and CVD.....p. 16

TABLE 4: Most frequent markers of antioxidant capacity and oxidative stress.....p. 19

TABLE 5: Nutrient composition of 100g of various nuts.....p. 26

TABLE 6: Mechanisms through which vitamin C may inhibit atherosclerosis.....p. 31

TABLES USED IN CHAPTER TWO

TABLE 1: Calculated and analyzed diets as well as the habitual diets.....p. 43

TABLE 2: Baseline characteristics.....p. 50

TABLE 3: Antioxidant profiles.....p. 53

TABLE 4: Inflammation markers during the intervention.....p. 56

FIGURES

FIGURES USED IN CHAPTER ONE

FIGURE 1: Progression of insulin resistance to type 2 diabetes parallels the progression of endothelial dysfunction to atherosclerosis.....p. 10

PREFACE

AIM AND OBJECTIVES

Main aim

The main aim of this dissertation was to investigate effects of indigenous South African crops of walnuts and cashew nuts on the antioxidant status of subjects with metabolic syndrome.

Objectives

The objectives were to investigate the effects of high walnut and unsalted cashew nut diets in subjects with metabolic syndrome on:

- **Antioxidant status:** Oxygen radical absorbance capacity (ORAC); diacron reactive oxygen metabolites (dRom); glutathione; oxidized glutathione (GSSG); reduced glutathione (GSH); glutathione redox state (GSH/GSSG).

- **Inflammation markers:** Fibrinogen; plasminogen activator inhibitor-1 activity ($PAI\text{-}1_a$)

BACKGROUND

The National Cholesterol Education Programme (NCEP) Adult Treatment Panel III (ATP III) defined the metabolic syndrome as a constellation of risk factors predisposing to coronary heart disease (CHD) (Anon, 2002a). These risk factors include abdominal obesity, atherogenic dyslipidemia (increased triacylglycerol (TG), decreased small dense low-density lipoprotein (LDL) particles, decreased high density lipoprotein (HDL) cholesterol, hypertension, insulin resistance, glucose intolerance, inflammation

and/or type 2 diabetes mellitus. Furthermore, clinical and epidemiological studies strongly associate these characteristics with increased cardiovascular disease risk (Grundy *et al.*, 2004a; Grundy *et al.*, 2004b; Smith *et al.*, 2005).

Subjects with the metabolic syndrome may be susceptible to oxidative stress (Maxwell *et al.*, 1997), which could be explained by the prolonged exposure of these subjects to elevated glucose levels (Maxwell *et al.*, 1997). A number of studies have investigated the effects of various antioxidants on insulin resistance and other aspects of metabolic syndrome. Recently the antioxidant vitamin C has been shown to restore insulin-impaired endothelial function (Arcaro *et al.,* 2002). In 36 healthy, non-diabetic volunteers it was found that variations in insulin-mediated glucose disposal in healthy individuals were significantly related to plasma concentrations of lipid hydroperoxides and liposoluble antioxidant vitamins alpha-carotene, beta carotene, alpha tocopherol, and delta tocopherol. The authors concluded that total plasma lipid peroxidation is increased in insulin-resistant individuals (Facchini *et al.,* 2000). Consequently, antioxidants may be useful in the dietary modification of the metabolic syndrome.

Nuts have become increasingly popular in the search for bioactive compounds that affect various diseases favourably, such as cardiovascular disease (CVD), diabetes and the metabolic syndrome. Nuts are rich sources of various nutrients, including unsaturated fat, protein, fibre, micronutrients, phytochemicals and antioxidants such as polyphenols (Kris-Etherton *et al.*, 1999). Due to their high antioxidant content, it can, therefore, be speculated that nuts may play a role in the prevention of oxidative stress in subjects with the metabolic syndrome.

The nuts chosen for this study include walnuts and unsalted cashew nuts. It has been reported that walnuts contain more than 1500mg/100g polyphenols (Macfarlane *et al.,* 1988). Polyphenols are antioxidants which may have protective effects on several diseases such as some cancers and heart disease (Mukhtar and Ahmad, 2000). They may protect LDL cholesterol from becoming oxidized (a key step in developing atherosclerosis), lower blood pressure in hypertensive subjects, and reduce the tendency

of the blood to clot. The family to which walnuts belong (Juglandaceae) is amongst the dietary plants that contain the most antioxidants (Halvorsen *et al.,* 2002). Furthermore, walnuts are rich in polyunsaturated fatty acids (PUFAs) (47.2g of PUFAs/100g of walnuts (U.S. Department op Agriculture ARS, 2001). Epidemiological evidence suggests that frequent nut consumption protects against CHD (Fraser *et al.,* 1992). Walnuts are unique in that they are a rich source of both ω-6 and ω-3 PUFAs, with a ω-6:ω-3 ratio of 4:1 (U.S. Department of Agriculture ARS, 2001). Unlike walnuts, cashew nuts have a higher concentration of mono-unsaturated fatty acids (MUFAs) (27.3g of MUFAs/100g of cashew nuts (U.S. Department of Agriculture ARS, 2001)) and high MUFA diets are recommended for subjects with metabolic syndrome (Sanz Paris, 2000). Furthermore, the effects of a high cashew nut diet on the markers of the metabolic syndrome have not been investigated before.

STRUCTURE OF THIS DISSERTATION

This dissertation is in article format. The empirical work consists of a controlled feeding trial with a randomized, controlled, parallel study design. This study investigated the effects of a high walnut and unsalted cashew nut diet on the antioxidant capacity, dRom, GSH, GSSG, GSH/GSSG, fibrinogen and PAI-1 in subjects with diagnosed metabolic syndrome. The study forms part of a larger trial that investigated the effects of nuts on markers of the metabolic syndrome (Mukuddem-Petersen, 2005).

Following this Preface, Chapter 1 provides background information necessary for the interpretation of the data in the article. An overview of risk factors for the metabolic syndrome is given. Furthermore, the general composition of nuts, including walnuts and cashew nuts, and the possible effects of the antioxidants in these nuts on the markers of metabolic syndrome, will be discussed.

Chapter 2 consists of the manuscript containing the results of the effects of a walnut and a high unsalted cashew nut diet on the antioxidant status of subjects with metabolic

syndrome. The manuscript will be submitted for publication to the European Journal of Nutrition.

The relevant references of Chapter 2 are provided at the end of the chapter according to the author's instructions of the European Journal of Nutrition. The references used in the Preface and Chapter 1 are provided according to the mandatory style stipulated by the North-West University at the end of this dissertation.

AUTHOR'S CONTRIBUTION

The study reported in this dissertation was planned and executed by a team of researchers. The contribution of each of the researchers is depicted in **Table 1**. Also included in this section is the statement from the co-authors confirming their individual roles in the study and giving their permission that the article may from part of this dissertation.

Table 1

Research team

NAME	CONTRIBUTION
Ms. L. Davis B.Sc Hons (Dietician)	Involved with the food preparation. Responsible, together with Du T Loots and F v.d. Westhuizen for polyphenol content and antioxidant analysis of the diets. Responsible for data management. Responsible, together with W Oosthuizen and J.C Jerling for statistical analysis. Responsible for nutrient analysis.
Prof. W. Oosthuizen Ph.D (Nutritionist)	Supervisor and study director. Assisting with all aspects of the study including the design, planning, approval of final protocol, execution and documentation of the study.
Dr. Du T. Loots	Co-supervisor. Responsible, together with L. Davis for the determination of the polyphenol content of the diets and the walnuts and cashew nuts. Assisted in the interpretation of the results.
Prof. J.C. Jerling Ph.D (Nutritionist)	Study Co-director. Assisting with all aspects of the study including the design, planning, approval of final protocol, execution and documentation of the study.
Mrs. J. Mukuddem-Petersen (Ph.D Dietetics student)	Involved with designing, planning and execution of the study.
Prof. C.S. Venter Ph.D (Dietician)	Assisting with diet history.
Dr. S.M. Hanekom Ph.D (Dietician)	Assisting with diet history. Designing of menu plan.
Dr. H Van't Riet (Nutritionist)	Giving advice concerning the design and planning of the study.
Dr. H.H Wright Ph.D (Dietician)	Assisting with diet history and designing of menu plan.
Sr. M.C. Lessing (Registered general nurse)	Assisting with recruitment of subjects and collection of blood samples.
Dr F.H. Van der Westhuizen (Biochemist)	Assisting with the testing of the antioxidant status. Determination of the antioxidant capacity of the diets and the walnuts and cashew nuts.
Ms. R. Breet (MSc. Nutrition student) Ms. M. Opperman (Ph.D Dietetics student) Mrs. Z White (MSc. Nutrition student)	Assisting with diet histories and the Nutcracker newsletter. Assisting with diet histories Assisting with nutrient analyses.
Anthropometrists: A. Greyling C. de Witt	Assisting with anthropometric measurements, biochemical analysis, and the Nutcracker newsletter.
Ms. A. Schutte (Physiologist)	Assisting with monitoring of blood pressure
Fourth year dietetic students E. Pienaar	Food preparation.
J.E Kotze	The supervising medical doctor on stand-by

I declare that I have approved the above mentioned article and that my role in the study as indicated above is representative of my actual contribution and that I hereby give my consent that it may be published as part of the M.Sc. dissertation of Lisa Davis.

Prof. W. Oosthuizen	Dr. Du T. Loots
Dr. J. Mukkudem-Petersen	Dr. F.H. Van der Westhuizen
Prof. J.C. Jerling	Dr. S.M. Hanekom

5.3 The effect of antioxidant nutrients and polyphenols in nuts, and glutathione on markers of the metabolic syndrome.....p. 28

6. SUMMARY.....p. 34

7. SUMMARY AND CONCLUSION.....p. 34

CHAPTER 2

THE EFFECTS OF HIGH WALNUT AND CASHEW NUT DIETS ON THE ANTIOXIDANT STATUS OF SUBJECTS WITH METABOLIC SYNDROME

- Title page.....p. 36
- Author's instructions.....p. 37
- Abstract.....p. 39
- Introduction.....p. 40
- Methods.....p. 41
- Results.....p. 48
- Discussion and conclusion.....p. 54
- Acknowledgements.....p. 59
- Literature cited.....p. 60

LITERATURE CITED.....p. 66

CHAPTER 1

LITERATURE REVIEW

1. INTRODUCTION

According to the National Cholesterol Education Programme (NCEP) Adult Treatment Panel III (ATP III), the metabolic syndrome is a constellation of risk factors predisposing to coronary heart disease (CHD) (Anon, 2002). Factors that are generally accepted as being characteristics of the metabolic syndrome include abdominal obesity, atherogenic dyslipidemia (increased triacylglycerol (TG), decreased small dense low-density lipoprotein (LDL) particles, decreased high density lipoprotein cholesterol (HDL-C), hypertension, insulin resistance, glucose intolerance, type 2 diabetes mellitus and inflammation. Clinical and epidemiological studies strongly associate these characteristics with increased cardiovascular risk (Grundy *et al.*, 2004a; Grundy *et al.*, 2004b; Smith *et al.*, 2005).

Subjects with the metabolic syndrome may be susceptible to oxidative stress (Maxwell *et al.*, 1997), which could be explained by the prolonged exposure of these subjects to elevated glucose levels (Maxwell *et al.*, 1997). However, a variety of natural antioxidants exists that may prevent oxidative damage to biological structures, including intracellular antioxidants such as glutathione and dietary antioxidants such as vitamin E, vitamin C, and polyphenols (Cao *et al.*; 1997, Barbagallo *et al.*, 1999a). Nutrition may, therefore, play a key role in the management of this disease. Regarding this, nuts have become increasingly popular in the search for bioactive compounds that affect various diseases favourably, such as cardiovascular disease (CVD), diabetes and the metabolic syndrome. Nuts are rich sources of various nutrients, including unsaturated fat, protein, fibre, micronutrients, phytochemicals and antioxidants (Kris-Etherton *et al.*, 1999). Due to their high antioxidant content, it can, therefore, be speculated that nuts may play a role in the prevention of oxidative stress in subjects with the metabolic syndrome.

In this review the concept of the metabolic syndrome, the relationship between oxidative stress, inflammation, CVD and the metabolic syndrome, as well as antioxidant markers will be discussed. Furthermore, the possible effects between

antioxidants, specifically in nuts, on oxidative stress, inflammation, the metabolic syndrome and atherosclerosis will be explored.

2. THE METABOLIC SYNDROME

2.1 Introduction

The metabolic syndrome is not a new concept. As early as 1933 an X syndrome was described, referring to a clustering of hypertension, obesity and gout. Insulin resistance syndrome, the cardiovascular metabolic syndrome and the deadly quartet all refer to the metabolic syndrome as it is known today. The World Health Organization (WHO) introduced the first unifying definition for the metabolic syndrome in 1998, describing it as insulin resistance together with any two of the following conditions (Groop & Orho-Melander, 2001):

i) Hypertension
ii) Dyslipidaemia
iii) Obesity and
iv) Microalbuminuria.

Insulin resistance can be defined as a defect in the ability of insulin to mediate glucose disposal by the muscle (Fletcher & Lamendola, 2004) and is the greatest risk factor for the development of type 2 diabetes mellitus (Keskin *et al.*, 2005). Many assays exist through which to measure insulin resistance. The hyperinsulinemic-euglycemic clamp technique and the hyperglycemic clamp technique are often referred to as the 'gold standard' tests (Wallace *et al.*, 2004). However, clamp techniques are complex invasive tests and may flux well outside the normal range (Wallace *et al.,* 2004). Another method to measure insulin resistance, the homeostatic model assessment (HOMA), compares well with the 'gold standard' tests (Wallace *et al.*, 2004). Regarding this, the literature as reviewed by Wallace (2004) is not clear on which method that measures insulin resistance can be considered the 'best'. Ultimately, the inclusion of insulin resistance in the criteria leads to extra laboratory testing beyond the

routine clinical examination. This makes the NCEP ATP III criteria more clinician friendly (Saylor, 2005).

A clinical identification of the metabolic syndrome, as stipulated by the NCEP ATP III criteria, involves the presence of at least three of the following criteria:

i) Abdominal obesity (waist circumference: men>102 cm; women>88 cm)
ii) High TG (≥ 1.7 mmol/L)
iii) Low HDL-C (men < 1.04 mmol/L; women < 1.3 mmol/L)
iv) High blood pressure (≥ 130/85 mmHg) and
v) High fasting blood glucose ≥6 mmol/L.

People with the metabolic syndrome are at increased risk to develop type 2 diabetes mellitus (Groop & Orho-Melander, 2001). Results from the Botnia study in Finland and Sweden (1996) showed that about 10% of subjects with normal glucose tolerance, 40% of subjects with impaired glucose tolerance (IGT) and 80% of subjects with type 2 diabetes mellitus would have the metabolic syndrome (Groop *et al.*, 1996).

Many different factors lead to the development of the metabolic syndrome (reviewed by Tenebaum *et al.*, 2004), including overweight, physical inactivity, diet, especially high carbohydrate (CHO) (> 60% of total energy) and high fat diets and genetic factors. Furthermore, inflammation may also be involved in the development of this disease (Nilsson *et al.,* 1994; Harris & Winter., 2004; Tenebaum *et al.*, 2004). These factors will be discussed in more detail in subsequent sections.

2.2 Prevalence of the metabolic syndrome and type 2 diabetes mellitus

The prevalence of the metabolic syndrome is age-dependent and is higher in males than in females (Isomaa *et al.*, 2001). Furthermore, the prevalence of the metabolic syndrome is associated with an increased risk of CHD, myocardial infarction and stroke (Grundy *et al.*, 2004b). In South Africa, the THUSA (acronym for Transition and Health in the Urbanization of South Africans) study showed that 12% of men and

28.4% of women of the Tswana speaking population of the North West Province had three or more characteristics of the metabolic syndrome. Moreover, the study revealed that the metabolic syndrome occurs mainly in undernourished men (body mass index (BMI) < 18.5) and over nourished men and women (BMI > 30) (Kruger, 2000:1-140). In 2002 it was estimated that approximately 22% of adults in the United States (24% after age adjustment) have the metabolic syndrome (Ford *et al.*, 2002). In 2004, Cameron *et al.* reported that the prevalence of the metabolic syndrome in the USA increased to approximately 60% in women and 45% in men.

The prevalence of type 2 diabetes mellitus has increased dramatically worldwide, posing a massive health problem in both developed and developing countries (Shaw & Chisholm, 2003). It is estimated that the number of diabetic subjects will more than double in the next 14 years (Ceriello & Motz, 2004). In developed countries, lower socio-economic groups are mostly affected, whereas in developing countries it is the higher socio-economic groups that are mostly affected. Worldwide, more than 150 million people are now living with diabetes mellitus (Shaw & Chisholm., 2003). Furthermore, it is speculated that this number would rise to 300 million by the year 2025 (Shaw & Chisholm, 2003).

In conclusion, numerous authors have identified the metabolic syndrome as a major health problem, affecting people all over the world and increasing gradually as the population becomes more obese and elderly (Cordain *et al.*, 2003; Mavri *et al.*, 2004). The metabolic syndrome is associated with various other diseases including polycystic ovarian syndrome (O'Brian & Dixon, 2002), nonalcoholic steatohepatitis (Farrell, 2003; O'Brian & Dixon, 2002), obstructive sleep apnea (O'Brian & Dixon, 2002), and asthma (O'Brian & Dixon, 2002). In addition, features of the metabolic syndrome are quite common in patients with schizophrenia (Ryan & Thakore, 2002). Therefore, the metabolic syndrome and related diseases have been classified as 'diseases of civilization' (Burkit, 1973; Eaton *et al.*, 1998; Seidell, 2000).

2.3 Components of the metabolic syndrome

As the definition of the metabolic syndrome suggests (Grundy *et al.*, 2004b), various clinical and non-clinical conditions are associated with this disease including:

- Abdominal obesity
- Physical inactivity
- Diet composition
- Atherogenic dyslipidemia
- Elevated blood pressure
- Insulin resistance
- Genetic factors
- Inflammation.

These factors will be discussed in the following section.

2.3.1 Abdominal obesity

Abdominal obesity is most strongly associated with the metabolic syndrome and presents itself as increased waist circumference (O'Brian & Dixon, 2002; Grundy *et al.*, 2004a). Furthermore, with obesity comes a higher risk for CHD, hypertension, dyslipidemia, diabetes, sleep apnea and respiratory problems (reviewed by Virgin & Scmitke, 2003), most of which can be associated with the metabolic syndrome. Leptin resistance could play a role in the epidemiology of obesity in the metabolic syndrome (Unger, 2003). The physiological role of high leptin concentrations in diet-induced obesity is to protect non-adipose tissue from lipotoxicity (over accumulation of lipids) (reviewed by Unger, 2003). When increased leptin concentrations (associated with obesity) fail to maintain normal lipid homeostasis in cells, lipitoxicity (an equivalent of the metabolic syndrome) ensues (Unger, 2003).

One of the focus areas of the treatment of the metabolic syndrome is the treatment of the presenting clinical outcomes of this disease (Anon, 2002). Weight loss is, therefore, an important part of the treatment of the metabolic syndrome. Regarding this, a 5% -

10% reduction in weight will result in an improvement in the lipid profile associated with CVD, blood pressure, insulin resistance and subsequent inflammation (Krauss *et al.*, 2000; Eckel *et al.*, 2005; Saylor, 2005; Wyszynski *et al.*, 2005). Furthermore, changes in the fat and energy intakes could also be beneficial in the prevention of lipotoxicity and the metabolic syndrome, including the treatment of this disease (Unger, 2003).

2.3.2 Physical activity

Approximately 70% of the United States population can be classified as being sedentary. Regular exercise has been shown to improve several metabolic risk factors. Physical inactivity should, therefore, be considered an important contributing factor to the development of the metabolic syndrome (Grundy *et al.*, 2004b). Furthermore, regular physical activity is one way through which to achieve and maintain weight loss (Grundy *et al.*, 2004b). The Diabetes Prevention Programme (Knowler *et al.*, 2002), demonstrated that a 7% reduction in body weight and 150 minutes of physical activity on a weekly basis, over a three year period, reduced the incidence of type 2 diabetes mellitus by 58% in individuals at significant risk to develop the disease. The same results were reported by the Finnish Diabetes Prevention Study and the US study in 2001 and 2002 respectively (Tuomilehto *et al.*, 2001; Knowler et al., 2002). Current guidelines recommend an exercise regimen of moderate-intensity exercise for at least 30 minutes a day (Thompson *et al.*, 2003). Results from a study done in 2003 on 105 sedentary subjects who were diagnosed as having the metabolic syndrome (NCEP ATP III criteria), showed a reduction in the prevalence of this disease (from 16.9% to 11.8%) after 20 weeks of aerobic exercise (Katzmarzyk *et al.*, 2003). In addition, previous studies have shown that physical activity without weight loss does not have any effect on the components of the metabolic syndrome (reviewed by Carrol & Dudfield, 2004).

2.3.3 Diet composition

It is widely agreed that dietary habits play a significant role in the prevention and management of the metabolic syndrome (Rosell *et al.*, 2004). Even so, the importance of different dietary components in the management of the metabolic syndrome is not

yet fully understood. The literature has linked excess dietary fat intake to insulin resistance and the metabolic syndrome (Grundy *et al.*, 2002; Riccardi *et al.*, 2004). However, the amount of fat in the diet remains controversial. Epidemiological studies show that a dietary fat intake of between 50% and 55% of the total energy intake had a negative effect on insulin resistance and increased the risk to develop the metabolic syndrome (reviewed by Riccardi *et al.*, 2004), while intervention trials remain inconclusive (reviewed by Riccardi *et al.*, 2004). A summary of ten intervention trials **(Table 1)** showed that a fat intake of between 30% and 83% of the total energy generally had no effect on insulin resistance.

With regard to the fatty acid composition, numerous animal studies have shown that a high intake of saturated fatty acids (SFA) may increase the risk to develop the metabolic syndrome, whereas mono-unsaturated fatty acids (MUFA's) and poly-unsaturated fatty acids (PUFA's) may delay the development of this disease (reviewed by Riccardi *et al.*, 2004). Epidemiological studies have reported similar results (reviewed by Riccardi *et al.*, 2004). However, these studies do not necessarily imply a cause/effect relationship, as this can only be proven by intervention studies. To date, the KANWU (acronym for the location of centres: Kuopio, Aarhus, Naples, Wollogong, and Uppsala) study (2001) is the only intervention trial performed using adequate methodologies and a sufficient sample size (Vessby *et al.*, 2001). Results from this study suggest that the total amount of fat can influence the development of the metabolic syndrome when it is consumed in amounts between 35% to 40% of the total energy intake (Vessby *et al.*, 2001). However, optimal dietary fat intake relative to CHO still remains a major unresolved issue. Previously, low-fat high-CHO diets were associated with an improvement in insulin resistance and glucose disposal. However, studies done in 1997 and 2000 failed to show any relationship between total CHO intake and the development of the metabolic syndrome (Salmeron *et al.*, 1997; Meyer *et al.*, 2000).

Table 1

Dietary fat and insulin sensitivity in healthy subjects or in individuals with type 2 diabetes or impaired glucose tolerance: high-fat vs. low-fat diets (adapted from Riccardi *et al.*, 2004).

Study	Fat content (%)	Subjects (n)	Duration (weeks)	Method	Relationship with insulin resistance
Chen *et al.*	55 vs. 0	H (18)	1 × 2	FSIGT	↑
Swinburn *et al.*	50 vs. 15	H (24)	2 × 2	FSIGT	↔
Borkman *et al.*	50 vs. 20	H (8)	3 × 2	Clamp	↔
Lovejouy *et al.*	50 vs. 20	H (31)	3 × 2	FSIGT	↑
Thomsen *et al.*	40 vs. 30	H (16)	4 × 2	FSIGT	↔
Bisshop *et al.*	83 vs. 41 vs. 0	H (6)	2 × 3	Clamp	↔
Parillo *et al.*	40 vs. 20	D (10)	2 × 2	Clamp	↓
Garg *et al.*	50 vs. 25	D (8)	2 × 3	Clamp	↔
Hughes *et al.*	30 vs. 20	D/IGT (10/10)	12	Clamp	↔
Sarkkinen *et al.*	40 vs. 34	D/IGT (17/14)	8	FSIGT	↔

H: Healthy subjects; D: Type 2 diabetes; IGT: Impaired glucose tolerance; FSIGT: Frequent sampling intravenous glucose tolerance test (minimal model); ↑: Increased insulin resistance; ↓: Decreased insulin resistance; ↔: Insulin resistance unchanged

2.3.4 Atherogenic dyslipidemia

This type of dyslipidemia is characterized by three primary abnormalities: increased TG concentrations, increased small dense LDL particles and decreased HDL-C concentrations (Grundy *et al.*, 2002), which are commonly present in obese individuals (Grundy, 2004). It is likely that most of the CHD risk associated with the metabolic syndrome is captured by increased blood pressure, increased total cholesterol (TC) concentrations and decreased HDL-C. In addition, increased TG concentrations and obesity further increase the risk for CHD (Grundy *et al.*, 2004a). In general, the increases in fatty acid flux to the liver increase the production of apo B-containing, TG-rich very low density lipoproteins (VLDL) (Eckel *et al.*, 2005). These particles may be atherogenic through their ability to produce a pro-inflammatory state (Grundy *et al.*,

2002). Insulin resistance also reduces lipoprotein lipase concentrations which may increase the TG concentrations even more (Eckel *et al.,* 2005). LDL particles associated with the metabolic syndrome and atherogenic dyslipidemia tend to be small and dense (Krauss, 1995). In fact, almost all individuals with fasting TG concentrations of > 2.0 mmol/L (normal ≤ 1.5 mmol/L) have a predominance of small, dense LDL particles (Eckel *et al.,* 2005). These particles display atherogenic properties through it's ability to transit through the endothelial basement membrane, it's ability to adhere well to glycosaminoglycans, it's toxicity to the endothelium, it's increased susceptibility to oxidation and/or it's ability to bind more readily to scavenger receptors on monocyte derived macrophages (Eckel *et al.,* 2005). Similarly, low HDL concentrations may be involved in the atherogenic process through various mechanisms (Grundy, 2004). Optimal high density lipoprotein cholesterol (HDL-C) concentrations are, among others, responsible for reverse cholesterol transport, they display anti-inflammatory properties, and have the ability protect against LDL modification (Grundy, 2004). In addition, obesity in itself reduces HDL-C concentrations (Anon, 1998) and is, therefore, considered an independent risk factor for CHD.

2.3.5 Elevated blood pressure

Elevated blood pressure is a key component of the metabolic syndrome (reviewed by Prabhakaran & Anand, 2004). Various proposed mechanisms exist through which elevated blood pressure leads to the metabolic syndrome. Firstly, high blood pressure could cause insulin resistance which may lead to the metabolic syndrome. Secondly, insulin resistance could cause elevated blood pressure and metabolic syndrome and thirdly, both can be responsible at the same time, as elevated blood pressure and insulin resistance could be consequences of genetic trade (Prabhakaran & Anand, 2004). Hyperinsulinemia causes elevated blood pressure through increased renal sodium and water reabsorption (Rowe *et al.,* 1981). Furthermore, insulin resistance may lead to vasoconstriction as a result of its vasoconstriction abilities (Sowers & Epstein, 1995). However, the relationship between blood pressure and the metabolic syndrome remains controversial, as not all the individuals who meet the diagnostic criteria of the metabolic syndrome have elevated blood pressure (Prabhakaran & Anand, 2004).

2.3.6 Insulin resistance

Three potential etiological categories have been associated with the metabolic syndrome (NCEP ATP III criteria): obesity, a constellation of independent factors with hepatic, vascular and immunologic origins and insulin resistance (Grundy *et al.*, 2004a). In addition, it is widely believed that insulin resistance is central to the development of the metabolic syndrome (Grundy *et al.*, 2004a) and may progress to the development of type 2 diabetes and atherosclerosis **(Figure 1)** (Hsueh *et al.*, 2004). Apart from glucose intolerance, little clinical evidence exists to substantiate the fact that an improvement in insulin resistance will improve the components of the metabolic syndrome (Grundy *et al.*, 2004b). Even so, insulin resistance is strongly associated with atherogenic dyslipidemia, endothelial dysfunction, inflammation and oxidative stress and may be considered an independent risk factor for the development of the CVD (Grundy *et al.*, 2004b).

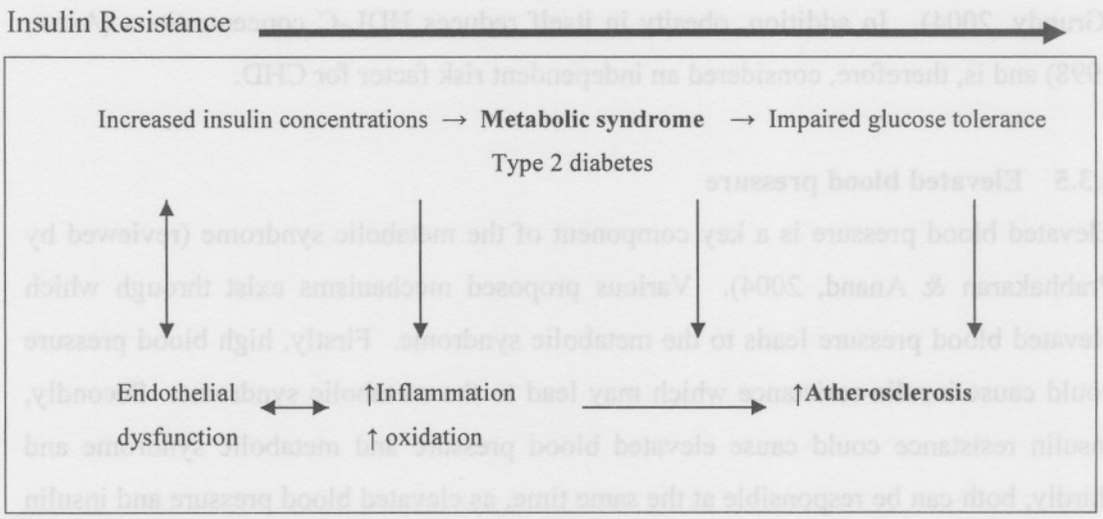


Figure 1
Progression of insulin resistance to type 2 diabetes parallels the progression of endothelial dysfunction to atherosclerosis (adapted from Hsueh *et al.*, 2004).

2.3.7 Genetic factors

Genetic factors influence the components of the metabolic syndrome. The literature reports that insulin resistance clusters in families (Groop *et al.*, 1996). Regarding this, 45% of first-degree relatives of patients with type 2 diabetes are insulin resistant compared to 20% of individuals without a family history of diabetes (Groop *et al.*,

1996). The estimation of the heritability in obesity has varied (20% - 90%), depending on whether the results were based on twin, adoption or family studies (Maes *et al.*, 1997). Heritability also influences other components of the metabolic syndrome, such as high blood pressure (Levy *et al.*, 2000) and TG and HDL-C concentrations (Snieder *et al.*, 1999).

2.3.8 Inflammation

Inflammation can be defined as the body's response to injury (Brink, 1997:349). Inflammation represents a central role in the pathophysiology of insulin resistance and atherosclerosis (Mavri *et al.*, 2004) **(Figure 1)**. Furthermore, inflammation is an additional feature associated with the metabolic syndrome that is not included in the definition (reviewed by Prabhakaran & Anand, 2004). Abnormalities in fibrinolysis (increased levels of plasminogen activator inhibitor (PAI-1), fibrinogen, and von Willebrand factor (acute phase proteins) have been attributed to the metabolic syndrome (reviewed by Isomaa, 2003). In addition, the inflammation associated with the metabolic syndrome is characterized by increases in serum high-sensitive C-reactive protein (CRP) (Isomaa, 2003). Observational trials confirmed these results by showing that insulin resistance is significantly related to higher concentrations of acute phase proteins (CRP, fibrinogen, and PAI-1) (Stentz *et al.*, 2004). The metabolic syndrome has also been linked to increased concentrations of other inflammation markers including pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), leptin, adiponectin and interleukin-10 (IL-10) (Garg *et al.*, 2003; Devaraj *et al.*, 2004). CRP is an inflammatory marker produced by the liver under the stimulation of cytokines including interleukin-1 (IL-1), Il-6 and TNF-α (Isomaa, 2003). Results from the Insulin Resistance and Atherosclerosis study (2000) showed a positive association between CRP and BMI, waist circumference, blood pressure, TG, cholesterol, LDL-C, plasma glucose, and fasting insulin. However, the study showed an inverse association between CRP and HDL-C (Festa *et al.*, 2002).

A relationship between adipose tissue and the immune system has been well described. However, many theories as to how such a link can exist have been mentioned. One of

these theories involves the triggering of insulin resistance by chronic inflammation (Pickup & Crook, 1998). β-cells are highly susceptible to oxidative damage due to low antioxidant concentrations. Therefore, oxidative stress may impair insulin action by changing the physical state of the β-cells (Ylönen *et al.,* 2003). Stimuli, such as overeating, result in cytokine hyper-secretion, which then lead to insulin resistance and subsequent diabetes. Overeating may also result in (visceral) obesity and may, therefore, be a contributory factor to the inflammatory response and ultimately the metabolic syndrome.

2.4 Clinical management of the metabolic syndrome

Effective treatment of the components of the metabolic syndrome will reduce the severity of all of the metabolic risk factors (Eckel *et al.,* 2005). However, if people are found to be at a particularly high risk or if a given component is severely abnormal, drug therapy may be necessary (Eckel *et al.,* 2005). In **Table 2**, approaches to the clinical management of each factor are discussed.

Table 2

Recommendations for the clinical management of the metabolic syndrome (adapted from Eckel *et al.*, 2005).

Component	Goals & recommendations	
	Goal	Recommendation
Abdominal obesity	10% weight loss during the first year after diagnosis. Thereafter, achieve or maintain a healthy body weight	Energy restriction, regular exercise, behaviour modification
Physical inactivity	Regular moderate-intensity physical activity	30 – 60 minutes moderate intensity exercise daily
Atherogenic diet	Reduced intakes of SFA, trans fatty acids and cholesterol	SFA intake should be ≤ 7% of TE, reduce trans fatty acid intake, daily dietary cholesterol intake should be < than 200mg, total fat intake should be between 25% and 35% of the TE
High LDL-C	*High risk patients: < 2.6 mmol/L #Moderately high and ΘModerate risk patients: < 3.4 mmol/L	≡Lifestyle therapies, LDL-C lowering medication
High TG or low HDL-C	Insufficient data to establish goal	Medication to improve TG and/or HDL-C concentrations
Elevated blood pressure	< 135/<85 mm Hg For diabetes: < 130/80 mm Hg	Lifestyle therapies, antihypertensive medication to achieve goal when necessary
Elevated glucose	Maintenance or reduction in fasting glucose if > 5.5 mmol/L	Lifestyle therapies, medication when necessary

*High risk patients: Those with established atherosclerotic cardiovascular disease, diabetes, or 10-year risk for coronary heart disease > 20%; #Moderately high risk patients: Those with a 10-year risk for coronary heart disease 10-20%; ΘModerate risk patients: Those with metabolic syndrome but 10-year risk for coronary heart disease < 10%; ≡Lifestyle therapies: Weight reduction, regular exercise, and antiatherogenic diet; SFA: Saturated fatty acids; TE: Total energy; LDL-C: Low density lipoprotein cholesterol; TG: Triacylglycerol; HDL-C: High density lipoprotein cholesterol.

3. OXIDANTS, ANTIOXIDANTS AND OXIDATIVE STRESS

3.1 Introduction

Oxidative stress (increased oxidation as a result of decreased antioxidants) may play a role in the pathophysiology of the metabolic syndrome and CVD (reviewed by Ford *et al.,* 2003). Findings from the Third National Health and Nutrition Examination Survey (NHANES III) (2003) showed a positive association between low vitamin E concentrations and the metabolic syndrome (Ford *et al.,* 2003). Furthermore, chronic hyperglycemia, as in diabetes mellitus, produces multiple biochemical effects, including oxidative stress (Martín-Gallán *et al.,* 2003). This diabetes-induced oxidative stress may play a role in the onset of the disease, where persistent hyperglycemia may cause elevated production of free radicals, generated in direct autoxidation processes of various compounds (Inoguchi *et al.,* 2000; Martín-Gallán *et al.,* 2003). Evidence also exists that hyperglycemia may disrupt natural antioxidant defenses (Dandona *et al.,* 2004). It has been suggested that prolonged exposure to elevated levels of glucose or free fatty acids (FFA), or a combination of both, may result in ß-cell dysfunction (Ceriello & Motz, 2004). Increased concentrations of FFAs are positively associated with both insulin resistance and the deterioration of ß-cell function in the presence of elevated glucose concentrations (Ceriello & Motz, 2004).

High-fat diets have been shown repeatedly to induce insulin resistance and increase FFA concentrations in subjects with euglycemia (reviewed by Krebs & Roden, 2004). However, at present it is not clear whether high lipid concentrations are the cause or the consequence of insulin resistance (Krebs & Roden, 2004). Insulin resistance and increased lipid concentrations are accompanied by a reduction of insulin stimulated glucose disposal and this may suggest a possible relationship between increased intracellular lipid concentrations and insulin resistance (Krebs & Roden, 2004). A recent study demonstrated that, in non-diabetic subjects, fat accumulation (as a result of high-fat diets) correlated closely with markers of oxidative stress (Furukawa *et al.,* 2004).

Lipid peroxidation plays a central role in the development of the metabolic syndrome, as poly-unsaturated fatty acids in the cell membrane are subjected to oxidation (Asplund, 2002). However, in *in vitro* experiments, LDL oxidation was prevented when antioxidant vitamins were added (Asplund, 2002). During this process, there was absorption of antioxidants starting with ubiquinone, followed by α-tocopherol and β-carotene, supporting the hypothesis that antioxidants may have different bioavailability and activities (Fukuda *et al.,* 2003). On depletion of these antioxidants, there was a rapid destruction of PUFA's in LDL (Mashima *et al.,* 2001). Information regarding different antioxidants and the effect on risk markers for the metabolic syndrome and CVD is presented in **Table 3**.

The question of whether antioxidants could have a beneficial effect on reducing the risk for these conditions has been investigated, but the results remain inconclusive (Asplund, 2002). However, if oxidative stress plays a role in the development of the metabolic syndrome and if antioxidants could have a beneficial effect on reducing the risk for this disease, understanding the pathophysiology of oxidative stress and the physiological status of antioxidant concentrations among people at risk to develop these conditions, is of interest.

Table 3

The relationship between antioxidants and some of the risk factors for the metabolic syndrome and CVD (adapted from Rice-Evans, 2001, Asplund, 2002)

Cardiovascular risk factors	Relationship to antioxidants		
	Carotene	Ascorbic acid	Tocopherol
Total cholesterol	Insufficient information	Reduction if TC is high and there is an ascorbic acid deficiency No effect in people with normal TC concentrations without ascorbic acid deficiency	No effect
LDL-C		Reduction if LDL-C is high and there is an ascorbic acid deficiency No effect in people with normal LDL-C concentrations without an ascorbic acid deficiency	No effect
LDL oxidation	May modify LDL oxidation when taken as a food supplement. However, does not occur under all conditions	Reduce LDL oxidation in combination with tocopherol	Reduce LDL oxidation in combination with ascorbic acid
HDL-C	Insufficient information	Increase HDL-C concentrations if concentrations are low. Effects are increased if there is an ascorbic acid deficiency. No effect in people with normal HDL-C concentrations without an ascorbic acid deficiency	No effect
TG	Insufficient information	Insufficient information	No effect
Blood pressure	Insufficient information	Reduction if intake is high (observational studies) Large doses as food supplements may lower BP	No apparent relationship

LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; TG: Triacylglycerol; BP: Blood pressure

3.2 Redox sensitive cell signaling

Free radicals (molecules with an odd number of electrons and high chemical activity) (Iamele *et al.,* 2002) are potentially toxic and are thus scavenged by antioxidants before they can inflict damage to lipids, proteins or nucleic acids (Yildiz *et al.,* 2002).

Excessive production of free radicals is involved in aging, atherosclerosis and diabetes, among others (Iamele *et al.,* 2002). It is possible that reactive free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) act as signals or mediators of change in cell function and/or cell death (Jackson *et al.,* 2002). Under conditions of oxidative stress, cellular responses to ROS are critical in helping to maintain cellular functions and making the decision between cell survival and cell death (Jackson *et al.,* 2002). A variety of stimuli lead to intracellular ROS production, including TNF-α (Jackson *et al.,* 2002). TNF-α provokes a rise in hydrogen peroxide production from the mitochondria, as well as other ROS via induction of NADPH oxidase (reviewed by Jackson *et al.,* 2002). In many respects, the effects of RNS parallel the effects of ROS and antioxidants on cellular function (Jackson *et al.,* 2002). For example, nitric oxide (a RNS) has beneficial physiological effects, such as enhancing vasodilatation and inhibiting the formation of platelet thrombi (Li & Forstermann, 2000). Furthermore, low concentrations of nitric oxide are constantly produced by the endothelium. Dietary antioxidants may enhance the formation of nitric oxide (Tomasian *et al.,* 2000), which then neutralizes lipid peroxidation (chain breaking activity) and can, therefore, be considered as an antioxidant (Jackson *et al.,* 2002). Chronic inhibition of nitric oxide (and other antioxidants) induces vascular inflammation (reviewed by Jackson *et al.,* 2000), that may increase the risk to develop the metabolic syndrome (as discussed in 2.3.8).

3.3 Cellular responses to oxidative stress

The effects of oxidative stress depend on the cell type, the level of oxidative stress experienced and the protective mechanism in place (intrinsic antioxidant system), particularly the glutathione (bodies natural defense against oxidation) concentrations within the cell (Jackson *et al.,* 2002). When cellular glutathione concentrations are low, the cell environment will be oxidized (Jackson *et al.,* 2002). Oxidative stress can be assessed by measuring reduced (GSH) and oxidized (GSSG) glutathione and is often expressed as the ratio between the two (Moskuag *et al.,* 2005). Free radicals are

recognized to play a part in cell death and cell necrosis. For example, ROS play an important part in the mediation of inflammatory responses (Jackson *et al.*, 2002).

3.4 Markers of antioxidant capacity and oxidative stress

Direct measurements of free radicals are confined to electron spin resonance, a complicated and expensive methodology not available for clinical purposes (Iamele *et al.*, 2002). Therefore, free radical production is normally estimated by indirect methods (Iamele *et al.*, 2002). Those most frequently used are summarized in **Table 4**.

Potentially harmful ROS are usually removed or inactivated by individual antioxidant defenses that work synergistically to form a team of antioxidants (Benzie & Strain, 1996). These antioxidants destroy potential oxidants, thus minimizing oxidative stress. However, a deficiency of these antioxidant defenses may lead to increased oxidative stress (reviewed by Benzie & Strain, 1996). Tests which measure the combined antioxidant effect of antioxidant defenses in biological fluids may be useful in providing an index of the ability to resist oxidative damage (antioxidant capacity). Most tests measuring the total antioxidant capacity have measured the ability of the plasma to withstand oxidative damage (Benzie & Strain, 1996). However, these measurements require specialized equipment and are time-consuming and technically demanding, limiting the amount of tests available.

Table 4

Most frequent markers of antioxidant capacity and oxidative stress (adapted from Raggi *et al.*, 1991; Rice-Evans & Miller, 1994; Benzie & Strain, 1996; Cao & Prior, 1998; Prior & Cao, 2000; Cornelli *et al.*, 2001; Iamele *et al.*, 2002; Urso & Clarcson, 2003)

MARKER	WHAT MEASURES	LIMITATIONS	STRENGTHS
dRoms (diacron reactive metabolites)	Presence of radical formation & estimates its magnitude; evaluates pro-oxidant status	Manual assay is clinically imprecise	Automatic assay is accurate; not influenced by age or gender; appropriate tool with which to determine the type and dosage of antioxidants
ORAC (oxygen radical absorbance capacity)	Antioxidant absorbance capacity (water-soluble antioxidants); ability to scavenge free radicals	Protein interference during measurement of antioxidant capacity	Combines inhibition time and inhibition degree of free radical action of antioxidants into a single quantity; different free radical generators or antioxidants can be used in assay; highly specific
FRAP (Ferric reducing antioxidant power)	Reducing capabilities ($Fe^{III} \rightarrow Fe^{II}$)	Does not measure SH-group containing antioxidants *(controversial?)*	Simple; reliable method to study antioxidants activity of various compounds; highly reproducible; inexpensive; reagents are easy to prepare
TRAP (total radical trapping ability of plasma)	Defenses against oxidative stress *in vivo*; measures antioxidants capacity	Produce radicals only in the aqueous compartment of the plasma; time consuming; requires high dilution of plasma to produce suitable lag phase; oxygen electrode will not maintain its stability over the period of time required	Sensitive; reliable
TBARS (thiobarbituric acid reactive substances)	Direct measure of oxidative stress; measures antioxidant activity	Unspecific	Easy; rapid; reliable; practical for routine measurements of total antioxidants activity in serum and other body fluids; works well on defined membrane systems (microsomes *in vitro*)
Glutathione	Measure bodies own antioxidant defense system	Under or overestimation	Rapid; sensitive; selective method for the quantification of GSSG and GSH in biological materials; accurate; precise

GSSG: Oxidized glutathione; GSH: Reduced glutathione

3.4.1 Diacron reactive metabolites (dRom) assay

The production of free radicals enhances the formation of reactive oxygen metabolites, which are responsible for damaging effects to lipids and DNA (Stohs, 1995). Since cell injury can be induced by oxidative stress, the measurements of reactive oxygen metabolites (ROM) may be useful in clinical settings to evaluate whether the antioxidant defense systems are adequate. This is done by a relatively new method, the Diacron reactive metabolites (dRom) assay (Iamele *et al.,* 2002), which is based on the ability of transition metals to catalyse the formation of free radicals in the presence of hydroperoxides, which are then trapped by an amine. The dRoms assay utilizes the N,N-diethyl-para-phenylendiamine (DEPPD), which reacts with the free radicals to form a coloured radical detectable at 505 nm and can be measured with a conventional spectrophotometer (Alberti *et al.,* 2000). dRoms measures ROM in metabolically active fluid. Compared to the automated assay, the manual assay may result in analytical imprecision (Alberti *et al.,* 2000). The dRoms assay is measured in Cartelli units (U.CARR), with the normal range between 250 and 300 U.CAR (healthy individuals), where 1 U.CARR corresponds to 0.8 mg/L H_2O_2 (Cornelli *et al.,* 2001). dRoms values > 300 U.CARR indicate a condition of oxidative stress (Cornelli *et al.,* 2001).

3.4.2 Oxygen radical absorbance capacity (ORAC) assay

ORAC (measured in Trolox equivalent/L) measures the total antioxidant capacity in both the hydrophilic and lipophilic compartments of the (blood/food) sample (Prior & Cao, 2000) and involves a pro-oxidant (free radical) and an oxidizable substrate (Prior & Coa, 1999). The effectiveness of the antioxidant network in the body depends on the normal functioning of each antioxidant component in the network (Prior & Cao, 2000). The ORAC assay depends on the detection of chemical damage to β- or *R*-phytoerythrin (PE) through decrease in its fluorescence emission (Prior & Cao, 2000). A loss of PE fluorescence in the presence of free radicals is an indication of oxidative damage (Prior & Cao, 2000). To date, the ORAC assay is the only method that takes free radical action to completion and uses an area-under-curve technique for quantification, thus combining the inhibition percentage and the length of inhibition

time of the free radical action by antioxidants into a single quantity (Cao & Prior, 1998). The ORAC assay is highly specific and it measures the capacity of an antioxidant to directly quench free radicals (Cao & Prior, 1998).

3.4.3 Ferric reducing antioxidant power (FRAP) assay

Tests which measure the combined antioxidant effect of the nonenzymatic (i.e. reductants) defenses in biological fluids may be useful in providing an index of ability to resist oxidative damage (Benzie & Strain, 1996). The inactivation of oxidants by reductants can be described as redox reactions in which one reactive species is reduced at the expense of the oxidation of another (Benzie & Strain, 1996). In this context, antioxidant power may be referred to as the reducing ability of antioxidants. The ferric reducing antioxidant power (FRAP) assay measures the ferric reducing ability of the sample, where ferrictripyridyltriazine (Fe^{III}) is reduced to the ferrous (Fe^{II}) form. With this, an intense blue colour develops at a low pH (reviewed by Benzie & Strain, 1996). However, the reaction is non-specific, but is easy to perform and gives fast, reproducible results (Benzie & Strain, 1996). One limitation may be the non-physiologically low pH (3.5) value used (Rice-Evans, 2000).

3.4.4 Total radical trapping antioxidant parameter (TRAP)

TRAP measures secondary antioxidant activities (i.e. α-tocopherol) and expresses the result as μmoles (reviewed by Ghiselli *et al.,* 1995). This assay is based on the time taken to prevent maximum oxygen uptake (lag phase) in a system containing a free radical generator, lipids and antioxidants (Prior & Cao, 1999). The lag phase induced by the TRAP assay is compared to that of an internal standard, Trolox (Aldrich, Milwaukee, WI, USA), and then quantitatively related to the antioxidant capacity of the plasma (Prior & Cao, 1999). However, the oxygen will not maintain its stability over the period of time required and thus poses a major problem (Rice-Evans & Miller, 1994).

3.4.5 Thiobarbituric acid reactive substances (TBARS) assay

The TBARS assay is used to measure oxidative stress and is based on the changes in malondialdehydes (MDA) to assess the rate of lipid peroxidation (Urso & Clarkson,

2003). This assay works well when used in defined membrane systems, such as microsomes *in vitro* (Halliwell & Chirico, 1993), but it lacks specificity and is, therefore, criticized for use in humans (Urso & Clarcson, 2003). However, the TBARS assay is easy, rapid, reliable and practical for routine measurements of total antioxidant activity in serum and other body fluids and only small samples of biological fluid are needed for analysis (Koracevic *et al.,* 2001).

3.4.6 Glutathione

A variety of high-performance liquid chromatography systems have been developed for the determination of glutathione in small quantities of tissue. Some involve the precolumn derivatization of the sulfhydryl group with fluorescent label (Newton *et al.,* 1981), or the trapping of the group with iodoacetic acid followed by reaction of the amino groups with a chromophore (reviewed by Alpert & Gilbert, 1985). However, these methods are not specific for glutathione and generally do not allow measurement of GSSG (Alpert & Gilbert, 1985). Other methods employ postcolumn reactions with chromogenic or fluorogenic reagents (Watanabe & Imai, 1983), or electrochemical detection (reviewed by Alpert & Gilbert, 1985). These methods lack sensitivity and are also not specific for glutathione (Alpert & Gilbert., 1985). The recycling assay (discovered by Owens & Belcher and developed by Tietze) is more sensitive and specific than the other assays (Alpert & Gilbert, 1985). The assay responds to both GSH and GSSG. However, GSSG in biological samples can be overestimated because of oxidation of GSH or underestimated because of reduction of GSSG by endogenous NADPH via glutathione reductase (Alpert & Gilbert, 1985).

4. SUMMARY

Considering the different aspects of the metabolic syndrome and the various factors involved in the development thereof, it can be concluded that an integral relationship between oxidative stress, inflammation and the metabolic syndrome exists. In addition, obesity is called an underlying risk factor for the metabolic syndrome (Grundy, 2004). Moreover, the prevalence of obesity is associated with decreased concentrations of

antioxidants (Ford *et al.,* 2003). In the presence of obesity, multiple products are released from the adipocytes, including FFA, inflammatory cytokines, PAI-1 and leptin (Grundy, 2004). Elevated glucose concentrations and FFA may influence endothelial and ß-cells through oxidative stress, producing endothelial dysfunction and eventually leading to atherosclerosis (Ceriello & Motz, 2004). An association between oxidative injury and the development of the metabolic syndrome has been reported, as enhanced oxidative stress may result from increased production of free radicals and/or impaired antioxidant systems (Gökkusu *et al.*, 2001; Palanduz *et al.,* 2001). However, antioxidant supplementation may reduce oxidative stress and improve insulin function (Gökkusu *et al.,* 2001) and it can be speculated that this may result in reduced inflammation and a lower risk for the metabolic syndrome. Many assays exist to measure the antioxidant capacity or oxdidative stress in biological fluid. Some of the most frequently used assays include ORACs, FRAP, dRoms, TRAP and TBARS. The ORAC assay measures total antioxidant capacity whereas dRoms measure end products of oxidation. Therefore, dRoms values should decrease with a rise in ORAC values (less oxidative stress) (Prior & Coa, 1999; Cornelli *et al.,* 2001).

5. NUTS

5.1 Introduction

Nuts have traditionally been considered foods with a high nutritional value (García-Lorda *et al.,* 2003). Nuts are low in saturated fatty acids (SFA), but high in unsaturated fatty acids (García-Lorda *et al.,* 2003). Furthermore, nuts are one of the most important sources of dietary fibre and good sources of plant proteins, antioxidants, vitamins, minerals, polyphenols (García-Lorda *et al.,* 2003), magnesium, potassium and arginin (Dreher *et al.,* 1996). The protective effects of nuts are mediated through several mechanisms. Clinical trials have shown that approximately two to three servings of nuts per day (30g/serving) decreases TC and LDL-C (Feldman, 2002). In addition, a diet rich in nuts may result in lower TG concentrations and may prevent decreases in HDL-C, when compared to a high-fat diet (Feldman, 2002). However, a recent clinical trial showed no effect on HDL-C, TG, TC and LDL cholesterol when

participants consumed between 63 and 108g (20% of total energy) of cashew nuts or walnuts per day (Mukkuddem-Petersen, 2005). In addition, nuts are rich sources of antioxidants (vitamin E and polyphenols) and to a lesser extent, vitamin C and, therefore, may have a protective effect against oxidative damage (Dreher *et al.,* 1996). Unsaturated fatty acids and fibre may improve plasma lipids by decreasing TC concentrations, decreasing platelet aggregation and preventing arrhythmias (Elin, 1993). Magnesium and potassium may improve blood pressure (Fraser, 1999). In addition, nuts are known to have a low glycemic index (LGI) (Forster-Powell & Brand Miller, 1995), which may be one of the mechanisms whereby they exert beneficial effects on markers of the metabolic syndrome.

For this discussion, the focus will be on the antioxidant properties of nuts and the possible effects thereof on markers of the metabolic syndrome.

5.2 Composition of nuts and proposed mechanism of action

5.2.1 Composition of nuts

Table 5 gives a summary of the nutrient content of walnuts, cashew nuts, hazelnuts and almonds. Generally nuts are high in unsaturated fats, protein, fibre, essential fatty acids and vitamin E (Hu *et al.,* 1998; Lovejoy *et al.,* 2002). The energy content of nuts ranges from 23.7 to 29.3 kJ/g, whereas the fat content ranges from approximately 0.51 to 0.73 g/g (Kris-Etherton *et al.,* 1999). Nuts are low in SFA and high in unsaturated fatty acids, predominantly MUFAs (≈62% of fat content) (Kris-Etherton *et al.,* 1999). Together, MUFAs and PUFAs contribute ≈91% of the total fat content (Kris-Etherton *et al.,* 1999). Walnuts are unique because they are a rich source of α-linolenic acid, an omega-3 PUFA (Kris-Etherton *et al.,* 1999). Compared to frequently used vegetable oils, nuts have less SFA than olive oil, but more SFA than canola oil (Kris-Etherton *et al.,* 1999). With regard to micronutrients, chromium and zinc are involved in carbohydrate and lipid metabolism. Both these micronutrients are part of the composition of nuts (Cabrera *et al.,* 2003). Moreover, the most frequent sign of chromium deficiency is altered glucose tolerance (Cabrera *et al.,* 2003). Furthermore,

chromium deficiency has been associated with diabetes and CVD (reviewed by Cabrera *et al.,* 2003). In addition, zinc has been recognized as a co-factor of the superoxide dismutase enzyme, which is involved in protection against oxidation (reviewed by Cabrera *et al.,* 2003).

Table 5

Nutrient composition of 100g of various nuts (Halvorsen *et al.*, 2002; Cabrera *et al.*, 2003; Wu *et al.*, 2004; FoodFinder 3, Medical Research Council of South Africa, Tygerberg).

Selected nutrients	Walnuts	Cashew nuts	Hazelnuts	Almonds	Pistachio nuts	Macadamia nuts
Energy						
(kJ)	2844	2533	2797	2604	2563	3101
(kcal)	677.14	603.10	655.95	620	610.24	738.33
Protein (g)	14.30	15.30	13.00	20.40	20.6	8.3
CHO (g)	13.50	26.60	9.20	7.50	14	8.4
Fibre (g)	4.80	6.10	6.10	11.00	10.8	5.3
Total fat (g)	61.90	46.40	62.60	52.50	48.4	73.7
MUFA (g)	14.18	27.32	49.09	34.11	32.67	58.17
PUFA (g)	39.13	7.84	6.00	11.02	7.32	1.72
SFA (g)	5.59	9.16	4.60	4.98	6.13	11.04
ω-6:ω-3	5:1	48:1	39:1	28:1	28:1	0*
Vitamin E (mg)	2.62	0.57	23.92	20.26	5.21	0
Antioxidant capacity (µmol TE/g)	>20	≈20	<1.0	44.5	79.8	17.0
Chromium (µg/g)	0.35	0.29	0.37	0.40	0	0
Zinc (µg/g)	28.50	40.00	42.50	38.80	1.34	1.71

CHO: Carbohydrate; MUFA: Mono-unsaturated fatty acid; PUFA: Poly-unsaturated fatty acid; SFA: Saturated fatty acid; Antioxidant capacity: Measured using the oxygen radical absorbance capacity (ORAC) method; *According to food finder 3 macadamia nuts has no ω-3 fatty acids

5.2.2 Poly-unsaturated fatty acids in nuts and inflammation

Walnuts are unique compared to other nuts because of their high content of PUFAs, including linoleic acid (ω-6) and linolenic acid (ω-3) with a ω-6:ω-3 ratio of 5:1 (Almario *et al.,* 2001; FoodFinder 3, Medical Research Council of South Africa, Tygerberg). Experimental and intervention studies have reported protective effects of ω-3 vs. ω-6 on autoimmune disease in animal models (Harbige, 1998). In experimentally-induced autoimmune disease, supplementation with ω-3 fatty acids appears to augment the disease, whereas ω-6 fatty acids prevent or reduce the severity of the disease (Harbige, 1998). The protective effects of ω-6 fatty acids on autoimmune disease remain unclear, but may include dihomo-gamma-linolenic acid and arachidonic acid-sensitive immunoregolatory responses (Harbige, 1998). It is often claimed that ω-6 fatty acids promote inflammatory diseases based on results obtained with linoleic acids only. However, linoleic acid does not reflect the functions of other ω-6 fatty acids. ω-3 fatty acids protect against inflammatory disease, in part, through reduced pro-inflammatory cytokine production (reviewed by Harbige, 1998). The interplay between signaling pathways associated with oxidative stress creates a complex system when considering the effects of PUFAs. For example, ω-3 fatty acids are more effective in reducing inflammation compared to ω-6 fatty acids, yet ω-6 fatty acids favour the production of prostaglandin (PG)-E_2 over PGE_3 (reviewed by Jackson *et al.,* 2002). PGE_2 acts primarily as an anti-inflammatory agent, inhibiting the production of pro-inflammatory cytokines. However, these effects only occur at high concentrations, making the anti-inflammatory effects of ω-3 fatty acids more effective at lower concentrations compared to ω-6 fatty acids (reviewed by Jackson *et al.,* 2002).

5.2.3 Antioxidant content and capacity of nuts

Antioxidants are needed to prevent the formation and oppose the actions of ROS and nitrogen species, which are generated *in vivo* and cause damage to DNA, lipids and proteins. Compared to pecan nuts (antioxidant capacity: 179.4 μmol TE/g), walnuts (>20 μmol TE/g), cashew nuts (≈20 μmol TE/100g), hazel nuts (< 1.0 μmol TE/100g) and almonds (<1.0 μmol TE/100g) have smaller antioxidant capacities to that of pecan

nuts (Wu *et al.,* 2004). In a sequence from the highest to the lowest antioxidant capacity, pecan nuts have the highest antioxidant capacity (179.4 µmol TE/g), followed by pistachio nuts (79.8 µmol TE/g), almonds (44.50 µmol TE/g), cashew nuts (20.00 µmol TE/g) and macadamia nuts (17.00 µmol TE/g) (Wu *et al.,* 2004). The antioxidant capacities were measured using the ORAC assay (Wu *et al.,* 2004; Ninfali *et al.,* 2005).

Endogenous antioxidant defenses are inadequate to prevent oxidative damage completely (Halliwell, 1996). Diet-derived antioxidants are, therefore, important in maintaining health. Much evidence exists to support the strong antioxidative action of vitamin E, polyphenols and to a lesser extent, vitamin C (Jackson *et al.,* 2002), which are present in nuts. Vitamin E is the most efficient chain breaking antioxidant that protects tissue membranes from oxidative damage (Montonen *et al.,* 2004), while polyphenols act as radical scavengers (Kris-Etherton *et al.,* 2004).

Although it has been suggested that antioxidant therapy protects against oxidative stress, results from intervention trials with a single compound (vitamin E and C or ß-carotene) could not show any positive effects on oxidative stress (Halvorsen *et al.,* 2001). Trials with certain antioxidants have resulted in adverse disease outcomes, such as an increased risk to develop lung cancer with ß-carotene supplementation (Blumberg & Block, 1994). A possible explanation could be that single antioxidant supplements may act as pro-oxidants (antioxidants that interact to negate pro-oxidant effects), contributing to the destructive effects of oxidative stress (reviewed by Abudu *et al.,* 2004).

5.3 The effects of antioxidant nutrients and polyphenols in nuts and glutathione on markers of the metabolic syndrome

As discussed earlier, free radicals contribute to autoimmune destruction of β-cells, leading to diminished secretion of insulin by the pancreas and subsequent hyperglycemia (Reunanen *et al.,* 1998). Furthermore, free radicals may be involved in

the development of microvascular and macrovascular complications of diabetes through hyperglycemia, insulin resistance and atherosclerosis (reviewed by Reunanen *et al.,* 1998). It has been shown that antioxidant therapy (carotenoids, vitamin E, and polyphenols) reduces oxidative stress (Jackson *et al.,* 2002). In addition, combination therapy, such as vitamin C and E, seems to be more advantageous than single antioxidants (Škrha *et al,* 1999), as a synergistic relationship between different antioxidants exists (Ghiselli *et al.,* 2000). As reported previously, nuts are sources of antioxidant nutrients (vitamin E and C) and non-nutrients (polyphenols). Furthermore, other nutrients such as PUFA's and MUFA's may also exert antioxidative effects (Jackson *et al.,* 2002). In this regard, the effects of these nutrients on markers of the metabolic syndrome will be discussed in the following section.

Even though glutathione is not an antioxidant nutrient found in nuts, it is an important part of the endogenous antioxidant defense system. Furthermore, glutathione concentrations are effected by vitamin E and polyphenol concentrations (Jackson *et al.,* 2002) and are, therefore, discussed as part of the antioxidant nutrients in nuts and their effects on markers of the metabolic syndrome.

5.3.1 Vitamin E

It is well documented that vitamin E improves the body's antioxidant defense system and may have a beneficial effect in improving glucose metabolism and insulin sensitivity (Paolisso *et al.,* 1993; Paolisso *et al.,* 1994; Barbagallo *et al.,* 1999a). Under ideal conditions, vitamin E reacts with a lipid peroxyl radical in a single electron reaction, forming a vitamin E radical. This radical breaks the chain reaction of lipid peroxidation. This prevents lipid peroxidation and the formation of oxidized LDL (reviewed by Jackson *et al.,* 2002), thereby decreasing the susceptibility of LDL to *ex vivo* oxidation (Carpenter *et al.,* 2003). Four weeks of vitamin E supplementation (600mg/d) resulted in increased insulin sensitivity, increased glucose disposal and improved GSH/GSSG ratio (Barbagallo *et al.,* 1999a). The relationship between vitamin E and glucose disposal may be mediated by the ability of vitamin E to stimulate glutathione (Barbagallo *et al.,* 1999a). A rise in the plasma GSH/GSSG ratio significantly improves total body

glucose disposal in healthy subjects and in diabetic patients by slightly improving the beta-cell response to glucose (Paolisso *et al.,* 1992). Furthermore, GSH may also improve glucose metabolism by potentiating magnesium synthesis, which in turn acts as a final common pathway to regulate glucose disposal (Barbagallo *et al.,* 1999b).

Some researchers suggest that there might be limitations to antioxidant infusion in subjects with insulin sensitivity and the metabolic syndrome. Results from the Insulin Resistance and Atherosclerosis Study show that the intake of vitamin E from food and supplements had no effect on insulin sensitivity after adjustment for BMI and age (Sanchez-Lugo *et al.*, 1997). In this case, vitamin E (as a pro-oxidant) may react with an aqueous radical from outside the lipoprotein, where the range of movement of the vitamin E molecule is limited. Vitamin E would, therefore, most likely react with another aqueous radical if it appears. If not, it will react with either another lipid soluble antioxidant within the lipoprotein, or an unsaturated fatty acid. In this case, a lipid peroxyl radical will be formed which can initiate a chain reaction of lipid peroxidation until it is terminated by another antioxidant (reviewed by Abudu *et al.,* 2004). Furthermore, α-tocopherol has been tested in large clinical trials for its capacity to prevent diseases believed to be associated with oxidative stress (Pfluger *et al.,* 2004). In most trials vitamin E was unable to prevent or delay such diseases. Many explanations have been forwarded, including a poor study design. However, it was claimed by the researchers that vitamin E needed the administration of co-antioxidants to exert an antioxidant function (reviewed by Pfluger *et al.,* 2004).

5.3.2 Vitamin C

Nuts are poor sources of vitamin C. Even so, it plays a role in the function of other antioxidants and remains an important part of the antioxidative function of nuts. The ability of vitamin C to provide electrons and be readily converted back to its reduced form by glutathione, accounts for its particular effectiveness as an *in vivo* antioxidant (Jacob, 2002). Vitamin C also provides antioxidant protection indirectly by regenerating other biological antioxidants such as glutathione, α-tocopherol and polyphenols to their active state (Jacob, 2002). In addition, vitamin C supplementation

may improve glycemic control, vascular health (Sauberlich, 1994) and insulin resistance (reviewed by Jacob, 2002). With regards to vascular disease, vitamin C, especially with tocopherol, inhibits LDL oxidation (reviewed by Jacob, 2002). Mechanisms through which vitamin C may inhibit atherosclerosis are presented in **Table 6**. Epidemiological studies show an inverse relationship between plasma vitamin C concentrations and the risk for atherosclerosis and CVD (reviewed by Jacob, 2002). However, the antioxidant supplementation in an atherosclerosis prevention (ASAP) study (2000) revealed that either vitamin C or Vitamin E had no effect on the risk for CVD when administered alone (Salonen *et al.,* 2000). The limited effects of vitamin C supplementation may be due to high concentrations of transition metals, which will increase pro-oxidation by vitamin C and other small water-soluble antioxidants such as glutathione (reviewed by Abudu *et al.,* 2004). However, a cocktail of vitamin C and vitamin E showed protective CVD effects (Salonen *et al.,* 2000).

Table 6

Mechanisms through which vitamin C may inhibit atherosclerosis (adapted from Prince *et al.,* 2001).

Vitamin C function	**Mechanism**	**Effect on atherosclerosis**
Antioxidant	Inhibits LDL oxidation	Decreases foam-cell formation
Collagen production	Decreases vascular permeability	Decreases subendothelial accumulation of LDL
Decrease infection	Decreases hemostatic factors	Decreases thrombosis
Anti-inflammatory	Decreases adherence of monocytes to endothelium Decreases acute phase proteins	Decreases inflammation (CVD risk factor)
mRNA regulation	Increases HDL	Favorable lipoprotein profile

LDL: Low density lipoprotein; CVD: Cardio vascular disease; HDL: High density lipoprotein

5.3.3 Polyphenols

Fruits, vegetables, beverages and grains are rich in the polyphenolic family of antioxidant phytochemicals, the polyphenols (Rice-Evans, 2001). Polyphenols can be divided into Non-flavonoid components (e.g. hydroxybenzoic acids, hydroxycinnamic acids, tannins), and flavonoids (Miller & Ruiz-Larrea, 2002). In turn, flavonoids can be divided into flavones (e.g. rutin, luteolin, chyrsin, apigenin), flavonols (e.g. quercitin, kaempferol), catechins (e.g. (epi)catechin, epigallocatechin, epigallocatechin gallate, epicatechin gallate), flavanones (e.g. taxifolin, narirutin, hesperidin), isoflavones (e.g. genistein), chalcones (e.g. phloretin) and anthocyanins (e.g. oenin, cyaniding, delphinidum) (Miller & Ruiz-Larrea, 2002). Polyphenols are known to be powerful hydrogen-donating antioxidants and scavengers of ROS and RNS (reviewed by Rice-Evans, 2000). Their ability to act as antioxidants by donating an electron to an oxidant critically depends on the reduction potentials of their radicals and their accessibility of the radicals (reviewed by Rice-Evans, 2001). However, polyphenols may also have an effect on endogenous antioxidant defense systems. *In vivo* experiments show that relatively low concentrations of polyphenols stimulated transcription for a critical gene for glutathione synthesis in cells (Myhrstad *et al.*, 2002).

Polyphenolic phytochemicals, such as polyphenols, may have an impact on inflammatory diseases due to their antioxidant and/or anti-inflammatory properties (Boersma *et al.*, 2001; Zdunezyk *et al.*, 2001; Choi *et al.*, 2003). Previous animal studies (Yamakoshi *et al.*, 1999) have shown beneficial effects after administering 1-2% polyphenol extracts into animal diets. However, polyphenols may act as pro-oxidants when administered in high doses. Skibola and Smith (2000) report the average daily flavonoid intake to be between 68 mg (flavonols) and 20-240 mg (isoflavones). Recently, a flavonoid containing walnut extract has shown to inhibit the oxidation of LDL *in vitro* (Fukuda *et al.*, 2003), possibly due to the favourable polyphenol content of walnuts (802 mg gallic acid equivalents/50g walnuts or 8-9 shelled walnuts), relative to other foods (Anderon *et al.*, 2001). In general, polyphenols may protect against CVD by decreasing TC, LDL-C oxidation and platelet aggregation, and by increasing HDL-C (reviewed by Kris-Etherton *et al.*, 2002). However, limitations may exist

when examining polyphenols as they seem to be unstable five to eight hours after consumption (Boulton *et al.*, 1999, Walle, 2004). The instability may be related to oxidative degradation (Boulton *et al.*, 1999).

5.3.4 Glutathione

The most important endogenous antioxidant defense systems are composed of the thiol-containing tripeptide glutathione and small thiol-containing proteins such as thioredoxin, glutaredoxin, and peroxiredoxin (Moskuag *et al.*, 2005). Of these, glutathione is the major contributor to the redox state of the cell (Moskuag *et al.*, 2005). Glutathione exists in two forms: GSH and GSSG. The redox state of the cells reflects the balance between the levels of oxidation and reduction.

One important task of cellular glutathione is to scavenge free radicals (e.g. ROS, RNS, dietary oxidants) and peroxides during normal cellular respiration, which would otherwise oxidize proteins, lipids and nucleic acids (Moskuag *et al.*, 2005). Glutathione in its reduced (GSH) form is responsible for the scavenging of free radicals and peroxides produced during normal cellular respiration (Moskuag *et al.*, 2005). GSH is oxidized to GSSG in an age dependent manner, possibly reflecting increased oxidative stress with an increase in age (reviewed by Moskuag *et al.*, 2005). Furthermore, GSH is vitally important to defend against physiologically as well as pathologically-generated oxidative stress, which can be caused by ROS , RNS or dietary oxidants. Severe oxidative stress thus depletes cellular GSH (Forman, 2003). The ratio of GSH to GSSG is determined by the overall redox state of the cell (Moskuag *et al.*, 2005). Changes in the GSH/GSSG (↑GSH:↓GSSG) ratio may affect the β-cell response to glucose and improve insulin action (Paolisso *et al.*, 1992). Furthermore, glutathione infusion may lower blood pressure and improve insulin resistance (reviewed by Barbagallo *et al.*, 1999a). Other dietary antioxidants (e.g. vitamin E and C) may affect the GSH/GSSG concentration. In this regard, vitamin E is regenerated through vitamin C which, in turn, converts to a free radical form. GSH promotes vitamin C regeneration through conversion to a thiyl radical (GS•). The latter, upon reacting with a similar molecule yields GSSG (Princemail *et al.*, 2001).

6. SUMMARY

Researchers have found that the concentrations of certain dietary antioxidants are lower among individuals with the metabolic syndrome (Ford *et al.*, 2003). In particular, lower concentrations of carotenoids and vitamin E were found in individuals with the metabolic syndrome (Seghrouchi *et al.*, 2002; Ford *et al.*, 2003). However, the literature is inconclusive as to whether dietary antioxidant treatment has a positive effect on the markers of the metabolic syndrome and it may even have negative effects on the markers of the metabolic syndrome. These negative effects may be explained by the pro-oxidative action of certain dietary antioxidants (Škrha *et al.*, 1999). Regardless, evidence in favour of the positive effects of dietary antioxidants on markers of the metabolic syndrome and the risk for CVD does exist, showing that a decrease in oxidative stress may diminish the risk for the metabolic syndrome and CVD (Škrha *et al.*, 1999). Nuts are rich sources of certain antioxidants (e.g. polyphenols and vitamin E) and poor sources of antioxidants such as vitamin C. One should, however, remember that antioxidants work synergistically to prevent oxidative damage and the mileu of antioxidants in nuts may be very effective in combating oxidative stress and the symptoms of metabolic syndrome.

7. SUMMARY AND CONCLUSION

In most human diseases, oxidative stress is a secondary phenomenon in the primary cause of various diseases. Evidence is growing that CVD can be prevented or delayed to some extent by dietary changes such as reduction in fat and the consumption of more fruits and vegetables (reviewed by Carpenter *et al.*, 2003). There is a considerable amount of data indicating that hyperglycemia simultaneously enhances both glycative and oxidative stress, which together synergistically contributes to the development of the metabolic syndrome (Robertson, 2004). In the absence of an appropriate compensatory response from the cell's endogenous antioxidant network, the antioxidant defense system becomes overwhelmed, resulting in oxidative stress. Evidence suggests that chronic inflammation may be involved in the pathogenesis of atherosclerosis, insulin resistance and the

metabolic syndrome (Cariello & Motz, 2004). However, inflammation is merely one manifestation of oxidative stress (Roebuck, 1999). In addition, nuts, as part of a healthy diet, contain several compounds that act as antioxidants in order to prevent oxidative stress (Halliwell, 1996), including vitamin E, β-carotene, vitamin C and polyphenols (for example ellagic acid, ellagitannins, quercetin). However, nuts are a poor source of β-carotene and vitamin C. Recent studies have, therefore, led to the conclusion that a cocktail of dietary antioxidants (for example vitamin E and C), prevents the initiation of oxidative stress (Mazière *et al.*, 2004).

Although the understanding of how hyperglycemia-induced oxidative stress leads to β-cell dysfunction and the metabolic syndrome has improved considerably in recent years, effective therapeutic strategies to lower the risk factors for this disease and ultimately the prevention thereof, remain limited. Therefore, further research regarding the effective treatment of the metabolic syndrome with single or combined antioxidants is needed. In addition, it is recommended that future research use postprandial blood samples, rather than fasting blood samples, to determine the serum polyphenol concentrations of individuals with the metabolic syndrome, as the plasma half-life of certain polyphenols is approximately two and a half to four hours (Boulton *et al.*, 1999; Walle, 2004). Fasting blood samples may, therefore, be a limiting factor when plasma polyphenol concentrations are examined. However, when investigating the chronic effect of antioxidants in the diet, fasting blood samples may serve to indicate the body's general antioxidant status. Furthermore, obesity may play a central role in the development of the metabolic syndrome (Montonen *et al.*, 2004; Wilkin & Voss, 2004) and future research regarding the treatment and prevention of this disease should focus on reducing body weight in addition to treating the other risk factors.

Even though this dissertation focuses only on the effects of nuts on the antioxidant status of subjects with the metabolic syndrome, it should be remembered that a holistic approach (e.g. weight loss, dietary habits, exercise patterns, etc.) should ideally be followed to reduce the risk for the metabolic syndrome and CVD effectively.

CHAPTER 2

THE EFFECTS OF HIGH WALNUT AND CASHEW NUT DIETS ON THE ANTIOXIDANT STATUS OF SUBJECTS WITH METABOLIC SYNDROME

THE EFFECTS OF HIGH WALNUT AND CASHEW NUT DIETS ON THE ANTIOXIDANT STATUS OF SUBJECTS WITH METABOLIC SYNDROME

Running title: Nuts and antioxidant status

Lisa Davis, Welma Oosthuizen, Du Toit Loots, Janine Mukuddem-Petersen, Francois H van der Westhuizen, Susanna M Hanekom, Johann C Jerling

L Davis, W Oosthuizen (✉), Du T Loots, J Mukuddem-Petersen, SM Hanekom, JC Jerling
School of Physiology, Nutrition and Consumer Science
North-West University
Private Bag X6001
Potchefstroom, South Africa
2520
Tel: +27 -18 / 299 2468
Fax: +27 -18 / 299 2464
E-Mail: vgewo@puk.ac.za

FH van der Westhuizen
School of Biochemistry
North-West University
Potchefstroom, South Africa

Will be submitted for publication to the European Journal of Nutrition.

European Journal of

NUTRITION

Aims and Scope

The **European Journal of Nutrition** publishes original papers, invited reviews, and short communications in nutritional sciences. The major focus of manuscripts submitted to the **Eur J Nutr** should consequently be on:

- cellular and molecular aspects of nutrition
- mechanistic studies on interactions between nutrients and non-nutrient food components on cell, organ, and body functions
- epidemiology with emphasis on the use of biomarkers
- nutrient metabolism in humans
- studies on the relation between individual genetic susceptibility, nutrition, and disease
- regulation of gene expression through nutrients or non-nutrient food components

Animal nutrition studies will only be considered for publication if a strong relation to actual problems in human nutrition is presented.

Guidelines for Authors

To enable an **effective and fast publication** process you can contact the editors by e-mail to introduce your future contribution by a 2 page structured abstract as follows:

- Background
- Aim of the study
- Methods
- Results
- Conclusions.

The editors are endeavored to answer within 3 or 4 days whether your contribution covers the Aims & Scope.

INSTRUCTIONS TO AUTHORS

The Publication Language will be exclusively English. The frequency of publication will be bimonthly (6 issues per year).

Papers may be submitted to one of the editors. All work should be original and not have been previously published in any journal, book, etc.

Each paper undergoes a process of review. It is, therefore, mandatory to submit 3 copies of your paper.

Please follow the instructions below to facilitate and speed up publication.

General rules (for all papers)

- The manuscript must be written in English,
- **Original contributions** should not exceed 21 manuscript pages (7 printed pages) including figures and tables, with max. 50 references.
  Invited reviews should not exceed 30 manuscript pages (10 printed pages) including figures and tables, with max. 100 references.
  Short communications are limited to 6–9 manuscript pages (2–3 printed pages) including maximum 2 figures and 1 table, with up to 15 references.
- The text portion must be prepared double-spaced throughout, 30 lines per page/60 characters per line and the manuscript pages numbered consecutively.
- The First page of the manuscript must contain
  - clear and concise title
  - name(s) of author(s) with complete first name
  - any footnotes of the title
  - the complete addresses of all authors, identifying the corresponding author (✉)
  - column title (abbreviated title as running pagehead)
- A **structured abstract** should be carefully prepared since it is the source for most documentation services. It must contain the following structure with bold sub-heading:
  - Background
  - Aim of the study
  - Methods
  - Results
  - Conclusions.
- Below the abstract place about **5 key words.**
- Please do not organize your manuscript according to a numeric system as all numbering will be deleted; references in the text to other sections should include the section heading or position in the work (e.g., in the preceding section, below, etc.).
- Changes in the text after acceptance of the paper create extra costs which will be charged to the author.
- To avoid unnecessary delays caused by lost mail, authors and reviewers should contact the editor if they do not receive prompt confirmation of their correspondence.

References

These must be cited in the text with numerals, e.g. [2], [2, 3, 7–9] and numbered consecutively in the order of textual appearance. Please compile the references on a separate sheet at the end of the paper.

EXAMPLES

Articles published in journals

Allison MC, Gallacher PJ (1984) Temporal artery biopsy and corticosteroid treatment. Ann Rheum Dis 43:416–417

Books

Dihlmann W (1973) Röntgendiagnostik der Iliosakralgelenke und ihrer nahen Umgebung. Thieme, Stuttgart, pp 57–59

Articles published in books

Gschwend N (1985) Synovectomy. In: Kelley WN, Harris ED, Ruddy S, Sledge CB (eds) Textbook of Rheumatology, 2nd edn. WB Saunders Comp, Philadelphia, pp 1793–1818

Tables

These must be typed on separate sheets and numbered with arabic numerals. They must have a short heading at the top. Please indicate the preferred placement of the tables in the margin of the paper.

Figures

The number and size of figures must be kept to the minimum required for clarification.

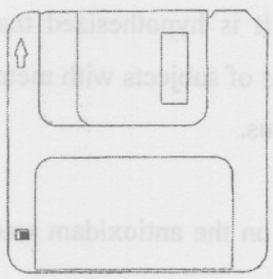
- Line drawings must be drawn clearly, preferably with Indian ink on transparent paper. Please make sure the lettering is large enough, and suitably related to the size of the figure, so that it would be clearly legible after reduction (e.g., a figure of about 16 cm in width would necessitate lettering of about 4 mm in height). In numerals, please ensure that in English manuscripts the English-style decimal point is used rather than the continental-style comma (e.g., 0.33 not 0,33).
- For halftone reproduction, good glossy photographs are required.
- The figure number and name of the first author should be written in pencil on the back.
- Figures must be numbered consecutively. Please make sure that each figure is referred to in the next and indicate preferred placement in the margin.
- Figure legends should be collected on a separate sheet, and not written onto the figures.
- Color illustrations will be accepted up to the discretion of the editors/the publisher.

Offprints

One issue of the journal will be sent automatically to the corresponding author. Offprints may be ordered when the corrected galley proofs are returned. In general, the reprints and the issue will arrive 4–6 weeks after publication.

For further information please contact

Production: Dr. Andreas Vogel
E-Mail: vogel.steinkopff@springer.de
Inhouse Editor: Dr. Maria Magdalene Nabbe
E-Mail: nabbe.steinkopff@springer.de



Manuscripts and illustrations submitted in electronic form

General advice

- Please send your manuscript only on the following data carriers:
  - Diskette
  - ZIP-Diskette
  - CD-ROM

Illustrations

- **General** Send us the illustrations separately from the text, i.e., not integrated into it. As well as data for the illustrations, always send us printouts. We regard the printouts as definitive.
- **Halftone illustrations** Store colored halftones as RGB (8 bit per channel) in TIFF format. Do not use color mode if an illustration is to be reproduced in black and white, as definition is lost in the conversion of colors into gray tones. Suitable image processing programs: Photoshop, Picture Publisher, Photo Paint, Paint Shop Pro.
- **Vector graphics** Vector graphics exported from a drawing program should be stored in EPS format. Fonts used in the graphics must be included (command: "Convert text objects [fonts] to path outlines").
  Please do not draw with hairlines. The minimum line width is 0.2 mm (i.e., 0.567 pt) measured at the final scale. Siutable drawing programs: Freehand, Illustrator, Corel Draw, Designer.
- **Spreadsheet graphics** Files generated with a presentation program (Excel, Power Point, Freelance) are only admitted, if the embedded graphics initially had been saved in EPS-format. Use patterns instead of colors to fill spreadsheet graphics, as monotone reproduction merges colors into gray tones.
- **Scans** Please check that your original, after scaling, has the resolution values in the table; only then will the print quality of the scan be sufficient. If in doubt, send us your originals.

Original	Scan mode	Final resolution	Format
Color illustration (photo/transparency)	RGB (24 bit)	300 dpi	TIFF
Monotone illustration (photo/transparency)	Grayscale (8 bit)	300 dpi	TIFF
Black/white line figure	Line	600–1200 dpi	EPS/TIFF

Text

- Please follow the **Instructions to Authors** when structuring the subject matter of your paper.
- If possible, please store your text in two versions:
  1. In the **standard data file format** offered by your word processing system and
  2. as RTF (Microsoft Rich Text Format)
- Always keep a copy of your diskette.
- Make sure your diskette is adequately packed when preparing it for dispatch.
- Enclose the required number of printouts of the **final text** with your diskette. The text file and the printout **must correspond** exactly. Any deviations may delay processing, involve high correction costs and make it uneconomical to use the diskette.
- Do not incorporate any special page layout in your text.
- Input your text continuously; in other words, only insert **hard returns** (¶) at the ends of paragraphs or headings, subheadings, lists, etc.
- Do not use the space bar to make **indents** (e. g. to indicate paragraphs or in lists). A tabulator or an indent command should be used for this purpose.
- Please use the automatic pagination function incorporated in your word processing system, i. e. do not insert page numbers manually.
- Any words or phrases in the text that you wish to **emphasize** should be indicated throughout the paper in *italic script* or by underlining.
- **Boldface type** should normally only be used in the running text for certain mathematical symbols, e. g. vectors.
- **Headings** can be in boldface for visual emphasis.
- Please place all tables at the end of your file. Always separate the individual columns using tabulators, not using the space bar.
- Please delete any annotations or comments from the final text file.
- Please send us only the **final updated version** on your diskette.

STEINKOPFF DARMSTADT

Springer

ABSTRACT

Background: Nut consumption is associated with a protective effect against coronary heart disease, partly due to its high antioxidant content. It is hypothesized that the inclusion of nuts in the diet will improve the antioxidant status of subjects with metabolic syndrome who may be vulnerable to impaired antioxidant status.

Aim: The effects of high cashew nut and high walnut diets on the antioxidant status of subjects with metabolic syndrome are investigated.

Methodology: Sixty-four volunteers (29 male and 35 female, 45±10y) with metabolic syndrome (diagnosed by using the ATP III criteria) received a prudent control diet, prepared in the metabolic kitchen of the North-West University for a period of 3 weeks (run-in). The participants were grouped according to gender and age and randomized into three groups, receiving either the walnut, cashew nut or the control diets for 8 weeks, while maintaining a stable body weight. Nuts provided 20% of daily energy intake. Fasting blood samples were taken after the run-in period (baseline) and at the end of the intervention period.

Results: The walnut and cashew nut diets had no significant effects on oxygen radical absorbance capacity (ORAC), reduced (GSH), oxidized (GSSG) glutathione, GSH:GSSG or hydroperoxides compared to the control group. However, all three groups showed significant improvements in antioxidant status from baseline to end (GSSG and dRom decreased; GSG:GSSG ratio and ORAC increased). This may be due to increased antioxidant intake from the prudent diet compared to the habitual diets.

Conclusion: Compared to the control group, the intake of walnuts and cashew nuts when incorporated into a prudent diet, did not improve the antioxidant profiles of subjects with metabolic syndrome.

Key words: Cashew nuts, walnuts, antioxidant status, polyphenols, redox status, metabolic syndrome

INTRODUCTION

Metabolic syndrome is characterized by a constellation of cardiovascular risk factors, including atherogenic dyslipidemia, abnormal glucose tolerance, hypertension and abdominal obesity. These factors are closely associated with insulin resistance and elevated insulin concentrations [1]. It has been suggested that hyperglycaemia and obesity may disrupt natural antioxidant defence systems [2] by increasing the generation of free radicals through the reduction of molecular oxygen [3]. There is also some evidence relating oxidative stress to the degree of insulin resistance [4]. It has been suggested that high oxidative stress promotes an impaired insulin action, which in turn may aggravate the degree of oxidation[5].

Little is known about the antioxidant status of subjects with metabolic syndrome. Obesity associated with a low antioxidant status [6], is high among subjects with metabolic syndrome and consequently, may be associated with a low antioxidant status [7]. Additionally, obesity may also contribute to an increased pro-inflammatory response, resulting in an up-regulation of interleukin (IL-6) production [8, 9], thereby linking the metabolic syndrome to inflammation. Findings from the third National Health And Nutrition Examination Survey (NHANES III) (2003) revealed low concentrations of vitamin C and E among subjects with metabolic syndrome [7]. This may be due to their involvement in antioxidant reactions in these individuals. Furthermore, research is beginning to highlight the potential health benefits of polyphenols in diets, which are known to be powerful scavengers of reactive oxygen species (ROS) and reactive nitrogen species (RNS) *in vitro* [10].

The nutritional composition of nuts suggests that they may reduce the risk for chronic diseases by various mechanisms [11]. Nuts are low in saturated fatty acids (SFA), high in unsaturated fatty acids [12], one of the most important sources of dietary fibre and good

sources of plant proteins, antioxidants, vitamins, minerals, polyphenols [12], magnesium, potassium and arginine [13]. The rich antioxidant content of nuts (e.g. vitamins and polyphenols) may offer protection against oxidative damage [13]. Compared to other nuts, walnuts are unique due to their high poly-unsaturated fatty acid (PUFA) content, specifically α-linolenic acid [14]. Walnuts are a good source of polyphenols [15] and to a lesser extent, vitamin E [13] that may further contribute to its antioxidant capacity.

There is no evidence of a trial investigating the effects of unsalted walnuts and cashew nuts on individuals with the metabolic syndrome. The main purpose of this paper is, therefore, to explore the effects of a high walnut and unsalted cashew nut diet on the antioxidant status of subjects with metabolic syndrome. Mukuddem-Petersen [16] provide a detailed discussion of the effects of the nut diets on markers of the metabolic syndrome and Pieters and co-workers [17] reported on the effects of these diets on haemostatic variables. They concluded that walnuts and cashew nuts (20% of the total energy) had no effect on any of the metabolic syndrome markers (TG, total cholesterol (TC), HDL-C, plasma glucose, low density lipoprotein cholesterol (LDL-C), serum insulin, insulin sensitivity, insulin resistance and fasting glucose) or the haemostatic variables. They further suggested that weight loss should probably be the main focus of dietary intervention for people with metabolic syndrome in order to reduce these markers.

METHODS

Subjects

Sixty-eight volunteers with the metabolic syndrome were recruited mainly from the Potchefstroom Campus of the North-West University and surrounding areas in Potchefstroom, South Africa. The power calculation for sample size was based on results of Dessein and co-workers [18]. To provide 80% power at 5% significance and by considering a 15% change in the quantitative insulin sensitivity check index (QUICKI) (due to the original research question of study) as significant, a total number of 22 subjects were calculated per group (i.e. a total of 66 subjects).

Sixty-four subjects completed the study (dropouts discussed later). The National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) criteria for the diagnosis of the metabolic syndrome were used. Subjects with the metabolic syndrome were defined as individuals with three or more of the following symptoms: abdominal obesity (waist circumference > 88 cm for women or > 102 cm for men), triacylglycerol (TG) ≥ 1.7 mmol/L, high density lipoprotein cholesterol (HDL-C) ≤ 1.3 mmol/L for women and ≤ 1.0 mmol/L for men, blood pressure≥ 130/85 mmHg (the use of antihypertensive medication was also an indication of high blood pressure) and fasting glucose ≥ 6.1 mmol/L. Additionally, subjects were included if they were able to comply with the specified feeding conditions, being able to eat walnuts and cashew nuts and being between the ages of 21 and 65 years. Exclusion criteria included the following: pregnancy or lactation, the use of thiazide diuretics (> 25 mg/day) or beta-blockers (non-specific, β_1 and β_2), subjects having nut allergies and subjects with diagnosed diabetes.

Study design

A controlled feeding trial with a randomized, controlled, parallel study design was used. The study protocol consisted of a three week run-in period during which the subjects consumed a control diet (percentage of total energy (%E) from protein: 20%, carbohydrate (CHO): 47%, and fat: 33%) (**Table 1**). After the run-in period, participants were grouped according to gender and age, after which they were randomly stratified by drawing numbers from a hat. Group one received walnuts (n=21), group 2 received unsalted cashew nuts (n=21) and group three (n=22) continued with the control diet without any nuts or nut-based ingredients. The intervention periods were followed for 8 weeks. Meals were prepared in the metabolic kitchen of the metabolic unit at the North-West University, Potchefstroom Campus. Fasting blood samples, anthropometric measurements and blood pressure measurements were taken before (after the three-week run-in period) and after the intervention period (8-week controlled feeding). The study participants were weighed twice a week throughout both the run-in and the intervention periods and the energy intake adjusted in order to maintain body weight. Body mass index (BMI) (kg/m^2) was calculated. The participants were informed of all the aspects of the study before commencement and gave consent. The Ethics Committee of the North-

West University, Potchefstroom Campus, approved the study. Participants were required to maintain their activity levels and subjects using chronic medication other than thiazide diuretics or β-blockers were to continue with the same dosage for the duration of the study. Due to practical reasons, the study was divided into three cohorts distributed over a one-year period.

TABLE 1
Calculated and analyzed diets as well as the habitual diets

Nutrients	Habitual diet prior to dietary intervention (n=64)	Walnut diet		Cashew nut diet		Control diet	
	FFQ	Planned[1]	Analyzed[2]	Planned[1]	Analyzed[2]	Planned[1]	Analyzed[2]
Protein (% of energy)	15.1	15.7	17.5 ± 1.54[#]	16.2	19.1 ± 1.69[#]	16.4	19.6 ± 1.74
Carbohydrate (% of energy)	49.2	48.9	42.1 ± 4.31[#]	46.8	44.4 ± 4.85	51.3	47.2 ± 6.55[#]
Fat (% of energy)	33.2	35.0	40.3 ± 4.95[#]	37.1	36.5 ± 4.06	32.8	33.2 ± 7.14[#]
Vitamin E (mg/day)	27.5	15.6	-	15.1	-	23.6	-
Vitamin C (mg/day)	147.3	157.9	-	145.9	-	154.4	-
Total polyphenol content (mg/g wet mass)	-	-	0.33 ± 0.02[#*]	-	0.28 ± 0.01[#*]	-	0.31 ± 0.02[#]
ORAC (mmol TE/g)	-	-	2270 ± 16.1	-	2140 ± 7.29	-	1580 ± 16.7

[1]: Determined by using the FoodFinder 2 Programme (Medical Research Council of South Africa, Tygerberg); [2]: Laboratory analysis; FFQ: Food Frequency questionnaire; Means with the same symbol differed significantly ($p < 0.05$, *t*-test for dependent samples)

Diet

The walnuts and cashew nuts provided 20% E of the diet (63 – 108g/day). In order to ensure that all groups received equal energy intakes from the meals, proportional reductions to all food substances in the walnut and cashew nut diet menus were made to accommodate the additional energy supplied by the respective nuts. Standardized recipes

over a 14-day cycle period were used. Five different energy intake diets were developed ranging from 8 000 kJ to 14 000 kJ with 1 500 kJ increments, using the FoodFinder 2 programme (Medical Research Council of South Africa, Tygerberg). Ten percent of the total energy intake was calculated in the form of "additional points" to be eaten daily. Validated lists of food with their associated number of points were provided to the participants. Validated food frequency questionnaires (FFQs) [19] and physical activity questionnaires (PAQ) [20] were analyzed in order to determine the correct energy intake requirements for the maintenance of body weight for each of the participants. Underreporting was established when the ratio of energy intake:basal metabolic rate was less than 1.2 [21, 22]. Participants were allowed to consume tea and coffee *ad libitum* during the intervention period. However, since tea and coffee may influence the antioxidant capacity of the participants [23], participants were requested to keep record of their tea and coffee intake during the intervention.

The macronutrient profiles and antioxidant capacity of the three diets were analyzed chemically to determine the actual composition of the diet. Duplicate portions of breakfast, lunch and dinner for the 14-day menu cycle were collected daily, homogenized in a container and frozen at -84°C until the analysis was done.

Compliance

Quality control with regard to the test meals was ensured by weighing food to the nearest gram before consumption and of the leftovers after consumption. Subjects ate lunch at the Metabolic ward of the North-West University, whereas dinner and the following day's breakfast were supplied in take-away format. A registered dietician supervised mealtimes and ensured the complete intake of the respective meals. Leftover foodstuffs were collected and weighed by researchers in order to determine the compliance. Any deviations from the study protocol was recorded in diaries and reviewed by the investigators during the study.

Blood sampling

After an overnight (12 hour) fast, a registered nurse collected venous blood samples from the antebrachial vein using a sterile winged infusion set and syringes. Samples were drawn with minimal stasis between 07:00 and 10:00 to avoid diurnal variation. For the preparation of serum hydroperoxides, 20ml of blood was drawn and left to clot. For the determination of plasma glucose concentrations 5ml blood was collected in tubes containing 10mg potassium oxalate and 12.5mg sodium fluoride. For the determination of coagulation factors samples of 10ml citrated blood was drawn. Blood was centrifuged at 2000 *g* to yield serum and plasma, respectively. For the determination of antioxidant variables (oxygen radical absorbance capacity (ORAC), reduced glutathione (GSH)/oxidized glutathione (GSSG)) serum was kept on ice, after which it was diluted 100-200 fold in a phosphate buffer. For the determination of ORAC the serum was firstly deproteinated by addition and thorough mixing with equal amounts of 0.5M perchloric acid, and then centrifuged at 4°C for 10 min at 16 000 *g*. The supernatant was recovered. Aliquots of serum, plasma and deproteinated samples were stored at -82°C until analysis.

Blood sample analyses

Markers for the metabolic syndrome:

Serum TG, TC, HDL-C and plasma glucose were measured using a Vitros DT60 II Chemistry Analyser (Ortho-Clinical Diagnostics, Rochester, New York, USA), with Vitros reagents (catalogue numbers 153 2159, 153 2175, 133 5504, and 153 2316) and controls (catalogue numbers 842 0317 and 144 8042). Serum LDL-C was calculated using the Friedewald equation (LDL-C (mmol/L) = TC-TG/2.2 –HDL-C) [24]. Fasting serum insulin was measured with an ELISA method on the Immulite 2000 Analyzer (Diagnostic Products Corporation, Los Angeles, CA, USA). The QUICKI formula was used to calculate insulin sensitivity as follows: QUICKI = 1/[log(fasting venous insulin(μIU/ml) + log(fasting venous glucose(mg/dL)] [25]. Furthermore, insulin resistance was calculated using the homeostasis model assessment (HOMA) formula: HOMA = (fasting insulin (μIU/mL) × fasting venous glucose (mmol/L)) / 22.5 [25]. During the recruitment of subjects, fasting capillary glucose was measured by fingerprick

with a SureStep™ blood glucose meter (Lifespan Inc., Milpitas, CA, USA), using Fine Point Lancets and SureStep™ test strips (code 11). Blood pressure was measured by a 7-minute continuous measurement of cardiovascular parameters using the Finometer™ device (Finapres Measurement Systems, Amsterdam, Netherlands). Serum high sensitivity C-reactive protein (hs-CRP) activity was measured rate turbidimetrically (immunoassay) using a Synchron LX® System (Beckman Coulter Inc., Fullerton, CA, USA). The coefficients of variance (CV) for all the abovementioned variables were less than 5%. PAI-1 activity was measured using a chromogenic assay (Spectrolyse®/pL PAI-1, Biopool, Umeå, Sweden, Cat.no. 101201, between-run CV=5.2%). Fibrinogen (between-run CV=5.0%) (modified Clauss method) was measured on the Automated Coagulation Laboratory 2000 (Instrumentation Laboratories, Milan, Italy).

Plasma antioxidant activity:

Plasma antioxidant capacity of deproteinated plasma was determined by the ORAC assay [26] on a BioTEK fluorescence plate reader at an excitation wavelength at 530nm and emission wavelength at 590nm [27]. AAPH (240 mM, 2,2'-azobis(2-amidinopropane) dihydrochloride) was used as a peroxyl radical generator. Trolox was used as a reference standard and the total plasma antioxidant capacity was expressed as μM trolox equivalents (TE).

Oxidative stress assessment:

The diacron reactive metabolites (dRoms) test (DIACRON International, Grosseto, Italy) was used by mixing 10 μl serum sample, 10 μl chromogen and 980 μl acetate buffer in a flat electron spin resonance cell. Various scansions were performed at an interval of 10mT at room temperature with a magnetic field modulation of 0.1mT and a microwave radiation power (-9.4 GHz) rate at 1 mW.

Serum glutathione:

The redox state of glutathione (GSH/GSSG) was measured using the spectrophotometric GSH/GSSG ratio assay kit (GSH/GSSG-412™) (OXIS Research Inc. Portland, USA).

Diet and nut sample analyses

Oxygen radical absorbance capacity (ORAC):

The hydrophilic and lipophilic components of the food samples were analyzed separately. ORAC analyses of these two components were done as previously described [28].

Macronutrient content:

The protein, carbohydrate and fat composition of the nuts and diets were measured by methods as described by Pieters and co-workers [17].

Total Polyphenols:

The total polyphenol content of the three diets and the walnuts and cashew nuts was determined according to the Folin-Ciocalteu method [29]. Unfractionated food and nut samples were diluted 62 times prior to analysis and filtered through a Whatman no 1 filter (Merck). Fractionated samples were used as collected from solid phase extraction. Samples (200ml) were introduced into test tubes followed by 1 ml Folin Ciocalteu's reagent (Sigma - F9252). This was allowed to stand for 8 minutes at room temperature. Next, 0.8ml sodium carbonate (7.5%) was added, mixed and allowed to stand for 30 minutes. Absorption was measured at 765 nm (Shimadzu UV - 1601 UV-vis). Total phenolic content was expressed as gallic acid (Aldrich) equivalents (GAE) in grams per litre (g/L).

Statistical analysis

The computer software package Statistica® (Statsoft Inc, Tulsa, OK, USA) was used for data analyses. Initially, variables were tested for normality using the Shapiro-Wilk's W-test. Non-normally distributed data were transformed into an approximately normal distribution using logarithmic transformations. Normally distributed data is expressed as mean (95% confidence interval (CI)) and data not normally distributed or logarithmic transformed as median (25, 75 percentiles). The *t*-test for dependent samples was used to

test for changes within groups, from baseline to end (parametric data). The Wilcoxon matched pairs test was used for non-parametric data. Differences between changes from baseline to end in the three groups were determined using the ANOVA for parametric data and the Kruskall Wallis ANOVA for non-parametric data. Since no significant overall effects were seen between groups with the ANOVA analysis, post-hoc comparisons were not performed. Lastly, because strong correlations were observed between the baseline values of the antioxidant variables and the differences in the changes in these variables, the differences between groups were determined while controlling for baseline values using the analysis of covariance (ANCOVA) test. Significance was set at $P \leq 0.05$. Multiple regression analysis was performed with insulin resistance, insulin sensitivity and insulin concentrations at baseline as dependent variables and GSH, GSSG, GSH/GSSG, ORAC and dRom as independent variables to determine the association between antioxidant and insulin markers.

RESULTS

Subjects

Four subjects failed to complete the study: 2 subjects had out of town job responsibilities, 1 subject had an unrelated illness and 1 subject went on holiday during the study period. Therefore 64 subjects (29 men and 35 women) completed the study. Most of the participants had a sedentary lifestyle prior to the intervention period and remained so for the duration of the study. A dietary compliance of ± 90% was calculated based on weighing of leftover food and food dairies. The baseline characteristics **(Table 2)** of the subjects did not differ between groups (ANOVA). Most of the subjects (91%) had waist circumference values > than indicated by the ATP III criteria. Fifty three percent of the subjects had high TG, 42% had high systolic blood pressure, 13% had high diastolic blood pressure, 91% had low HDL-C and only 5% had high glucose concentrations as indicated by the ATP III criteria. Evidently, the subjects' characteristics at baseline were clear indications of the metabolic syndrome. Only four participants were smokers. At baseline only 13% of subjects had high hs-CRP concentrations greater than 7.5mg/L (as indicated by the producers of the diagnostic kit). Weight, BMI and waist circumference

remained unchanged during the intervention period. The responses in variables for men and women, as well as smokers and non-smokers did not differ significantly. The data were, therefore, analyzed and reported for the combined study group, irrespective of gender and smoking habits.

TABLE 2
Baseline characteristics

Variables	Walnut diet (n=21)		Cashew nut diet (n=21)		Control diet (n=22)	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Men/women	10/11	-	8/13	-	11/11	-
Age (yr)	45.0	40.4, 50.2	46.0	40.7, 50.7	45.0	40.8, 49.3
Weight (kg)	107.0	99.4, 115	99.0	92.5, 106	106.0	99.2, 113
BMI (kg/m^2)	36.0	33.3, 38.7	34.4	32.2, 36.6	35.1	32.8, 37.4
WC (cm)	109.0	103, 115	105.0	98.5, 111	108.0	102, 113
Cigarette smokers	1	-	0	-	3	-
Blood pressure (mmHg)						
Systolic	128	126, 131	131	126, 135	131	126, 137
Diastolic	78.7	76.3, 81.2	77.0	74.0, 79.9	79.2	76.1, 82.2
Serum TG (mmol/L)	1.90	1.48, 2.32	1.81	1.33, 2.30	1.86	1.55, 2.16
Serum HDL-C (mmol/L)	0.94	0.83, 1.05	1.02	0.88, 1.16	0.85	0.77, 0.93
	Median	25, 75 Percentiles	Median	25, 75 Percentiles	Median	25, 75 Percentiles
Serum TC (mmol/L)	4.80	4.54, 5.18	4.49	3.98, 5.34	4.90	4.39, 5.52
Serum LDL-C (mmol/L)	2.99	2.63, 3,29	2.64	2.33, 3.16	3.21	2.79, 3.79
Serum insulin (µIU/mL)	14.0	10, 18	12.0	9, 16	12.5	11, 17
Plasma glucose (mmol/L)	4.50	4.30, 5.20	4.70	4.30, 5.10	4.55	4.30, 5.40
Insulin sensitivity (QUICKI)	0.57	0.50, 0.59	0.58	0.52, 0.62	0.56	0.52, 0.58
Insulin resistance (HOMA)	2.61	2.24, 4.48	2.35	1.84, 3.82	2.81	2.35, 3.76
Serum-hs CRP (mg/L)	2.40	0.60, 4.80	4.30	1.80, 5.50	3.05	1.30, 6.10

BMI: Body mass index; CI: Confidence interval; HDL-C: High density lipoprotein cholesterol; hs-CRP: High sensitivity C-reactive protein; LDL-C: Low density lipoprotein cholesterol; TC: Total cholesterol; TG Triacylglycerol; WC: Waist circumference; $p \leq 0.05$; No significant p-values were obtained; QUICKI: Quantitative insulin sensitivity check index; HOMA: Homeastasis model assessment

Diet composition

The habitual diets of the participants consisted of between 5 500 and 13 000 kJ/day (1 310 – 3 095 kcal/d). The chemical analysis of the diets compared well to the calculated compositions, with the exception of the total fat and CHO content of the walnut diet **(Table 1)**. The analyzed fat content was higher because the actual fat content of the walnuts was higher than indicated in the food composition tables (73g/100g vs. 62g/100g). The vitamin E content was higher in the control diet than both the walnut and cashew nut diets. The vitamin C content was the same in all three diets. The antioxidant capacity (ORAC) of both the walnut and cashew nut diets tended to be higher than the control diet ($p = 0.07$ and $p = 0.06$, respectively) **(Table 1).** The total polyphenol content of the walnut and control diets was significantly higher than the cashew nut diet ($p<0.05$). The total polyphenol content of walnuts tended to be higher compared to cashew nuts (0.83 ± 0.02 vs. 0.76 ± 0.01mg wet mass, $p=0.06$). ORAC concentration was also higher in the walnuts compared to cashew nuts, but the difference was not statistically significant (202 ± 6.2 vs. 146 ± 15.85mmol TE/g, $p=0.19$).

Markers of metabolic syndrome

The markers of the metabolic syndrome (TC, HDL-C, LDL-C, TG, serum insulin, insulin sensitivity, insulin resistance and blood pressure) displayed no significant differences between the walnut, cashew nut and control diets at baseline and in response to the intervention. Plasma glucose concentrations increased significantly ($p=0.04$) in the cashew nut group compared to the control group (results discussed by Mukuddem-Petersen [16]).

Antioxidant profiles

In **Table 3**, significant improvements from baseline to end were reported for GSSG, GSH/GSSG, dRom and ORAC for all three diets. GSH showed no significant changes when comparing baseline and end values of all three groups **(Table 3)**. Similarly, no significant differences in the changes from baseline to end between the three groups were

seen (ANOVA) for any of the markers. The results did not change when the markers were controlled for baseline concentrations.

TABLE 3
Antioxidant profiles

		Walnut diet (n=21)			Cashew nut diet (n=21)			Control diet (n=22)			P-value between groups (ANOVA)
Markers		Mean	95% CI	P-value (B *vs* E)	Mean	95% CI	P-value (B *vs* E)	Mean	95% CI	P-value (B *vs* E)	
GSH	B	655	610, 700	0.54	626	588, 664	0.30	668	613, 724	0.68	0.44
(mmol/L)	E	636	602, 671		647	614, 681		673	639, 706		
	△	-18.6	-75.3, 38.1		21.0	-22.5, 64.4		4.40	-49.0, 57.8		0.34
GSSG	B	2.87	2.37, 3.35	0.01	2.89	2.50, 3.29	0.02	2.89	2.43, 3.34	0.03	0.99
(mmol/L)	E	2.09	1.81, 2.36		2.27	1.93, 2.62		2.30	1.95, 2.64		
	△	-0.78	-1.38, -0.19		-0.62	-1.14, -0.10		-0.59	-1.12, -0.06		0.72
GSH/GSSG	B	250	214, 286	<0.001	241	201, 281	0.01	256	215, 297	0.01	0.82
(mmol/L)	E	328	285, 371		319	265, 374		324	276, 372		
	△	78	29, 128		78	22, 134		68	14, 122		0.86
dRom	B	502	434, 569	<0.001	524	476, 572	<0.001	531	454, 608	<0.001	0.79
(mg/g wet	E	388	347, 429		397	355, 440		416	353, 479		
mass)	△	-114	-179, -49		-126	-168, -85		-115	-153, -77		0.92
ORAC	B	1073	951, 1195	<0.001	1105	939, 1271	<0.001	1067	921, 1213	<0.001	0.87
(mg TE/g)	E	1489	1312, 1667		1315	1139, 1491		1340	1179, 1501		
	△	417	216, 617		209	-18, 437		273	97, 449		0.10

B: Baseline (after 3 week run-in period); CI: Confidence interval; dRom: Diacron reactive metabolites; E: End; GSH: Reduced glutathione; GSSG: Oxidized glutathione; ORAC: Oxygen radical absorbance capacity; △: Change from baseline to end

Inflammation markers

Fibrinogen and PAI-1 activity did not change from baseline to end in the three groups. In addition, no significant differences were observed for the changes in these markers between groups (ANOVA). Hs-CRP showed a significant deterioration from baseline to end in the walnut group and a non-significant deterioration (approximately to the same extent) in the control group and cashew nut groups **(Table 4).** The difference in change from baseline to end between groups (ANOVA) in hs-CRP activity concentrations was also not significant **(Table 4)**. The increase in inflammation markers in the walnut group is, therefore, probably not an independent effect of walnuts.

Associations between insulin and markers of antioxidant status

Multiple regression analysis showed that only 7% of the variance in insulin sensitivity ($R^2 = 0.07$, $p = 0.54$) and 10% of the variance for both insulin resistance ($R^2 = 0.101$, $p = 0.30$) and insulin concentrations at baseline ($R^2 = 0.104$, $p = 0.276$) could be explained by the effect of GSH, GSSG, GSH/GSSG, ORAC and dRom..

DISCUSSION AND CONCLUSION

The effects of whole nuts on the antioxidant status of vegetarians compared to omnivores were examined in a recent review [30]. The review concluded that vegetarians have higher plasma and/or serum concentrations of antioxidant vitamins (vitamin C, E, and beta-carotene) compared to omnivores, which may be explained by high fruit, vegetable and nuts intakes by vegetarians.

This study was unique in the sense that the effect of the diet on various antioxidant capacity markers was investigated in subjects with diagnosed metabolic syndrome, a condition associated with disrupted antioxidant defence systems [4]. The main findings of this study were that markers of antioxidant status improved significantly from baseline to end in both the intervention groups and the control group. No significant difference

was observed between the walnut, cashew nut and control diets, however. These results are in accordance with results from a study by Cooney and co-workers who reported no change in oxidative stress after consuming muffins with walnuts as primary oil compared to muffins with sesame seeds or soy as primary oil in healthy subjects [31]. However, only the tocopherol content, and not the total polyphenol content, were analysed in this study.

TABLE 4
Inflammation markers during the intervention

Marker		Walnut diet (n=21)			Cashew nut diet (n=21)			Control diet (n=22)			P-value between groups (ANOVA)
		Mean	95% CI	P-value (B vs. E)	Mean	95% CI	P-value (B vs. E)	Mean	95% CI	P-value (B vs. E)	
Plasma fibrinogen (g/L)	B	3.03	2.79, 3.26	0.23	3.11	2.87, 3.36	0.84	3.21	2.93, 3.49	0.14	0.56
	E	3.10	2.86, 3.33		3.12	2.84, 3.41		3.05	2.81, 3.28		
	Δ	0.07	-0.05, 0.19		0.01	-0.13, 0.16		-0.16	-0.39, 0.06		0.11
Plasma PAI-1 act (U/ml)	B	22.2	10.8, 35.5	0.39	14.1	10.6, 20.5	0.15	14.6	9.9, 30.8	0.88	0.64
	E	23.3	15.2, 35.9		18.9	12.2, 25.1		17.7	10.9, 25.6		
	Δ	0.58	-1.73, 2.75		1.6	-2.21, 5.99		0.95	-4.73, 6.50		0.17
		Median	25, 75 Percentiles	P-value (B vs. E)	Median	25, 75 Percentiles	P-value (B vs. E)	Median	25, 75 Percentiles	P-value (B vs. E)	P-value between groups (ANOVA)
Serum hs-CRP (mg/L)	B	2.40	0.60, 4.80	0.01	4.30	1.80, 5.50	0.07	3.05	1.30, 6.10	0.21	0.17
	E	3.00	1.10, 5.50		4.20	2.10, 6.40		3.65	2.20, 7.30		
	Δ	0.50	0.10, 1.50		0.20	-0.50, 3.20		0.65	-0.30, 2.20		0.38

B: Baseline; E: End; hs-CRP act: High sensitivity C-reactive protein activity; PAI-1 act: Plasminogen activator inhibitor-1 activity; Δ: Change from baseline to end; Fibrinogen refence range: 2.43 – 3.23 g/L; PAI reference range: 2.6 – 13.2 U/ml; hs-CRP reference range: <7.5mg/L (reference ranges as given by the producers of the diagnostic kits)

It was expected that the antioxidant profiles of the participants consuming the walnut and cashew nut diets would improve compared to those not consuming nuts. Walnuts have favourable phenolic concentrations compared to apple juice [32], a milk chocolate bar [33], or a glass of red wine [34], all of which are known for their high phenolic and antioxidant concentrations [35]. The number of servings of these foods that would have to be consumed to equal the phenolics in a serving of walnuts is as follows: 2.2 servings of red wine, 3.9 servings of milk chocolate and 4.6 servings of apple juice [35]. Recent findings showed that cashew nut kernel oil had an ability to increase the antioxidant status of animals [36]. Furthermore, antioxidants given in amounts close to the recommended daily allowance may also reduce oxidative stress [37].

A walnut extract containing polyphenols, ellagic acid, and gallic acid was reported to inhibit the oxidation of human plasma low density lipoproteins (LDL) *in vitro* [34]. Cornelli *et al.,* (2001) indicated that antioxidants (vitamin A, vitamin E, β-carotene, selenium, zinc, and L-cysteine, carotene, citrus flavonoids, vitamin C, coenzyme Q_{10}, vitamin D and pyridoxine hydrochloride) given in combinations close to the recommended daily allowance (RDA), reduced oxidative stress. However, a fluid form was shown to be more active and bioavailable than a dry form. Considering this, the antioxidants in the nuts may have been more bioavailable had they been consumed in a liquid form as opposed to their natural state.

Recent *in vitro* studies have revealed that polyphenols in almond skins work synergistically with vitamin E and C to decrease the susceptibility of LDL to oxidation [38]. The lack of effect reported in this study may be attributed to the fact that the incorporation of walnuts and cashew nuts into the diets resulted in a lower vitamin E content compared to the habitual and control diets. The lower vitamin E content is probably due to the fact that predetermined amounts of spreads and fats (high in vitamin E) were omitted, in order to compensate for the inclusion of the nuts in these diets. Despite the lower vitamin E and similar polyphenol concentrations in the nut diets compared to the control and habitual diets, the antioxidant capacity in the walnut and

cashew nut diets tended to be higher compared to the control diet. Furthermore, different antioxidants may have different activities [15] and using different methods, such as the ferric reducing antioxidant power (FRAP) assay in order to determine the antioxidant status, may lead to different results, showing different antioxidant capacities of the various diets. This may be because various different polyphenols have different activities (e.g. FRAP analysis may show different results) [15]. The antioxidant capacity of the nut diets may, however, still have been insufficient to make a significant difference in the serum antioxidant capacity of the subjects receiving nuts compared to the control diet. Bioavailability studies [39, 40] have shown that the maximum polyphenol concentrations are reached one to two hours after ingestion, followed by a rapid reduction in these concentrations. It may, therefore, be worthwhile to investigate the effects of nuts on the antioxidant capacity in the postprandial state. Despite the higher ORAC values of the intervention diets compared to the control diet, the serum antioxidant capacity of the subjects in the different groups did not differ. This may be due to the fact that fasting blood samples were used for these analyses. It is, therefore, possible that the measurements did not reflect the true antioxidant capacity, which may have been displayed two to three hours after nut ingestion.

Subjects had to maintain their initial body weight in order to prevent confounding results due to weight loss. In view of recent findings indicating that increased body weight may induce oxidative stress [41], these findings may be masked by the high prevalence of android obesity as well as physical inactivity in this study population.

Since the power calculation for this study was based on QUICKI, the possibility that the number of subjects to investigate effects on antioxidant status might have been insufficient cannot be excluded. However, it is clear that there were no conspicuous differences between the groups for ORAC, GSH, GSSG, GSH/GSSG and dRom. With regard to ORAC, the increases in the walnut group were noticeably greater than in the cashew nut and control groups ($p=0.10$) and a larger study group might have resulted in a statistically significant change. However, in order to make conclusions regarding the effect on antioxidant status, one would expect to see the change in more than one

antioxidant variable, since different antioxidant systems work synergistically in order to improve the total antioxidant capacity [30].

There are indications from the literature that oxidative stress may be related to the degree of insulin resistance through prolonged exposure to high glucose levels. This may result in further deterioration of glucose intolerance and insulin resistance [5, 4]. However, in this study only a small percentage of the variance in insulin resistance and insulin sensitivity could be explained by antioxidant markers as most of the participants presented with normal plasma glucose levels at baseline. Only 5% (n=3) of the subjects had fasting blood glucose concentrations > 6.1mmol/L.

In conclusion, compared to the control group, the intake of walnuts and cashew nuts when incorporated into a prudent diet did not improve the antioxidant profiles of subjects with metabolic syndrome. It is recommended that further studies investigate the effects of nut diets on antioxidant status in the postprandial state instead of the fasting state.

ACKNOWLEDGEMENT

The authors would like to thank Sister Chrissie Lessing for the outstanding role she played in the handling of the subjects and the blood collection as well as the subjects for their devoted participation in this study. We also wish to thank the entire Nutrition Department who all made significant contributions to this study. Funding was provided by the National Research Foundation of South Africa as well as the Technology and Human Resources for Industry Programme. We would also like to thank the food companies, Tiger brands, Pick 'n Pay, Clover and Unilever-Bestfoods-Robertsons, who donated various foods for the controlled feeding trial.

LITERATURE CITED

1. Hansel B, Giral P, Nobecourt E, Chantepie S, Bruckhurt E, Chapman M.J, Kontush A (2004) Metabolic syndrome is associated with elevated oxidative stress and dysfunctional dense high-density lipoprotein particles displaying impaired antioxidative activity. J Clin Endocrinol Metab 89(10):4963-4971

2. Obrosova I.G, Van Hysen C, Fathallah L, Cao X, Greene D.A, Stevens M.J (2002) An aldose reductase inhibitor reverses early diabetes-induced changes in peripheral nerve function, metabolism and antioxidative defence. FASEB J 16:123-125

3. Paolisso G, Giugliano D (1996) Oxidative stress and insulin action. Is there a relationship? Diabetologia 39:357–363

4. Sentí M, Tomás M, Fitó M, Weinbrenner T, Covas M, Sala J, Masiá R, Marrugat J (2005) Antioxidant paraoxonase 1 activity in the metabolic syndrome. J Clin Endocrinol Metab 88(11):5422-5426

5. Giugliano D, Ceriello A, Paolisso G (1999) Oxidative stress and diabetic vascular complications. Diabetes care 19:257-263

6. Reitman A, Friedrich I, Ben-Amotz A, Levy Y (2002) Low plasma antioxidants and normal plasma B vitamins and homocysteine in patients with severe obesity. Isr Medic Assoc J 4:590-593

7. Ford E.S, Mokdad A.H, Giles W.H, Brown D.W (2003) The metabolic syndrome and antioxidant concentrations. Findings from the third national health and nutrition examination survey. Diabetes 52:2346-2352

8. Tracy R.P (2003) Thrombin, inflammation, and cardiovascular disease. Chest 24:49S-57S

9. Kriketos A.D, Greenfield J.R, Campbell L.V, Peake P.W, Charlesworth J.A, Denyer G.S (2004) Inflammation, insulin resistance, and adiposity. Diabetes care 27:2033-2040

10. Rice-Evans C.A, Miller N.J, Panganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Medic. 20:933-956

11. Sabaté J, Fraser G.E (1994) Nuts: a new protective food against coronary heart disease. Curr Opin Lipidol 5:11-16

12. García-Lorda P, Rangil M, Salas-Salvadó J (2003) Nut consumption, body weight and insulin resistance. Eur J Clin Nutr 57:S8-S11

13. Dreher M.L, Maher C.V, Kearney P (1996) The traditional and emerging role of nuts in healthful diets. Nutr Rev 54:241-245

14. Tapsell L.C, Gillen L.J, Patch C.S, Batterham M, Owen A, Bare M, Kennedy M (2004) Including walnuts in a low-fat/modified-fat diet improves HDL cholesterol-to-total cholesterol ratios in patients with type 2 diabetes. Diabetes care 27(12):2777-2783

15. Fukuda T, Ito H, Yoshida T (2003) Antioxidative polyphenols from walnuts (Juglans regia L.). Phytochemistry 63(7):795-801

16. Mukuddem-Petersen J (2005) The effects of nuts on markers of the metabolic syndrome. Potchefstroom : North-West University. (Thesis – PhD) 180 p.

17. Pieters M, Oosthuizen W, Jerling J.C, Loots Du T, Mukkuddem-Petersen J, Hanekom S.M (2005) Clustering of haemostatic variables and the effect of high cashew and walnut diets on these variables in metabolic syndrome patients. Blood Coagul Fibrinolysis 16(6):429-436

18. Dessein P.H, Joffe B.I, Stanwix A.E (2002) Effects of disease modifying agents and dietary intervention on insulin resistance and dyslipidemia in inflammatory arthritis: a pilot study. Arthritis Care Res 4:R12

19. Macintyre U.E, Venter C.S, Vorster H.H (2001) A culture-sensitive quantitative food frequency questionnaire used in an African population: Development and reproducibility. Public Health Nutr 4:53-62

20. Kruger H.S, Venter C.S, Steyn H.S (2004) A stadarized physical activity questionnaire fora population in transition: the Thusa study. African journal for physical, health education, recreation and dance 6:54-64

21. Bingham S. A (1991) Limitations of the various methods for collecting dietary intake data. Ann Nutr Metab 35(3): 117-27

22. Briefel R. R, Sempos C. T, Mcdowell M. A, Chien S, Almaimo (1997) Dietary methods research in the third national health and nutrition examination survey: underreporting of energy intake. Am J Clin Nutr 65(4): 1203S-1209S

23. Natella F, Nardini M, Giatnnetti I, Dattilo C, Scaccini C (2002) Coffee drinking influences plasma antioxidant capacity in humans. J Agric Food Chem 50(21):6211-6216

24. Friedewald W.T, Levy R.I, Fredrickson D.S (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499-502

25. Katz A, Nambi S.S, Mather K, Baron A.D, Follman D.A, Sullivan G, Quon M.J (2000) Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 85:2402-2410

26. Cao G, Prior R.L (1998) Measurement of oxygen radical absorbance capacity in biological samples. In: Methods in Enzymology, Academic Press, New York Vol 299, pp 50-62

27. Cao G, Verdon C, Wu A.H.B, Wang H, Prior R.L (1995) Automated oxygen radical absorbance capacity assay using the COBAS FARA II. Clin Chem 41:1738-1744

28. Janse van Rensburg C, Erasmus E, Loots D.T, Oosthuizen W, Jerling J.C, Kruger H.S, Louw R, Brits M, Van der Westhuizen F.H (2005) Rosa roxburghii supplementation in a controlled feeding study increases plasma antioxidant capacity and glutathione redox state. Eur J Nutr 44(7):452-457

29. Singleton V.L, Rossi J.A (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 16:144-158

30. Rauma A.L, Mykkanen H (2000) Antioxidant status in vegetarians versus omnivores. J Nutr 16(2):111-119

31. Cooney R.V, Custer L.J, Okinaka L, Franke A.A (2001) Effects of dietary sesame seeds on plasma tocopherol levels. Nutr Cancer 39(1):66-71

32. Pearson D.A, Tan C.H, German J.B, Davis P.A, Gershwin M.E (1999) Apple juice inhibits human low density lipoprotein oxidation. Life sci 64: 1913-1920

33. Waterhouse A.L, Shirley J.R, Donovan J.L (1996) Antioxidants in chocolate. Lancet 348:834

34. Frankel E.N, Waterhouse A.L, Teissedre P.L (1995) Principal phenolic phytichemicals in selected California wines and their antioxidant activity in inhibiting oxidation of low-density lipoproteins. J Agric Food Chem 43:890-894

35. Anderson K.J, Teuber S.S, Gobeille A, Cremin P, Waterhouse A.L, Steinberg F.M (2001) Walnut polyphenolic inhibit in vitro human plasma and LDL oxidation. J Nutr 131:2837-2842

36. Singh B, Kale R.K, Rao A.R (2004) Modulation of antioxidant potential in liver of mice by kernel oil of cashew nut (Anacardium occidentale) and its lack of tumour promoting ability in DMBA induced skin papillomagenesis. Indian J Exp Biol 42(4):373-377

37. Cornelli U, Terranova R, Luca S, Cornelli M, Alberti A (2001) Bioavailability and antioxidant activity of some food supplements in men and women using the dRom test as a marker of oxidative stress. J Nutr 131:3208-3211

38. Chen C, Milbury P.E, Lapsley K, Blumberg J.B (2005) Flavonoids from almond skins are bioavailable and act synergistically with vitamins C and E to enhance hamster and human LDL resistance to oxidation. J Nutr 135:1366-1373

39. Unno T, Kondo K, Itakura H, Takeo T (1996) Analysis of (-)-epigallocatechin gallate in human serum obtained after ingesting green tea. Biosci Biotechnol Biochem 60:2066-2068

40. Aziz A.A, Edwards C.A, Lean M.E.J, Crozier A (1998) Absorption and excretion of conjugated flavonols, including quercetin-4'-O-β-glucoside and isorhamnetin-4'-O-β-glucoside by human volunteers after the consumption of onions. Free radic res 29:257-269

41. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I (2004) Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 114(12):1752-1761

LITERATURE CITED

ABUDU N., MILLER J.J., ATTAELMANNAN M., LEVINSON S.S. 2004. Vitamins in human arteriosclerosis with emphasis on vitamin C and E. *Clinica chimica acta,* 339:11-25.

ALBERTI A., BOLOGNINI L., MACCIANTELLI D., CARATELI M. 2000. The radical cation N,N-diethyl-para-phenylendiamine: a possible indicator of oxidative stress in biological samples. *Research on chemical intermediates* , 26:253-267.

ALPERT A.J & GILBERT H.F. 1985. Detection of oxidized and reduced glutathione with a cycling postcolumn reaction. *Analytical biochemistry,* 144:553-562.

ALMARIO R.U., VONGHAVARAVAT V., WONG R., KASIM-KARAKAS S.E. 2001. Effects of walnut consumption on plasma fatty acid and lipoproteins in combined hyperlipidemia. *American journal of clinical nutrition,* 74:72-79.

ANDERSON K.J., TEUBER S.S., GOBEILLE A., CREMIN P., WATERHOUSE A.L., STEINBERG F.M. 2001. Walnut polyphenolic inhibit in vitro human plasma and LDL oxidation. *Journal of nutrition*, 131:2837-2842.

ANON. 2002. Third report of the National cholesterol education program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *Circulation*, 106:3143-3421.

ANON. 1998. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults – the evidence report . National institutes of health. *Obesity research,* 6:51S-209S.

ARCARO G., CRETTI A., BALZANO S., LECHI A., MUGGEO M., BONORA E., BONADONNA R.C. 2002. Insulin causes endothelial dysfunction in humans: sites and mechanisms. *Circulation*, 105(5):576-82.

ASPLUND K. 2002. Antioxidant vitamins in the prevention of cardiovascular disease: a systematic review. *Journal of international medicine,* 251:372-392.

BARBAGALLO M., DOMINGUEZ L.J., TAGLIAMONTE M.R., RESNICK L.M., PAOLISSO G. 1999a. Effects of vitamin E and glutathione on glucose metabolism: role of magnesium. *Hypertension,* 34(2):1002-1006.

BARBAGALLO M., DOMINGUEZ L.J., TAGLIAMONTE M.R., RESNICK L.M., PAOLISSO G. 1999b. Effects of glutathione on red blood cell intracellular magnesium: relation to glucose metabolism. *Hypertension,* 34(1):76-82.

BENZIE F.F & STRAIN J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry,* 239:70-76.

BLUMBERG J & BLOCK G. 1994. The alpha-tocopherol, beta-carotene cancer prevention study in Finland. *Nutrition reviews,* 52(7):242-245.

BOERSMA B.J., PATEL R.P., BOTTING N., WHITE C.R., PARKS D., BARNES S., DARLEY-USMAR V.M. 2001. Formation of novel bioactive metabolites from the reactions of pro-inflammatory oxidants with polyphenolics. *BioFactors*, 15:79-81.

BOULTON D.W., WALLE U.K., WALLE T. 1999. Fate of flavonoid quercetin in human cell lines: chemical instability and metabolism. *Journal of pharmacy and pharmacology,* 51(3):353-359.

BRINK A.J. 1997. Woordeboek van afrikaanse geneeskunde-terme. Kaapstad.: Nasionale boekdrukkery. p:349.

BURKIT D.P. 1973. Some diseases characteristics of modern western civilization. *British medical journal*, 1:274-278.

CABRERA C., LLORIS F., GIMÉNEZ R., OLALLA M., LÓPEZ C. 2003. Mineral content in legumes and nuts: contribution to the Spanish dietary intake. *The science of the total environment,* 308:1-14.

CAO G & PRIOR R.L. 1998. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clinical chemistry,* 44(6):1309-1315.

CAO G., SOFIC E., PRIOR R.L. 1997. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free radical biology & medicine,* 22(5):749-760.

CARPENTER K.L.H., KIRKPATRICK P.J., WEISSBERG P.L/. CHALLIS I.R., DENNIS I.F., FREEMAN M.A., MITCHINSON M.J. 2003. Oral α-tocopherol supplementation inhibits LDL oxidation in established human atherosclerotic lesions. *Free radical research,* 37(11):1235-1244.

CARROL S & DUDFIELD M. 2004. What is the relationship between exercise and metabolic abnormalities? A review of the metabolic syndrome. *Sports medicine,* 34(6):371-418.

CERIELLO A & MOTZ E. 2004. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arteriosclerosis, thrombosis and vascular biology,* 24:816-823.

CHOI Y., KANG J., PARK J.H.Y., LEE Y., CHOI J., KANG Y. 2003. Polyphenolic flavonoids differ in their antiapoptotic efficacy in hydrogen peroxide-treated human vascular endothelial cells. *Journal of nutrition,* 133:985-991.

CORDAIN L., EADES M.R., EADES M.D. 2003. Hyperinsulinemic diseases of civilization: more than just syndrome X. *Comparative biochemistry and physiology part A,* 136:95-112.

CORNELLI U., TERRANOVA R., LUCA S., CORNELLI M., ALBERTI A. 2001. Bioavailability and antioxidant activity of some food supplements in men and women using the D-ROMs test as a marker of oxidative stress. *Journal of nutrition,* 131:3208-3211.

DANDONA P., ALJADA A., BANDYOPADHYAY A. 2004. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends in immunology,* 25(1):4-7.

DEVARAJ S., ROSENSON R.S., JIALAL I. 2004. Metabolic syndrome: an appraisal of the pro-inflammatory and procoagulant status. *Endocrinology and metabolism clinics of North America,* 33:431-453.

DREHER M.L., MAHER C.V., KEARNEY P. 1996. The traditional and emerging role of nuts in healthful diets. *Nutrition review*, 54:241-245.

EATON S.B, KONNER M., SHOSTAK M. 1998. Stone agers in the fast lane: chronic degenerative diseases in evolutionary perspective. *American journal of medicine*, 84:739-749.

ECKEL R.H., GRUNDY S.M., ZIMMET P.Z. 2005. The metabolic syndrome. *Lancet*, 365:1415-1428.

ELIN R.J. 1993. Is the magnesium content of nuts a factor for coronary heart disease? *Archives of internal medicine,* 153:779-780.

FACCHINI F.S., HUMPHREYS H.M., DoNASCIMENTO C.A., ABBASI F., REAVEN G.M. 2000. Relation between insulin resistance and plasma concentrations

of lipid hydroperoxides, carotenoids, and tocopherols. *American journal of clinical nutrition,* 72(3):776-779.

FARRELL G.C. 2003. Non-alcoholic steatohepatitis: what is it, and why is it important in the Asia-Pacific region? *Journal of gastroenterology and hepatology,* 18:124-138.

FELDMAN, E.B. 2002. LSRO report: the scientific evidence for a beneficial health relationship between walnuts and coronary heart disease. *Journal of nutrition,* 132:1062S-1101S.

FESTA A., D'AGOSTINO R., HOWARD G., MYKKÄNEN L., TRACY R.P., HAFFNER S.M. 2002. Chronic subclinical inflammation as part of the insulin resistance syndrome: the insulin resistance atherosclerosis study (IRAS). *Circulation,* 102:42-47.

FLETCHER B & LAMENDOLA C. 2004. Insulin resistance syndrome. *Journal of cardiovascular nursing,* 19(5):339-345.

FORD E.S., GILES W.H., DIETZ W.H. 2002. Prevalence of the metabolic syndrome among US adults. Findings from the third national health and nutrition examination survey. *Journal of the American medical association,* 287(2):356-359.

FORD E.S., MOKDAD A.H., GILES W.H., BROWN D.W. 2003. The metabolic syndrome and antioxidant concentrations: findings from the third national health and nutrition examination survey. *Diabetes,* 52:2346-2352.

FORMAN H.J & DICKINSON D.A. 2003. Oxidative signaling and glutathione synthesis. *Biofactors,* 17(1-4):1-12.

FOSTER-POWELL K& BRAND MILLER J. 1995. International tables of glycaemic index. *American journal of clinical nutrition,* 62:817S-893S.

FRASER G.E., SABATE J., BEESON W.L., STRAHAN T.M. 1992. A possible protective effect of nut consumption on risk of coronary heart disease. The adventist health study. *Archives of internal medicine,* 152(7):1416-1424.

FUKUDA T., ITO H., YOSHIDA T. 2003. Antioxidative polyphenols from walnuts (*Juglans regia* L.). *Phytochemistry,* 63:795-801.

FURUKAWA S., FUJITA T., SHIMABUKURO M., IWAKI M., YAMADA Y., NAKAJIMA Y., NAKAYAMA O., MAKISHIMA M., MATSUDA M., SHIMOMURA I. 2004. Increased oxidative stress in obesity and its impact on metabolic syndrome. *Journal of clinical investigation,* 114(12):1752-1761.

FUKUDA T., ITO H., YOSHIDA T. 2003. Antioxidative polyphenols from walnuts (*Juglans regia* L.). *Phytochemistry,* 63:795-801.

GARCÍA-LORDA P., RANGIL M., SALAS-SALVADÓ J. 2003. Nut consumption, body weight and insulin resistance. *European journal of clinical nutrition,* 57:S8-S11.

GARG R., TRIPATHY D., DANDONA P. 2003. Insulin resistance as a proinflammatory state: mechanismetabolic syndrome, mediators, and therapeutic interventions. *Current drug targets,* 4:487-492.

GHISELLI A., SERAFINI M., MAIANI G., AZZININ E., FERRO-LUZZI A. 1995. A fluorescence-based method for measuring total plasma antioxidant capability. *Free radical biology and medicine,* 18(1):29-36.

GHISELLI A., SERAFINI M., NATELLA F., SCACCINI C. 2000. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. *Free radical biology & medicine,* 29(11):1106-1114.

GÖKKUSU C., PALANDUZ S., ADEMOGLU E., TAMER S. 2001. Oxidant and antioxidant systemetabolic syndrome in niddm patients: influence of vitamin E supplementation. *Endocrine research,* 27(3):377-386.

GROOP L & ORHO-MELANDER M. 2001. The dysmetabolic syndrome. *Journal of international medicine,* 250:105-120.

GROOP L., FORSBLOM C., LEHTOVIRTA M., TUOMI T., KARANKO S., NISSEN M., EHRNSTROM B.O., FORSEN B., ISOMAA B., SNICKARS B., TASKINEN M.R. 1996. Metabolic consequences of a family history of NIDDM (the Botnia study): evidence for sex-specific parental effects. *Diabetes,* 45(11):1585-93.

GRUNDY S.M., BREWER B., CLEEMAN J.I., SMITH S.C., LENFANT C. 2004a. Definition of the metabolic syndrome. Report of the national heart, lung, and blood institute/American heart association conference on scientific issues related to definition. *Circulation,* 109:433-438.

GRUNDY S.M., HANSEN B., SMITH S.C., CLEEMAN J.I., KAHN R.A. 2004b. Clinical management of metabolic syndrome. Report of the American heart association/national heart, lung, and blood institute/American diabetes association conference on scientific issues related to management. *Circulation,* 109:551-556.

GRUNDY S.M., ABATE N., CHANDALIA M. 2002. Diet composition and the metabolic syndrome: what is optimal fat intake? *American journal of medicine,* 113(9B):25S-29S.

GRUNDY S.M. 2004. Obesity, metabolic syndrome, and cardiovascular disease. *Journal of endocrinology & metabolism,* 89:6):2595-2600.

HALLIWELL B. 1996. Antioxidants in human health and disease. *Annual Review of Nutrition,* 16:33-50.

HALLIWELL B & CHIRICO S. 1993. Lipid peroxidation: its mechanism, measurement, and significance. *American journal of clinical nutrition,* 57:715S-724.

HALVORSEN B.L., HOLTE K., MYHRSTAD M.C.W., BARIKMO I., HVATTUM E., REMBERG S.F., WOLD A., HAFFNER K., BAUGEROD H., ANDERSEN L.F., MOSKAUG J., JACOBS, Jr D.R., BLOMHOFF R. 2002. A systematic screening of total antioxidants in dietary plants. *Journal of nutrition,* 132:461-471.

HARBIGE L.S. 1998. Dietary n-6 and n-3 fatty acids in immunity and autoimmune disease. *The proceedings of the nutrition society,* 57(4):555-562.

HARRIS N & WINTER W.E. 2004. The chemical pathology of insulin resistance and the metabolic syndrome. *Medical laboratory observer: an electronic journal.* [Web:] www.mlo-online.com [Date used: 21 Apr. 2005].

HSUEH W.A., LYON C.J., QUIÑONES M.J. 2004. Insulin resistance and the endothelium. *American journal of medicine,* 117:109-117.

HU F.B., STAMPFER M.J., MANSON J.E., RIMM E.B., COLDITZ G.A., ROSNER B.A., SPEIZER F.E., HENNEKENS C.H., WILLETT W.C. 1998. Frequent nut consumption and the risk of coronary heart disease in women: prospective cohort study. *British medical journal,* 317:13141345.

IAMELE L., FIOCCHI R., VERNOCCHI A. 2002. Evaluation of an automated spectrophometric assay for reactive oxygen metabolites in serum. *Clinical chemistry and laboratory medicine,* 40(7):673-676.

INOGUCHI T., LI P., UMEDA F., YU H.Y., KAKIMOTO M., IMAMURA M., AOKI T., ETOH T., HASHIMOTO T., NARUSE M., SANO H., UTSUMI H., NAWATA H. 2000. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cells. *Diabetes*, 49:1939-1945.

ISOMAA B. 2003. A major health hazard: the metabolic syndrome. *Life sciences*, 73:2395-2411.

ISOMAA B., ALMGREN P., YUOMI T., FORSÉN B., LATHI K., NISSÉN M., TASKINEN M., GROOP L. 2001. Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes care*, 24(4):683-689.

JACKSON M.J., PAPA S., BOLAÑOS J., BRUCKDORFER R., CARLSEN H., ELLIOTT R.M., FLIER J., GRIFFITH H.R., HEALES S., HOLST B., LORUSSO M., LUND E., MOSKAUG J.O., MOSRE U., DI PAOLA M., POLIDORI M.C., SIGNORILE A., STAHL W., VIÑA-RIBES J., ASTLEY S.B. 2002. Antioxidants, reactive oxygen and nitrogen species, gene induction and mitochondrial function. *Molecular aspects of medicine*, 23:209-285.

JACOB R.A. 2002. Vitamin C function and status in chronic disease. *Nutrition in clinical care*, 5(2):66-74.

KATZMARZYK P.T., LEON A.S., WILMORE J.H., SKINNER J.S., RAO D.C., RANKINEN T., BOUCHARD C. 2003. Targeting the metabolic syndrome with exercise: evidence from the heritage family study. *Medicine and science in sports and exercise*, 35(10):1703-1709.

KESKIN M, KURTOGLU S, KENDIRCI M, ATABEK E, YAZICI C. 2005. Homeostasis model assessment is more reliable than the fasting glucose/insulin ratio and quantitive sensitivity check index for assessing insulin resistance among obese children and adoloscents. *Pediatrics*, 115(4):e500-e503.

KNOWLER W.C., BARRETT-CONNOR E., FOWLER S.E., HAMMAN R.F., LANCHIN J.M., WALKER E.A., NATHAN D.M. 2002. Reduction in incidence of type 2 diabetes with lifestyle intervention or metformin. *New England journal of medicine*, 346(6):393-403.

KORACEVIC D., KORACEVIC G., DJORDJEVIC V., ANDREJEVIC S., COSIC V. 2001. Methods for the measurements of antioxidant activity in human fluids. *Journal of clinical pathology,* 54(5):356-361.

KRAUSS R.M., ECKEL R.H., HOWARD B., APPEL L.J., DANIELS S.R., DECKELBAUM R.J., ERDMAN J.W., KRIS-ETHERTON P., GOLDBERG I.J., KOTCHEN T.A., LICHTENSTEIN A.H., MITCH W.E., MULLIS R., ROBINSON K., WYLIE-ROSETT J., St. JEOR S., SUTTIE J., TRIBBLE D.L., BAZZARRE T.L. 2000. AHA dietary guidelines. Revision 2000: a statement for health care professionals from the nutrition committee of the American heart association. *Circulation,* 102:2284-2299.

KRAUSS R.M. 1995. Dense low density lipoproteins and coronary artery disease. *American journal of cardiology*, 75:53B-57B.

KREBS M & RODEN M. 2004. Nutrient-induced insulin resistance in human skeletal muscle. *Current medical chemistry,* 11:901-908.

KRIS-ETHERTON P.M., LEFEVRE M., BEECHER G.R., GROSS M.D., KEEN C.L., ETHERTON T.D. 2004. Bioactive compounds in nutrition and health-research methodologies for establishing biological function: the antioxidant and anti-inflammatory effects of flavonoids on atherosclerosis. *Annual Review of Nutrition*, 24:511-538.

KRIS-ETHERTON P.M., YU-POTH S., SABATÉ J., RATCLIFFE H.E., ZHAO G., ETHERTON T.D. 1999. Nuts and their bioactive constituents: effects on serum lipids and other factors that affect disease risk. *American journal of clinical nutrition,* 70:504S-511S.

KRUGER A. 2000. Metabolic syndrome in Africans. Potcehfstroom : Potcehfstroom University for Christian Higher Education. (Thesis – M.Soc.Sc.) 149 p.

LI H & FORSTERMANN U. 2000. Nitric oxide in the pathogenesis of vascular disease. *Journal of pathology,* 190 (3), 244-254.

LOVEJOY J.C., MOST M.M., LEFEVRE M., GREENWAY F.L., ROOD J.C. 2002. Effects of diets enriched in almonds on insulin action and serum lipids in adults with normal glucose tolerance or type 2 diabetes. *American journal of clinical nutrition,* 76:1000-1006.

MACFARLANE B.J., BEZWODA W.R., BOTHWELL T.H., BAYNES R.D., BOTHWELL J.E., MACPHAIL A.P., LAMPARELLI R.D., MAYET F. 1998. Inhibitory effect of nuts on iron absorption. *American journal of clinical nutrition,* 47(2):270-274.

MAES H.H., NEALE M.C., EAVES L.J. 1997. Genetic and environmental factors in relative body weight and human adiposity. *Behavior genetics,* 27:325-351.

MARTÍN-GALLÁN P., CARRASCOSA A., GUSSINYÉ M., DOMÍNGUEZ C. 2002. Biomarkers of diabetes-associated oxidative stress and antioxidant status in young diabetic patients with or without subclinical complications. *Free radical biology and medicine,* 34(12):1563-1574.

MASHIMA R., WITTING P.K., STOCKER R. 2001. Oxidants and antioxidants in atherosclerosis. *Current opinion in lipidology,* 12:411-418.

MAVRI A., ALESSI M.C., JUHAN-VAGUE I. 2004. Hypofibrinolysis in the insulin resistance syndrome: implications in vascular disease. *Journal of international medicine,* 255:448-456.

MAXWELL S.R.J., THOMASON H., SANDLER D., LEGUEN C., BAXTER M.A., THORPE G.H.G., JONES A.F., BARNETT A.H. 1997. Antioxidant status in patients with uncomplicated insulin-dependent and non-insulin-dependent diabetes mellitus. *European journal of clinical investigation,* 27:484-490.

MAZIÈRE C., MORLIÈRE P., SANTUS R., MARCHEUX V., LOUANDRE C., CONTE M., MARIÈRE J. 2004. Inhibition of insulin signaling by oxidized low density lipoprotein: protective effect of the antioxidant vitamin E. *Atherosclerosis,* 175:23-30.

MEYER K.A., KUSHI L.H., JACOBS D.R., SLAVIN J., SELLERS T.A., FOLSORN A.R. 2000. Carbohydrates, dietary fiber, and incidence of type 2 diabetes in older women. *American journal of clinical nutrition,* 71:921-930.

MOSKUAG J., CARLSEN H., MYHRSTAD C.W., BLOMHOFF R. 2005. Polyphenols and glutathione synthesis regulation. *American journal of clinical nutrition,* 81:277S-283S.

MONTONEN J., REUNANEN A., KNEKT P., JÄRVINEN R. 2004. Dietary antioxidant intake and risk of type 2 diabetes. *Diabetes care*, 27:362-366.

MUKHTAR & AHMAD. 2000. Tea polyphenols: prevention of cancer and optimizing health. *American journal of clinical nutrition,* 71(6): 1698S-1702S.

MUKUDDEM-PETERSEN, J. 2005. The effects of nuts on markers of the metabolic syndrome. Potchefstroom: North-West University. (Thesis – P.hD.) 180 p.

MYHRSTAD M.C., CARLSEN H., NORDSTROM O., BLOMHOFF R., MOSKUAG J.O. 2002. Flavonoids increase the intracellular glutathione level by transactivitaion of γ-glutamylcysteine sythetase catalytical subunit promoter. *Free radical biology & medicine,* 32:386-393.

NEWTON G.L., DORAIN R., FAHEY R.C. 1981. Analysis of biological thiols: derivatization with monobromobimane and separation by reverse-phase high-performance liquid chromatography. *Analytical biochemistry,* 114:383-387.

NILSSON T.K., BOMAN K., BJERLE P., HALLMANS G., HELLSTEN G. 1994. von Willebrand factor and fibrinolytic variables are differently affected in insulin resistance syndrome. *Journal of international medicine,* 235:419-423.

NINFALI P., MEA G., GIORGINI S., ROCCHI M., BACCHIOCCA M. 2005. Antioxidant capacity of vegetables, spices and dressings relevant to nutrition. *British journal of nutrition,* 93:257-266.

O'BRIAN P.E & DIXON J.B. 2002. The extent of the problem of obesity. *The American journal of surgery,* 184:4S-8S.

OWENS C.W.I & BELCHER R.V. 1965. A colorimetric micro-method for the determination of glutathione. The b*iochemical journal,* 94:705-711.

PALANDUZ S., ADEMOGLU E., GÖKKUSU C., TAMER S. 2001. Plasma antioxidants and type 2 diabetes mellitus. *Research communications in molecular pathology and pharmacology*, 109(5&6):309-318.

PAOLISSO G., DI MARO G., PIZZA G., D'AMORE A., SGAMBATO S., TESAURO P., VARRICCHIO M., D'ONOFRIO F. 1992. Plasma GSH/GSSG affects glucose homeostasis in healthy subjects and non-insulin dependent diabetics. *American journal of physiology,* 263:E435-E440.

PAOLISSO G., D'AMORE A., GIUGLIANO D., CERIELLO A., VARRICCHIO M., D'ONOFRIO F. 1993. Pharmacologic doses of vitamin E improve insulin action in healthy subjects and non-insulin-dependent diabetic patients. *American journal of clinical nutrition: an electronic journal*, 57(5)0-656. [Web:] http://www.ajcn.org/cgi/content/abstract/57/5/650 [Date used: 20 Apr. 2005].

PAOLISSO G., DI MARO G., GALZERANO D., CACCIAPUOTI F., VARRICCHIO G., VARRICCHIO M., D'ONOFRIO F. 1994. Pharmacological doses of vitamin E and insulin action in elderly subjects. *American journal of clinical nutrition,* 59(6):1291-1296. [Web:] http://www.ajcn.org/cgi/content/absract/59/6/1291 [Date used: 20 Apr. 2005].

PFLUGER P., KLUTH D., LANDES N., BUMKE-VOGT C., BRIGELIUS-FLOHÉ R. 2004. Vitamin E: underestimated as an antioxidant. *Redox report,* 9(5):249-254.

PICKUP J.C., CROOK M.A. 1998. Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia,* 41:1241-1248.

PRINCEMAIL J., MEURISSE M., LIMET R., DEFRAIGNE J.O. 2001. Measurements and use of antioxidants in human medicine. *In press*.

PRABHAKARAN D & ANAND S.S. 2004. The metabolic syndrome: an emerging risk state for cardiovascular disease. *Vascular medicine*, 9:55-68.

PRINCE K.D., PRICE C.S.C., REYNOLDS R.D. 2001. Hyperglycemia-induced ascorbic acid deficiency promotes endothelial dysfunction and the development of atherosclerosis. *Atherosclerosis*, 158:1-12.

PRIOR R.L & CAO G. 1999. In vivo total antioxidant capacity: comparison of different analytical methods. *Free radical & biology medicine*, 27(11/12):1173-1181.

PRIOR R.L & CAO G. 2000. Analysis of botanicals and dietary supplements for antioxidant capacity: a review. *Journal of AOAC international*, 83(4):950-956.

RAGGI M.A., NOBILE L., GIOVANNINI A.G. 1991. Spectrophotometric determination of glutathione and of its oxidation product in pharmaceutical dosage form. *Journal of pharmaceutical and biochemical analysis*, 9:1037-1040.

REUNANEN A., KNEKT P., AARAN R.K., AROMAA A. 1998. Serum antioxidants and risk of non-insulin dependent diabetes mellitus. *European journal of clinical nutrition*, 52:89-93.

RICCARDI G., GIACCO R., RIVELLESE A.A. 2004. Dietary fat, insulin sensitivity and the metabolic syndrome. *Clinical nutrition*, 23:447-456.

RICE-EVANS C & MILLER N.J. 1994. Total antioxidant status in plasma and body fluids. *Methods in enzymology*, 243:279-293.

RICE-EVANS C.A. 2000. Measurements of total antioxidant activity as a marker of antioxidant status *in vivo*: procedures and limitations. *Free radical research,* 33:559-566.

RICE-EVANS C.A. 2001. Flavonoid antioxidants. *Current medical chemistry,* 8:797-807.

ROBERTSON R.P. 2004. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet β-cells in diabetes. *The journal of biological chemistry,* 279(41):42351-42354.

ROEBUCK K.A. 1999. Oxidant stress regulation of IL-8 and ICAM-1 gene expression: differential activation and binding of the transcription factors AP-1 and NF-kappaB. *International journal of molecular medicine,* 4:223-230.

ROSELL M., JAHANSSON G., BERGLUND L., VESSBY B., DE FAIRE U., HELLÉNIUS M.L. 2004. Association between the intake of diary fat and calcium and abdominal obesity. *International journal of obesity,* 28:1427-1434.

ROWE J.W., YOUNG J.B., MINAKER K.L., STEVENS A.L., PALOTTA J., LANDSBERG L. 1981. Effect of insulin and glucose infusion on sympathetic nervous system activity in normal man. *Diabetes*, 30:19-25.

RYAN M.C.M & THAKORE J.H. 2002. Physical consequences of schizophrenia and its treatment: the metabolic syndrome (mini review). *Life sciences,* 71:239-257.

SALMERON J., ASCHERIO M., RIMM E.B., COLDITZ G.A., SPEIGELMAN D., JENKENS D.J., STAMPFER M.J., WING A.L., WILLETT W.C. 1997. Dietary fiber, glycemic load, and risk of NIDDM in men. *Diabetes care*, 20:545-550.

SALONEN J.T., NVYSSONEN K., SALONEN R., LAKKA H.M., KAIKKONEN J., PORKKALA-SARATAHO E., VOUTILAINEN S., LAKKA T.A., RISSANEN T., LESKINEN L., TUOMAINEN T.P., VALKONEN V.P., RISTONMAA U., POULSEN H.E. 2000. Antioxidant supplementation in arthrosclerosis prevention (ASAP) study: a randomized trial of the effect of vitamins E and C on 3-year progression of carotid atherosclerosis. *Journal of international medicine,* 248(5):377-386.

SANCHEZ-LUGO L ., MAYER-DAVIS E.J ., HOWARD G ., SELBY J.V ., AYAD M.F ., REWERS M ., HAFFNER S. 1997. Insulin sensitivity and intake of vitamin E and C in African American, Hispanic, and non-Hispanic white men and women: the insulin resistance and atherosclerosis study (IRAS). *American journal of clinical nutrition,* 66(5):1224-1231.

SANZ PARIS A. 2000. Diabetes and nutrition. *Nutricion hospitalaria,* 15(1):58-68.

SAUBERLICH H.E. 1994. Pharmacology of vitamin C. *Annual review of nutrition,* 14:371-391.

SAYLOR J. 2005. Risk factors clusters for metabolic syndrome in coronary heart disease. *Dimensions of critical care nursing,* 24(2):64-69.

SEGHROUCHNI I., DRAI J., BANNIER E., RIVIÈRE J., CALMARD P., GARCIA I., ORGIAZZI J., REVOL A. 2001. Oxidative stress parameters in type I, type II and insulin-treated type 2 diabetes mellitus; insulin treatment efficiency. *Clinica chimica acta,* 321:89-96.

SEIDELL J.C. 2000. Obesity, insulin resistance and diabetes – a worldwide epidemic. *British journal of nutrition,* 83:S5-S8.

SHAW J.E & CHISHOLM D.J. 2003. Epidemiology and prevention of type 2 diabetes and the metabolic syndrome. *Medical journal of Australia*: an electronic journal, 179:379-383. [Web:] http://www.mja.com.au/public/issues/179_07_061003/sha10375_fm.html [Date used: 5 Apr. 2005].

SKIBOLA C.F & SMITH M.T. 2000. Potential health impacts of excessive flavonoid intake. *Free radical biology & medicine*, 29(3&4):375-383.

ŠKRHA J., ŠINDELKA G., KVASNICKA J., HILGERTOVÁ J. 1999. Insulin action and fibrinolysis influenced by vitamin E in obese type 2 diabetes mellitus. *Diabetes research and clinical practice*, 44:27-33.

SMITH S.C., CLARK L.T., COOPER R.S., DANIELS S.R., KUMANYIKA S.K., OFILI E., QUINONES M.A., SANCHEZ E.J., SAUNDERS E., TIUKINHOY S.D. 2005. Discovering the full spectrum of cardiovascular disease. *Circulation*, 111:e134-e139.

SNIEDER H., VAN DOORNEN L.J., BOOMSMA D.I. 1999. Dissecting the genetic architecture of lipids, lipoproteins, and apiloproteins: lessons from twin studies. *Arteriosclerosis thrombosis and vascular biology*, 19:2826-2834.

SOWERS J.R & EPSTEIN M. 1995. Diabetes mellitus and associated hypertension, vascular disease, and nephropathy. *Hypertension*, 26:869-79.

STENTZ F.B., UMPIERREZ G.E., CUERVO R., KITABCHI A.E. 2004. Proinflammatory cytokines, markers of cardiovascular risks, oxidative stress, and lipid peroxidation in patients with hyperglycemic crises. *Diabetes*, 53:2079-2086.

STOHS S.J. 1995. The role of free radicals in toxicity and disease. *Journal of basic clinical physiology and pharmacology*, 6:205-228.

TENEBAUM A., MOTRO M., SCHWAMMENTHAL E., FISMAN E.Z. 2004. Macrovascular complications of metabolic syndrome: an early intervention is imperative. *International journal of cardiology,* 97:167-172.

THOMPSON P.D., BUCHNER D., PIÑA I.L., BALADY G.J., WILLIAMS M.A., MARCUS B.H., BERRA K., BLAIR S.N., COSTA F., FRANKLIN B., FLETCHER G.F., GORDON N.F., PATE R.R., RODRIGUEZ B.L., YANCEY A.K., WENGER N.K. 2003. Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease. *Circulation,* 107:3109-3116.

TIETZE F. 1969. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Analytical biochemistry,* 27:502-522.

TOMASIAN D., KEANEY J.F., VITA J.A. 2000. Antioxidants and the bioactivity of endothelium-derived nitric oxide. *Cardiovascular research,* 47(3):426-435.

TUOMILEHO J., LINDSTROM J., ERIKSSON J.G., VALLE T.T., HAMALAINEN H., ILANNE-PARIKKA P., KEINANEN-KIUKAANIEMI S., LAAKSO M., LOUHERANTA A., RASTAS M., SALMINEN V., UUSITUPA M. 2001. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *New England journal of medicine,* 344(18):1343-1350.

UNGER R.H. 2003. Lipid overload and overflow: metabolic trauma and the metabolic syndrome. *Trends in endocrinology and metabolism,* 14(9):398-403.

URSO M.L & CLARKSON P.M. 2003. Oxidative stress, exercise, and antioxidant supplementation. *Toxicology,* 189:41-54.

VESSBY B., UUSITUPA M., HERMANSEN K. 2001. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: the KANWU study. *Diabetologia,* 44(3):312-319.

VIRGIN S.E & SCHMITKE J.A. 2003. Metabolic syndrome. *Official journal of the American association of occupational health nurses*, 51(1):28-37.

WALLACE T.M., LEVY J.C., MATTHEWS D.R. 2004. Use and abuse of HOMA modeling. *Diabetes care*, 27(6):1487-1485.

WALLE T. 2004. Serial review: flavonoids and isoflavones (phytoestrogens): absorption, metabolism, and bioactivity. *Free radical biology & medicine,* 36(7):829-837.

WATANABE Y & IMAI K. 1983. Liquid chromatographic determination of amino and imino acids and thiols by postcolumn derivatization with 4-fluoro-7-nitrobenzo-2,1,3-oxadiazole. *Analytical chemistry,* 55:1786-1791.

WILKIN T.J & VOSS L.D. 2004. Metabolic syndrome: maladaptation to a modern world. *Journal of the royal society of medicine,* 97:511-520.

WU X., GU L., HOLDEN J., HAYTOWITZ D.B., GEBHARDT S.E., BEECHER G., PRIOR R.L. 2004. Development of a database for total antioxidant capacity in foods: a preliminary study. *Journal of food consumption and analysis,* 17L407-422.

WYSZYNSKI D.F., WATERWORTH D.M., BARTER P.J., COHEN J., KESÄNIEMI Y.A., MAHLEY R.W., McPHERSON R., WAEBER G., BERSOT T.P., SHARMA S.S., NOLAN V., MIDDLETON L.T., SUNDSETH S.S., FARRER L.A., MOOSER V., GRUNDY S.M. 2005. Relation between atherogenic dyslipidemia and the adult treatment program-III definition of metabolic syndrome (genetic epidemiology of metabolic syndrome project). *The American journal of cardiology,* 95:194-198.

YAMAKOSHI J., KATAOKA S., KOGA T., ARIGA T. 1999. Proanthocyanidin-rich extract from grape seeds attenuates the development of arthrosclerosis in cholesterol-fed rabbits. *Atherosclerosis,* 142(1):139-149.

YILDIZ L., KAYAOGLU N., AKSOY H. 2002. The changes of superoxide dismutase, catalase and glutathione peroxidase activities in erythrocytes of active and passive smokers. *Clinical chemistry and laboratory medicine,* 40(6):612-615.

YLÖNEN K., ALFTHAN G., GROOP L., SALORANTA C., ARO A., VIRTANEN S.M., BOTNIA RESEARCH GROUP. 2003. Dietary intakes and plasma concentrations of carotenoids and tocopherols in relation to glucose metabolism in subjects at high risk of type 2 diabetes: the Botnia Dietary Study. *American journal of clinical nutrition,* 77:1434-1441.

ZDUNEZYK Z., FREJNAGEL S., WRÓBLEWSKA M., JUSKIEWICZ J., OSZMIANSKI J., ESTRELLA I. 2000. Biological activity of polyphenol extracts from different plant sources. *Food research international,* 53:183-186.