

The effects of nuts on markers of the metabolic syndrome



J Mukuddem-Petersen RD
M.Sc. in Dietetics *Cum Laude*

Thesis submitted for the degree in
Philosophia Doctor
in
Dietetics at the
School for Physiology,
Nutrition and Consumer Science
of the
North-West University (Potchefstroom Campus)

Promotor : Prof W Oosthuizen
Co-Promotor : Prof JC Jerling

Potchefstroom
South Africa
2005



*To my husband Mark Adam Petersen and daughter Daena Blythe
Kendra Petersen for their love, support and unwavering faith in
me throughout this study and the completion of my thesis.*

Acknowledgements

Firstly, I would like to thank God for His grace in enabling me to complete this thesis.

A special word of thanks to the Potchefstroom Campus of North-West University (NWU-PC) for the use of their facilities like, for instance, the Metabolic Ward of the Nutrition Department. My sincere gratitude to the National Research Foundation (NRF) and the South African governments' Technology and Human Resources for Industry Program (THRIP) for their financial support and to the food companies (Tiger brands, Pick 'n Pay, Clover, Unilever-Bestfoods-Robertsons) who donated various foods for our controlled feeding trial henceforth known as the Nut Study.

I would also like to express my appreciation to the following people who made the completion of this study possible:

- My wonderful promoter, Prof. W. Oosthuizen for her guidance, brilliant advice and pleasing disposition, and to my co-promoter Prof. J.C. Jerling for his intellectual inputs and for obtaining financial support from the industry.
- Dr. Grieta Hanekom for her hard work and commitment to the Nut study.
- The subjects/participants (primarily NWU-PC staff members) for their willingness to participate and enthusiasm shown towards the Nut study protocol.
- Sr. M.C. Lessing for screening participants, performing the oral glucose tolerance test (OGTT) and for drawing the blood samples.
- Prof. C.S. Venter, Prof. H.S. Kruger and Dr. H. van't Riet for their contributions during the planning phase of the Nut study.
- Prof. C.S. Venter, Dr. H.H. Wright, Prof. H.S. Kruger, R. Breet and M. Opperman for their assistance with the interviewing of participants and completing of Food Frequency Questionnaires (FFQ) and Physical Activity Questionnaires (PAQ).
- Z. White, C. de Witt, A. Greyling and R. Breet for their creative contribution to the study's newsletter, viz., the NUTCRACKER. This team together with K. Moruisi also regularly weighed the participants.
- Z. White and R. Breet for meticulously checking the compliance forms.
- E. Pienaar for her dedication and accuracy in running the kitchen and supervising all aspects of the food inventory and preparation.
- Z. White and A. Greyling for doing various blood analyses.

- Dr. F. van der Westhuizen and his students for doing some of the biochemical analyses.
- A. Van Graan, Dr. H.H. Wright, Dr. M. van Lieshout, Dr. D. Loots, Dr. M. Pieters-Loots, Z. White, C. de Witt and M. Phometsi for their “hands-on” assistance in the kitchen with food preparation and weighing.
- The physiology team, viz., Dr. A. Schutte, Dr. H. Huisman, Dr. J. van Rooyen and their students for accurately determining the arterial thickness and various blood pressure (BP) parameters of the participants. S. Jordaan for accurate and speedy analysis of BP measurements.
- 4th year dietetic students (J. Wheeler, J. Bekker, C. Jansen van Rensburg, M. Bailey, L. Loots, V. van Scheltinga, L. Wiggett, L. Davies, L. van Wyk, E. Snyman) for their commitment in preparing the food for the controlled feeding trial.
- F. Mpho and T. Holcle for maintaining a high standard of hygiene in the metabolic kitchen as well as assisting with the preparation and weighing of food.
- To the willing staff of the Nutrition Department at the NWU-PC for their hands-on assistance during critical periods of the study.
- To Prof. H.H. Vorster for her words of encouragement.
- To H. van der Waldt at the Ferdinand Postma Library for her invaluable and friendly assistance.
- Last but not least, to my loving husband and mentor, Mark and daughter Daena for their love and support. To my mother, Daphne Mukuddem and grandmother Edna Rensburg for their constant prayers and encouragement throughout the years. Thanks to my sister, Gail Bester and family for their love and interest shown. To my mother-in-law, Ruth Petersen for her love and support and to my sister-in-law Joy Fourie and family for their interest shown. To Sheilah and Clive Carr and family for their loving words of encouragement.

Jer. 29 v 11

*“For I know the plans I have for you,
declares the Lord, plans to prosper you and not to harm you, plans to give you a hope and a future.”*

Abstract

Motivation: The metabolic syndrome is characterized by a group of risk factors for cardiovascular disease (CVD) that includes obesity, dyslipidemia, high blood pressure, insulin resistance, glucose intolerance or non-insulin dependant diabetes mellitus, prothrombotic state and proinflammatory state. The NHANES III study showed the prevalence of this syndrome to be 24.0% in men and 23.4% in women in the USA. These figures translate to more than 47 million US residents having the metabolic syndrome. In the THUSA (acronym for Transition and Health in the Urbanization of South Africans) study in South Africa it was found that 12% and 28.4% of men and women, respectively, of the black population of the North West Province had three or more disturbances characterizing this syndrome. Therefore, it is evident that the metabolic syndrome is a health problem not only for developed countries but also for developing countries. As a result, this syndrome has been identified as a target for dietary therapies to reduce the risk of CVD and type 2 diabetes.

Epidemiological studies have consistently demonstrated an inverse association between nut consumption and coronary heart disease (CHD) morbidity and mortality in different population groups. Nut consumption may not only offer protection against heart disease, but also increase longevity. Recently, the benefits of nuts consumption were acknowledged by the U.S. Food and Drug Administration when they approved a qualified health claim that eating nuts (1.5 ounces/day $\approx$ 42.8 g/day) may reduce the risk of CHD. In this regard, the most comprehensively studied mechanism involved the favourable lipid lowering effects of nuts. There is, however, a lack of data in the literature regarding the effect of nuts on the metabolic syndrome.

Objective: The main objective of this study was to examine the effects of a high walnut diet and a high unsalted cashew nut diet on markers of the metabolic syndrome in humans. In order to provide a foundational body of evidence for the aforementioned, a secondary objective included conducting a systematic review that investigates the effects of nuts on the lipid profile.

Methods: The main project consisted of a controlled feeding trial with a parallel, randomized controlled study design on participants having the metabolic syndrome. Sixty-four subjects having this syndrome (29 men, 35 women) with a mean ($\pm$ SD) age of 45 ± 10 y and who met with the selection criteria were all fed a 3-week run-in control diet. After this period, participants were grouped according to gender and age and then randomized into three groups, namely, those that received a controlled feeding diet including walnuts (20% energy

(E), 60-100g/day; protein:carbohydrate:fat=18:42:40%E), or unsalted cashew nuts (20%E 66-115g/day; protein:carbohydrate:fat=19:44:37%E) or no nuts (protein:carbohydrate:fat=20:47:33%E) for 8 weeks. The participants' physical activity and weight were maintained for the duration of the study.

For the systematic review, human intervention trials that investigated the independent effects of nuts on lipid concentrations were included. Medline and Web of Science databases were searched from the start of the database to August 2004 and supplemented by cross-checking reference lists of relevant publications. These papers received a rating based upon the methodology as it appeared in the publication. No formal statistical analysis was performed due to the large differences in study designs of the dietary intervention trials. The main outcome measures for the systematic review, were percentage differences between treatment and control groups for total blood cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triacylglycerols (TG).

Results: Regarding the main objective, we found that both the walnut and unsalted cashew nut intervention diets had no significant effect on the lipid profile, serum fructosamine, insulin, insulin sensitivity, insulin resistance, serum high sensitivity C-reactive protein, blood pressure and serum uric acid concentrations when compared to the control diet. All three groups experienced highly significant increases in serum insulin concentrations when comparing the baseline to end ($P<0.05$). In turn, insulin resistance increased while insulin sensitivity decreased in all three groups. Plasma glucose concentrations increased significantly in the cashew nut group compared to the control group ($P<0.05$). By contrast, serum fructosamine was unchanged in the cashew nut group while the control group had significantly increased concentrations of this short-term marker of glycaemic control.

The literature search for the systematic review yielded 415 publications. After screening, 23 nut studies were included in the review with most of these studies including heart-healthy diets. The majority of the studies were short (4-6 weeks) with only one study lasting 6 months. The number of subjects in most of the studies was sufficient to study the effects on TC and LDL-C but not for HDL-C and TG. The results of three almond (50-100g/day), two peanut (35-68g/day), one pecan nut (72g/day) and four walnut (40-84g/day) studies showed convincing evidence for a lipid lowering effect of TC between 2-16% and LDL-C between 2-19%, when compared to their control diets. Currently, there are indications from inadequately designed intervention studies that hazelnuts (1g/day/kg body weight) and pistachios (20%E) may have a lipid lowering effect. At this stage the evidence for macadamia nuts is less convincing. Furthermore, it is apparent that the components in nuts further reduce TC and

LDL-C concentrations beyond the effects predicted by equations based solely on dietary fatty acid profiles.

Conclusions: In the controlled feeding trial, subjects displayed no improvement in the markers of the metabolic syndrome after following a walnut or unsalted cashew nut diet compared to a control diet while maintaining body weight (8 weeks). Finally, we suspect that the dramatic increase in insulin resistance may have masked the protective effects of the walnut and cashew nut diets in our subjects with the metabolic syndrome. Further research is warranted before a consensus can be reached.

From the systematic review it was concluded that the consumption of ≈ 50 -100g (≈ 1.5 -3.5 *servings*) of nuts five or more times/week as part of a heart-healthy diet with total fat content (high in mono- and /or polyunsaturated fatty acids) of $\approx 35\%$ of energy may significantly decrease TC and LDL-C in normo- and hyperlipidemic individuals.

Recommendations:

A similar nut controlled feeding trial with some form of calorie restriction, should be done on participants having the metabolic syndrome.

Future research should use randomized controlled studies with larger sample sizes and longer duration to investigate the effects of nuts on HDL-C and TG concentrations. Also, studies should investigate the effects on the lipid profile of mixed nuts and those individual nuts not yet considered. In addition, the unique nutrient and non-nutrient composition of nuts requires further research in order to elucidate the possible mechanisms responsible for the LDL-C lowering effect

Key words: Metabolic syndrome, nuts, insulin sensitivity, insulin resistance, hyperlipidemia, hypertension, lipids, lipoproteins, triacylglycerol, fatty acids

Afrikaanse titel: Die effekte van neutre op merkers van die metabooliese sindroom

Opsomming

Motivering: Die metabooliese sindroom word gekenmerk deur die aanwesigheid van verskeie risikofaktore vir kardiovaskulêre siektes (KVS) naamlik oorgewig, dislipidemie, hoë bloeddruk, insulienweerstand, glukose-onverdraagsaamheid of nie-insulienafhanklike diabetes mellitus (DM), protrombotiese en -inflammatoriese toestande. In die VSA is deur die NHANES III studie gerapporteer dat die voorkoms van metabooliese sindroom in mans en vrouens 24% en 23.4%, onderskeidelik was. Die syfers dui daarop dat 47 miljoen Amerikaners aan die sindroom lei. In die THUSA (akroniem vir "Transition and Health in the Urbanization of South Africans") studie in Suid-Afrika is gevind dat 12% van mans en 28.4% van vrouens in die swart gemeenskap van die Noordwes Provinsie aan drie of meer van die afwykings wat die metabooliese sindroom kenmerk, lei. Dit is dus duidelik dat die metabooliese sindroom 'n gesondheidsprobleem vir ontwikkelende sowel as ontwikkelde lande is. As gevolg hiervan, word hierdie sindroom geteiken vir dieetbehandelinge om die risiko vir KVS en tipe 2 diabetes te verlaag.

Epidemiologiese studies het oortuigend bewys dat daar 'n omgekeerde verwantskap tussen die inname van neutre en morbiditeit en mortaliteit as gevolg van koronêre hartsiekte (KHS) in verskillende bevolkingsgroepe bestaan. Neutrinname beskerm nie net teen hartsiektes nie, maar kan ook tot langer lewensverwagting bydra. Die voordelige effekte van neutrinname word deur die "U.S. Food and Drug Administration" (FDA) erken deurdat hulle onlangs 'n kwalitatiewe gesondheidsaanspraak vir die inname van neutre (1.5 onse/dag = 42.8 g/dag) en KHS goedgekeur het. Die mees bestudeerde meganisme in die verband is die hipokolesterolemiese effek van neutre. Daar bestaan egter nog 'n tekort in die literatuur aangaande die effekte van neutre op die metabooliese sindroom.

Doelwitte: Die hoofdoelwit van die studie was om die uitwerking van 'n hoë okkerneut- en 'n hoë ongesoute kasjoeneutdieet op merkers van die metabooliese sindroom in mense te ondersoek. 'n Sekondêre doelwit was om 'n sistematiese oorsig oor die effek van neutre op die lipiedprofiel uit te voer wat gedeeltelik as basis kan dien vir die interpretering van die resultate uit die eersgenoemde doelwit.

Metodes: Die hoofondersoek was 'n gekontroleerde voedingstudie met 'n parallelle ewekansig gekontroleerde studie-ontwerp op proefpersone wat die metabooliese sindroom onder lede gehad het. Vier-en-sestig proefpersone met hierdie sindroom (29 mans, 35 vrouens) met 'n gemiddelde ($\pm$ SA) ouderdom van 45 ± 10 jr en wat aan die insluitingskriteria

voldoen het, het vir 'n inloop-periode van drie weke lank die kontroledieet gevolg. Die proefpersone is daarna volgens geslag en ouderdom gegroepeer en ewekansig in drie groepe verdeel wat vir 8 weke een van die volgende diëte gevolg het: 'n gekontroleerde dieet wat óf okkerneute (20% van totale energie (E); 60-100 g/dag; proteïene:koolhidrate:vet = 18:42:40%E) óf ongesoute kasjoeneute (20%E; 66-115 g/dag; proteïene:koolhidrate:vet = 19:44:37%E) óf geen neute (proteïene:koolhidrate:vet = 20:47:33%E) ingesluit het. Die proefpersone se fisieke aktiwiteit en gewig het vir die duur van die studie konstant gebly.

Vir die sistematiese oorsig, is menslike intervensiestudies wat die onafhanklike effekte van neute op die lipiedkonsentrasies ondersoek het, ingesluit. *Medline* en *Web of Science* databasisse van die begin van die databasis tot Augustus 2004, is vir die literatuursoektog gebruik en aangevul deur die bronnelyste van relevante publikasies na te gaan. Hierdie artikels is gekeur volgens die metodologie soos dit in die publikasies voorgekom het. Geen formele statistiese analise was uitgevoer nie as gevolg van die groot verskille in studicontwerpe van die dieetintervensies. Die hoofuitkomste van die sistematiese oorsig was die persentasie verskille tussen die behandeling- en kontrolegroepe wat betref totale bloedcholesterol (TC), lae-digtheidslipoproteïencholesterol (LDL-C), hoë-digtheidslipoproteïencholesterol en triasielgliserol (TG).

Resultate: Met betrekking tot die hoofdoelwit, is bevind dat beide die okkerneut- en ongesoute kasjoeneutdieet geen effek op die lipiedprofiel, serumfruktosamien, -insulien, insuliensensitiwiteit, insulienweerstand, serum-hoë-sensitiwiteit-C-reaktieweproteïen, bloeddruk en serumuriensuurkonsentrasies gehad het nie in vergelyking met die kontroledieet. Al drie groepe het hoogsbetekenisvolle toenames in seruminsulienkonsentrasies, insulienweerstand en verlaging in insuliensensitiwiteit, van basislyn na end, getoon ($P < 0.05$). Die plasmaglukosekonsentrasies het betekenisvol verhoog in die kasjoeneutgroep in vergelyking met die kontrole groep. In teenstelling hiermee, het die serumfruktosamien, 'n korttermynmerker vir glukemiese beheer, in die kasjoeneutgroep onveranderd gebly terwyl dit in die kontrolegroep betekenisvol verhoog het van basislyn na end.

Die literatuursoektog vir die sistematiese oorsig het 415 publikasies opgelewer. Na 'n deeglike siftingsproses, was slegs 23 studies geskik om in die oorsig in te sluit. Die meerderheid van die neutdiëte het aan die riglyne vir die voorkoming van KHS voldoen. Die meeste studies was kort (4-6 weke) met net een studie wat 6 maande geduur het. Die proefpersoongetalle in meeste van die studies was voldoende om die effek op TC en LDL-C te ondersoek, maar nie in die geval van HDL-C en TG nie. Die resultate van drie amandel- (50-100 g/dag), twee grondboontjie- (36-68 g/dag), een pekanneut- (72 g/dag) en vier okkerneut- (40-84 g/dag)

studies het oortuigende bewyse gelewer vir 'n lipiedverlagende effek tussen 2-16% vir TC en 2-19% vir LDL-C, in vergelyking met die kontrolegroep. Huidiglik is daar aanduidings, uit studies met onvoldoende studie-ontwerpe, dat hazelneute (1 g/dag/kg liggaamsgewig) en pimperneute (20%E) ook moontlik 'n hipocholesterolemiese effek kan hê. Die bewyse vir 'n hipocholesterolemiese effek deur macadamianeute is tans onoortuigend. Dit is verder duidelik dat die verskillende komponente in neute die TC en LDL-C konsentrasies tot 'n groter mate verlaag as wat deur die dieetvetsuurprofiel voorspel word.

Gevolgtrekkings: Die gekontroleerde voedingsintervensie met okkerneute en kasjoeneute het geen verbetering in terme van die merkers van die metaboliese sindroom teweeggebring in vergelyking met 'n kontroledieet terwyl liggaamsgewig vir 8 weke gehandhaaf is nie. Ons vermoed dat die drastiese toename in insulienweerstand die voordelige effekte van die okkerneut- en kasjoeneutdiëte kon verberg het. Verdere navorsing is nodig om konsensus oor hierdie saak te verkry.

Die sistematiese oorsig het bewys dat die inname van ≈ 50 -100 g (≈ 1.5 -3.5 porsies) neute vyf of meer keer per week as deel van 'n KHS-voorkomingsdieet met 'n totale vetinhoud (hoog in mono- en/of polionversadigde vetsure) van ≈ 35 %E, 'n beduidende effek op die verlaging van TC en LDL-C konsentrasies in normo- en hiperlipidemiese persone kan hê.

Aanbevelings: 'n Soortgelyke gekontroleerde neutintervensiestudie tesame met 'n mate van energiebeperking moet uitgevoer word op proefpersone met die metaboliese sindroom. Verdere navorsing behoort ewekansig gekontroleerde voedingintervensiestudies met groter proefpersoongetalle en langer duur in te sluit om die effekte van neute op HDL-C en TG te ondersoek. Die effekte van gemengde neute en neute wat nie voorheen bestudeer is nie op die lipiedprofiel is ook noodsaaklik. Die unieke nutriënt-en nie-nutriëntsamestelling van neute as moontlike meganismes verantwoordelik vir die verlaging van LDL-C, behoort ook verder ondersoek te word.

Table of Contents	Page
Dedication	i
Acknowledgements	ii
Abstract	iv
Opsomming	vii
Table of Contents	x
Abbreviations	xii
 Chapter 1: Introduction	 1
1 Background and motivation	2
1.1 Definition, pathogenesis and diagnosis of the metabolic syndrome	2
1.2 Prevalence	3
1.3 Treatment	3
1.4 Nuts	6
2 The problem	8
3 Aims, goals and objectives of the study	9
3.1 Aims	9
3.1 Goals and objectives	9
3.2 Secondary objective	9
4 Hypothesis	9
4.1 Hypothesis developed for the study	9
4.2 Overall design of the study	10
4.3 Approach to test hypothesis	10
5 Structure of thesis	10
6 Co-authors and co-workers	11
7 References	13
 Chapter 2: Dietary intervention trials investigating the effects of nuts on the lipid profiles: A systematic review	 21
Abstract	21
Introduction	22
Subjects and methods	22
Results	24
Discussion	26
Conclusions	30
Recommendations for future research	31

Acknowledgements	31
References	31
 Chapter 3: The effects of a high walnut and high cashew nut diet on markers of the metabolic syndrome: A controlled feeding trial	 46
Abstract	46
Introduction	47
Subject and methods	48
Results	52
Discussion	53
Acknowledgements	57
References	57
 Chapter 4: Discussion and conclusions	 69
1 Introduction	70
2 Strengths of the study	70
3 Limitations and problems experienced in the study	71
4 Summary of main findings	72
5 Conclusions	72
6 Recommendations	73
7 References	74
 Addendum	 75
Example of the Nut Study calendar	76
The Food Frequency Questionnaire (FFQ)	77
The Physical Activity Questionnaire (PAQ)	81
Clinical assessment form for subjects	84
Example of “Additional Points” system	85
Illness diary	89
Example of the 14 day menu cycle	90
Example of the newsletter –The Nutcracker	94
Information for Authors	98

List of abbreviations

Abbreviation	Description
Δ	change in
$\Uparrow$	increase in
$\Downarrow$	decrease in
$\square$	no change in
% E	percentage energy
AACE	American Association of Clinical Endocrinologists
AAD	average American diet
ALA	α -linolenic acid
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ATP III	National Cholesterol Education Program's Adult Treatment Panel III
B	baseline
BMI	body mass index
BMR	basal metabolic rate
BP	blood pressure
CHD	coronary heart disease
CHO	carbohydrate
CI	confidence interval
CRP	c-reactive protein
Cu	copper
CV	coefficient of variance
CVD	cardiovascular disease
DBP	diastolic blood pressure
E	end
EI	energy intake
FDA	Food and Drug Administration
FFQ	Food Frequency Questionnaire
GI	glycemic index
GL	glycemic load
HDL-C	high density-lipoprotein cholesterol
HOMA	homeostasis model assessment
IFG	impaired fasting glucose
IR	insulin resistance
IVGTT	intravenous glucose tolerance test
K	potassium
LA	linoleic acid
LDL-C	low density-lipoprotein cholesterol
LOG	logarithm
M	men
Mg	magnesium
MUFA	monounsaturated fatty acid
N	number
n-3	omega-3
n-6	omega-6
NO	nitric oxide
NR	not reported
NS	non-significant
OGTT	oral glucose tolerance test
P	phosphorous
PAI-1	plasminogen activator inhibitor 1
PAQ	physical activity questionnaire
PUFA	polyunsaturated fatty acid
QUICKI	quantitative insulin sensitivity check index
SBP	systolic blood pressure
Se	selenium
SFA	saturated fatty acid
S-hs CRP	serum high sensitivity C-reactive protein
Step I	American Heart Association / National Cholesterol Education Program Step I – 30% energy from fat
Step II	American Heart Association / National Cholesterol Education Program Step II – 25% energy from fat
t	time
TC	total cholesterol
TG	triacylglycerol
Vit. E	vitamin E
W	women
WC	waist circumference
WHO	World Health Organization
Zn	zinc

1

Introduction

1 Background and motivation

1.1 Definition, pathogenesis and diagnosis of the metabolic syndrome

Metabolic syndrome is a term linking the clinical profiles of some of the world's major health problems today, viz., obesity, heart disease, and diabetes. In particular, this syndrome is characterized by a group of metabolic risk factors for cardiovascular disease (CVD) that includes android obesity, atherogenic dyslipidemia (high triacylglycerols (TG), decreased high density-lipoprotein cholesterol (HDL-C), high apolipoprotein B and small low density-lipoprotein cholesterol (LDL-C) particles), elevated blood pressure (BP), insulin resistance (IR), glucose intolerance or non-insulin dependant diabetes mellitus (NIDDM), prothrombotic state (high fibrinogen and plasminogen activator inhibitor 1 (PAI-1)) and proinflammatory state (elevated C-reactive protein (CRP)) (Scott 2003; Roman & Hancu, 2004). In addition, studies with simple linear regression analysis revealed that serum uric acid concentration was inversely correlated with insulin sensitivity (rate of glucose utilization) (Vuorinen-Markkola & Yki-Jarvinen, 1994; Temelkova-Kurktschiev *et al.*, 2002). Not only is CVD the primary clinical outcome of metabolic syndrome (Isomaa *et al.*, 2001) but the risk for type 2 diabetes is also higher. Diabetes is a major risk factor for CVD (Grundy *et al.*, 2004).

The pathogenesis of this syndrome appears to have 3 potential etiological categories: obesity and disorders of adipose tissue; insulin resistance and a constellation of independent factors (eg, molecules of hepatic, vascular, and immunologic origin) that mediate specific components of the metabolic syndrome (Grundy *et al.*, 2004; Wolever, 2000). Contributing factors includes aging, proinflammatory state and hormonal changes (Grundy *et al.*, 2004).

There are no universally accepted criteria for diagnosing the metabolic syndrome. However, the criteria proposed by the National Cholesterol Education Program-Adult Treatment Panel III (ATP III) are the most current and widely used (Grundy *et al.*, 2004). This criteria provides a practical tool to diagnose patients having the metabolic syndrome by identifying 5 diagnostic traits, that include:- abdominal obesity, waist circumference > 88 cm for women or > 102 cm for men; high fasting TG, ≥ 1.7 mmol/L; low HDL-C, ≤ 1.0 mmol/L for men, ≤ 1.3 mmol/L for women; high BP, $\geq 130/85$ mmHg and high fasting glucose, ≥ 6.1 mmol/L (Ford *et al.*, 2002). The ATP III report considers the presence of any 3 of these factors sufficient for diagnosis. The World Health Organization (WHO) and American Association of Clinical Endocrinologists (AACE) criteria require further oral glucose testing if impaired fasting glucose (IFG) and diabetes are absent. Impaired glucose tolerance (IGT) on oral glucose tolerance test (OGTT) denotes greater-risk for diabetes than does metabolic syndrome without elevated fasting glucose (Roman & Hancu, 2004).

1.2 Prevalence

The NHANES III study showed the prevalence of metabolic syndrome to be 24.0% in men and 23.4% in women in the USA (Ford, 2004). These authors found that the prevalence of metabolic syndrome increased from 6.7% among participants aged 20-29 years to 43.5% for participants aged 60-69 years. It is estimated that about 47 million U.S. adults have this syndrome (Ford, 2004). In the THUSA (acronym for Transition and Health in the Urbanization of South Africans) study in South Africa it was found that 12% of men and 28.4% of women of the black population of the North West Province had 3 or more metabolic disturbances characterizing the metabolic syndrome. The THUSA study showed that the metabolic syndrome occurred in undernourished men (body mass index (BMI) $<18.5\text{kg/m}^2$) and overnourished men and women (BMI $>30\text{ kg/m}^2$) (Kruger A *et al.*, 2004). Therefore, it is evident that the metabolic syndrome is a health problem not only for developed but also for developing countries.

1.3 Treatment

Underlying causes of the metabolic syndrome include overweight/obesity, physical inactivity and genetic factors. While insulin resistance may be a unifying factor in this syndrome, obesity is also central. Obesity increases the tendency for clustering and is associated with increased incidence of hypertension, diabetes, and dyslipidemia (Wilson *et al.*, 1999). Therefore, the treatment of this syndrome is a multifactorial process that includes diet, exercise, and pharmacological therapy (Scott, 2003). Despite, the role of diet in the etiology of the metabolic syndrome being poorly understood, ATP III has identified this syndrome as a target for vigorous lifestyle intervention. In particular, dietary intervention has been targeted in order to reduce the risk of CVD and development of diabetes mellitus (Pan *et al.*, 1997; Isomaa *et al.*, 2001).

Table 1 Summary of the effects of diet/lifestyle on markers of the metabolic syndrome (adapted from Grundy *et al.*, 2004; Riccardi & Rivellese, 2000; Nestel, 2004; Tapsell, 2004).

Dietary /lifestyle factors	Markers of the metabolic syndrome				
	TG	HDL-C	BP	IR	Glucose
Decreased energy intake resulting in weight loss (stabilized)	↓	↑	↓	↓	↓
Increased energy expenditure (increased physical activity)	↓	↑	↓	↓	↓
SFA	—	↑	↑	↑	↑ ?
Trans fatty acids	↑	↓	↑	↑ ?	↑ ?
MUFA	↓	— / ↑	↓	↓	↓ ?
n-3 PUFA	↓	—	↓	↓	?
n-6 PUFA	↓	↓	↓	↓	?
CHO - high GI	↑	↓	?	↑	↑
CHO - low GI	↓	—	?	↓	↓
CHO - fibre	— / ↓	—	↓ ?	↓	↓
Salt	?	?	↑	↑	— ?
Alcohol	↑	↑	↑	↓	↓ ?
Antioxidants	↓ ?	?	↓ ?	↓	— ?

Abbreviations: SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids;

CHO: carbohydrate; GI: glycemic index.

↓ decrease

↑ increase

— no change

? uncertainties, inconsistencies and/or a lack of data in the literature

From Table 1 it is apparent that dietary or lifestyle factors play an important role in markers of the metabolic syndrome. However, some uncertainties, inconsistencies and a lack of data in the literature regarding the effect of most dietary factors on this syndrome still exist. One of the most common and potent causes of insulin resistance is obesity (Riccardi *et al.*, 2004) with weight loss improving glucose tolerance, decreasing TG concentrations, and lowering BP (Wilson *et al.*, 1999; Abbasi *et al.*, 2002). All 5 components of the metabolic syndrome are improved by even modest amounts of weight loss achieved with diet and exercise (Avenell *et al.*, 2004). Ultimately, the first-line of therapy should be weight reduction reinforced with increased physical activity.

In a recent study, it was concluded that reducing saturated fat intake will lower insulin resistance in individuals having the metabolic syndrome (Deen, 2004). Furthermore, reduction of saturated fat and trans fatty acids is beneficial for lowering LDL-C (Stamler & Shekelle, 1988; Mensink *et al.*, 2003). In the KANWU Study, (Vessby *et al.*, 2001) replacement of saturated fat with monounsaturated fat was associated with improvement in insulin sensitivity, which in turn could translate into reduced risk of developing type 2 diabetes since resistance to the action of insulin is the underlying abnormality in most cases of type 2 diabetes (Mann, 2001). Studies have shown that a higher intake of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) improves insulin sensitivity (Vessby *et al.*, 2001; Houtsmuller *et al.*, 1980; Heine *et al.*, 1989). Emerging research has suggested possible health benefits associated with modest increases in dietary α -linolenic acid (ALA) (PUFA), including reduced BP (Hunter, 1990; Ferrara *et al.*, 2000; Hermansen, 2000). Numerous studies conducted in healthy and hypertensive individuals have shown a beneficial effect of MUFA on a number of outcomes related to cardiovascular risk, including BP (Roche *et al.*, 1998). Zhao and colleagues (Zhao *et al.*, 2004), concluded that a diet high in ALA, obtained from walnuts, walnut oil and flaxseed oil, elicited cardioprotective effects and vascular anti-inflammatory effects. Recently, some evidence has been presented for a beneficial effect of MUFA on a number of outcomes related to cardiovascular risk, including reduced inflammation (Hunter, 1990; Ferrara *et al.*, 2000).

Pereira and collaborators have suggested that diets with a lower GI and glycemic load (GL) are associated with lower insulin resistance as determined using the homeostasis model assessment index (HOMA) method (Pereira *et al.*, 1998). Consequently, participants with the highest GI intakes were 40% more likely to have the metabolic syndrome than were participants with the lowest dietary GI (Perez-Jimenez *et al.*, 2001). Deen and investigators (Deen, 2004) recently showed that reducing high glycemic-index (GI) carbohydrate (CHO)

intake lowered TG concentrations and reducing sodium intake lowered BP. Soluble fibre has shown to reduce total- and LDL-C concentrations and improve glycemic control (Mann, 2001; Mann, 2002).

According to what is outlined above, a diet for individuals having the metabolic syndrome should have the following requirements. This includes calorie restriction (if overweight), reduced saturated fat, and increased high fibre/low-GI foods (e.g. vegetables, legumes, whole grains, low-fat dairy products) should be used without specific limitations. The dietary guidelines for the metabolic syndrome includes percentage energy (%E) from protein:CHO:fat=15:45-55:30-40%. In addition the diet should have a low salt content (<4g/day) and limited alcohol intake (<30g/day) (Riccardi & Rivellese, 2000; Mann, 2002; Deen, 2004; Mann, 2001; van Dam *et al.*, 2000). Furthermore, moderate amounts of monounsaturated fat could be permitted, as they do not induce detrimental metabolic effects (Riccardi & Rivellese, 2000). If the diet has these characteristics, it may not be necessary to drastically reduce the total amount of fat, as advocated in the past in order to provide CVD protection. Therefore, it is clear that within certain limits (not more than 40% E from fat), the quality rather than the total amount of fat really matters. In essence, provided that saturated fat is reduced and most of the CHO-rich foods have a high fibre/low-GI, some flexibility might be allowed in the amount of fat and CHO, e.g. exchanging about 10% energy between monounsaturated fat and CHO-rich foods with high GI (Riccardi & Rivellese, 2000).

Ultimately, the proper management of the individual abnormalities of this syndrome can reduce morbidity and mortality (Isomaa *et al.*, 2001).

1.4 Nuts

Recently, it has been confirmed that the inclusion of nuts in the diet may result in a variety of changes in the dietary composition (Jaceldo-Siegl *et al.*, 2004). In this context, nuts may change the dietary profile by decreasing the SFA, trans fatty acids, GI and increasing the MUFA, PUFA, fibre and antioxidant concentrations (Table 1).

The health benefits of nuts are apparent in numerous epidemiological studies. In particular, they have consistently demonstrated an association of nut consumption on CHD morbidity and mortality in different population groups (Sabate *et al.*, 1999; Kris-Etherton *et al.*, 2001). The Adventist Health Study reported that men and women who consumed nuts 5 times/wk lowered their risk of heart disease by 50% and increased their life expectancy by several years compared with those who hardly ever ate nuts (Fraser, 1999a; Fraser *et al.*, 1992).

Nuts have a low GI and they are a rich source of protein, unsaturated fatty acids, vitamin E (which may act as an antioxidant), B₆, folic acid, niacin, fibre, magnesium, potassium, arginine, phytosterols and other phytochemical compounds (such as flavonoids, phenolic components and ellagic acid). Most nuts are rich in MUFA (oleic acid), however, walnuts are unique in that they are rich in PUFA (linoleic acid (LA) and ALA) (Dreher *et al.*, 1996). The cardio-protective effects of nuts may be mediated through several mechanisms. These include, a decrease in TC and LDL-C as well as the beneficial effects of the bioactive components in nuts (e.g. fibre, vit E, arginine, phytosterols, antioxidants, magnesium).

Firstly, in a recent systematic review (Mukuddem-Petersen *et al.*, 2005) (Chapter 2) we found that randomized, controlled intervention trials (3 almond, 2 peanut, 1 pecan nut and 4 walnut studies) showed convincing evidence for a lipid lowering effect of TC between 2-16% and LDL cholesterol between 2-19%, when compared to their control diets. The results for macadamia nuts (50-100g/day) are less convincing. We concluded from this systematic review that consumption $\approx$ 50-100g ($\approx$ 1.5-3.5 *servings*) of nuts 5 or more times/week as part of a heart-healthy diet with total fat content of $\approx$ 35% of energy may significantly decrease TC and LDL-C in normo-and hyperlipidemic individuals. Secondly, unsaturated fatty acids and fibre may improve plasma lipids (by decreasing TG and cholesterol concentrations), decrease platelet aggregation and prevent arrhythmias (Alper & Mattes, 2003; Elin & Hosseini, 1993). Brown and colleagues conducted a meta-analysis (Brown *et al.*, 1999) of 67 controlled trials to quantify the cholesterol-lowering effect of major dietary fibres. They concluded that soluble fibre of 2-10g/day was associated with small but significant decreases in TC. Arginine, the second most abundant amino acid found in nut proteins, may account for the hypocholesterolemic effect observed in animal studies (Kurowska & Carroll, 1994). In addition, this effect has been seen in human intervention trials when the dosage of arginine in the diet appeared to be very high (e.g one study had 6-21g/day of arginine) (Maxwell *et al.*, 2000). Phytosterols and other phytochemical compounds such as flavonoids, phenolic components and ellagic acid (Anderson *et al.*, 2001) may have serum cholesterol modulating effects (Law M, 2000; Vorster *et al.*, 2003). In particular, well-designed studies have shown that 2-2.2g of plant sterols significantly reduces cholesterol absorption which in turn decreases plasma and LDL-C concentrations (Law, 2000; Richelle *et al.*, 2004; Law, 2000). A study by Anderson and collaborators demonstrated that walnut polyphenolics are effective inhibitors of in vitro plasma and LDL oxidation and therefore adds to nuts antiatherogenic potential (Anderson *et al.*, 2001). It is apparent, that fibre, antioxidants (vitamin E), arginine, phytosterols and other phytochemical compounds may have an important cholesterol lowering

effect. However, from practical intakes of nuts it is not sufficient to exert an individual hypocholesterolemic effect (Lampe, 1999). Therefore, it is possible that there are multiple small effects that add up, and these are mediated by more than cholesterol levels (Lampe, 1999).

Some studies have investigated the effects of nuts on diabetes mellitus (or on markers of this disease e.g. glucose, insulin resistance), blood pressure and inflammation. In this context, data from the Nurses' Health Study (a large prospective cohort study of women, combined with other clinical and epidemiological data) supports a nut diets' potential for reducing type II diabetes risk (Jiang *et al.*, 2002). In particular, components of nuts, such as fibre and magnesium, together with the low GI of nuts may decrease insulin demand and resistance and have been inversely associated with the risk of type II diabetes (Resnick, 1993; Salmeron *et al.*, 1997). Also, cations, such as magnesium and potassium, may improve BP and arginine by promoting the production of nitric oxide (NO) (Fraser, 1999b), a potent vasodilator that can inhibit platelet adhesion and aggregation. Also, evidence suggests a beneficial effect of MUFA (nuts are high in MUFA) on a number of outcomes related to cardiovascular risk, including reduced inflammation (Hunter, 1990). In turn, a decrease in inflammation may decrease insulin resistance (Temelkova-Kurktschiev *et al.*, 2002).

When all of these beneficial effects of nuts are taken together it is comprehensible, although not scientifically proven, that a cholesterol lowering diet including nuts may facilitate the prevention or treatment of the metabolic syndrome.

2 The problem

Based on the results of the THUSA study (Kruger *et al.*, 2004) it is clear that the metabolic syndrome is likely to be an important health issue in South Africa as the prevalence is similar to that found in developed countries (Ford, 2004). Recently, the benefits of nuts were acknowledged by the U.S. Food and Drug Administration (FDA) when they approved a qualified health claim that eating nuts (1.5 ounces/day $\approx$ 42.8 g/day) may reduce the risk of CHD (Brown, 2003).

It was evident from the literature that nuts may reduce cardiovascular risk. However, data on the effects of nut diets on patients having the metabolic syndrome required further study. In response to this, the main project (The Nut study) was designed to probe this issue in South Africa. Studies to date focused mainly on the effects of nuts on lipids. Therefore, as background information for the main study, the effects of nuts on lipids are discussed in the systematic review in Chapter 2.

The nuts chosen for the main study were walnuts and unsalted cashew nuts. Walnuts are rich in PUFAs (47.2g of PUFAs/100g of walnuts) and they are unique because they are rich sources of both n-6 and n-3 PUFAs, with a n-6:n-3 ratio of 4 (US Department of Agriculture, 2003). The inclusion of cashew nuts was motivated by the fact that no clinical studies have been done using this type of nut before. Unlike walnuts, cashew nuts have a higher concentration of MUFAs (27.3g of MUFAs/100g of cashew nuts) (US Department of Agriculture, 2003) by comparison to the composition of most other nuts. Finally, cashew and walnut crops are cultivated in South Africa with the result that studies done on these nuts may stimulate interest on a broad front (viz., consumers, nut industry, farmers, nut crackers industry and health professionals) which in turn may impact the local economy.

3 Aims, goals and objectives of the study

3.1 Aims

The aim of this study was to examine for the first time the effect of a high walnut diet and a high-unsalted cashew nut diet on markers of the metabolic syndrome in humans (Chapter 3).

3.2 Goals and objectives

To investigate the effects of a high walnut and a high unsalted cashew nut diet on:

- ❑ Markers of CHO metabolism: serum insulin, plasma glucose, OGTT, insulin sensitivity (quantitative insulin sensitivity check index (QUICKI)), insulin resistance (HOMA) and serum fructosamine
- ❑ Serum lipids: TC, TG, HDL-C and LDL-C
- ❑ BP
- ❑ Serum uric acid
- ❑ Serum high sensitivity C-reactive protein (S-hs CRP).

3.3 Secondary objective

To conduct a systematic review that investigates the effects of nuts on the lipid profile (Chapter 2).

4 Hypothesis

4.1 Hypothesis developed for the study

A high walnut and unsalted cashew nut diet will improve the markers of the metabolic syndrome compared to the control diet. In this regard, the markers, viz., insulin, glucose, oral glucose tolerance, insulin sensitivity, serum fructosamine, serum TG, serum HDL, serum LDL-C, BP, serum uric acid and S-hs CRP will improve.

4.2 Overall design of the study

This project was a parallel, randomized, controlled feeding trial on patients having the metabolic syndrome that included a control group (Chapter 3). In this regard, because of a large study population in relation to a small metabolic kitchen, the study was done in 3 cohorts over a one year period. It was preceded by a pilot study (n=9) aimed at testing the controlled feeding protocols and eradicating any unanticipated problems.

4.3 Approach to test hypothesis

A multidisciplinary team of scientists participated in this project. It was, however, planned, managed and controlled by the author of this thesis in collaboration with 2 study leaders. To test the hypothesis of the project the following team was recruited: nutritionists, Ph.D student in Dietetics, a general practitioner, dietitians, clinical sisters, pharmacists, biochemists, physiologists, a home economist, a research assistant and 4th year dietetic students at the Potchefstroom Campus of the North-West University (NWU-PC).

5 Structure of thesis

This thesis is constituted by chapters written specifically to comply with the requirements of the NWU-PC and journals to which manuscripts were submitted for possible publication. In particular, directives in terms of the English language usage, formatting and bibliography styles were strictly adhered to. All chapters and manuscripts will have their own reference index.

The current chapter is introductory in nature and presents the background and motivation of the study, as well as its objectives, goals and hypothesis. Chapter 2 is a systematic review about dietary intervention trials investigating the effects of nuts on lipid profiles. The said chapter provides an overview of the effects of nuts on lipids and sketches the background necessary for the interpretation of some of the data in the main study (Chapter 3). Also, this chapter highlighted the role of nuts in healthy or diseased (hypercholesterolemic, hyperlipidemic, type 2 diabetic, polygenic hypercholesterolemic) subjects. In Chapter 3, we investigate the effects of walnuts and unsalted cashew nuts on markers of the metabolic

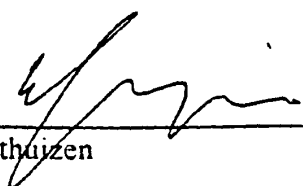
syndrome. As far as we know, this was the first controlled feeding trial of its kind in South Africa. Lastly, Chapter 4 integrates the results of Chapters 2 and 3 in a general discussion and conclusion. Recommendations regarding further research and practical applications are made. Attached as addendums are the *Instructions to the Authors* concerning the requirements of the specific journal for the 2 manuscripts (Chapter 2 and 3) as well as examples of forms, questionnaires and a newsletter (the Nutcracker) used in the main study.

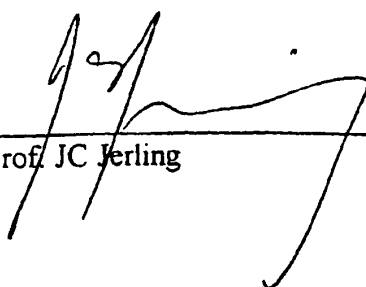
6 Co-authors and co-workers

The principal author of this thesis is Ms J Mukuddem-Petersen. In Table 2 contributions of the co-authors and co-workers are summarized.

The following is a statement from the co-authors confirming their role in the study and giving their permission that the manuscripts may form part of this thesis.

I declare that I have approved the above mentioned manuscripts, that my role in the study, as indicated, is representative of my actual contribution and that I hereby give my consent that it may be published as part of the Ph.D thesis of Janine Mukuddem-Petersen.


Prof. W Oosthuizen


Prof. JC Jerling


Dr. SM Hanekom

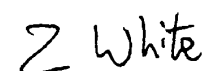

Ms Z White

Table 2 Co-authors and co-workers and their contributions

Chapter	Co-author	Co-worker	Contribution
Chapter 2	J. Mukuddem-Petersen (Dietitian)	-	Responsible for literature searches, designing, planning, execution and documentation of the study
	Prof. W. Oosthuizen (Nutritionist)	-	Promotor, assisting in all aspects: designing, planning, execution and documentation of the study.
	Prof. J.C. Jerling (Nutritionist)	-	Co-promotor, assisting in designing, planning, execution and documentation of the study.
Chapter 3	J. Mukuddem-Petersen (Dietitian)	-	Responsible for literature searches, designing, planning, execution, statistical analysis and documentation of the study
	Prof. W. Oosthuizen (Nutritionist)	-	Promotor, assisting in all aspects: designing, planning, execution, statistical analysis and documentation of the study.
	Prof. J.C. Jerling (Nutritionist)	-	Co-promotor, assisting in designing, planning, execution, statistical analysis and documentation of the study.
	Dr. S.M. Hanekom (Dietitian)	-	Assisting with the dietary histories (FFQ), designing of menu plan & supervision of catering team
	Z. White (Nutritionist)	-	Research assistant:- analysis of diet histories (FFQ), nutrient analyses, analyses of food diaries, biochemical analyses and assisting with the Nutcracker newsletter
	-	Sr. M.C. Lessing (registered general nurse)	Assisting with recruitment of subjects and collection of blood samples
	-	Dr. H. H. Vorster (Dietitian)	Assisting with dietary histories and designing of menu plan
	-	R. Breet (Dietitian) M Opperman (Dietitian)	Assisting with diet histories (FFQ) and the Nutcracker newsletter Assisting with diet histories (FFQ)
	-	Anthropometrist/Nutritionists A. Greyling C. de Witt	Assisting with anthropometric measurements, biochemical analyses and the Nutcracker newsletter Assisting with anthropometric measurements and the Nutcracker newsletter
	-	Prof. C.S. Venter (Dietitian)	Assisting with dietary histories (FFQ)
	-	Prof. H.S. Kruger (Dietitian & Pharmacist)	Assisting with planning, dietary histories (FFQ) & pharmacological advice
	-	E. Pienaar, J. Wheeler, J. Bekker, C. Jansen van Rensburg, M. Bailey, I. Loots, V. van Scheltinga, L. Wiggett, L. Davies, I. van Wyk, F. Snyman, F. Mpho and T. Holele,	Catering team
	-	A. Van Graan, M. van Lieshout, D. Loots, M. Pieters-Loots, K. Moruosi, M. Opperman and M. Phometsi	Assisting the catering team when necessary
	-	Physiologist Dr. A. Schutte Dr. H. Huisman Dr. J. van Rooyen S. Jordaan	Assisting with monitoring BP
	-	Dr. J.E. Kotze (General Practitioner)	The supervising medical doctor on stand-by
	-	Dr. H. Van't Riet (Nutritionist)	Giving advice concerning the design and planning of the study

7 References

- ABBASI, F., BROWN, B.W., JR., LAMENDOLA, C., MCLAUGHLIN, T. & REAVEN, G. M. 2002. Relationship between obesity, insulin resistance, and coronary heart disease risk. *Journal of the American College of Cardiology*, 40(5):937-943.
- ALPER, C.M. & MATTES, R.D. 2003. Peanut consumption improves indices of cardiovascular disease risk in healthy adults. *Journal of American College of Nutrition*, 22(2):133-141.
- ANDERSON, K.J., TEUBER, S.S., GOBEILLE, A., CREMIN, P., WATERHOUSE, A.L. & STEINBERG, F.M. 2001. Walnut polyphenolics inhibit in vitro human plasma and LDL oxidation. *Journal of nutrition*, 131(11):2837-2842.
- AVENELL, A., BROOM, J., BROWN, T.J., POOBALAN, A., AUCOTT, L., STEARNS, S. C., SMITH, W.C., JUNG, R.T. , CAMPBELL, M.K. & GRANT, A.M. 2004. Systematic review of the long-term effects and economic consequences of treatments for obesity and implications for health improvement. *Health technology assessment*, 8(21):iii-182.
- BROWN, D. 2003. FDA considers health claim for nuts. *Journal of American Dietetic Association*, 103(4):426.
- BROWN, L., ROSNER, B., WILLETT, W.W. & SACKS, F.M. 1999. Cholesterol-lowering effects of dietary fiber: a meta-analysis. *American journal of clinical nutrition*, 69(1):30-42.
- DEEN, D. 2004. Metabolic syndrome: time for action. *American family physician*, 69(12):2875-2882.
- DREHER, M.L., MAHER, C.V. & KEARNEY, P. 1996. The traditional and emerging role of nuts in healthful diets. *Nutrition reviews*, 54(8):241-245.
- ELIN, R.J. & HOSSEINI, J.M. 1993. Is the magnesium content of nuts a factor for coronary heart disease? *Archives of internal medicine*, 153(6):779-780.

FERRARA, L.A., RAIMONDI, A.S., D'EPISCOPO, L., GUIDA, L., DELLO, R.A. & MAROTTA, T. 2000. Olive oil and reduced need for antihypertensive medications. *Archives of internal medicine*, 160(6):837-842.

FORD, E.S. 2004. Prevalence of the metabolic syndrome in US populations. *Endocrinology and metabolism clinics of North America*, 33(2):333-350.

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 American Medical Association*, 287(3):356-359.

FRASER, G.E. 1999a. Associations between diet and cancer, ischemic heart disease, and all-cause mortality in non-Hispanic white California Seventh-day Adventists. *American journal of clinical nutrition*, 70(suppl. 3):532S-538S.

FRASER, G.E. 1999b. Nut consumption, lipids, and risk of a coronary event. *Clinical cardiology*, 22(suppl. 7):III11-III15.

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.

GRUNDY, S.M., BREWER, H.B., JR., CLEEMAN, J.I., SMITH, S.C., JR. & LENFANT, C. 2004. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation*, 109(3):433-438.

HEINE, R.J., MULDER, C., POPP-SNIJDERS, C., VAN DER, M.J. & VAN DER VEEN, E.A. 1989. Linoleic-acid-enriched diet: long-term effects on serum lipoprotein and apolipoprotein concentrations and insulin sensitivity in noninsulin-dependent diabetic patients. *American journal of clinical nutrition*, 49(3):448-456.

HERMANSEN, K. 2000. Diet, blood pressure and hypertension. *British journal of nutrition*, 83(suppl. 1):S113-S119.

- HOUTSMULLER, A.J., HAL-FERWERDA, J., ZAHN, K.J. & HENKES, H.E. 1980. Favourable influences of linoleic acid on the progression of diabetic micro- and macroangiopathy. *Nutrition and metabolism*, 24(suppl. 1):105-118.
- HUNTER, J.E. 1990. n-3 fatty acids from vegetable oils. *American journal of clinical nutrition*, 51(5):809-814.
- ISOMAA, B., ALMGREN, P., TUOMI, T., FORSEN, B., LAHTI, K., NISSEN, M., TASKINEN, M.R. & GROOP, L. 2001. Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes care*, 24(4):683-689.
- JACELDO-SIEGL, K., SABATE, J., RAJARAM, S. & FRASER, G.E. 2004. Long-term almond supplementation without advice on food replacement induces favourable nutrient modifications to the habitual diets of free-living individuals. *British journal of nutrition*, 92(3):533-540.
- JIANG, R., MANSON, J.E., STAMPFER, M.J., LIU, S., WILLETT, W.C. & HU, F.B. 2002. Nut and peanut butter consumption and risk of type 2 diabetes in women. *Journal of American Medical Association*, 288(20):2554-2560.
- KRIS-ETHERTON, P.M., ZHAO, G., BINKOSKI, A.E., COVAL, S.M. & ETHERTON, T.D. 2001. The effects of nuts on coronary heart disease risk. *Nutrition reviews*, 59(4):103-111.
- KRUGER, A., VORSTER, H.H., OOSTHUIZEN, W. & MARGETTS, B.M. 2004. The metabolic syndrome and insulin resistance in the black population of the North West Province of South Africa - the THUSA-study. *Submitted for publication to South African journal of cardiovascular disease*.
- KUROWSKA, E.M. & CARROLL, K.K. 1994. Hypercholesterolemic responses in rabbits to selected groups of dietary essential amino acids. *Journal of nutrition*, 124(3):364-370.
- LAMPE, J.W. 1999. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. *American journal of clinical nutrition*, 70(suppl. 3):475S-490S.

LAW, M. 2000. Plant sterol and stanol margarines and health. *British medical journal*, 320(7238):861-864.

MANN, J.I. 2001. Dietary fibre and diabetes revisited. *European journal of clinical nutrition*, 55(11):919-921.

MANN, J.I. 2002. Diet and risk of coronary heart disease and type 2 diabetes. *Lancet*, 360(9335):783-789.

MAXWELL, A.J., ANDERSON, B., ZAPIEN, M.P. & COOKE, J.P. 2000. Endothelial dysfunction in hypercholesterolemia is reversed by a nutritional product designed to enhance nitric oxide activity. *Cardiovascular drugs and therapy*, 14(3):309-316.

MENSINK, R.P., ZOCK, P.L., KESTER, A.D. & KATAN, M.B. 2003. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *American journal of clinical nutrition*, 77(5):1146-1155.

MUKUDDER-PETERSEN, J., OOSTHUIZEN, W. & JERLING, J.C. 2005. Dietary intervention trials investigating the effects of nuts on lipid profiles: A systematic review. *Submitted for publication to American journal of clinical nutrition*.

NESTEL, P. 2004. Nutritional aspects in the causation and management of the metabolic syndrome. *Endocrinology and metabolism clinics of North America*, 33(3):483-492.

PAN, X.R., LI, G.W., HU, Y.H., WANG, J.X., YANG, W.Y., AN, Z.X., HU, Z.X., LIN, J., XIAO, J.Z., CAO, H.B., LIU, P.A., JIANG, X.G., JIANG, Y.Y., WANG, J.P., ZHENG, H., ZHANG, H., BENNETT, P.H. & HOWARD, B.V. 1997. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. *Diabetes care*, 20(4):537-544.

PEREIRA, M.A., KRISKA, A.M., COLLINS, V.R., DOWSE, G.K., TUOMILEHTO, J., ALBERTI, K.G., GAREEBOO, H., HEMRAJ, F., PURRAN, A., FAREED, D., BRISSENETTE, G. & ZIMMET, P.Z. 1998. Occupational status and cardiovascular disease risk factors in the rapidly developing, high-risk population of Mauritius. *American journal of epidemiology*, 148(2):148-159.

PEREZ-JIMENEZ, F., LOPEZ-MIRANDA, J., PINILLOS, M.D., GOMEZ, P., PAZ-ROJAS, E., MONTILLA, P., MARIN, C., VELASCO, M.J., BLANCO-MOLINA, A., JIMENEZ PEREPEREZ, J.A. & ORDOVAS, J.M. 2001. A Mediterranean and a high-carbohydrate diet improve glucose metabolism in healthy young persons. *Diabetologia*, 44(11):2038-2043.

RESNICK, L. M. 1993. Ionic basis of hypertension, insulin resistance, vascular disease, and related disorders. The mechanism of "Syndrome X". *American journal of hypertension*, 6(4):123S-134S.

RICCARDI, G., GIACCO, R. & RIVELLESE, A.A. 2004. Dietary fat, insulin sensitivity and the metabolic syndrome. *Clinical nutrition*, 23(4):447-456.

RICCARDI, G. & RIVELLESE, A.A. 2000. Dietary treatment of the metabolic syndrome--the optimal diet. *British journal of nutrition*, 83(suppl. 1):S143-S148.

RICHELLE, M., ENSLEN, M., HAGER, C., GROUX, M., TAVAZZI, I., GODIN, J.P., BERGER, A., METAIRON, S., QUAILE, S., PIGUET-WELSCH, C., SAGALOWICZ, L., GREEN, H. & FAY, L.B. 2004. Both free and esterified plant sterols reduce cholesterol absorption and the bioavailability of beta-carotene and alpha-tocopherol in normocholesterolemic humans. *American journal of clinical nutrition*, 80(1):171-177.

ROCHE, H.M., ZAMPELAS, A., KNAPPER, J.M., WEBB, D., BROOKS, C., JACKSON, K.G., WRIGHT, J.W., GOULD, B.J., KAFATOS, A., GIBNEY, M.J. & WILLIAMS, C.M. 1998. Effect of long-term olive oil dietary intervention on postprandial triacylglycerol and factor VII metabolism. *American journal of clinical nutrition*, 68(3):552-560.

ROMAN, G. & HANCU, N. 2004. Metabolic syndrome - new insights into a growing entity. *Romanian journal of internal medicine*, 42(2):257-266.

SABATE, J., FRASER, G.E., BENNETT, H.W., JACELDO, K.B. & SABATE, J. 1999. Nut consumption, vegetarian diets, ischemic heart disease risk, and all-cause mortality: evidence from epidemiologic studies. *American journal of clinical nutrition*, 70(suppl. 3):500S-503S.

SALMERON, J., MANSON, J.E., STAMPFER, M.J., COLDITZ, G.A., WING, A.L. & WILLETT, W.C. 1997. Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. *Journal of American Medical Association*, 277(6):472-477.

SCOTT, C.L. 2003. Diagnosis, prevention, and intervention for the metabolic syndrome. *American journal of cardiology*, 92(1A):35i-42i.

STAMLER, J. & SHEKELLE, R. 1988. Dietary cholesterol and human coronary heart disease. The epidemiologic evidence. *Archives of pathology and laboratory medicine*, 112(10):1032-1040.

TAPSELL, L.C. 2004. Diet and metabolic syndrome: where does resistant starch fit in? *The journal of AOAC international*, 87(3):756-760.

TEMELKOVA-KURKTSCHIEV, T., SIEGERT, G., BERGMANN, S., HENKEL, E., KOEHLER, C., JAROSS, W. & HANEFELD, M. 2002. Subclinical inflammation is strongly related to insulin resistance but not to impaired insulin secretion in a high risk population for diabetes. *Metabolism*, 51(6):743-749.

US Department of Agriculture, A.R.S. 2003. Nutrient Database for Standard Reference Release 15 Nutrient Data. [Web:] <http://www.nal.usda.gov/fnic/foodcomp> [Date of access: 15 August 2004].

VAN DAM, R.M., VISSCHER, A.W., FESKENS, E.J., VERHOEF, P. & KROMHOUT, D. 2000. Dietary glycemic index in relation to metabolic risk factors and incidence of coronary heart disease: the Zutphen Elderly Study. *European journal of clinical nutrition*, 54(9):726-731.

VESSBY, B., UNSITUPA, M., HERMANSEN, K., RICCARDI, G., RIVELLESE, A.A., TAPSELL, L.C., NALSEN, C., BERGLUND, L., LOUHERANTA, A., RASMUSSEN, B.M., CALVERT, G.D., MAFFETONE, A., PEDERSEN, E., GUSTAFSSON, I.B. & STORLIEN, L.H. 2001. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. *Diabetologia*, 44(3):312-319.

VORSTER, H.H., RAAL, F.J., UBBINK, J.B., MARAIS, A.D. & RAJPUT, M.C. 2003. Phytosterols - a new dietary aid for the treatment of hypercholesterolaemia. *South African medical journal*, 93(8):581-582.

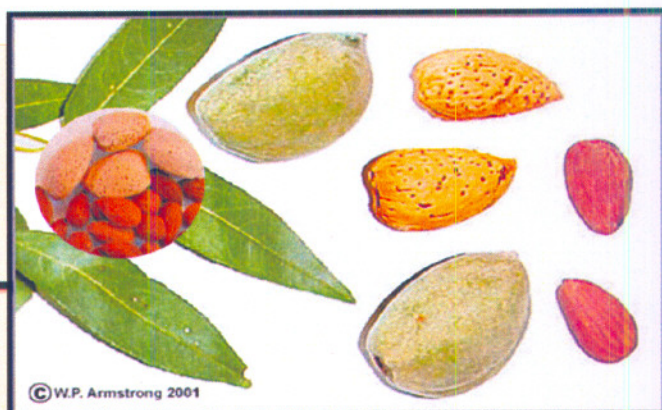
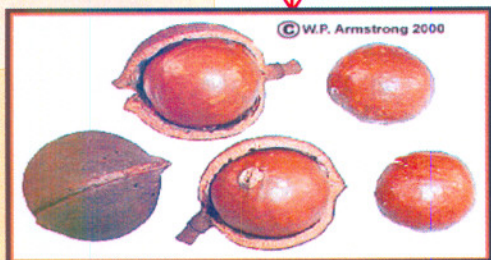
VUORINEN-MARKKOLA, H. & YKI-JARVINEN, H. 1994. Hyperuricemia and insulin resistance. *Journal of clinical endocrinology and metabolism*, 78(1):25-29.

WILSON, P.W., KANNEL, W.B., SILBERSHATZ, H. & D'AGOSTINO, R.B. 1999. Clustering of metabolic factors and coronary heart disease. *Archives of internal medicine*, 159(10):1104-1109.

WOLEVER, T.M. 2000. Dietary carbohydrates and insulin action in humans. *British journal of nutrition*, 83(suppl. 1):S97-102.

ZHAO, G., ETHERTON, T.D., MARTIN, K.R., WEST, S.G., GILLIES, P.J. & KRIS-ETHERTON, P.M. 2004. Dietary α -Linolenic Acid Reduces Inflammatory and Lipid Cardiovascular Risk Factors in Hypercholesterolemic Men and Women. *Journal of nutrition*, 134(11):2991-2997.

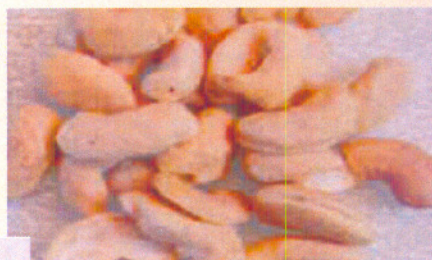
Macadamia



Almond



Walnut



Pecans



Pistachio

2

Dietary intervention trials investigating the effects of nuts on lipid profiles: A systematic review

Janine Mukuddem-Petersen M.Sc, Welma Oosthuizen Ph.D, Johann C. Jerling Ph.D

ABSTRACT

Background: The inverse association of nut consumption and risk markers of coronary heart disease (lipids) has sparked the interest of the scientific and lay community.

Objective: To conduct a systematic review that investigates the effects of nuts on the lipid profile.

Design: Medline and Web of Science databases were searched from the start of the database to August 2004 and supplemented by cross-checking reference lists of relevant publications. Human intervention trials with the objective to investigate independent effects of nuts on lipid concentrations were included. From the literature search, 415 publications were screened and 23 studies were included. These papers received a rating based upon the methodology as it appeared in the publication. No formal statistical analysis was performed due to the large differences in study designs of the dietary intervention trials.

Results: The results of 3 almond (50-100g/day), 2 peanut (35-68g/day), 1 pecan nut (72g/day) and 4 walnut (40-84g/day) studies showed convincing evidence for a lipid lowering effect of TC between 2-16% and LDL cholesterol between 2-19%, when compared to their control diets. The results for macadamia nuts (50-100g/day) are less convincing.

Conclusions: Consumption of $\approx 50-100\text{g}$ ($\approx 1.5-3.5$ servings) of nuts 5 or more times/week as part of a heart-healthy diet with total fat content (high in mono- and/or polyunsaturated fatty acids) of $\approx 35\%$ of energy may significantly decrease TC and LDL-C in normo- and hyperlipidemic individuals.

KEY WORDS lipids, lipoprotein, nuts, triacylglycerol, diet

Submitted to American Journal of Clinical Nutrition

INTRODUCTION

It is undisputed that dietary habits affect coronary risk factors and hence the risk of a coronary event. In the search for bioactive components in foods that favourably affect cardiovascular disease (CVD) risk, nuts have begun to attract attention (1). Nuts come from many different plant families and are classified as either tree nuts (a one-seeded fruit in a hard shell e.g. almonds, walnuts, macadamia nuts, hazelnuts, pecans, coconuts) or ground nuts e.g. peanuts (a member of the Leguminosae family) (2).

Epidemiological studies have consistently demonstrated an association of nut consumption on coronary heart disease (CHD) morbidity and mortality in different population groups (3). Also, numerous studies demonstrated that the isocaloric replacement of nuts for other foods in the diet does not lead to weight gain (4-9). Compared to people who ate nuts less than once weekly, those who ate them one to four times a week had a 25% reduced risk of dying from CHD; people who ate nuts five or more times a week experienced an approximate 50% reduction in risk (10). Nut consumption may not only offer protection against heart disease, but also increase longevity (11). Recently, the benefits of nuts were acknowledged by the U.S. Food and Drug Administration (FDA) when they approved a qualified health claim that eating nuts (1.5 ounces/day $\approx$ 42.8 g/day) may reduce the risk of CHD (12).

There is significant evidence that nuts may have favourable effects on CHD through a variety of mechanisms. In this regard, the most extensively studied mechanism involves the lipid lowering effects. Nuts are good sources of unsaturated fatty acids (monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA)) that are known for their favourable effects on blood lipids (**Figure 1**). Furthermore, evidence suggests that components in nuts further reduce total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) concentrations beyond the effects predicted by equations based solely on fatty acid profiles (13;14). In particular, the magnitude of the cholesterol-lowering effect has been shown to be 25% greater than would be predicted based on the fatty acid profiles of the test diets studied (3). Therefore, the possible mechanisms whereby nuts may improve lipid profiles are not exclusively due to the beneficial action of unsaturated fatty acids (PUFA and MUFA) but may include the effects of fibre, micronutrients such as vitamin E and C, folic acid, copper, magnesium, plant protein (e.g. arginine), plant sterols and phenolic components (**Table 1**) (3).

This systematic review evaluates the scientific evidence that is related to the effects of nut consumption on lipid profiles.

SUBJECTS AND METHODS

Subjects

Dietary intervention studies with healthy or diseased (hypercholesterolemia, hyperlipidemia, type 2 diabetes) subjects were included in this systematic review.

Studies

Studies were included if the objective was to investigate the independent effect of nuts on lipid concentrations and the study was conducted on humans. Trials were excluded when these independent effects could not be assessed and studies had incomplete or missing data.

Outcome measures

The main outcome measures were percentage differences between treatment and control groups for blood TC, LDL-C, high-density lipoprotein cholesterol (HDL-C) and triacylglycerols (TG).

Data sources

Dietary intervention trials with the primary objective to identify the effects of nuts on lipid concentrations were identified through Medline (since inception to August 2004) and Web of Science (from 1994 to August 2004) research databases, supplemented by contact with authors of papers, and reference lists of relevant publications. Search terms used included MeSH terms (Medline) – Nuts [MeSH] AND (“Lipoproteins”[MeSH] OR “Cholesterol”[MeSH]) OR “Triacylglycerol”[MeSH]) and key words (Web of Science) - (nuts OR walnut* OR almond* OR pecan* OR macadamia* OR hazelnut* OR peanut*OR pistachio*) AND (cholesterol OR triacylglycerol OR lipoprotein).

Study selection and data extraction

Two reviewers independently assessed studies to determine eligibility, executed data extraction by using a prepiloted standardized form, and determined the quality of all the identified intervention studies. Differences in data extracted or quality score was adjudicated by a third reviewer and then finalized in discussion between the three authors.

Data synthesis

After careful scrutiny of selected studies, they received a rating based upon the methodology as it appeared in the publication. The following criteria for quality assessment of the dietary intervention trials were used (adapted from Verhagen et al.) (15):

Study was controlled

Randomization was performed

Justified sample size (20 participants or < 20 with power calculation for TC or LDL-C)

Good compliance

Researchers were blinded (single-blind)

Groups were similar at baseline regarding the most important prognostic indicators or differences in baseline characteristics were statistically controlled for
No order of treatment effect

The following quality scores were assigned:

- 1= if all of the above criteria items were present
- 2= if controlled and two criteria items were missing
- 3= if not controlled or three or more criteria items were missing

Furthermore, the letter *a* was assigned for controlled feeding trials and *b* for studies conducted under free-living conditions.

Also, we did not consider the nut studies that received a 3 rating when drawing the conclusions. No formal statistical analysis was performed due to large differences in study designs of the dietary intervention trials.

RESULTS

Of the 415 articles screened (titles and abstracts, original research and review papers), 71 were considered in depth for inclusion. Forty-eight were excluded as a result of incomplete or missing data and where the independent effects of nuts could not be assessed. Consequently, from this comprehensive literature search, 23 original research papers were identified that were suitable for inclusion in this systematic review. Sixteen out of 23 studies received a 1 or 2 rating. In **Table 2** (4;6-9;16-33) we summarized all the dietary intervention trials investigating the effects of nut consumption on lipid profiles. The experimental designs of these studies were variable; subject characteristics differed (normolipidemic, hypercholesterolemic and diabetic), as did the degree of dietary control (free-living, controlled feeding), the type and amount of fat (ranging from 20% to 45%), dose/mode of nuts consumed, duration of the studies, the control diets (high-fat, low-fat, Step I, Step II, low-fat high complex carbohydrate (CHO), average American, olive-oil, habitual, Mediterranean, Japanese) and sample sizes. In this systematic review, most of the nut intervention diets were in fact heart-healthy diets, being low in saturated fatty acids, trans fatty acids, dietary cholesterol, high in unsaturated fatty acids and dietary fibre (**Table 3**). Only studies with a 1 or 2 rating will be discussed. Exceptions to this rule applied in cases when the only studies available for a specific type of nut were studies with a rating of 3. The majority of the studies were short (4-6 weeks) with only one study lasting 6 months (9). Most of the studies (12 out of 16) included > 20 subjects/group. This is enough to detect clinical significant changes in TC and LDL-C. However, according to power calculations (80% power at a level of 5% significance) based on data from studies by Chisholm et al; Ros et al; Sabate et al; Lovejoy et al and Jenkins et al. (19-21;27;33), it was clear that all the studies did not have adequate

power to detect clinical significant changes in HDL-C and TG. At least 109 participants are needed to detect an increase in HDL-C of 0.13mmol/L that may reduce the risk of CVD by 10% (34). For TG at least 67 participants are needed to detect a decrease in TG of 0.31mmol/L that may in turn decrease the risk of CVD by 10% (35). Regarding the latter it is important to take into account the large (12.9% to 40.8%) intra-individual variation (36;37) in the TG concentrations. In essence, this intra-individual variation may result in a larger number (> 67) of participants needed to have sufficient power for TG concentrations.

In Table 2, three out of four highly rated almond studies showed that a diet containing 50-100g of almonds per day significantly decreased TC (between 4%-16%) and LDL-C (between 7%-19%) in hypercholesterolemic (18;19) and normocholesterolemic (21) subjects compared to subjects on a control diet (control, low-fat and Step I diet). The intervention diets that provided less than 50g of almonds per day did not induce any significant change in the LDL-C concentrations (19;21). No significant improvement in the lipid profile was seen when comparing the almond intervention diet with the olive-oil based diet (18). Results of the study by Jenkins and investigators (19), where hyperlipidemic subjects consumed 50-100g of almonds per day displayed a significant increase in HDL-C of 2% compared to a low-fat control diet (26% fat vs. 36% fat provided by the almond diet). Lovejoy and collaborators (20) showed that 57-113g of almonds per day had no significant beneficial effect on the lipid profile of diabetic subjects when compared to a high- and low-fat control group. This study showed a significant decrease in HDL-C concentrations when compared to the aforementioned control groups. None of the 4 almond studies showed any significant effect on TG concentrations when comparing the nut intervention diet to the respective control diets (18-21).

Two good macadamia nut studies have been summarized in Table 2. This nut diet significantly lowered TC (5%), LDL-C (5%) and TG (9%) concentrations when compared to the average American diet (AAD) (7), but not when compared to a low-fat high complex CHO (23), or Step I diet (7). The HDL-C concentration was not affected when compared to a Step I diet or to a low fat high complex CHO diet (7;23). When compared to the AAD, the macadamia nut diet showed a significant decrease in HDL-C concentrations (4%) (7).

Two peanut studies showed a significant decrease in TC concentrations when compared to a low-fat control diet (7%) (9) and to the AAD (11%) and Step II diet (2%) (4). The LDL-C concentrations were significantly decreased when compared to the AAD (14%) (4) and low-fat diet (9%) (9). The HDL-C concentrations were significantly decreased when compared to the low-fat diet (0.3%) (9). In the latter study (9), the modest reduction in HDL-C concentration was due to changes in the cholesterol content of the HDL₃ subfraction. The ratio of TC:HDL-C and LDL-C:HDL-C decreased by 0.05-0.11 in the peanut intervention group compared to the low-fat control group (9). The TG concentrations decreased

in both studies but this was only significant when compared to the AAD (13%) and Step II diet (22%) (4). There was no significant change in the lipid profile when the peanut diet was compared to the olive-oil diet (4).

A feeding trial conducted by Rajaram et al (8) showed that intakes of 72g of pecans/day by subjects with normal-to-high cholesterol concentrations decreased serum TC (7%), LDL-C (10%), TG (11%) and increased HDL-C (6%), beyond that seen in the Step I diet (Table 2).

Walnuts are unique compared to other nuts because they are predominant in n-6 (linoleic acid) and n-3 (α -linolenic acid) PUFA's rather than the MUFA's that are dominant in most other nuts (Figure 1) (2). Of all the nuts presented in this review, walnuts have been studied the most extensively. Four out of 7 walnut studies (40-84g/day) displayed a significant decrease in TC (4-12%) and LDL-C (6-16%) when compared to Step I (6), Mediterranean (28;33) and Japanese (32) diets. The smallest dosage of nuts correlated with the smallest percentage change in TC and LDL-C and vice versa (6;28). Three studies showed no significant change in the lipid profile of hyperlipidemic subjects who followed a walnut intervention diet (41-78g/day) when compared to a low-fat (27), Mediterranean (30) and Step I diet (31). In one walnut study the HDL-C concentrations decreased significantly when compared to a Step I diet (6). Despite the aforementioned significant decrease in HDL-C (6), the mean ratio of LDL-C:HDL-C was 2.5 ± 0.6 during the reference diet and decreased to 2.2 ± 0.7 during the walnut diet ($P < 0.001$). The ratio of TC:HDL-C also decreased from 4.0 ± 1.0 during the reference diet to 3.7 ± 1.0 during the walnut diet ($P < 0.001$) (6). In most of the walnut studies HDL-C was not significantly affected, as seen in the other nut studies. In addition, TG was not significantly affected in the walnut diets compared to their respective control diets.

Only one hazelnut study and one pistachio nut study investigated the effects on lipid concentrations. Both these nut studies received a 3b rating, therefore, the results should be interpreted with caution. Firstly, in an uncontrolled study, Durak and collaborators showed that hazelnuts, resulted in a significant decrease in TC (6%), LDL-C (19%) and a significant increase in HDL-C (7%) and TG (25%) when compared to the baseline diet (Table 2) (22). Secondly, the small ($n=10$) pistachio nut intervention study that substituted 20% of daily calories with pistachio nuts significantly improved the TC (4%) and HDL-C (8%) concentrations and had no significant effect on LDL-C or TG in subjects with hypercholesterolemia when compared to the habitual diet (26).

DISCUSSION

Convincing evidence for a lipid lowering (decrease in TC and LDL-C) effect has been documented in experimental studies with almonds (18;19;21) peanuts (a legume) (4;9), pecan nuts (8) and walnuts

(6;28;32;33) (Table 2). There are indications from inadequately designed intervention studies that hazelnut (22) and pistachio (26) nut studies may have a lipid lowering effect. However, the evidence for macadamia nuts (7) are not convincing enough.

The changes in plasma lipids and lipoproteins with diet stabilize within 2-3 weeks. Therefore, the length of the diet periods of the clinical trials in this systematic review was sufficient to achieve stabilization of blood lipids (38). The sustainability of the outcomes can, however, not be evaluated with duration periods of 4-6 weeks (39).

Inconsistent trial results could possibly be explained in a number of ways.

The beneficial effects of almonds (18;19;21) on the lipid profile appeared to be dose dependant (Table 2). It could be suggested that the reason for no significant change in the lipid profile when the almond diet was compared to the olive-oil based diet could be as a result of the similarities in fatty acid composition of these two diets (18) (Table 2). The possible explanation for the increase in HDL-C in the Jenkins study (19) could be as a result of the low-fat (26%) content of the control diet compared to the intervention diet (36%). Low-fat diets have been shown to decrease HDL-C concentrations (4). The absence of an effect by Lovejoy and investigators may be linked to the similarities in fatty acid distribution of the almond diets and the control diets (MUFA provided by olive and canola oil) as seen in the study by Spiller et al (18). Also, it was the only nut study done on diabetic subjects (20), which may suggest that other mechanisms came into play. Notably, the 2 aforementioned almond studies had no significant effect when compared to the olive-oil control diets (18;20). In this context, it could be speculated that like the nut diets, the olive-oil control diets may also have additional cholesterol lowering effects that may extend beyond its fatty acid composition.

The macadamia nut intervention that was compared to the AAD showed significant decreases in the lipid profile (7). This nut diet and the AAD diet had the same percentage total fat (35% TF), however, the control diet was high in SFA (14% vs. 9%) and low in MUFA (12% vs. 20%) (Table 3). The absence of a beneficial lipid effect when comparing the macadamia nut diet to the low-fat high complex CHO (23), or Step I diet (7) may in part be due to the high concentration of palmitoleic acid (C16 :1); a metabolite of palmitic acid, in these nuts. In this regard, macadamia nuts contain the largest amount of palmitoleic acid (C16 :1, 12.7g/100g of nuts) compared to the other nuts (C16:1, 0-0.5g/100g of nuts) discussed in this systematic review (2). A study by Nestel and collaborators showed that palmitoleic acid behaves like a saturated and not a monounsaturated fatty acid in its effect on LDL-C in hypercholesterolemic men (40). The non-significant outcome of the nut diet by Colquhoun and researchers may be explained by the large contrast between the very high concentration of total fat (42%), SFA (11%) and low concentrations of CHO (39%) compared to the low-fat high complex CHO control diet (21% TF, 9% SFA and 57% CHO) (23) (Table 3). Curb and collaborators concluded that

the interpretation of the decrease in HDL-C may be difficult and further investigation of HDL-C effects in longer-term macadamia nut studies with close attention to other dietary components is warranted (7). Even though this study received a high rating, the authors did not provide information about the exact amount of nuts eaten daily (7).

It is evident from the two peanut intervention studies (4;9) that the TC and LDL-C concentrations of both hyper- and normocholesterolemic subjects were significantly improved except when compared to an olive-oil control diet (4). As mentioned previously, the olive-oil control diet may be similar to the nut diet with regards to its hypocholesterolemic properties. Epidemiological and clinical data indicate that the inverse relationship seen between HDL-C and premature atherosclerosis is stronger for HDL₂-C than for HDL₃-C (41;42). Although HDL₃-C decreased the concentration of HDL₂-C and apo A-I did not change in O'Byrne's study. The resultant ratio of LDL-C:HDL-C, therefore, showed a beneficial downward trend. Consequently, the protective role of HDL-C was probably not compromised by the peanut intervention diet compared to the low-fat control diet (9). The significant decrease in TG could be ascribed to the Step II control diet that caused a significant increase in TG concentrations (4). Also, Kris-Etherton and investigators concluded that a high-MUFA, cholesterol-lowering diet that includes peanuts is an acceptable dietary approach towards favourably affecting CVD risk status (4). Unfortunately, these authors did not report the dosage of peanuts ingested daily (4).

Pecans decreased TC and LDL-C concentrations compared to a Step I diet. The increase in HDL-C and decrease in TG concentrations may in part be ascribed to the decreasing effect of the HDL-C and increasing effect of TG with the low-fat Step I diet (28% vs. 40% provided by the pecan diet) (Table 3).

It is clear from the walnut interventions that the TC and LDL-C (**Figure 2**) concentrations decreasing effect extended beyond the cholesterol decreasing effect seen with the Step I (6), Mediterranean (28;33) and Japanese (32) diets (Table 3). These favourable changes in the TC and LDL-C appeared to be dose related. It can be speculated that the study by Sabate and investigators (6) had the most pronounced improvement because it had the largest dosage of walnuts (i.e. 84g/day). However, these dose-related speculations need to be confirmed by nut intervention studies investigating dose-response before quantitative claims can be made. In two walnut intervention trials (64-78g/day compared to a low-fat (27), and Step I diet (31)), no significant improvements in the lipid profiles were seen that may possibly be due to the large difference in the percentage fat between the nut diet (38%, 45% respectively) and the control diets (30%, 33% respectively). Also, it is evident from all the walnut studies that the significant favourable change in the lipid profile was amiss when the total percentage fat in the diet was >37% (27;31). Nevertheless, the beneficial results in TC and LDL-C concentrations

were evident when the consumption ranged from $\approx 40\text{g}$ - 84g walnuts daily (≈ 1.5 - 3 servings daily). In the study by Munoz et al, the walnut diet compared to the Mediterranean diet displayed no significant change in the lipid profile probably as a result of the small sample size ($n=10$) or other mechanisms that may come into play in subjects having polygenic hypercholesterolemia (30). Generally, most of the walnut diets appeared to have no significant effect on HDL-C concentrations when compared to the aforementioned control diets (27;28;30-33). The study by Sabate and investigators showed a significant decrease in HDL-C, however, it still displayed favourable TC:HDL-C and LDL-C:HDL-C ratio's (6). The decrease in HDL-C may be ascribed to the high PUFA (17% vs 10%), low SFA (6% vs. 9%) and MUFA (7% vs. 9%) content of the walnut diet compared to the control diet. Saturated fatty acids and MUFA are known to increase HDL-C, whereas high intakes of PUFA ($> 10\%$) are known to decrease HDL-C (43).

It has been confirmed that the inclusion of nuts results in a change in the fatty acid composition of the diet (44), and this change may in turn favourably affect lipid concentrations. It may therefore, be debated that the improvements seen in the lipid profiles with the intake of nuts may be owing to the changes in dietary fatty acid content and not necessarily to the nuts per se (Table 2). In many of the studies that showed a beneficial effect the nut diets were higher in MUFA (4;7-9;18;19;21) or PUFA (6;28;32;33) compared to the control diets. In addition, no effect occurred when the almond (18;20) and peanut (4) diets were compared to an olive-oil control diet (with similar fatty acid distributions). However, Figure 2 confirms the beneficial effect of nuts beyond its fatty acid profile. By following the lead of Kris-Etherton and collaborators (1), we compared the actual observed changes to predicted changes in LDL-C and TC concentrations based on differences in the dietary fatty acid composition in the nut and control diets using the predictive equation of Mensink and Katan (13). The nut diets combined resulted in a significant greater reduction in LDL-C and TC concentrations of 0.11mmol/L and 0.12mmol/L compared to the predicted changes (Wilcoxon matched pairs test, $P=0.01$). In Figure 2, all 3 macadamia nut interventions showed greater predicted decreases than the actual decrease in LDL-C concentrations and this may be due to the effects of palmitoleic acid as discussed earlier. The studies that did not show a greater reduction in LDL-C compared to the predicted change were studies that used $< 50\text{g}$ of almonds/day (19;21); the study of Lovejoy and colleagues on diabetic subjects (20); studies that used control diets high in olive-oil (4;20) or a Step II control diet (4) and the walnut study of Chisholm and investigators that was compared to a low-fat control diet (27).

Although the fatty acid profile of the nuts contributes to the hypocholesterolemic effect, the other components in nuts also have favourable effects on the lipid profile (1). In this regard, nuts are comprised of $\approx 7\text{g}/100\text{g}$ of dietary fibre, of which, $\approx 25\%$ is soluble fibre (45). Soluble fibre has shown to reduce total- and LDL-C concentrations (46). This was confirmed by Brown and colleagues who conducted a meta-analysis (45) of 67 controlled trials to quantify the cholesterol-lowering effect

of major dietary fibres. They concluded that soluble fibre of 2-10g/day was associated with small but significant decreases in TC. Furthermore, the practical range for increasing soluble fibre only made a small contribution to dietary therapy to lower cholesterol (45). Rimm and Stampfer (47) suggested that plasma alpha tocopherol concentrations $\geq 27.5\mu\text{mol/L}$ may be necessary to reduce cardiovascular risk by an approximate dietary intake of 15-30mg of vitamin E. Even though nuts are significant sources of vitamin E, there is not sufficient evidence to confirm a hypocholesterolemic effect. Arginine, the second most abundant amino acid found in nut proteins, may account for the hypocholesterolemic effect observed in animal studies (48). In addition, this effect has been seen in human intervention trials when the dosage of arginine in the diet appeared to be very high (e.g. one study had 6-21g/day of arginine) (49). Nuts are also sources of phytosterols (Table 1) and other phytochemical compounds such as flavonoids, phenolic components and ellagic acid with possible serum cholesterol modulating effects (50-54). In particular, studies have shown that 2-2.2g of plant sterols significantly reduces cholesterol absorption which in turn decreases plasma and LDL-C concentrations (50;52). Furthermore, a study by Anderson and collaborators demonstrated that walnut polyphenolics are effective inhibitors of in vitro plasma and LDL oxidation and therefore adds to nuts antiatherogenic potential (50). It is apparent that fibre, vitamin E, arginine, phytosterols and phenolic components from practical intakes of nuts is not sufficient to exert an individual hypocholesterolemic effect (55). In essence, it is possible that there are multiple small effects that contribute, and these are mediated by more than the lipid lowering fatty acid composition.

Some of the limitations of this review could include the fact that the independent effects of the type of nut diets and the type of control diets may mask potential effects or accentuate others. Also, differences in the study populations whose lipid profile may be altered due to underlying disease, poorly designed studies (sample size, control, duration, size of intervention) publication bias and insufficient reporting of results.

CONCLUSIONS

This systematic review highlights the evidence from well-designed nut intervention studies. As eluded to previously, most of these nut intervention diets are in fact heart-healthy diets. In this regard, moderate-fat diets (35% of energy) containing $\approx 50\text{-}100\text{g/day}$ of nuts, especially almonds or peanuts or pecan nuts or walnuts significantly lowered TC (2-16%) and LDL-C (2-19%) concentrations to a greater degree than observed for lower-fat, cholesterol-lowering diets without nuts or changes in dietary fatty acid profiles. Currently, there are indications from inadequately designed intervention studies that hazelnuts (1g/day/kg body weight) and pistachios (20% of daily energy intake) may have a lipid lowering effect. At this stage the evidence for macadamia nuts is less convincing. This systematic rating of the dietary intervention trials has confirmed the benefits of nut consumption with respect to

the lipid profile. Since nuts are extremely versatile and can be consumed in many different ways, consumers can easily incorporate them into their diets. Based on the results of these nut intervention studies, it could be suggested that normo- and hyperlipidemic individuals may consume a variety of nuts approximately 50-100g (≈ 1.5 -3.5 *servings*) at least 5 times per week. Nevertheless, emphasis should still be placed on providing dietary guidance (56) to help people understand how to plan heart-healthy diets that include nuts. Therefore, future food-based dietary strategies for improving plasma lipid concentrations should take the lipid lowering effect of nuts into account.

Recommendations for future research

Future research should use quality-feeding studies with larger sample sizes and longer duration to investigate the effects of nuts on HDL-C and TG concentrations. In particular, this should apply to mixed nuts and those individual nuts not yet considered.

The unique nutrient and non-nutrient composition of nuts requires further research in order to elucidate the possible mechanisms responsible for the LDL-C lowering effect.

Acknowledgements

We would like to acknowledge our colleague Wilmarie Dercksen who despite her battle against cancer showed exceptional courage and perseverance and made invaluable contributions to this document right to the end of her life.

REFERENCES

1. Kris-Etherton PM, Yu-Poth S, Sabate J, Ratcliffe HE, Zhao G, Etherton TD. Nuts and their bioactive constituents: effects on serum lipids and other factors that affect disease risk. *Am.J.Clin.Nutr.* 1999;70:504S-11S.
2. US Department of Agriculture ARS. Nutrient Database for Standard Reference Release 15 Nutrient Data. Available at: <http://www.nal.usda.gov/fnic/foodcomp>. Accessed August 2004, 2003.
3. Kris-Etherton PM, Zhao G, Binkoski AE, Coval SM, Etherton TD. The effects of nuts on coronary heart disease risk. *Nutr.Rev.* 2001;59:103-11.
4. Kris-Etherton PM, Pearson TA, Wan Y et al. High-monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. *Am.J.Clin.Nutr* 1999;70:1009-15.
5. Fraser GE, Bennett HW, Jaceldo KB, Sabate J. Effect on body weight of a free 76 Kilojoule (320 calorie) daily supplement of almonds for six months. *J.Am.Coll.Nutr* 2002;21:275-83.

6. Sabate J, Fraser GE, Burke K, Knutsen SF, Bennett H, Lindsted KD. Effects of walnuts on serum lipid levels and blood pressure in normal men. *N.Engl.J.Med.* 1993;328:603-7.
7. Curb JD, Wergowske G, Dobbs JC, Abbott RD, Huang B. Serum lipid effects of a high-monounsaturated fat diet based on macadamia nuts. *Arch.Intern.Med.* 2000;160:1154-8.
8. Rajaram S, Burke K, Connell B, Myint T, Sabate J. A monounsaturated fatty acid-rich pecan-enriched diet favorably alters the serum lipid profile of healthy men and women. *J.Nutr.* 2001;131:2275-9.
9. O'Byrne DJ, Knauff DA, Shireman RB. Low fat-monounsaturated rich diets containing high-oleic peanuts improve serum lipoprotein profiles. *Lipids* 1997;32:687-95.
10. Dreher ML, Maher CV, Kearney P. The traditional and emerging role of nuts in healthful diets. *Nutr.Rev.* 1996;54:241-5.
11. Sabate J. Nut consumption, vegetarian diets, ischemic heart disease risk, and all-cause mortality: evidence from epidemiologic studies. *Am.J.Clin.Nutr* 1999;70:500S-3S.
12. Brown D. FDA considers health claim for nuts. *J.Am.Diet.Assoc.* 2003;103:426.
13. Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. *Arterioscler.Thromb.* 1992;12:911-9.
14. Hegsted DM, Ausman LM, Johnson JA, Dallal GE. Dietary fat and serum lipids: an evaluation of the experimental data. *Am.J.Clin.Nutr.* 1993;57:875-83.
15. Verhagen AP, de Vet HC, de Bic RA et al. The Delphi list: a criteria list for quality assessment of randomized clinical trials for conducting systematic reviews developed by Delphi consensus. *J.Clin.Epidemiol.* 1998;51:1235-41.
16. Spiller GA, Jenkins DJ, Cragen LN et al. Effect of a diet high in monounsaturated fat from almonds on plasma cholesterol and lipoproteins. *J.Am.Coll.Nutr.* 1992;11:126-30.
17. Abbey M, Noakes M, Belling GB, Nestel PJ. Partial replacement of saturated fatty acids with almonds or walnuts lowers total plasma cholesterol and low-density-lipoprotein cholesterol. *Am.J.Clin.Nutr.* 1994;59:995-9.
18. Spiller GA, Jenkins DA, Bosello O, Gates JE, Cragen LN, Bruce B. Nuts and plasma lipids: an almond-based diet lowers LDL-C while preserving HDL-C. *J.Am.Coll.Nutr.* 1998; 17:285-90.

19. Jenkins DJ, Kendall CW, Marchie A et al. Dose response of almonds on coronary heart disease risk factors: blood lipids, oxidized low-density lipoproteins, lipoprotein(a), homocysteine, and pulmonary nitric oxide: a randomized, controlled, crossover trial. *Circulation* 2002;106:1327-32.
20. Lovejoy JC, Most MM, Lefevre M, Greenway FL, Rood JC. Effect of diets enriched in almonds on insulin action and serum lipids in adults with normal glucose tolerance or type 2 diabetes. *Am.J.Clin.Nutr.* 2002;76:1000-6.
21. Sabate J, Haddad E, Tanzman JS, Jambazian P, Rajaram S. Serum lipid response to the graduated enrichment of a Step I diet with almonds: a randomized feeding trial. *Am.J.Clin.Nutr.* 2003;77:1379-84.
22. Durak I, Koksali I, Kacmaz M, Buyukkocak S, Cimen BM, Ozturk HS. Hazelnut supplementation enhances plasma antioxidant potential and lowers plasma cholesterol levels. *Clin.Chim.Acta* 1999;284:113-5.
23. Colquhoun DM, Humphries JA, Moores D, Somerset SM. Effects of a macadamia nut enriched diet on serum lipids and lipoproteins compared to a low-fat diet. *Food Aust.* 1996;48:216-22.
24. Garg ML, Blake RJ, Wills RB. Macadamia nut consumption lowers plasma total and LDL cholesterol levels in hypercholesterolemic men. *J.Nutr.* 2003;133:1060-3.
25. Morgan WA, Clayshulte BJ. Pecans lower low-density lipoprotein cholesterol in people with normal lipid levels. *J.Am.Diet.Assoc.* 2000;100:312-8.
26. Edwards K, Kwaw I, Matud J, Kurtz I. Effect of pistachio nuts on serum lipid levels in patients with moderate hypercholesterolemia. *J.Am.Coll.Nutr.* 1999;18:229-32.
27. Chisholm A, Mann J, Skeaff M et al. A diet rich in walnuts favourably influences plasma fatty acid profile in moderately hyperlipidaemic subjects. *Eur.J.Clin.Nutr.* 1998;52:12-6.
28. Zambon D, Sabate J, Munoz S et al. Substituting walnuts for monounsaturated fat improves the serum lipid profile of hypercholesterolemic men and women. A randomized crossover trial. *Ann.Intern.Med.* 2000;132:538-46.
29. Almario RU, Vonghavaravat V, Wong R, Kasim-Karakas SE. Effects of walnut consumption on plasma fatty acids and lipoproteins in combined hyperlipidemia. *Am.J.Clin.Nutr.* 2001;74:72-9.

30. Munoz S, Merlos M, Zambon D et al. Walnut-enriched diet increases the association of LDL from hypercholesterolemic men with human HepG2 cells. *J.Lipid Res.* 2001;42:2069-76.
31. Morgan JM, Horton K, Reese D, Carey C, Walker K, Capuzzi DM. Effects of walnut consumption as part of a low-fat, low-cholesterol diet on serum cardiovascular risk factors. *Int.J.Vitam.Nutr.Res.* 2002;72:341-7.
32. Iwamoto M, Imaizumi K, Sato M et al. Serum lipid profiles in Japanese women and men during consumption of walnuts. *Eur.J.Clin.Nutr.* 2002;56:629-37.
33. Ros E, Nunez I, Perez-Heras A et al. A walnut diet improves endothelial function in hypercholesterolemic subjects: a randomized crossover trial. *Circulation* 2004;109:1609-14.
34. Gordon DJ, Probstfield JL, Garrison RJ et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation* 1989;79:8-15.
35. Austin MA, Hokanson JE, Edwards KL. Hypertriglyceridemia as a cardiovascular risk factor. *Am.J.Cardiol.* 1998;81:7B-12B.
36. Demacker PN, Schade RW, Jansen RT, Van 't LA. Intra-individual variation of serum cholesterol, triglycerides and high density lipoprotein cholesterol in normal humans. *Atherosclerosis* 1982;45:259-66.
37. Warnick GR, Albers JJ. Physiological and analytical variation in cholesterol and triglycerides. *Lipids* 1976; 11:203-8.
38. Ginsberg HN, Kris-Etherton P, Dennis B et al. Effects of reducing dietary saturated fatty acids on plasma lipids and lipoproteins in healthy subjects: the DELTA Study, protocol 1. *Arterioscler.Thromb.Vasc.Biol.* 1998;18:441-9.
39. Feldman EB. The scientific evidence for a beneficial health relationship between walnuts and coronary heart disease. *J.Nutr* 2002;132:1062S-101S.
40. Nestel P, Clifton P, Noakes M. Effects of increasing dietary palmitoleic acid compared with palmitic and oleic acids on plasma lipids of hypercholesterolemic men. *J.Lipid Res.* 1994;35:656-62.
41. Assmann G, Funke H. HDL metabolism and atherosclerosis. *J.Cardiovasc.Pharmacol.* 1990;16 Suppl 9 :S15-S20.
42. Tribble DL, Krauss RM. HDL and coronary artery disease. *Adv.Intern.Med.* 1993;38:1-29.

43. Nutrition and Metabolism. Oxford, UK: Blackwell Publishing, 2003.
44. Jaceldo-Siegl K, Sabate J, Rajaram S, Fraser GE. Long-term almond supplementation without advice on food replacement induces favourable nutrient modifications to the habitual diets of free-living individuals. *Br.J.Nutr* 2004;92:533-40.
45. Brown L, Rosner B, Willett WW, Sacks FM. Cholesterol-lowering effects of dietary fiber: a meta-analysis. *Am.J.Clin.Nutr.* 1999;69:30-42.
46. Anderson JW, Smith BM, Gustafson NJ. Health benefits and practical aspects of high-fiber diets. *Am.J.Clin.Nutr.* 1994;59:1242S-7S.
47. Rimm EB, Stampfer MJ. The role of antioxidants in preventive cardiology. *Curr.Opin.Cardiol.* 1997;12:188-94.
48. Kurowska EM, Carroll KK. Hypercholesterolemic responses in rabbits to selected groups of dietary essential amino acids. *J.Nutr.* 1994;124:364-70.
49. Maxwell AJ, Anderson B, Zapien MP, Cooke JP. Endothelial dysfunction in hypercholesterolemia is reversed by a nutritional product designed to enhance nitric oxide activity. *Cardiovasc.Drugs Ther.* 2000;14:309-16.
50. Anderson KJ, Teuber SS, Gobeille A, Cremin P, Waterhouse AL, Steinberg FM. Walnut polyphenolics inhibit in vitro human plasma and LDL oxidation. *J.Nutr* 2001;131:2837-42.
51. Mojzisova G, Kuchta M. Dietary flavonoids and risk of coronary heart disease. *Physiol Res.* 2001;50:529-35.
52. Law M. Plant sterol and stanol margarines and health. *BMJ* 2000;320:861-4.
53. Vorster HH, Raal FJ, Ubbink JB, Marais AD, Rajput MC. Phytosterols--a new dietary aid for the treatment of hypercholesterolaemia. *S.Afr.Med.J.* 2003;93:581-2.
54. Richelle M, Enslin M, Hager C et al. Both free and esterified plant sterols reduce cholesterol absorption and the bioavailability of beta-carotene and alpha-tocopherol in normocholesterolemic humans. *Am.J.Clin.Nutr* 2004;80:171-7.
55. Lampe JW. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. *Am.J.Clin.Nutr.* 1999;70:475S-90S.
56. Mann J. Importance of dietary management and practical patient counseling, the European/Australasian perspective. *Atheroscler.Suppl* 2002;3:23-9.

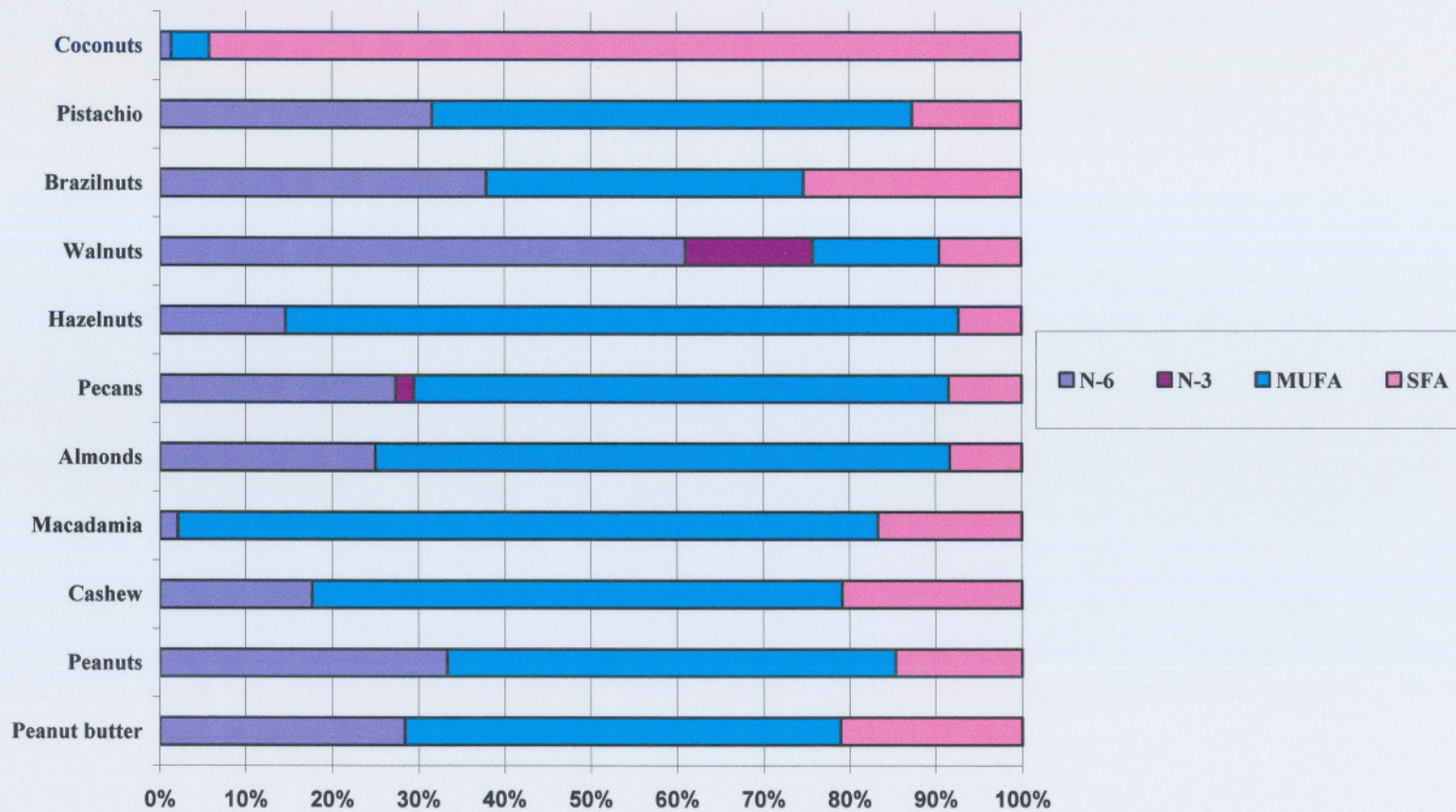


FIGURE 1. Fatty acid composition of nuts (2)

TABLE 1

Selected nutritional information of nuts and peanuts (serving size is 1oz = 28.5g) (2)

Nuts and peanuts	Energy (kJ)	Protein (g)	Total fat (g)	CHO (g)	Fibre (g)	Phytosterols (mg)	Minerals, vitamin & amino acids								
							Mg (mg)	P (mg)	K (mg)	Zn (mg)	Cu (mg)	Se (mcg)	Folate (mcg)	Vit. E (mg)	Arginine (g)
Almonds	685.5	6.0	14.4	5.6	3.4	34.0	78.0	134.4	206.4	1.0	0.3	0.8	8.2	7.3	0.7
Brazil nuts, dried, unblanched	777.6	4.1	18.8	3.5	2.1	-	106.6	205.5	186.8	1.2	0.5	543.5	6.2	1.6	0.6
Cashew nuts, raw	671.6	5.2	13.3	7.7	0.9	-	82.8	168.1	187.1	1.6	0.6	5.6	7.1	0.3	0.6
Hazelnuts	745.3	4.2	17.2	4.7	2.8	27.2	46.2	82.2	192.8	0.7	0.5	0.7	32.0	4.3	0.6
Peanuts, raw	673.0	7.3	14.0	4.6	2.4	62.4	47.6	106.6	199.9	0.9	0.3	2.0	68.0	2.4	0.9
Pecans	819.0	2.6	20.4	3.9	2.7	28.9	34.3	78.5	116.2	1.3	0.3	1.1	6.2	0.4	0.3
Pistachio nuts, raw	661.1	5.8	12.6	7.9	2.9	60.7	34.3	138.9	290.6	0.6	0.4	2.0	14.5	0.7	0.6
Macadamia nuts, raw	851.6	2.2	21.5	3.9	2.4	32.9	36.9	53.3	104.3	0.4	0.2	1.0	3.1	0.2	0.4
Walnuts, English	776.2	4.3	18.5	3.9	1.9	20.4	44.8	98.1	125.0	0.9	0.5	1.4	27.8	0.2	0.7

Abbreviations: CHO, carbohydrates; Mg, magnesium; P, phosphorous; K, potassium; Zn, zinc; Cu, copper; Se, selenium; Vit. E, vitamin E.

TABLE 2

Summary and quality assessment of dietary intervention trials - Investigating the effects of nuts on the lipid profile

Study & subjects	Study design	Compared to	Mean amount of nuts/day	Duration of intervention	% Total fat from		Nut diet end vs the control diet end *				Quality score of publication
					Nut diet	Control diet	TC	LDL-C	HDL-C	TG	
ALMONDS											
Spiller et al, 1992 (16) N=26 13M, 13W (hypercholesterolemic)	Single intervention	Baseline diet	100g/day	9 weeks	36.9	28.5	-8.9% p< 0.05	-12.4% p< 0.01	NS	-4.4% NS	3b
Abbey et al, 1994 (17) N=16M (normolipidemic)	Sequential intervention periods	Control	84g/day	3 weeks	35.9	35.7	-7% p < 0.01	-10% p < 0.001	NS	+1.4% NS	3b
Spiller et al, 1998 (18) N=45 12M, 33W (hypercholesterolemic)	Randomized, controlled, parallel design	Control	100g/day	4 weeks	39	35	-15.6% p < 0.001	-19% p < 0.001	NS	NS	2b
		Olive-oil based diet	100g/day	4 weeks	39	35	-8.7% NS	-9.8% NS	NS	NS	
Jenkins et al. 2002 (19) N=27 15M (hyperlipidemic), 12W (postmenopausal, hyperlipidemic)	Randomized, crossover design	Muffins-control	25-50g/day (half almond dose)	4 weeks	32.1	26.3	-2%** p< 0.05	-1.9%** NS	+2.2%** NS	-3.2%** NS	2b
		Muffins-control	50-100g/day (full almond dose)	4 weeks	36	26.3	-4.4%** p≤0.01	-7.1%** p≤0.01	+2.2%** p< 0.05	-5.3%** NS	
Lovejoy et al, 2002 (20) (Study 2) N=30 13M, 17W (type 2 diabetes)	Randomized, double-blind crossover design	High-fat control	57-113g/day	4 weeks	39	36.8	-1.3% NS	-2.7% NS p=0.06	-3.4% p=0.002	+5.4% NS	1a
		Low-fat control	57-113g/day	4 weeks	27.2	26	NS	NS	-2.6% p=0.002	+5% NS	
Sabate et al, 2003 (21) N= 25 14M, 11W (healthy)	Randomized cross-over design,	Step I diet	±27g/day (10% of total energy, low almond diet)	4 weeks	35	30	-0.9% NS	-1.1% NS	-0.9% NS	NS	2a
		Step I diet	±54g/day (20% of total energy, high almond diet)	4 weeks	39	30	-4.4% p< 0.05	-7% p< 0.05	+1.7% NS	-3.3% NS	
HAZELNUT											
Durak et al, 1999 (22) N=30 18M, 12W (healthy)	Single intervention	Baseline diet	1g/day/kg body weight	1 month	NR	NR	-6.1% p<0.005	-18.7% p<0.001	+7.2% p<0.05	+24.8% p<0.001	3b

TABLE 2

Summary and quality assessment of dietary intervention trials - Investigating the effects of nuts on the lipid profile (cont.)

Study & subjects	Study design	Compared to	Mean amount of nuts/day	Duration of intervention	% Total fat from		Nut diet end vs the control diet end *				Quality score of publication
					Nut diet	Control diet	TC	LDL-C	HDL-C	TG	
MACADAMIA											
Colquhoun et al. 1996 (23) N=14 7M, 7W (hypercholesterolemic)	Dietary advice, randomised, crossover design	Low-fat high-complex CHO diet	50-100g/day (20% of daily energy intake)	4 weeks	42.4	21.3	+0.2% NS	-0.3% NS	+9.1% NS	-13.3% NS	2b
Curb et al. 2000 (7) N=30 15M, 15W (normo- and hyperlipidemic)	Randomized, controlled, crossover design	Step 1 diet Average American diet	NR NR	4 weeks 4 weeks	35 35	30 35	-0.8% NS -4.8% p<0.01	+0.3% NS -4.5% p<0.05	+2.2% NS -4.2% p<0.01	-16% NS -9.2% p<0.05	1a
Garg et al. 2003 (24) N=17M (hypercholesterolemic)	Single intervention	Baseline diet	40-90g/day (15% of daily energy intake)	4 weeks	37.6	31.2	-3.2% p<0.05	-6% p<0.05	+6.7% p<0.05	-2.8% NS	3b
PEANUTS											
O'Byrne et al. 1997 (9) N=25W (hypercholesterolemic, postmenopausal)	Controlled parallel design	Low-fat diet	35-68g/day	6 months	26	17	-7.4% p≤0.01	-9.1% p≤0.01	-0.3% p≤0.01	-11% NS	2b
Kris-Etherton et al. 1999 (4) N=22 9M, 13W (normocholesterolemic)	Randomized, double-blind controlled, crossover design	Average American diet Step II diet	NR NR	24 days 24 days	36 36	34 25	-10.9% p<0.05 -2% p<0.05 +0.6% NS	-13.9% p<0.05 +0.7% NS +1.7% NS	-2.3% NS +1.61% NS -1.6% NS	-12.8% p<0.05 -21.6% p<0.05 +0.9% NS	1a
PECANS											
Morgan et al. 2000 (25) N=19 4 M, 15W (normolipidemic)	Randomized, controlled, parallel design	Control	68g/day	8 weeks	20	17	-10.7% p<0.05	-16.3% p<0.05	+1.2% p<0.05	-9.3% NS	3b
Rajaram et al. 2001 (8) N=23 14M, 9W (normal-high cholesterol)	Single-blind, randomised, controlled, crossover design	Step 1 diet	72g/day	4 weeks	39.6	28.3	-6.7% p≤0.01	-10.4% p≤0.01	+5.6% p≤0.01	-11.1% p≤0.01	1a
PISTACHIO NUTS											
Edwards et al. 1999 (26) N= 10, 4M, 6W (hypercholesterolemic)	Controlled, randomised, crossover design	Habitual diet	20% of daily energy intake	3 weeks	39	37	-3.7% p<0.04	-6.1% NS	+8% p<0.09	-5.3% NS	3b

TABLE 2

Summary and quality assessment of dietary intervention trials - Investigating the effects of nuts on the lipid profile (cont.)

Study & subjects	Study design	Compared to	Mean amount of nuts/day	Duration of intervention	% Total fat from		Nut diet end vs the control diet end *				Quality score of publication
					Nut diet	Control diet	TC	LDL-C	HDL-C	TG	
WALNUTS											
Sabate et al, 1993 (6) n=18M (healthy)	Randomized, controlled, single-blind, crossover design	Step I diet	84g/day (20% of daily energy intake)	4 weeks	31.3	29.3	-12.4% p< 0.001	-16.3% p< 0.001	-4.9% p= 0.009	-8.3% NS p=0.10	2a
Abbey et al, 1994 (17) N=16 M (normolipidemic)	Consecutive supplemental periods	Control	68g/day	3 weeks	36.5	35.7	-5% p< 0.01	-9% p< 0.001	+3.13% NS	+4.3% NS	3b
Chisholm et al, 1998 (27) N=21M (hyperlipidemic)	Randomized, crossover design	Low-fat diet	78g/day	4 weeks	38	30	-2% NS	-3.9% NS	+2.5% NS	+7.5% NS	2b
Zambon et al, 2000 (28) N=49 26 M, 23W (hypercholesterolemic)	Randomized, crossover design	Mediterranean diet	41-56g/day (18% of daily energy intake)	6 weeks	33.2	31.2	-4.1% p< 0.001	-5.9% p< 0.001	+3.2% NS p=0.13	-6.1% NS p=0.10	2b
Almario et al, 2001 (29) N=18 5M (hyperlipidemic), 13W (hyperlipidemic, postmenopausal)	Sequential intervention periods	Habitual diet (4 weeks)	48g/day	6 weeks	37.2	31.4	-3% NS	-1.7% NS	-10.2% p< 0.01	-10.1% NS	3b
		Low-fat diet	48g/day	6 weeks	33.7	19.7	-7.7% p< 0.01	-12.3% p< 0.01	+1.8% NS	-1.3% NS	
Munoz et al, 2001 (30) N=10M subset of the Zambon study (polygenic hypercholesterolemic)	Randomized, crossover design	Mediterranean diet	41-56g/day	6 weeks	31.8	30.9	-4.2% NS p=0.18	-6% NS p=0.09	0% NS p>0.2	-5.1% NS p>0.2	2b
Morgan et al, 2002 (31) N=42 17M, 25W (borderline hypercholesterolemic)	Randomized, cross-over design	Step I	64g/day	6 weeks	45	33	-3.3% NS	-3% NS	+6.7% NS	-6.3% NS	2b
Iwamoto et al, 2002 (32) N=40 20M, 20W (healthy)	Randomized, crossover design	Japanese diet	44-58g/day (12.5% of daily energy intake)	4 weeks	26	24	-4.5% p=0.001	-9.8% p=0.001	-1.3% NS p=0.49	0% NS	1a

TABLE 2

Summary and quality assessment of dietary intervention trials - Investigating the effects of nuts on the lipid profile (cont.)

Study & subjects	Study design	Compared to	Mean amount of nuts/day	Duration of intervention	% Total fat from		Nut diet end vs the control diet end *				Quality score of publication
					Nut diet	Control diet	TC	LDL-C	HDL-C	TG	
Ros et al, 2004 (33) N=20 8M, 12W (hypercholesterolemic)	Randomized, crossover design	Mediterranean diet	40-65g/day (18% of the total energy)	4 weeks	33	33.2	-4.3% p= 0.02	-6.7% p=0.01	-1.3% NS	+8.3% NS	1b

Abbreviations: N, number of subjects; NR, not reported; NS, non-significant; M, men; W, women; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triacylglycerol; Step I, American Heart Association/National Cholesterol Education Program Step I diet – 30% energy from fat; Step II, American Heart Association/National Cholesterol Education Program Step II diet – energy from fat 25%; CHO, carbohydrate.

*In cases where the % difference is not indicated, we used the formulae:-

$$\frac{\text{End value nut diet} - \text{End value control diet}}{\text{End value control diet}} \times 100$$

or

where baseline values are compared to nut intervention end values

$$\frac{\text{End value nut diet} - \text{Baseline value}}{\text{Baseline value}} \times 100$$

In cases where no values were given the changes were not reported

**In cases where baseline values were different, change from baseline to end with the nut diet was compared to the change from baseline to end in the control diet

TABLE 3

The fat, cholesterol, carbohydrate and fibre composition of nut and control diets of clinical trials*

Nut Diets	TF %	SFA %	MUFA %	PUFA %	chol mg/d	CHO %	fibre g/d	Control diets	TF %	SFA %	MUFA %	PUFA %	chol mg/d	CHO %	fibre g/d
ALMONDS															
Spiller et al. 1998 (18)	39	5	28	7	72	47	25	Control	35	17	15	3	269	47	26
	39	5	28	7	72	47	25	olive-oil based diet	35	6	27	3	119	48	29
Jenkins et al. 2002 (half-dose)	32	8	15	8	189	48	32	muffins control (25-50g/d)	26	7	9	8	170	55	31
(full-dose) (19)	36	7	19	8	161	45	33	muffins control (50-100g/d)	26	7	9	8	170	55	31
Lovejoy et al. 2002 (high)	39	7	23	11	226	46	31	high-fat control	37	7	21	9	270	49	17
(low) (20)	27	5	16	8	167	57	31	low-fat control	26	5	15	7	257	60	17
Sabate et al. 2003 (low)	35	8	17	8	163	51	30	Step I (27g/d)	30	8	12	6	202	56	28
(high) (21)	39	8	19	9	140	46	32	Step I (54g/d)	30	8	12	6	202	56	28
MACADAMIA															
Colquhoun et al. 1996 (23)	42	11	27	4	189	39	31	low-fat complex CHO	21	9	8	5	218	57	33
Curb et al. 2000 (7)	35	9	20	6	300	48	NR	Step I diet	30	9	15	7	297	54	NR
	35	9	20	6	300	48	NR	AAD	35	14	12	9	305	48	NR
PEANUTS															
O'Byrne et al. 1997 (9)	26	5	14	2	130	55	22	low-fat diet	17	5	6	2	129	63	17
Kris-Etherton et al. 1999 (4)	36	8	18	10	200	47	NR	AAD	34	16	11	7	400	50	NR
	36	8	18	10	200	47	NR	Step II	25	7	12	6	200	59	NR
	36	8	18	10	200	47	NR	olive-oil based diet	34	7	21	6	200	50	NR
PECANS															
Rajaram et al. 2001 (8)	40	8	19	11	175	47	30	Step I	28	8	11	6	210	57	25
WALNUTS															
Sabate et al. 1993 (6)	31	6	7	17	125	54	37	Step I	29	9	9	10	237	56	36
Chisholm et al. 1998 (27)	38	10	10	16	230	40	30	low-fat diet	30	12	10	5	320	46	30
Zambon et al. 2000 (28)	33	6	14	12	166	48	9**	Mediterranean	31	7	18	5	221	50	8**
Munoz et al. 2001 (30)	32	6	13	11	167	49	8**	Mediterranean	31	6	18	4	223	49	7**
Morgan et al. 2002 (31)	45	13	NR	NR	213	41	NR	Step I	33	13	NR	NR	NR	50	NR
Iwamoto et al. 2002 (32)	26	5	7	15	252	60	22	Japanese diet	24	7	10	7	279	62	20
Ros et al. 2004 (33)	33	5	14	12	147	49	8**	Mediterranean	33	6	20	4	183	49	8**

Abbreviations: TF, total fat; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; chol, cholesterol; CHO, carbohydrate Step I, American Heart Association/National Cholesterol Education Program Step I diet – 30% energy from fat; Step II, American Heart Association/National Cholesterol Education Program Step II diet – energy from fat 25%.

AAD, average American diet; NR, not reported.

*Only clinical trials that received a 1 or 2 rating were included in this table.

**Grams (g) of soluble fibre/day

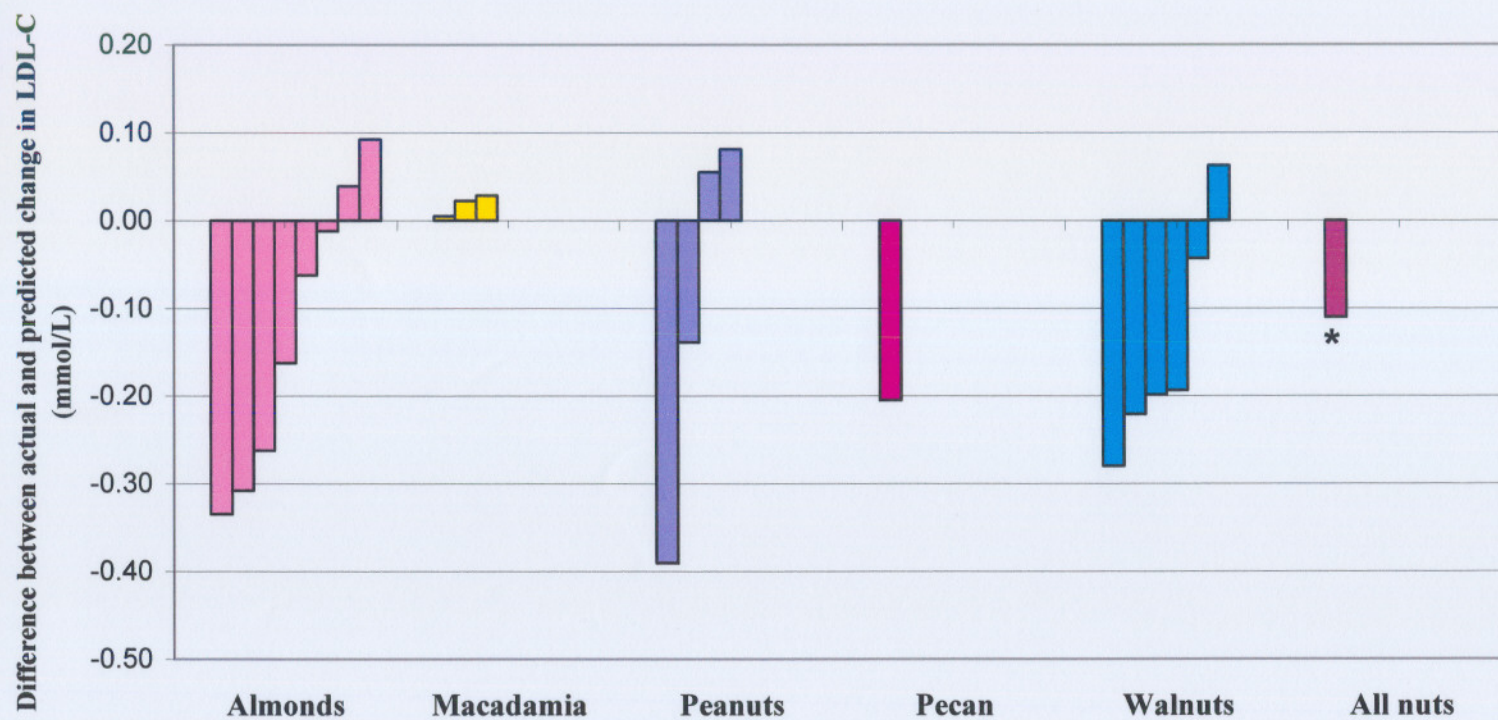


FIGURE 2. The difference between the actual and predicted changes (based on fatty acid distribution) (13) in LDL-C with nut diets compared to control diets. Only clinical trials that received a 1 or 2 rating were included in this figure. *Actual change was significantly different from the predicted change, $P < 0.05$ (Wilcoxon matched pairs test).

The NUT study- Controlled feeding trial



Nut Study welcoming tea

Signing
consent
forms

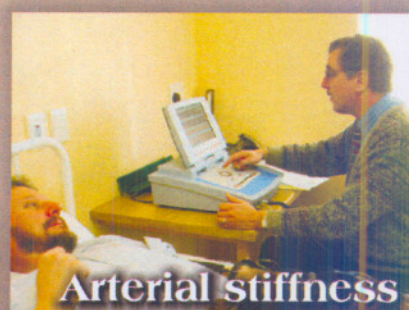


Studying
information
booklet



Anthropometric
measurments

The LIPID clinic
waiting room



Arterial stiffness
and
Blood pressure
measurements





**Blood Glucose -
finger pricks and Oral Glucose
Tolerance Test**



**LIPID Clinic Kitchen Staff -
accurately weighing portion sizes and
tastefully preparing food also
take-away parcels for breakfast and supper**



**Lunch time meals
at the
LIPID Clinic**



3

The effects of a high walnut and high cashew nut diet on markers of the metabolic syndrome: A controlled feeding trial

Janine Mukuddem-Petersen, Welma Oosthuizen, Johann C. Jerling, Grieta M. Hanekom and Zelda White

ABSTRACT

Background: Numerous questions surround the prevention and treatment of the metabolic syndrome. Despite this, it is clear that nuts have shown to be beneficial to health.

Objective: We investigated the effects of a high walnut diet and a high unsalted cashew nut diet on markers of the metabolic syndrome.

Design: In a randomized, parallel, controlled study design, 64 subjects having the metabolic syndrome (29 men, 35 women) with a mean ($\pm$ SD) age of 45 ± 10 y and who met the selection criteria were all fed a 3-week run-in control diet. Hereafter, participants were grouped according to gender and age and then randomized into three groups receiving a controlled feeding diet including walnuts, or unsalted cashew nuts or no nuts for 8 weeks.

Results: Both the walnut and the unsalted cashew nut intervention diets had no significant effect on the HDL-C, TG, TC, LDL-C, serum fructosamine, insulin, insulin sensitivity, insulin resistance, S-hs CRP, BP and serum uric acid concentrations when compared to the control diet. All 3 groups experienced highly significant increases in serum insulin concentrations when comparing the baseline to end. In turn, insulin resistance increased while insulin sensitivity decreased. Plasma glucose concentrations increased significantly in the cashew nut group compared to the control group. By contrast, serum fructosamine was unchanged in the cashew nut group while the control group had significantly increased concentrations of this short-term marker of glycaemic control.

Conclusions: Subjects displayed no improvement in the markers of the metabolic syndrome after following a walnut diet or a cashew nut diet compared to a control diet while maintaining body weight.

KEY WORDS Metabolic syndrome, cashew nuts, walnuts, insulin sensitivity, insulin resistance, hyperlipidemia, hypertension, lipids, fatty acids.

Submitted to American Journal of Clinical Nutrition

INTRODUCTION

The National Cholesterol Education Program's Adult Treatment Panel III (ATP III) defines the metabolic syndrome by easily measured clinical parameters that include: increased abdominal circumference, elevated triacylglycerol (TG), low high-density lipoprotein-cholesterol (HDL-C), elevated fasting blood glucose and / or elevated blood pressure (BP). Three of these five are required for diagnosis (1). Other abnormalities associated with this syndrome include insulin resistance with hyperinsulinaemia, prothrombotic and pro-inflammatory state, hyperinsulinaemia and microalbuminuria (2).

From the NHANES III (3) and THUSA study (Kruger A, Vorster HH, Oosthuizen W, Margetts BM, 2004) it is evident that the increasing prevalence of the metabolic syndrome is a health problem not only for developed countries but also for developing countries.

This syndrome has been identified as a target for dietary therapies (4) to reduce the risk of cardiovascular disease (CVD) (5) and type 2 diabetes (6). However, the role of diet in the etiology of the metabolic syndrome is still poorly understood (7). Riccardi and co-workers (8) concluded that the diet for the treatment of the metabolic syndrome should be limited in the intake of saturated fat, while high fibre/low-glycaemic-index (GI) foods should be used without specific limitations (9). Recently, in the Framingham Offspring cohort, the researchers found GI and glycemic load (GL: a measure of both carbohydrate (CHO) quality and quantity) were positively associated with both insulin resistance (homeostasis model assessment (HOMA)) and the prevalence of the metabolic syndrome (10). The so-called prudent dietary pattern is increasingly favoured for a beneficial effect on a number of health outcomes (11-13). The prudent diet does not have to be a low-fat one, because a variety of vegetable foods rich in monounsaturated fatty acids (MUFA) that are good sources of antioxidants, such as high-oleic acid oils and nuts can be incorporated to increase palatability and compliance, with a good chance of furthering health benefits (14).

Epidemiological findings indicate that frequent nut consumption offers protection from fatal and non-fatal coronary heart disease (CHD) events (15). Nuts have a low GI (16) and are a rich source of protein, unsaturated fatty acids (MUFA and polyunsaturated fatty acids (PUFA)), vitamin E, B₆, folic acid and niacin, fibre, magnesium, potassium, arginine, phytosterols and other phytochemical compounds (such as flavonoids, phenolic components and ellagic acid) (17). The protective effects of nuts are mediated through several mechanisms. There are no studies to date that investigated the effects of nuts on markers of the metabolic syndrome. Therefore, the primary objective of our study was to determine the effects of a high walnut diet and a high unsalted cashew nut diet on markers of the metabolic syndrome compared to a control diet. In particular these markers included: serum lipids

(total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), HDL-C and TG), serum fructosamine, serum insulin, plasma glucose, insulin sensitivity, insulin resistance, BP, serum uric acid and serum high sensitivity C-reactive protein (S-hs CRP).

SUBJECTS AND METHODS

Subjects

All participants gave written informed consent and the study was approved by the Ethics Committee of the Potchefstroom Campus of the North-West University (NWU-PC). Sixty-eight volunteers with the metabolic syndrome were recruited mainly from the NWU-PC and surrounding areas in Potchefstroom, South Africa. The power calculation was based on the results of Dessein and co-workers (18). To provide 80% power at 5% significance and taking a 15% change in the quantitative insulin sensitivity check index (QUICKI) as significant, a total number of 22 subjects were needed per group (i.e. a total of 66 subjects). Sixty-four subjects completed the study (dropouts discussed later). The ATP III criteria for the diagnosis of the metabolic syndrome was used. In this regard, the metabolic syndrome was defined as the presence of 3 or more of the following criteria (19): abdominal obesity (waist circumference (WC) > 88 cm for women or > 102 cm for men); fasting TG ≥ 1.7 mmol/L; HDL-C ≤ 1.0 mmol/L for men, ≤ 1.3 mmol/L for women; BP $\geq 130/85$ mmHg (the use of anti-hypertensive medication was also an indication of high BP) and fasting glucose ≥ 6.1 mmol/L (fasting finger prick blood glucose concentrations were measured with a SureStep™ blood glucose meter (Lifescan Inc., Milpitas, CA, USA), using Fine Point lancets, and SureStep™ test strips (code 11).

Additional inclusion criteria included subjects being able to comply with controlled feeding conditions; being willing and able to eat walnuts and cashew nuts and participants had to be older than 21 and younger than 65 years. Pregnancy or lactation, thiazide (> 25mg/day) and beta-blocker (non-specific, β_1 and β_2) use, subjects having nut allergies and diagnosed diabetes formed part of the exclusion criteria.

Study design

A randomized, controlled, parallel, study design was used. The study protocol consisted of a 3-wk run-in period during which the subjects consumed a control diet (percentage of total energy (%E) intake from protein:CHO:fat = 20:47:33%E (Table 1)). After the run-in period participants were grouped according to gender and age and then into three groups by randomly drawing numbers from a hat. Group one received walnuts (n=21), group two, unsalted cashew nuts (n=21), while group three continued with the control diet without any nuts or nut-based ingredients (n=22). Furthermore, these 3 intervention diets were followed for 8 weeks. Due to practical reasons the study was divided into three

cohorts distributed over a one year period. All the food was provided to the participants for the duration of the trial. Fasting blood samples, oral glucose tolerance tests (OGTT), anthropometric measurements, and BP measurements were taken before (after the 3 week run-in period) and after the intervention period (8-weeks controlled feeding). Body mass index (BMI) (kg/m^2) was calculated. In addition, the weighing of subjects were done twice weekly throughout the run-in-period and the experimental phase. The subjects were informed of all aspects of the study before commencement.

Diet

Twenty percent of the total energy (ranging from 63-108g/day) came from nuts in group one and two. Except for the nuts, the diets were identical. This was achieved by making proportional reductions to all food portions in the walnut and the unsalted cashew nut diet menus to accommodate the energy supplied by the respective nuts (Table 1 & Table 2). The study featured a highly controlled feeding protocol. In this regard, all subjects were required to have their lunch at the metabolic ward of the Department of Nutrition at the North-West University (Potchefstroom Campus). Breakfast and dinner was provided in take-away format. Using pre-packed food parcels and a variety of set menu options ensured compliance over weekends. Ten percent of the total energy intake was calculated in the form of “additional points” to be eaten daily. In this regard, a list of foods with their associated number of points were provided to the participants. In order to ensure total energy intake and some freedom of choice, participants were advised to choose any foods from the list, provided they added up to the allotted number of points for their respective energy intakes for that day. A validated food frequency questionnaire (FFQ) and physical activity questionnaire (PAQ), measuring activity index, were analyzed in order to determine the correct energy intake (EI) requirements for the maintenance of body weight for each participant. The validated PAQ is based on the Baecke physical activity questionnaire (20). Underreporting was established when the ratio of energy intake (EI): basal metabolic rate (BMR) was less than 1.2 (21;22).

A 14-day menu-cycle was designed for 5 amounts of energy intake, ranging from 8000 to 14000 kJ/day (1905 to 3333kcal/day). It was planned by using the FoodFinder 2 program (Medical Research Council of South Africa, Tygerberg), which is based on the South African food composition tables (23). The macronutrient profiles and fatty acid distribution of the 3 diets were analyzed chemically to validate the diet composition. Duplicate portions of breakfast, lunch and dinner for the 14-day menu cycle were collected daily, homogenized and pooled in a container and frozen at -84°C until the analysis was done.

Compliance to dietary intervention

Quality control and compliance with the protocol were ensured among study participants by the following means: 1) foods were weighed to the nearest gram before being served to the participants; 2)

the principal investigator, a registered dietitian, supervised mealtimes and ensured the complete intake of all study foods; 3) participants kept food diaries of the additional points used and possible left-overs were collected and weighed (by researchers). In addition, any deviation from the study protocol were recorded in these diaries (these diaries were reviewed by the investigators during the study); 4) participants were weighed twice weekly and the caloric intake was adjusted (especially during the first 3 week run-in period) in order to maintain body weight; and 5) participants were urged to maintain the same activity level throughout the study. Lastly, those individuals who used chronic medication at baseline were instructed to continue use and to maintain the same dosage for the duration of the trial.

Blood sampling and oral glucose tolerance test

The subjects were required to fast overnight (12 hours). A qualified nursing sister collected venous blood samples. For the preparation of serum, 20ml of blood was drawn and left to clot. For determination of plasma glucose concentrations 5ml blood was collected in tubes containing potassium oxalate (10mg) and sodium fluoride (12.5mg).

After the fasting blood samples were collected, the OGTT was continued. Seventy-five grams of glucose was dissolved in 300ml of water. Blood samples for the measurement of glucose and insulin were drawn again after 2 hours. Blood was centrifuged for 15 minutes at 2000g to yield serum and plasma respectively. Aliquots of serum and plasma were stored at -82°C until the analysis was performed.

Insulin sensitivity and insulin resistance

The QUICKI method is said to provide a reproducible and robust estimate of insulin sensitivity and we used it primarily because of its good correlation with the “gold standard”, the hyperinsulinemic euglycemic glucose clamp method (24).

The formula is as follows: $QUICKI = 1/[\log(\text{fasting insulin}) + \log(\text{fasting glucose})]$ (24).

Insulin resistance was determined by using the HOMA method (24). The use of the current HOMA model performed well in comparison with the hyperglycaemic clamp, the frequently sampled intravenous glucose tolerance test (IVGTT) or the oral glucose tolerance test (OGTT) (25-28). The formula is as follows (24): $HOMA = (\text{Insulin } (\mu\text{IU/ml}) \times \text{glucose (mmol/l)})/22.5$.

Analytical methods

The fatty acid composition of the nuts and diets was measured by gas chromatography as described by Van Jaarsveld and colleagues (29). The percentage protein was analyzed by a general combustion method (AOAC Method 992.23) (30) by using a LECO FP 528 (LECO Corporation, Michigan, USA), the percentage fat by a GAVIEZEL® method using the Büchi B 820 fat determination system with the Büchi B 815 extraction unit (Büchi Labortechnik AG, Flawil, Switzerland), the percentage fiber by the

filter bag technique using the ANKOM 220 fiber analyzer with F57 filter bags (ANKOM Technologies, Fairport, New York, USA), the percentage moisture with the air-oven (aluminium plate) method (AACC method 44-16) (31) and percentage ash with the AOAC Official Method 942.05 (30). The CHO content was then calculated as the sum of the protein, fat, fiber, moisture and ash subtracted from 100.

Coefficients of variance (CV) for all the laboratory analyses of blood samples, were less than 5%. Serum TC, HDL-C, TG, uric acid and plasma glucose were measured on a Vitros DT60 II Chemistry System (Ortho-Clinical Diagnostics, Rochester, New York, USA) with Vitros reagents (catalogue numbers 153 2175, 133 5504, 153 2159, 153 2134 and 1532316, respectively) and controls (catalogue numbers 842-0317, 144-8042). Serum LDL-C was calculated using the Friedewald formula: (LDL-C (mmol/L) = TC-TG/2.2 -HDL). Serum insulin was determined with an ELISA method on the Immulite 2000 Analyzer (Diagnostic Products Corporation, Los Angeles, USA). Serum high sensitivity C-reactive protein was measured using the Synchron LX20® clinical system, (Beckman Coulter, Inc., Fullerton, CA, USA). Serum fructosamine was measured with a calorimetric method, (Roche Basel, Switzerland, catalogue number 1930010). Blood pressure measurements were obtained by taking a 7-minute continuous measurement of cardiovascular parameters using the Finometer™ device (FMS, Finapres Measurement Systems, Arnhem, Netherlands).

Statistical analyses

The computer software package Statistica® (Statsoft Inc, Tulsa, OK, USA) was used for the analyses of the data. The statistical analysis was done in five steps. Initially, the variables were tested for normality using the Shapiro-Wilk's W-test. Non-normally distributed data were transformed into an approximately normal distribution by logarithmic and square-root transformations and again tested for normality. Thereafter, descriptive statistics were done. Data that were normally distributed are expressed as mean [95% confidence intervals (CI)]. Data that are not normally distributed or logarithmic and square-root transformed are expressed as median (25, 75 percentiles). Furthermore, changes within groups, from baseline to end, were tested for significance by using the *t*-test for dependent samples in the case of parametric data and the Wilcoxon matched pairs test in the case of nonparametric data. Also, differences in baseline and delta (change from baseline to end) between the three groups were determined by using the analysis of variance (ANOVA) for parametric data and the Kruskal Wallis ANOVA for nonparametric data. When significance between the changes in the three groups was indicated with the ANOVA, the Tukey honest significant difference test for unequal *N* for parametric data were used to determine between which groups the differences occurred. Lastly, weight adjusted differences in baseline and delta between the three groups were determined by using the analysis of covariance (ANCOVA). Significance was set at $P \leq 0.05$.

RESULTS

Subjects

Four subjects discontinued the study for the following reasons: 2 had work obligations outside Potchefstroom, 1 had an unrelated medical condition, and 1 went on a holiday during the study period. The remaining 64 subjects (29 men and 35 women, aged 45 ± 10 y) all completed one of the three intervention diets. Ninety percent compliance with the experimental diets was calculated. Weight, waist circumference (WC) and BMI did not change from baseline to end for all three intervention groups. Also, activity level was maintained throughout the study. In this regard, most of the participants had a sedentary lifestyle. The baseline characteristics of the subjects did not differ between groups (ANOVA) (Table 3). Most of the subjects were obese with a high WC, approximately two-thirds of the subjects had high TG, BP and low HDL-C and less than a third had high fasting glucose concentrations. Therefore, the subject characteristics at baseline were indicative of the metabolic syndrome. Also, of the entire study population 4 were smokers (Table 3).

Diet composition

The participants' habitual energy intakes ranged from 5500 to 13000 kJ/d (1310 to 3095 kcal/d), however, 52% of the subjects underreported their energy intake. The chemical analysis of the composition of the diets was comparable to the calculated diets, except for 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 (Table 2). All 3 experimental diets had a low to moderate GI and GL (32;33) (Table 1). The composition of walnuts (high in α -linolenic acid (ALA) and linoleic acid (LA)) and cashew nuts (high in oleic acid) (Table 2) were reflected in the diets and resulted in the anticipated increase in PUFA and MUFA concentrations, in the diets respectively. In particular, the fatty acid composition of the walnut, cashew nut and control diets (expressed as % of total fatty acids) was 6.42% ALA, 46.31% LA, 25.65% oleic acid; 1.05% ALA, 27.82% LA, 43.65% oleic acid and 0.86% ALA, 27.13% LA, 32.88% oleic acid respectively.

Lipid profiles

In Table 4, the serum lipid concentrations displayed no significant changes between walnut, cashew nut and control groups at baseline and in response to the intervention. The subjects in the control diet group showed a small significant increase in HDL-C compared to baseline (Table 4). Results from both nut diets displayed no significant change in HDL-C, TG, TC or LDL-C concentrations when compared to the baseline.

Markers of CHO metabolism

In **Table 5**, serum fructosamine, serum insulin (t=0min and t=120min), plasma glucose (t=0min and t=120min), insulin sensitivity and insulin resistance showed no significant difference at baseline between groups. Also, there was no significant difference in serum fructosamine, serum insulin, insulin sensitivity and insulin resistance between groups after the intervention diets. Plasma glucose concentrations (t=0) increased significantly by 0.70mmol/L ($P=0.04$) in the cashew nut group compared to the control group. Unlike the nut diets, fructosamine significantly increased in the control diet group at the end of the study period, when compared to the baseline concentrations. Both fasting serum insulin and serum insulin measured 2 hours later significantly increased in all 3 intervention groups when compared to the baseline. Therefore, the resultant insulin sensitivity was significantly decreased and in turn, the insulin resistance was significantly increased when compared to baseline. Insulin sensitivity had a tendency ($P=0.07$) to decrease more in the cashew nut compared to the control group. The 2-hours OGTT showed that there was no significant difference between the groups and when comparing baseline to end values.

BP, uric acid and S-hs CRP

Of all 3 intervention diets, systolic and diastolic BP as well as uric acid concentrations, displayed no significant change between groups at baseline and from baseline to end. The changes in S-hs CRP did not differ significantly between groups (**Table 6**). There was a significant increase in S-hs CRP concentrations in the walnut intervention group from baseline to end and no significant change in the cashew nut and control groups when comparing baseline to end values. Although not statistically significant, S-hs CRP was also increased, approximately to the same extent, in the control and cashew nut groups. The increase in the walnut group is therefore, probably not an independent effect of walnuts.

DISCUSSION

As far as we know this well-designed parallel, randomized controlled feeding trial investigating the effects of nuts on participants having the metabolic syndrome is the first study of its kind. Regarding our main objective, we found that both the walnut and the unsalted cashew nut intervention diets had no significant effect on the HDL-C, TG, TC, LDL-C, serum fructosamine, insulin, insulin sensitivity, insulin resistance, S-hs CRP, BP and serum uric acid concentrations when compared to the control diet. All 3 groups experienced highly significant increases in serum insulin concentrations when comparing the baseline to end. This in turn implied that insulin resistance increased and insulin sensitivity decreased in all three groups. Plasma glucose concentrations increased significantly in the cashew nut group compared to the control group resulting in a tendency for insulin sensitivity to decrease more in this group compared to the control group.

In a recent systematic review (Mukuddem-Petersen J, Oosthuizen W, Jerling JC, 2005) it was concluded from randomized controlled intervention trials that the consumption of 50-100g (≈ 1.5 -3.5 *servings*) of nuts 5 or more times/week as part of a heart healthy diet with a total fat content (high in mono- and /or polyunsaturated fatty acids) of $\approx 35\%$ E may significantly decrease TC and LDL-C.

Contrary to the outcomes of our controlled feeding trial, 4 out of 7 well-designed walnut studies (40-84g/day) displayed a significant decrease in TC and LDL-C when compared to Step I (healthy) (34), Mediterranean (hypercholesterolemic) (35;36) and Japanese (healthy) (37) diets. The average %E from fat in the aforementioned nut diets and the control diets were 31% and 29%, respectively. Two of the studies that showed no significant change in the lipid profile of hyperlipidemic subjects who followed a walnut intervention diet (41-78g/day) when compared to a low-fat (30%E from fat) and Step I diet (33%E from fat) provided $\geq 38\%$ E from fat (38). Similarly, in the current study, the walnut diet (60-100g/day) was high in fat (41%E from fat) compared to the control diet (33%E from fat). Therefore, these large differences in the percentage fat in the nut and control diet could possibly account for the absence of an effect on the lipid profile.

As no clinical trials have been done on cashew nuts before, we considered almond studies as they are similar in composition to cashew nuts. We expected the cashew nut study to have a beneficial effect on TC and LDL-C concentrations as seen in previous almond nut studies. In this context 3 out of 4, well-designed almond studies (54-100g/day) ranging from 32% to 39%E from fat, significantly decreased TC and LDL-C in hypercholesterolemic (39;40) and normocholesterolemic (41) subjects compared to subjects on a control diet (35%E from fat), low-fat (26.3%E from fat) and Step I diet (30%E from fat) (41). In this study, the cashew nut (66-115g/day) diet (37%E from fat) had no significant beneficial effect on the lipid profile (Table 4) when compared to the control diet that is lower in fat (33%E from fat). This outcome was very similar to results found by Lovejoy and collaborators (42) who showed that 57-113g of almonds per day (39%E from fat) had no significant beneficial effect on the lipid profile of diabetic subjects when compared to a high fat (37%E from fat) control group. The same outcome was achieved when the same amount of almonds (27%E) as part of a low fat diet was compared to a low fat (26%E from fat) control diet (42). Therefore in this context, reasons other than a high fat diet causing non-significance in lipid concentrations needs to be explored.

No direct studies have been done to investigate the effects of nuts on BP. Despite this, we anticipated an improvement in the BP readings with the walnut and cashew nut diets based on its fatty acid composition. In particular, emerging research has suggested possible health benefits associated with modest increases in dietary ALA (walnuts), including reduced BP (43-45). Also, numerous studies conducted in healthy and hypertensive individuals have shown a beneficial effect of MUFA (cashew nuts) on a number of outcomes related to cardiovascular risk, including BP (46). However, no

improvement in BP readings was seen after the nut intervention diets in the current controlled feeding trial.

Zhao and colleagues (47), concluded that a diet high in ALA, obtained from walnuts, walnut oil and flaxseed oil, elicited cardioprotective effects and vascular anti-inflammatory effects. Regarding the latter, it has been reported that walnuts are amongst the dietary plants that contain the most antioxidants (48) and several health effects have been ascribed to flavonoids (an antioxidant) including reduced inflammation (49). In our study, the walnut diet (high in ALA) resulted in an increase in S-hs CRP concentrations, although this was probably not an independent effect of walnuts. Recently, some evidence has been presented for a beneficial effect of MUFA on a number of outcomes related to cardiovascular risk, including reduced inflammation (44). Consequently, a cashew nut diet (high in MUFA) could be expected to improve the inflammatory parameter CRP. However, this was not evident in our study.

Numerous studies compared a low fat diet (21% ; 23%; 29%E from fat) (50-53) to a high-MUFA diet (35%; 40%E from fat) (50). The results of these studies provided similar glycemic control, as assessed by fasting glucose and insulin concentrations. Those authors concluded that provided the intake of saturated fatty acids (SFA) is low, a MUFA diet with a total fat content of up to 40%E has effects on glycemic control that are similar to those of the traditional high-CHO diet with fat limited to 25–30%E. An almond nut study conducted by Lovejoy and colleagues shown no effect on plasma glucose and insulin concentrations compared to a low-fat (26%E from fat) control group (olive and canola oil) in diabetics (42). In contrast to these findings, the significant increase of the plasma blood glucose ($t=0$) seen in the cashew nut diet (high MUFA (36.5%E from fat) group was unexpected. Even though serum fructosamine is a short-term marker of glycaemic control, its concentrations remained unchanged in the cashew nut group compared to the control group that increased. The unanticipated increase in insulin concentrations and consequent increased insulin resistance and decreased insulin sensitivity state in all 3 groups is difficult to explain. No other variables associated with insulin (e.g. TG), displayed significant changes after the experimental diets. The increased insulin concentrations cannot be ascribed to measurement errors. In particular, factors such as seasonal variation, timing of baseline and end analyses, differences in reagent batches used, temperatures of the fridges, duration of storage, method of analyses etc, were systematically excluded as possible explanations.

It can be speculated that the controlled feeding intervention may have contributed to the increase in insulin as a result of either an increase in CHO (quality and quantity), increase in fat or an increase in protein compared to the habitual diet. Increased dietary GL can create a self-perpetuating insulin resistance state and predicts greater CHD risk (54). However, this does not appear to be the reason for the increase in insulin resistance in this study, as all 3 dietary interventions had a low/moderate GI and

a moderate GL (Table 1). No comparisons can be made with the habitual diet, because its GI and GL were not determined. Secondly, the dramatic increase in insulin in our 2 nut diet groups may be explained by an increase in dietary fat compared to their habitual diet. Evidence from both animal and human studies show that insulin resistance is associated with an oversupply of lipids regardless of the origin (55;56). However, the aforementioned “dietary fat theory” is not applicable to the control group because they received a relatively lower fat diet (33%) which appears to be the same as the participants habitual diet. Thirdly, a recent, 5-week intervention study on type 2 diabetics (57) showed that an increase in dietary protein (from 15% to 30%E) and a decrease in CHO content (from 55% to 40%E) resulted in a significant reduction in total glycohemoglobin, a decrease in postprandial glucose concentrations and a modest increase in insulin concentrations. Notably, the aforementioned (high protein) study (40) only showed a modest increase in insulin compared to our dramatic increase. It could be speculated that this difference could in part be due to the fact that our subjects had the metabolic syndrome and not only Type 2 diabetes.

In our (moderate protein) study (17.5% to 19.6%E from protein), after the 3-week run-in period very high insulin concentrations (as seen after 8 weeks) was not evident (in the control group). This is possibly as a result of short duration, and therefore the dramatic increase was only visible after an additional 8 weeks.

One of the most common and potent causes of insulin resistance is obesity (8). In addition, it could be argued that the balanced caloric intake and the lack of physical activity provided the necessary impetus for the increased insulin resistance in our subjects having the metabolic syndrome (58). Furthermore, ATP III recommends that obesity should be the primary target of intervention for the metabolic syndrome. In turn, the first-line of therapy should be weight reduction reinforced with increased physical activity. Consequently, as most of the study population was obese (average BMI=35) and sedentary therefore it could be speculated that with such a high degree of obesity that even a “good diet including nuts” would not suffice in inducing beneficial effects without weight loss. Future nut research on this aspect is needed.

We have exhausted all possible explanations for this increase in insulin concentrations but a plausible one still eludes us. However, it must be noted that investigating insulin resistance was not the main aim of this study.

In conclusion, individuals having the metabolic syndrome showed no improvement in the markers of this syndrome after following a walnut diet or a cashew nut diet (8 weeks) compared to a control diet while maintaining body weight. The possibility that the dramatic increase in insulin resistance may

have masked the protective effects of the walnut and cashew nut diets in people having the metabolic syndrome can however not be excluded. Further research will be needed before a consensus can be reached.

Acknowledgements

We would like to extend a sincere word of thanks to the National Research Foundation (NRF) and the South African governments' Technology and Human Resources for Industry Program (THRIP) for their financial support as well as the food companies (Tiger brands, Pick 'n Pay, Clover, Unilever-Bestfoods-Robertsons) who donated various foods for the controlled feeding trial. To M.C. Lessing, E. Pienaar, Z. White, A. Greyling, C. de Witt, J. Wheeler, J. Bekker, C. Jansen van Rensburg, M. Bailey, L. Loots, V. van Scheltinga, L. Wiggett, L. Davics, L. van Wyk, E. Snyman, F. Mpho and T. Holele, A. Schutte, H. Huisman, J. van Rooyen, S. Jordaan, F. van der Westhuizen, C.S. Venter, H.H. Wright, H.S. Kruger, H. van't Riet, R. Breet, A. Van Graan, M. van Lieshout, D. Loots, M. Pieters-Loots, K. Moruisi, M. Opperman and M. Phometsi for contributing in various ways to the successful execution and completion of the controlled feeding trial – The Nut Study.

REFERENCES

1. Executive Summary of The 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). JAMA 2001;285:2486-97.
2. Grundy SM, Brewer HB, Jr., Cleeman JI, Smith SC, Jr., Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004;109:433-8.
3. Ford ES. Prevalence of the metabolic syndrome in US populations. Endocrinol. Metab Clin. North Am. 2004;33:333-50.
4. Wirfalt E, Hedblad B, Gullberg B et al. Food patterns and components of the metabolic syndrome in men and women: a cross-sectional study within the Malmo Diet and Cancer cohort. Am. J. Epidemiol. 2001;154:1150-9.
5. Shirai K. Obesity as the core of the metabolic syndrome and the management of coronary heart disease. Curr. Med. Res. Opin. 2004;20:295-304.
6. Jimenez-Cruz A, Seimandi-Mora H, Bacardi-Gascon M. [Effect of low glycemic index diets in hyperlipidemia.]. Nutr Hosp. 2003;18:331-5.

7. Esposito K, Marfella R, Ciotola M et al. Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *JAMA* 2004;292:1440-6.
8. Riccardi G, Giacco R, Rivellese AA. Dietary fat, insulin sensitivity and the metabolic syndrome. *Clin.Nutr* 2004;23:447-56.
9. Giacco R, Parillo M, Rivellese AA et al. Long-term dietary treatment with increased amounts of fiber-rich low-glycemic index natural foods improves blood glucose control and reduces the number of hypoglycemic events in type 1 diabetic patients. *Diabetes Care* 2000;23:1461-6.
10. McKeown NM, Meigs JB, Liu S, Saltzman E, Wilson PW, Jacques PF. Carbohydrate nutrition, insulin resistance, and the prevalence of the metabolic syndrome in the Framingham Offspring Cohort. *Diabetes Care* 2004;27:538-46.
11. Hu FB, Willett WC. Optimal diets for prevention of coronary heart disease. *JAMA* 2002;288:2569-78.
12. Balkau B, Charles MA, Drivsholm T et al. Frequency of the WHO metabolic syndrome in European cohorts, and an alternative definition of an insulin resistance syndrome. *Diabetes Metab* 2002;28:364-76.
13. Franz MJ. 2002 diabetes nutrition recommendations: grading the evidence. *Diabetes Educ.* 2002;28:756-4, 766.
14. Kris-Etherton PM, Zhao G, Binkoski AE, Coval SM, Etherton TD. The effects of nuts on coronary heart disease risk. *Nutr.Rev.* 2001;59:103-11.
15. Sabate J. Does nut consumption protect against ischaemic heart disease? *Eur.J.Clin.Nutr.* 1993;47 Suppl 1:S71-S75.
16. Foster-Powell K, Holt SH, Brand-Miller JC. International table of glycemic index and glycemic load values: 2002. *Am.J.Clin.Nutr* 2002;76:5-56.
17. Dreher ML, Maher CV, Kearney P. The traditional and emerging role of nuts in healthful diets. *Nutr.Rev.* 1996;54:241-5.
18. Dessein PH, Joffe BI, Stanwix AE. Effects of disease modifying agents and dietary intervention on insulin resistance and dyslipidemia in inflammatory arthritis: a pilot study. *Arthritis Res.* 2002;4:R12.

19. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA* 2002;287:356-9.
20. Kruger HS, Venter CS, Steyn HS. A standardized physical activity questionnaire for a population in transition: the Thusa study. *African journal for physical, health education, recreation and dance* 2004;6:54-64.
21. Bingham SA. Limitations of the various methods for collecting dietary intake data. *Ann.Nutr Metab* 1991;35:117-27.
22. Briefel RR, Sempos CT, McDowell MA, Chien S, Alaimo K. Dietary methods research in the third National Health and Nutrition Examination Survey: underreporting of energy intake. *Am.J.Clin.Nutr* 1997;65:1203S-9S.
23. Langenhoven ML, Kruger M, Gouws E, Faber M. MRC Food composition tables.3rd Ed. Parow: National Reserach Institute for Nutritional Diseases of the South African Medical Research Council. 2001:-245.
24. Katz A, Nambi SS, Mather K et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J.Clin.Endocrinol.Metab* 2000;85:2402-10.
25. Foley JE, Chen YD, Lardinois CK, Hollenbeck CB, Liu GC, Reaven GM. Estimates of in vivo insulin action in humans: comparison of the insulin clamp and the minimal model techniques. *Horm.Metab Res.* 1985;17:406-9.
26. Beard JC, Bergman RN, Ward WK, Porte D, Jr. The insulin sensitivity index in nondiabetic man. Correlation between clamp-derived and IVGTT-derived values. *Diabetes* 1986;35:362-9.
27. Galvin P, Ward G, Walters J et al. A simple method for quantitation of insulin sensitivity and insulin release from an intravenous glucose tolerance test. *Diabet.Med.* 1992;9:921-8.
28. Swan JW, Walton C, Godsland IF. Assessment of insulin sensitivity in man: a comparison of minimal model- and euglycaemic clamp-derived measures in health and heart failure. *Clin.Sci.(Lond)* 1994;86:317-22.
29. van Jaarsveld PJ, Smuts CM, Tichelaar HY, Kruger M, Benade AJ. Effect of palm oil on plasma lipoprotein concentrations and plasma low-density lipoprotein composition in non-human primates. *Int.J.Food Sci.Nutr* 2000;51 Suppl:S21-S30.

30. Official methods of analysis of AOAC International. Gaithersburg, Maryland, USA: AOAC International, 2002.
31. Abstracts of the 55th Annual Meeting of the AACC (American Association of Clinical Chemistry). Philadelphia, Pennsylvania, USA. July 20-24, 2003. Clin.Chem. 2003;49:A2-182.
32. Wolever TM, Jenkins DJ. Application of glycaemic index to mixed meals. Lancet 1985;2:944.
33. Foster-Powell K, Holt SH, Brand-Miller JC. International table of glycemic index and glycemic load values: 2002. Am.J.Clin.Nutr 2002;76:5-56.
34. Sabate J, Fraser GE, Burke K, Knutsen SF, Bennett H, Lindsted KD. Effects of walnuts on serum lipid levels and blood pressure in normal men. N.Engl.J.Med. 1993;328:603-7.
35. Zambon D, Sabate J, Munoz S et al. Substituting walnuts for monounsaturated fat improves the serum lipid profile of hypercholesterolemic men and women. A randomized crossover trial. Ann.Intern.Med. 2000;132:538-46.
36. Ros E, Nuncz I, Perez-Heras A et al. A walnut diet improves endothelial function in hypercholesterolemic subjects: a randomized crossover trial. Circulation 2004;109:1609-14.
37. Iwamoto M, Imaizumi K, Sato M et al. Serum lipid profiles in Japanese women and men during consumption of walnuts. Eur.J.Clin.Nutr. 2002;56:629-37.
38. Morgan JM, Horton K, Reese D, Carey C, Walker K, Capuzzi DM. Effects of walnut consumption as part of a low-fat, low-cholesterol diet on serum cardiovascular risk factors. Int.J.Vitam.Nutr.Res. 2002;72:341-7.
39. Spiller GA, Jenkins DA, Bosello O, Gates JE, Cragen LN, Bruce B. Nuts and plasma lipids: an almond-based diet lowers LDL-C while preserving HDL-C. J.Am.Coll.Nutr. 1998; 17:285-90.
40. Jenkins DJ, Kendall CW, Marchie A et al. Dose response of almonds on coronary heart disease risk factors: blood lipids, oxidized low-density lipoproteins, lipoprotein(a), homocysteine, and pulmonary nitric oxide: a randomized, controlled, crossover trial. Circulation 2002;106:1327-32.
41. Sabate J, Haddad E, Tanzman JS, Jambazian P, Rajaram S. Serum lipid response to the graduated enrichment of a Step I diet with almonds: a randomized feeding trial. Am.J.Clin.Nutr. 2003;77:1379-84.

42. Lovejoy JC, Most MM, Lefevre M, Greenway FL, Rood JC. Effect of diets enriched in almonds on insulin action and serum lipids in adults with normal glucose tolerance or type 2 diabetes. *Am.J.Clin.Nutr.* 2002;76:1000-6.
43. Hunter JE. n-3 fatty acids from vegetable oils. *Am.J.Clin.Nutr* 1990;51:809-14.
44. Ferrara LA, Raimondi AS, D'Episcopo L, Guida L, Dello RA, Marotta T. Olive oil and reduced need for antihypertensive medications. *Arch.Intern.Med.* 2000;160 :837-42.
45. Hermansen K. Diet, blood pressure and hypertension. *Br.J.Nutr* 2000;83 Suppl 1:S113-S119.
46. Roche HM, Zampelas A, Knapper JM et al. Effect of long-term olive oil dietary intervention on postprandial triacylglycerol and factor VII metabolism. *Am.J.Clin.Nutr* 1998;68:552-60.
47. Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ, Kris-Etherton PM. Dietary {alpha}-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. *J.Nutr* 2004;134:2991-7.
48. Halvorsen BL, Holte K, Myhrstad MC et al. A systematic screening of total antioxidants in dietary plants. *J.Nutr* 2002;132:461-71.
49. Nijveldt RJ, van Nood E, van Hoon DE, Boelens PG, van Norren K, van Leeuwen PA. Flavonoids: a review of probable mechanisms of action and potential applications. *Am.J.Clin.Nutr* 2001;74:418-25.
50. Luscombe ND, Noakes M, Clifton PM. Diets high and low in glycemic index versus high monounsaturated fat diets: effects on glucose and lipid metabolism in NIDDM. *Eur.J.Clin.Nutr* 1999;53:473-8.
51. Rodriguez-Villar C, Manzanares JM, Casals E et al. High-monounsaturated fat, olive oil-rich diet has effects similar to a high-carbohydrate diet on fasting and postprandial state and metabolic profiles of patients with type 2 diabetes. *Metabolism* 2000;49:1511-7.
52. Thomsen C, Rasmussen O, Christiansen C et al. Comparison of the effects of a monounsaturated fat diet and a high carbohydrate diet on cardiovascular risk factors in first degree relatives to type-2 diabetic subjects. *Eur.J.Clin.Nutr* 1999;53:818-23.
53. Perez-Jimenez F, Lopez-Miranda J, Pinillos MD et al. A Mediterranean and a high-carbohydrate diet improve glucose metabolism in healthy young persons. *Diabetologia* 2001;44:2038-43.

54. Liu S, Willett WC. Dietary glycemic load and atherothrombotic risk. *Curr.Atheroscler.Rep* 2002;4:454-61.
55. Storlien LH, Tapsell LC, Fraser A et al. Insulin resistance. Influence of diet and physical activity. *World Rev.Nutr Dict.* 2001;90:26-43.
56. Ouyang P, Sung J, Kelemen MD et al. Relationships of insulin sensitivity with fatness and fitness and in older men and women. *J.Womens Health (Larchmt.)* 2004;13:177-85.
57. Nuttall FQ, Gannon MC. Metabolic response of people with type 2 diabetes to a high protein diet. *Nutr Metab (Lond)* 2004;1:6.
58. Bessesen DH. The role of carbohydrates in insulin resistance. *J.Nutr* 2001;131:2782S-6S.
59. Langenhoven M, Kruger, M., Gouws E, and Faber M. MRC food composition tables. Supplement third edition (1991). 1-245. 1991. Tygerberg, South Africa, Medical Research Council.
60. Kruger M, Langenhoven M, and Faber M. Fatty acid and amino acid composition tables. Supplement to MRC food composition tables (1991). 1-205. 1992. Tygerberg, South Africa, Medical Research Council.

TABLE 1

Planned and analyzed composition of diets as well as the habitual diets

Nutrients, glycemic index & glycemic load	Habitual diet prior to dietary intervention (n=64)	Walnut diet (n=21)		Cashew nut diet (n=21)		Control diet (n=22)	
	FFQ ¹	Planned ¹	Analyzed ²	Planned ¹	Analyzed ²	Planned ¹	Analyzed ²
Protein (% of energy)	15.1	15.7	17.5	16.2	19.1	16.4	19.6
Carbohydrate (% of energy)	49.2	48.9	42.1	46.8	44.4	51.3	47.2
Fat (% of energy)	33.2	35.0	40.3	37.1	36.5	32.8	33.2
SFA (% of energy)	11.0	7.49	8.2	9.25	9.4	7.7	12.2
MUFA (% of energy)	11.3	10.6	10.7	15.9	16.3	9.4	11.3
PUFA (% of energy)	7.5	15.1	21.4	7.83	10.8	8.1	9.5
Cholesterol (mg/d)	316	226	-	224	-	246	-
Fibre (g/d)	30.7	34.1	-	33.4	-	27.8	-
Glycemic index (33) ³	-	54 ³	-	53 ³	-	56 ³	-
Glycemic load (33) ³	-	14 ³	-	14 ³	-	15 ³	-

¹Determined by using the FoodFinder 2 Program (Medical Research Council of South Africa, Tygerberg) - Mean of 14-day menu²Laboratory analysis³Calculated with the aid of the GI and GL tables by Foster-Powell et al., 2002 (33)

FFQ: Food Frequency Questionnaire; SFA: Saturated fatty acid; PUFA: Polyunsaturated fatty acid; MUFA: Monounsaturated fatty acid.

TABLE 2

Macronutrient composition of 100g of walnuts and unsalted cashew nuts

Selected Nutrients	Walnuts		Cashew nuts	
	Laboratory analysis	Food composition tables (59;60)	Laboratory analysis	Food composition tables (59;60)
Energy				
(kJ)		2686		2402
(kcal)		642		574
Protein (g)	13.0	14.3	20.5	15.3
CHO (g)	3.89	13.5	19.4	26.6
Fibre (g)	5.75	6.10	5.62	4.80
Total fat (g)	73.2	61.9	48.4	46.4
MUFA (g)	13.4 (18.3 ¹)	14.2	25.6 (52.9 ¹)	27.3
Oleic acid (C18:1) (g)	13.2 (18.0 ¹)	21.5	25.5 (52.6 ¹)	57.8
PUFA (g)	53.8 (73.5 ¹)	39.1	15.3 (31.6 ¹)	7.84
Linoleic acid (C18:2) (g)	44.7 (61.0 ¹)	51.3	15.0 (31.0 ¹)	16.5
α -Linolenic acid (C18:3) (g)	9.15 (12.5 ¹)	11.0	0.247 (0.51 ¹)	0.34
SFA (g)	6.0 (8.20 ¹)	5.59	7.51 (15.5 ¹)	9.16
Palmitic acid (C16:0) (g)	4.41 (6.02 ¹)	6.85	4.08 (8.43 ¹)	9.38
Stearic acid (C18:0) (g)	1.47 (2.01 ¹)	1.74	3.09 (6.38 ¹)	6.40

CHO: Carbohydrate, MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid.

SFA: Saturated fatty acid.

¹Percentage of total fatty acids.

TABLE 3
 Characteristics at baseline for all subjects

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 (y)	45	40.4, 50.2	46	40.7, 50.7	45	40.8, 49.3
Weight (kg)	107	99.4, 115	99	92.5, 106	106	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	103, 115	105	98.5, 111	108	102, 113
Cigarette smokers	1	-	0	-	3	-

CI: Confidence interval; BMI: Body mass index; WC: Waist circumference; TC: Total cholesterol.

TABLE 4

Serum lipids during intervention

Serum lipids (mmol/L)	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)	P-value (B vs. E)	Mean	95% CI	P-value (B vs. E)	P-value (B vs. E)	Mean	95% CI	P-value (B vs. E)	P-value (B vs. E)	
HDL-C	B	0.94	0.83, 1.05	0.344	1.02	0.88, 1.16	0.706		0.85	0.77, 0.93	0.04	0.08	
	E	0.91	0.80, 1.01		1.01	0.90, 1.12			0.91	0.82, 1.0			
	Δ	-0.03	-0.10, 0.04		-0.01	-0.09, 0.06			0.06	0, 0.11		0.11	
TG	B	1.90	1.48, 2.32	0.798	1.81	1.33, 2.30	0.364		1.86	1.55, 2.16	0.41	0.96	
	E	1.86	1.44, 2.28		1.64	1.23, 2.05			1.96	1.62, 2.31			
	Δ	-0.04	-0.35, 0.27		-0.16	-0.52, 0.20			0.11	-0.16, 0.38		0.45	
TC	B	4.80	4.54, 5.18	0.833	4.49	3.98, 5.34	0.619		4.90	4.39, 5.52	0.09	0.55	
	E	4.72	4.28, 5.76		4.51	3.87, 5.64			5.39	4.44, 5.83			
	Δ	-0.03	-0.30, 0.37		0.07	-0.22, 0.49			0.07	-0.06, 0.60		0.46	
LDL-C	B	2.99	2.63, 3.29	0.357	2.64	2.33, 3.16	0.247		3.21	2.79, 3.79	0.22	0.11	
	E	3.19	2.38, 3.94		2.84	2.26, 3.60			3.36	2.86, 3.84			
	Δ	0.18	-0.21, 0.50		0.12	-0.25, 0.55			0.19	-0.11, 0.46		1.00	

B: Baseline (after 3-week run-in period); E: End; Δ: Change from baseline to end; HDL-C: High-density lipoprotein cholesterol; TG: Triacylglycerol; TC: Total cholesterol;

LDL-C: Low-density lipoprotein cholesterol; B vs E: P-values for change from baseline to end of intervention period.

Significance was set at $P \leq 0.05$.

TABLE 5.
Markers of carbohydrate metabolism during the interventions

Markers	Walnut diet				Cashew nut diet				Control diet				P-value between groups (ANOVA)
	Mean	95% CI	P-value (B vs. E)	Mean	95% CI	P-value (B vs. E)	Mean	95% CI	Mean	95% CI	P-value (B vs. E)	P-value between groups (ANOVA)	
Serum fructosamine ($\mu\text{mol/L}$)	B	210	202, 218	0.79	212	206, 218	0.32	204	204	198, 210	0.04	0.23	0.24
	E	209	201, 217		214	206, 222		210	210	203, 218			
	Δ	-0.95	-8.16, 6.26		2.29	-2.38, 6.96		6.00	6.00	0.20, 11.8			
Serum insulin ($\text{t}=0$ $\mu\text{U/mL}$)	B	14.0	10.0, 18.0	<0.001	12.0	9.00, 16.0	<0.001	12.5	12.5	11.0, 17.0	<0.001	0.70	0.74
	E	50.0	42.0, 72.0		52.0	36.0, 88.0		48.0	48.0	24.0, 87.0			
	Δ	38.0	19.0, 55.0		43.0	25.0, 66.0		35.5	35.5	16.0, 73.0			
Serum insulin ($\text{t}=120$ $\mu\text{U/mL}$)	B	16.0	12.0, 20.0	<0.001	11.0	7.00, 18.0	<0.001	12.0	12.0	10.0, 19.0	<0.001	0.25	0.73
	E	57.0	40.0, 67.0		50.0	34.0, 67.0		55.0	55.0	35.0, 94.0			
	Δ	43.0	29.0, 55.0		41.0	24.0, 58.0		43.0	43.0	15.0, 66.0			
Plasma glucose ($\text{t}=0$ (mmol/L))	B	4.50	4.30, 5.20	0.64	4.70	4.30, 5.10	0.03	4.55	4.55	4.30, 5.40	0.18	0.73	0.04
	E	4.70	4.00, 5.40		5.30	4.70, 6.20		4.55	4.55	3.80, 5.40			
	Δ	0.40	-0.60, 0.70		0.70 ¹	-0.30, 1.60		-0.75 ¹	-0.75 ¹	-1.40, 0.80			
Plasma glucose ($\text{t}=120$ (mmol/L))	B	4.70	4.50, 5.40	0.09	4.60	4.10, 5.30	0.25	4.60	4.60	4.30, 5.10	0.87	0.94	0.80
	E	5.50	4.40, 6.30		5.00	4.20, 5.90		4.80	4.80	3.90, 6.10			
	Δ	0.50	-0.20, 1.30		0.40	0.70, 1.40		-0.20	-0.20	-0.90, 1.10			
Insulin sensitivity	B	0.57	0.50, 0.59	<0.001	0.58	0.52, 0.62	<0.001	0.56	0.56	0.52, 0.58	<0.001	0.75	0.07
	E	0.42	0.38, 0.46		0.41	0.36, 0.43		0.42	0.42	0.38, 0.53			
	Δ	-0.14	-0.17, 0.11		-0.18	-0.19, -0.14		-0.13	-0.13	-0.17, -0.06			
Insulin Resistance	B	2.61	2.24, 4.48	<0.001	2.35	1.84, 3.82	<0.001	2.81	2.81	2.35, 3.76	<0.001	0.73	0.84
	E	11.1	6.65, 18.5		12.4	9.20, 26.9		10.1	10.1	3.41, 18.2			
	Δ	8.52	3.08, 15.3		10.4	6.76, 22.4		7.50	7.50	1.69, 12.7			

B: Baseline; E: End; Δ : Change from baseline to end; B vs E: P-values for change from baseline to end of intervention period; t: Time; OGTT: Oral glucose tolerance test.

¹Significant difference between cashew nut group and control diet group (significance was set at $P \leq 0.05$).

TABLE 6

Blood pressure, uric acid and C-reactive protein changes within and between groups during intervention

Markers		Walnut diet			Cashew nut diet			Control diet			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)	
Blood pressure (mmHg)											
	Systolic	B	128	126, 131	0.26	131	126, 135	0.28	131	125, 137	0.66
		E	130	126, 133		128	123, 134		133	128, 137	
		Δ	2.21	-1.74, 6.16		-2.45	-7.09, 2.19		1.74	-2.45, 5.92	0.22
Diastolic		B	78.7	76.3, 81.0	0.49	77.0	74.0, 80.0	0.72	79.2	76.1, 82.2	0.79
		E	79.1	75.9, 82.0		76.0	73.1, 80.0		79.6	76.9, 82.4	
		Δ	1.21	-2.39, 4.81		-0.59	-4.01, 2.83		0.48	-3.11, 4.07	0.76
Serum uric acid (μmol/L)		B	388	347, 429	0.30	373	329, 418	0.65	412	368, 456	0.57
		E	401	361, 440		377	328, 426		416	376, 457	
		Δ	12.6	-12.2, 37.3		3.86	-13.5, 21.2		4.64	-12.1, 21.4	0.78
		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)
S-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
		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; Δ: Change from baseline to end; B vs E : P-values for change from baseline to end of intervention period S-hs CRP: Serum high sensitivity C-reactive protein.

Significance was set at $P \leq 0.05$.

4

Discussion and Conclusions

1 Introduction

This chapter, discusses the strengths, limitations and problems experienced in the study, a summary of the main findings, conclusions and recommendations. In particular, more details of some of the aforementioned issues are given in Chapters 2 and 3.

The overall purpose of this study was to investigate the role of a nut diet in individuals having the metabolic syndrome. In this regard, the main objective was to explore the effects of walnuts and unsalted cashew nuts on markers of this syndrome. This objective was realized by conducting a randomized, controlled feeding trial (The Nut study) (n=64).

Our secondary objective was to conduct a systematic review on dietary intervention trials that investigates the effects of nuts on the lipid profile. This review served as a background to facilitate the interpretation of some of the data from the main study.

2 Strengths of the study (Chapter 2 & 3)

- The topic addressed by the systematic review is unique (has not been discussed in full detail before) and pertinent to both the scientific and lay community (Chapter 2).
- A systematic review has the following advantages (adapted from Greenhalgh, 1997):-
 - It allows for a large number of studies on a topic to be identified, summarized and evaluated.
 - Both the reliability and accuracy of conclusions are enhanced by the review procedure.
 - Bias is limited by using explicit methods when identifying and rejecting studies.
 - There is a reduction in elapsed time between research discoveries and implementation of effective diagnostic and therapeutic strategies.
 - New hypotheses can be generated and reasons for heterogeneity identified about particular subgroups.
 - The findings of different studies can be compared formally to establish generalisability and consistency of results.

- ▣ This systematic review encapsulates the findings of various clinical trials and suggested guidelines are given (ideal quantity and frequency of nut consumption per day) for normo- and hyperlipidemic individuals (Chapter 2).
- All clinical trials that comply to the inclusion criteria were included in this systematic review (Chapter 2).
- ▣ The main study is the first to investigate the effects of nuts (walnuts and unsalted cashew nuts) on the markers of the metabolic syndrome (Chapter 3).
- The fact that this study was well-designed (controlled, randomized and in the form of a controlled feeding trial) adds to the reliability of the results (Chapter 3).
- The duration (3 weeks run-in period, 8 weeks experimental period) and sample size (n=64) enhances the study design (Chapter 3).

3 Limitations and problems experienced in the study

The following limitations were identified in the systematic review (Chapter 2):

- The independent effects of the control diets (high-fat, low-fat, Step I, Step II, low-fat high complex carbohydrate (CHO), average American, olive-oil, habitual, Mediterranean, Japanese) may have masked potential effects or accentuated others.
- The differences in the study populations due to underlying disease, may have resulted in altered lipid metabolism.
- The results of all the studies were included, however, no discussions were done or conclusions drawn for those that were poorly designed and had insufficient reporting of results.
- Due to small sample sizes, all the studies did not have adequate power to detect clinical significant changes in high-density lipoprotein cholesterol (HDL-C) and triacylglycerol (TG). In this regard, conclusions about HDL-C and TG could not be drawn.
- The length of the diet periods of the clinical trials in this systematic review was sufficient to achieve stabilization of blood lipids. However, sustainability of the outcomes could not be evaluated with duration periods of 4-6 weeks.
- Publication bias (the tendency on the part of investigators, reviewers, and editors to submit or accept manuscripts for publication based on the direction or strength of the study findings) may have taken place.

The following limitation was identified in the main study (Chapter 3):

- Despite the fact that the 3 dietary interventions were planned to be the same, the chemical analyses of the walnuts used in the study showed the fat composition to be much higher than that found in the food composition tables (used to calculate the dietary composition). Therefore, the varying composition of these 3 dietary intervention groups makes comparisons a bit more difficult than if they had similar macronutrient distributions.

4 Summary of the main findings

Firstly, it was apparent from the systematic review that the results of the almond, peanut, pecan nut and walnut studies showed convincing evidence for a lipid lowering effect of total cholesterol (TC) between 2-16% and low-density lipoprotein cholesterol (LDL-C) between 2-19%, when compared to their control diets. Moreover, there are indications from inadequately designed intervention studies that hazelnuts and pistachio nuts may also have a lipid lowering effect. Currently, the evidence for macadamia nuts is less convincing. In addition, it seems that the components in nuts further reduce TC and LDL-C concentrations beyond the effects predicted by equations based solely on fatty acid distribution of dietary interventions.

Secondly, with regard to our main objective, we found that both the walnut and unsalted cashew nut intervention diets had no significant effect on the lipid profile, serum fructosamine, insulin, insulin sensitivity, insulin resistance, serum high sensitivity C-reactive protein (S-hs CRP), blood pressure (BP) and serum uric acid concentrations when compared to the control diet. All 3 groups experienced highly significant increases in serum insulin concentrations when comparing the baseline to end ($P < 0.05$). In turn, insulin resistance increased while insulin sensitivity decreased in all three groups. Plasma glucose concentrations increased significantly in the cashew nut group compared to the control group ($P < 0.05$). By contrast, serum fructosamine was unchanged in the cashew nut group while the control group had significantly increased concentrations of this short-term marker of glycaemic control.

5 Conclusions

The results of this study met the main aims and secondary objective set in Chapter 1.

From the systematic review it was concluded that the consumption of ≈ 50 -100g (≈ 1.5 -3.5 *servings*) of nuts 5 or more times/week as part of a heart-healthy diet with total fat content of $\approx 35\%$ of energy may significantly decrease TC and LDL-C in normo-and hyperlipidemic

individuals. This review provided the necessary background for the interpretation of some of the data from the main study. Regarding the latter, subjects displayed no improvement in the markers of the metabolic syndrome after following a walnut diet or a cashew nut diet compared to a control diet while maintaining body weight (8 weeks). Firstly, we suspect that the effects of nuts on people having the metabolic syndrome may be different to the effects on hyperlipidemic individuals. Secondly, it may be possible that the dramatic increase in insulin resistance may have masked the protective effects of the walnut and cashew nut diets in people having the metabolic syndrome. The two nut intervention diets, especially the walnut diet, did not prove to have any detrimental effects. However, in this context, additional research is needed before reaching a verdict.

The results of our studies are therefore valuable in augmenting the current knowledge about nuts and how they may impact our health. Ultimately, the strength of our research lies in its novelty, topicality and results achieved. Notably, our studies have 3 *firsts* associated with them. The topic addressed has not been presented in the peer reviewed literature in the form of a systematic review before. The Nut Study was the first study to examine the effects of nuts on markers of the metabolic syndrome. Lastly, it was the first controlled feeding trial in South Africa.

Our studies make a contribution to the improvement of economic and therapeutic activities related to nuts. In particular, cashew and walnut crops are cultivated in South Africa with the nut industry, farmers and the nut cracking industry having a vested interest in the outcomes of nut-related research. Furthermore, studies of this nature may stimulate interest among consumers, which in turn impacts the profitability of the nut industry and the state of the local economy. Furthermore, an increase in the quantity and quality of research studies on metabolic syndrome positively affect the standard of dietary treatment by the medical community. In this context, based on conclusions from the systematic review, health professionals will be able to encourage hyperlipidemic patients to have the frequent consumption of nuts form part of their heart-healthy lifestyle.

6 Recommendations

The results from our investigations provide the impetus for conducting future research. In this regard, improved feeding studies with larger sample sizes and longer duration should investigate the effects of nuts on TG and HDL-C concentrations. This should apply to mixed

nuts and those individual nuts not yet considered. In addition, the unique nutrient and non-nutrient composition of nuts requires further research in order to elucidate the possible mechanisms responsible for the LDL-C lowering effect.

It is well known that all 5 components of the metabolic syndrome are improved by even modest amounts of weight loss achieved with diet and exercise (Avenell *et al.*, 2004). Therefore, the first-line of therapy should be weight reduction reinforced by increased physical activity. In particular, a similar nut controlled feeding trial with caloric restriction should be conducted on participants having the metabolic syndrome. In this regard, chemical analyses of the intervention diet should be done during a pilot study in order to ensure that the calculated dietary composition based on Food Composition Tables correspond to the actual dietary composition. However, more scientific investigations related to the metabolic syndrome remain to be done.

7 References

AVENELL, A., BROOM, J., BROWN, T.J., POOBALAN, A., AUCOTT, L., STEARNS, S.C., SMITH, W.C., JUNG, R.T., CAMPBELL, M.K. & GRANT, A.M. 2004. Systematic review of the long-term effects and economic consequences of treatments for obesity and implications for health improvement. *Health technology assessment*, 8(21):iii-182.

GREENHALGH, T. 1997. Papers that summarise other papers (systematic reviews and meta-analysis). *British medical journal*, 315:572-675.

Addendum

The Nut Study: CALENDAR – Cohort 1

Day	August		September		October	
Wed					1	
Thur					2	
Fri	1				3	
Sat	2				4	
Sun	3				5	
Mon	4		1		6	
Tue	5		2	** A M  BP 	7	
Wed	6		3		8	
Thur	7		4		9	
Fri	8		5		10	
Sat	9		6		11	
Sun	10		7		12	
Mon	11		8		13	
Tue	12		9		14	
Wed	13		10		15	
Thu	14		11		16	
Fri	15		12		17	
Sat	16		13		18	
Sun	17		14		19	
Mon	18		15		20	
Tue	19		16		21	
Wed	20		17		22	
Thur	21		18		23	
Fri	22		19		24	
Sat	23		20		25	
Sun	24		21		26	
Mon	25		22		27	
Tue	26		23		28	** A M  BP 
Wed	27		24		29	
Thur	28		25		30	
Fri	29		26		31	
Sat	30		27			
Sun	31		28			
Mon			29			
Tue			30			

Days that blood samples will be drawn

BP

Blood pressure

A

Arterial stiffness

M

Measurements/anthropometry

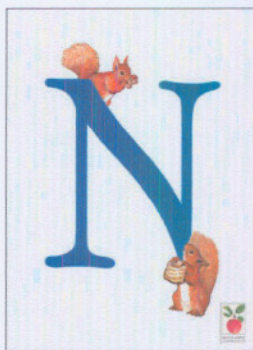


Weighing

*** All meals will be received (including weekends)**

*	3 week run-in period: 12 August to 1 September
*	8 week experimental period: 2 September to 31 October

**** Participants need to book one of these days for the drawing of blood samples, etc**



Nuts about nuts

The Nut Study – Food Frequency Questionnaire (FFQ) or FREKWENSIEVRAELYS OM GEBRUIKLIKE VOEDSELINNAME TE MEET

Proefpersoonnummer: _____

Naam _____ Geslag _____

Adres: _____ Geboortedatum: _____

Etniese groep: _____ Taal: _____ Datum van opname: _____

Beroep: _____ Beroep van gesin se broodwinner: _____

BEANTWOORD ASSEBLIEF DIE VOLGENDE VRAE:

1. Hoeveel persone eet gewoonlik by die huis? _____
Volwassenes (u ingesluit): _____ 5 of 16-jariges: _____ 1 tot 4-jariges _____
jonger as 1-jaar: _____
2. Is u op 'n spesiale dieet? (spesifiseer) _____
3. Gebruik u een van die volgende dieetaanvullings? Onderstreep / spesifiseer:
vitamiene (1), tonikums (2), gesondheidskosse (3), liggaamsboumiddels (4), dieetvesel (5),
gewigsverliesmiddels (6), ander (spesifiseer) _____
4. Gebruik u addisionele sout aan tafel? (hoeveel) _____
5. Hoe gereeld eet u kerrievoedsel: (sterk/matig) _____
6. Hoe gereeld gebruik u knoffel en ander kruie? _____
7. Sny u die vet van vleis af voor voorbereiding? _____
8. Hoeveel keer per week eet u dierlike proteïen (vleis/vis/vark/hoender) _____

HIERDIE VRAELYS HANDEL OOR DIE VOEDSEL WAT U GEWOONLIK EET.

Dui asseblief die hoeveelhede* en die hoeveelheid kere wat u 'n voedsel eet, aan. As daar meer as een opsie by 'n vraag op u van toepassing is, onderstreep dit wat van toepassing is en merk die voedsel en sy hoeveelhede met 'n teken, byvoorbeeld: boontjies, ertjies, brusselse spruitjies, sodat die hoeveelhede maklik met die tipe voedsel in verband gebring kan word.

*Hoeveelhede word as volg aangedui (gebruik simbool in hakies)

gram (g), koppie (k), teelepel (t), eetlepel (E), milliliters (ml), snye (s), porsies (p).

Die dikte van snye brood is as volg:

dun: 1 cm en minder

medium: tussen 1 en 2 cm

dik: 2 cm en meer

VOEDSELITEM EN BESKRYWING	Hoeveelheid per keer (k, t ens.)	Kere geëet				Kode
		per dag	per week	per maand	nooit/selde	
BROOD/BROODROLLE: Volgraan/bruin/wit; dun/medium/dik						
Ander: brosbrood, provita, matzos, cream crackers ens.						
PAP: Hawermout, Maltebella, Mielie (slap, styf of putupap)						
GRAANKOS: Pro Nutro, 'Rice Crispies', Muesli, All Bran, ander:						
Suikerbedekte (bv. 'Sugar Puffs')						
Ander:						
SUIKER: (saam met pap of graankos gebruik)						
GRAAN: Rys (Bruin/wit), Koring						
Mielierys, stampmielies, heel mielies						
Stampmielies en bone (gee die verhouding)						
PASTA: Noem tipe, asook gereg bv. noedels, spaghetti bolognaise, macaroni en kaas						
KOEK EN TERT:						
Soet: Skons, pannenkoek, vetkoek, laagkoek, terte, ens.						
Sout: Worsrolletjies, samoesas, ens.						
BESKUIT: (noem tipe)						
SKYFIES: (aartappelskyfies, springmielies, ens.)						
NAGEREG: jellies en waterbasisnageregte						
Melkbasis bv. kitspoedings en vla						
Gebakte/gestoomde poedings						
LEKKERS: Suiglekkers (noem soort)						
Toffies 'fudge' (noem soort)						
Sjokolade (stafies en blokke) (noem soort)						
KONFYT, HEUNING, STROOP (noem soort)						
BROODSMEER: grondboontjebotter, 'marmite', vissmeer, ens.						
MELK: As 'n drank (vars./suur/dikmelk), (vol./ lae-vet,/ afgeroom)						
MELKDRANKE: milo, melkskommels, ens.						
MELK: (by tee of koffie, pap of ontbytkosse)						
SUIKER (bygevoeg by tee/koffie)						
POEIERMELK (volroom/afgeroom) droë poeier/aangemaak?						
MENGSELS VAN VARS EN POEIERMELK (noem verhouding)						
INGEDAMPTE OF KONDENSEMELK						
NIE-SUIWELVERROMERS: bv. 'Cremora'						
KAAS: noem tipe bv. cheddar, soetmelk, ens						
MAASKAAS/GEROOMDE KAAS: (vetvry/laevet/gewoon)						
ROOM: Vars/'orley whip'						
ROOMYS: (noem soort)						
EIERS: gekook, geposjeer, gebak (vet of olie) omelet, Roereiers, soufflés, ens.						

VOEDSELITEM EN BESKRYWING	Hoeveelheid per keer (k, t ens.)	Kere geëet				Kode
		per dag	per week	per maand	nooit/selde	
<u>VLEIS: HOVEEL KEER PER WEEK EET U VLEIS?</u>						
<u>ROOIVLEIS:</u> (spesifiseer gerooster, gebak, gekook, gebraai; dit sluit gemaalde vleis in)						
Beesvleis (met vet/sonder vet)						
Skaapvleis (met vet/sonder vet)						
Varkvleis/sppek (met vet/sonder vet)						
Wors (spesifiseer soort)						
Maalvleis						
<u>PLUIMVEE:</u> (spesifiseer gerooster, gebak, gekook, gebraai) wit vleis / donker vleis						
<u>ORGAANVLEIS:</u> Afval, lewer, niertjies, spesifiseer						
<u>KOUEN EN GEPROSESEERDE VLEIS</u> spesifiseer						
<u>VLEISPASTEIE EN BREDIES</u> (spesifiseer soort)						
<u>SOUSE EN VLEISEKSTRAK</u> (spesifiseer soort)						
<u>SOUSE:</u> bv. tamatiesous, atjar, bruinsous, spesifiseer						
<u>VIS:</u> bv. Haring, tuna, visvingers. (Spesifiseer vars, gevries/geblik en gerooster/ gebak of gestoom , gebraai)						
<u>SKULPVIS:</u> (spesifiseer)						
<u>GEDROOGDE PEULGROENTES:</u> (bone, erte lensies) spesifiseer						
<u>NEUTE:</u> (spesifiseer)						
<u>PLANTPROTEÏENPRODUKTE:</u> Toppers, TVP, Sossies, ens.						
<u>SOP:</u> water- of melkbasis (geblik of in pakkies of tuisgemaak). spesifiseer , bv. ertjiesop						
<u>GROENTE:</u> (vars/gevries/geblik) Tipe:						
<u>HOVEEL PORSIES GROENTE EET U PER DAG?</u>						
Rou slaagroentes bv. tamatie kropslaai ens						
Gekookte slaagroentes bv. beet en aspersies						
Groen blaagroentes bv. spinasie ens						
Groen groentes bv. ertjies, groenbone, ens						
Uie/tamaties (gekook)						
Geelgroentes, bv. pampoen, wortels, ens.(soet/sout)						
Wit groentes, bv. blomkool, radyse, ens						
Groenmielies						
Gemengde groente (soet/sout)						
Eiervrug of sampioene						
Aartappels (gekook, gebak, fyn, aartappelslaai)						
Witsous/kaassous by groente						
<u>VRUGTE:</u> (vars, geblik, gedroog)						
<u>HOVEEL PORSIES VRUGTE EET U PER DAG?</u>						
Appels en pere						
Steenvrugte (perskes, pruime, ens.)						
Sitrusvrugte						
Piesangs						
Koejawels						

VOEDSELITEM EN BESKRYWING	Hoeveelheid per keer (k, t ens.)	Kere geëet				Kode
		per dag	per week	per maand	nooit/selde	
Ander (spesifiseer)						
Avokado's of olywe						
Bessievrugte						
Gestoofde of geblikte vrugte (met/sonder suiker)						
Vrugterolle of verglansde vrugte						
<u>VETTE EN OLIES:</u> op brood, in slaai, groente						
Botter						
Margarien: (sag/hard) Tipe:						
Vet, olie, kailings, ens.						
Mayonnaise, slaaisous, (volvet/ lae-vet/ vetvry)						
<u>DRANKE:</u>						
Koffie of tee						
Suiker gebruik per koppie						
Vrugtesap (vars, 'liquifruit', ens noem soort)						
Ander: koeldranke bv Oros, ens						
Gaskoeldranke ('Coca Cola', 'Lemon Twist', ens)						
Magou						
<u>ALKOHOLIESE DRANKE:</u>						
Sorghumbier (Tlokwe ens)						
Bier						
Sjerrie, Vermoet, Portwyn, ens. (soet, medium, droog)						
Tafelwyn (medium, soet of droog)						
Spiritualieë bv brandewyn, whisky, jenewer						
Likeur (noem soort)						
Mengeldrankies (spesifiseer)						



The Nut Study – Physical activity questionnaire (PAQ)

Date: _____

Place:

Interviewer:

The information on this questionnaire is confidential

Name: _____

1.	Subject number					(1-4)		
2.	Gender					(5)		
3.	What is your main occupation?.....							
	Low level: office work, housework, scholar					1		
	Middle level: factory work, carpentry, farming, hospital nurse, plumber					2		
	High level ("sweat work"): construction work, digging, manual labour					3		
4.	At work I sit	1. never	2. seldom	3. sometimes	4. often	5. always	(7)	
5.	At work I stand	1. never	2. seldom	3. sometimes	4. often	5. always	(8)	
6.	At work I walk	1. never	2. seldom	3. sometimes	4. often	5. always	(9)	
7.	At work I lift heavy loads	1. never	2. seldom	3. sometimes	4. often	5. always	(10)	
8.	At work I am tired	1. never	2. seldom	3. sometimes	4. often	5. always	(11)	
9.	At work I sweat	1. never	2. seldom	3. sometimes	4. often	5. always	(12)	
10.	Do you commute?				yes	1	(13)	
					no	2		
11.	If yes, how do you get to work/school?				walk	1		
					cycle	2		
					car/taxi	3		
12.	How long does it take you to walk/cycle to work/school? (or to the taxi rank/ bus stop/ train station)				0-15 min	1	(14)	
					16-30 min	2		
					31-60 min	3		
					1-2 hours	4		
13.	If you walk or cycle to work/school, what is your usual pace? (or to taxi rank/bus stop/ train station)				casual strolling		1	(15)
					fairly brisk		2	
					brisk/fast		3	
14.	Do you climb stairs often?				yes		1	(16)
					no		2	
15.	If yes, how many flights of stairs do you climb each day? (1 flight = 10							(17)

steps)							
16.	How many days per week do you climb steps?			(18)			
17.	Do you play sport?	yes	1				
		no	2	(19)			
18.	Which sport do you play most frequently?	low level: bowling, golf, billiards	1	0.76* ¹			
		middle level: tennis, athletics, cycling	2	1.26			
		high level: soccer, rugby, netball, boxing	3	1.76(20)			
		If other, specify					
19.	How many hours per week do you practice? <1/ 1-2/ 2-3/ 3-4/ >4 (Write appropriate code in space) 0.5, 1.5, 2.5, 3.5, 4.5* ²			(21-23)			
20.	How many months per year ? <1/ 1-3/ 4-6/ 7-9/ >9 (Write appropriate code in space) 0.04, 0.17, 0.42, 0.67, 0.92* ³	.		(24-26)			
* ¹ intensity code of sport, * ² time code for sport, * ³ proportion of year							
21.	If you play a second sport, which is it?	low level: bowling, golf, billiards	1	0.76* ¹			
		middle level: tennis, athletics, cycling	2	1.26			
		high level: soccer, rugby, netball, boxing	3	1.76(27)			
		Other, specify					
22.	How many hours per week do you practice? <1/ 1-2/ 2-3/ 3-4/ >4 0.5, 1.5, 2.5, 3.5, 4.5* ²		.	(28-30)			
23.	How many months per year? <1/ 1-3/ 4-6/ 7-9/ >9 0.04, 0.17, 0.42, 0.67, 0.92* ³	.		(31-33)			
24.	During leisure time I watch TV/ do sitting activities (read, study, play cards)	1.never	2.seldom	3.sometimes	4.often	5.always	(34)
25.	During leisure time I walk/ do standing activities (gardening, housework)	1.never	2.seldom	3.sometimes	4.often	5.always	(35)
26.	Other leisure-time activities:..... (leisure-time = time off from work/school).		2.seldom	3.sometimes	4.often	5.always	(36)

Definitions and explanation of the questionnaire (interviewer's notes)

Item 1: Write in the subject number as on the name label provided at the recruitment station.

Item 2: Circle gender: male or female

Item 3. Occupation: paid job or unpaid duties for most of the day; including school, housework, childminding

Write in the occupation stated and circle 1,2 or 3 (low level, middle level or high level)

Item 4-9: never: ⊕: never, almost never

seldom: ⊕ one-quarter of the workday or workweek

- NB: *leisure-time* is time after work, school, or housework is finished.



Potchefstroomse Universiteit

vir Christelike Hoër Onderwys

THE NUT STUDY

CLINICAL ASSESSMENT FORM FOR SUBJECTS

Name of subject: _____

Subject number: _____

Birth date: _____

		Visits:			
		1	2		
Date:					
Blood sample taken:					
Blood pressure (mmHg):					
Temperature:					
Clinical signs:					
Thyroid:					
Hair:					
Skin:					
Eyes:					
OGTT: glucose concentrations (finger prick):					
Date		T0 (baseline)	T30	T60	T120
1					
2					

Signature:



The Nut Study – Example of “additional points” system

Additional Foods to Select

**Following is a list of foods and its nutritional value translated in terms of points.
You are allowed to select any food/beverage that adds up to your allocated number of additional points.**

Subject name: _____ Subject no.: _____ Date: _____

Type of diet: _____

Additional points

The following table provides the number of additional points for the different types of diets:
Each participant will be informed about his or her respective type of diet. Furthermore, all subjects should utilise this table in conjunction with the table provided below containing the different food options. It is imperative to try and use the correct number of points for your respective diet:-

Addendum

Date Type of diet - Walnuts	Fruit					Starch, fat, alcohol, sugar					Milk					Protein				
	8000 kJ	9500 kJ	11000 kJ	12500 kJ	14000 kJ	8000 kJ	9500 kJ	11000 kJ	12500 kJ	14000 kJ	8000 kJ	9500 kJ	11000 kJ	12500 kJ	14000 kJ	8000 kJ	9500 kJ	11000 kJ	12500 kJ	14000 kJ
Day 1	1	1	1	2	2	2.5	3.5	4.5	5.5	6.5	-	-	-	-	-	-	3	5	8	12
Day 2	-	-	1	1	1	0.5	3	4	4.5	3	-	-	-	-	-	-	-	2	-	-
Day 3	1	1	1	2	2	1	2	2.5	3.5	4.5	-	-	-	-	-	-	-	2	-	2
Day 4	1	1	1	2	2	5.5	7	7	6	4	-	-	-	-	-	-	2	3	5	6
Day 5	1	1	1	2	2	4	4	4	9	8	-	-	-	-	-	-	-	-	-	-
Day 6	1	1	1	2	2	6.5	10.5	12	13	11	-	-	-	-	-	-	4	2	4	8
Day 7	-	-	1	2	2	5	5.5	6.5	11.5	8	-	-	-	-	-	-	-	2	2	6
Day 8	1	1	1	2	2	2.5	5.5	6.5	7.5	5.5	-	-	-	-	-	-	-	2	4	8
Day 9	1	1	1	2	2	1.5	3	7	10	7.5	-	-	-	-	-	-	-	-	2	6
Day 10	1	1	1	2	2	4	6	7.5	8.5	6.5	-	-	-	-	-	-	-	-	-	-
Day 11	1	1	3	2	4	4	3	5	4	3	-	-	-	-	-	-	2	4	6	10
Day 12	1	1	1	2	2	1.5	4.5	4	2	4	-	-	-	-	-	-	-	2	-	4
Day 13	-	-	-	1	1	2	3.5	4.5	5.5	7.5	-	-	-	-	-	3	2	6	8	12
Day 14	1	1	1	2	2	4.5	6	8	12	10	-	-	-	-	-	1	-	-	2	4

Addendum

Walnut Diet

The following table should be completed daily throughout the study period. Please tick off the number of points of the various foods listed below that you ingested on a given day.

Subject name:

Subject no.:

Date:

Type of diet:

Fruit	Points	Tick	Starch, fat, alcohol and sugar	Points	Tick	Protein	Points	Tick
250 ml strawberries	1		Starch:					
80 g apple	1		2 thin slices (60g) bread	2		1 egg	2	
2 medium (100g) apricots	1		3 Provita's	1		15g cheddar cheese	1	
2 heel (15g) dried dates	1		3 Cream crackers	1		1 vienna	2	
12 grapes	1		6 Salticracks	2		20g cottage cheese (low fat)	1	
4 medium grenadilas	1		2 (25g) dry biscuits	3				
2 small guavas	1		1 small (20g) rusk	3				
1 medium (100g) orange	1		750 ml popcorn (Ster-Kinekor)	3				
5 whole litchies	1		1 packet Fruit Gummi	4				
1 medium mango	2		45 g Pretzels	5				
1 large tangerine	1		1 Energy bar	5				
100g paw-paw	1		1 Stay Trim bar	5				
1 small (100g) pear	1		13 g Fizzers	2				
			Powerade/Energade	4				
1 medium (100g) peach	1		Fat:					
1 medium (100g) banana	2		1 tsp margarine/butter/oil	1				
			5 small olives	1				
½ medium (125g) grapefruit	1		2 tsp low-fat sandwich spread e.g. Sandwhich spread	1				
½ cup of pineapple cubes	1		1 Tbs cream/cream cheese	3				
30 ml raisins	1		1 small packet chips	4				
			Alcohol:					
200 g sweet melon	1		340 ml beer, usual	4				
2 large (50g) figs	1		340 ml beer, lite	2				
250 ml watermelon cubes	1		45 ml strong drink*	3				
			30 ml Brandy*	2				
			120 ml Wine:					
			Dry white	2				
			Red	2				
			Sweet	3				
12 g dried fruit sweets	1		Light	1				
			100 ml Champagne, sweet 120 ml	2				
Milk	Points	Tick	Champagne, dry	2				
½ cup ice-cream	4		60 ml Cherry	2				
			50 ml Port	2				
250 ml low-fat milk	4		90 ml Vermoet, dry	3				
			90 ml Vermoet, sweet	4				
			Sugar:					
½ cup low-fat flavored yoghurt	2		2 tsp honey/jam/syrup/sugar (white or brown sugar)	1				
			10 g Jelly babies	1				
			10 g Peppermints	1				
			10 g Sparkles	1				

Addendum

*Mix with : Tab/ Coke lite/ sodawater / tap water

NB! Tea, coffee (except milk and sugar are not free points), Sprite lite, Tab, Coke lite, spices and herbs are all free points

- ☐ How much tea did you have and specify the amount and type?
(number of cups / mugs and the type of tea) e.g. 2 mugs rooibos tea

Rooibos

Normal

Green tea

Other: specify

- ☐ How many cups or mugs of coffee did you have?
-

- ☐ Was there any change in your activity?
-

- ☐ Mention any foods eaten that was not given in this study or in the above list. Discuss why?
-

Name: _____

Instructions:

[illegible]

14-day menu cycle**or****Spyskaarte en porsies vir 13 dae plus een braaidag**

Week 1	Maaltydplan	D1	D2	D3	D4	D5	D6	D 7
	ONTBYT ***							
1	Ontbytgraan/Pap	Keuse	Keuse	Keuse	Keuse	Keuse	Keuse	Keuse
2	Melk, afgeroom	→	→	→	→	→	→	→
3	Suiker	→	→	→	→	→	→	→
4	Brood	→	→	→	→	→	→	→
5	Margarien	→	→	→	→	→	→	→
6	Konfy/Smeer	Keuse	Keuse	Keuse	Keuse	Keuse	Keuse	Keuse
7	Bygereg	Ontbytskons	Plaatkoekies	Ongelooflike muffins	Volgraanbeskuit	Rosyntjie muffins	Ontbytskons	Wortelbroodjie
8	Vrugtesap	Keuse	Keuse	Keuse	Keuse	Keuse	Keuse	Keuse
9	MIDDAGETE ***	D1	D2	D3	D4	D5	D6	D7
10	Stysel	Mielies	Geurige rys	Noedels	Geroomde aartappels	Skondeeg	Worsbroodjie	Mieliepap / Aartappelslaai
11	Proteïen	Gebakte stokvisgereg	Varktjops	Noedelhoender	Potgebraaide beesvleisstukke	Visrol	Boerewors	Gebakte / gebraaide skaaptjops
12	Sous		Appelsous		Soet-suursous		Tamatie, mayonnaise en blatjangsous	Tamatie en uiesmoor
13	Groente A	Brokkoli met neut	Komkommer-skywe	Gestoofde kool	Gestoofde groenbone	Tamatiewiggies	Blaarslaai, appel en uie	Koolsaai met mayonnaise
14	Groente B	Beetslaai	Patats in stroop	Erte	Soetpampoen	Wortel en pynappelslaai	Groenboontjie-slaai	Pampoenkoekies met kaneelsuiker
15	Nagereg (Wo)			Ingemaakte perskes				Vrugteslaai
16	Sous vir nagereg			Vlasous				Gegeurde youghurt

Addendum

	AANDETE ***	D1	D2	D3	D4	D5	D6	D7
17	Stysel	Pasta	Skons	Hamburger-broodjie	Soetpatats	Geurige rys	Macaroni en kaas	Witrys
18	Proteïen	Ham en pastagereg	Mexican Quiche	Maal vleiskoekies	Gebakte wors	Gemarineerde Hoenderdye		Geurige kerriemaalvleis
19	Sous			Mostert of mayonnaise	Chutney	Marinadesous		
20	Groente/Slaai	Wortel en lemoenslaai	Drieboneslaai	Tamatie- en uieskywe & blaarslaai	Gemengde groente	Blomkool met neut	Somerslaai	
21	Vrug	Keuse	Keuse	Keuse	Keuse	Keuse	Keuse	Piesangslaai met mayonnaise
22	Brood	→	→	→	→	→	→	→
23	Margarien	→	→	→	→	→	→	→
24	Konfyt/smeer	→	→	→	→	→	→	→

Addendum

Week 2	Maaltydplan	D8	D9	D10	D11	D12	D13	Naweek / braaidag
	ONTBYT ***							
1	Ontbytgraan/Pap	Keuse	Keuse	Keuse	Keuse	Keuse	Keuse	Keuse
2	Melk, afgeroom	→	→	→	→	→	→	→
3	Suiker	→	→	→	→	→	→	→
4	Brood	→	→	→	→	→	→	→
5	Margarien	→	→	→	→	→	→	→
6	Konfy/Smeer	Keuse	Keuse	Keuse	Keuse	Keuse	Keuse	Keuse
7	Bygereg	Plaatkoekies	Ongelooflike muffins	Botterbroodjies	Volgraanbroodjie	Rosynemuffins	Croissants	Karringmelkbeskuit
8	Vrugtesap	Keuse	Keuse	Keuse	Keuse	Keuse	Keuse	Keuse
9	MIDDAGETE ***	D8	D9	D10	D11	D12	D13	
10	Stysel	Noedelslaai	Kapokaartappel	Witrys	Kapokaartappel met pietersielie	Geurige rys	Geelrosynerys	Mieliepap / roosterkoek /broodjie
11	Proteïen	Gebakte soutvleis	Frikkadelle	Smelt-in-die-mond biefstuk	Mexikaanse vleisskywe	Gebakte hoender	Bobotie	T-been skyf /steak
12	Sous	Mosterdsous	Tamatie en uiesous		Sous I	Soet-suur sous		Tamatie en uiesous
13	Groente A	Groenmengelslaai	Ertjie	Tamatiewiggies	Koolslaai	Tamatie, uie en blaarslaai	Groenbone met uie	Mengelslaai
14	Groente B	Blomkool met neut	Gestoofde wortels	Gestoofde groenbone	Butternuts met kaneelsuiker	Soetertjies	Pynappel en blaarslaai	
15	Nagereg (Wo)				Asynpoeding			Gebakte sjokolade nagereg
16	Sous vir nagereg				Vlasous			Vlasous

Addendum

	AANDETE ***	D8	D9	D10	D11	D12	D13	Naweek / braaidag
17	Stysel	Volgraanbroodrolletjie met tunaslaai	Noedels	Souttert	Wors en rysgereg	Skelvis roulade	Franse brood met pizza-vulsel	Broodrolletjie
18	Proteïen		Geroosterde hoender met mayonnaise					Kaas
19	Sous							
20	Groente/Slaai	Komkommer-skywe	Lemoen-pampoentjies	Tropiese slaai	Mielies	Mengelslaai	Baby marrow	Tamatieskyfies en blaarslaai
21	Vrug/sap	Keuse	Keuse	Keuse	Keuse	Keuse	Keuse	Keuse
22								
23								
24								

The



2nd edition

... from the editor

The 3 week run-period has been completed and served its purpose well - allowing participants to adapt and familiarize themselves with the procedures at hand.

Tuesday 02 March'04 we commenced with the experimental period of the Nut study. That morning the air loomed thick with warm anticipation and excitement as "nutcrackers" (researchers) and "nutcases" (participants) gathered together for the commencement of the experimental phase. Hereafter, the verdict was soon announced as to the fate of the nutcases or just cases without nuts. Participants received the news revealing their type of diet with mixed emotions. Well, whether you are in a nut group or not I hope that you will continue to be a wonderful, compliant participant. As a reminder to the control group the countdown to you receiving your bag of nuts upon completion of this phase has begun.

Remember that being part of this study means that you are one of the pioneers with regards to control feeding trials of this nature in South Africa. Therefore, during the remaining 4 and a half weeks it is imperative that all the subjects remain compliant in order to ensure accurate and meaningful results to benefit us all.

Read all about some of the interesting facts about insulin resistance.

Zoom into the expressions of some of the subjects during the experimental phase and share the joy of Mexico with the rest of your family.

I hope that you will find this publication (2nd edition) of The **NUTCRACKER** entertaining, educational and enlightening.

By:
Janine Mukuddem-Petersen



Kiekies vir elke okkasie



Ag hartjie, ek weet jy het nou 'n Oscar en als, maar jy moet nogsteeds anderkant om lê hoor, ek kan nie bloed uit jou elmboog uit trek nie!!!!

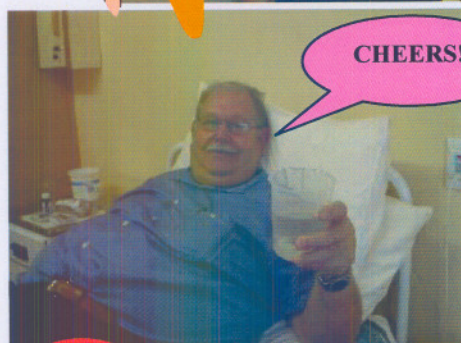
Deur Christelle De Witt



Goed, maar net vir 1 drankie, ek gaan nie gewoonlik saam met vreemde meisies uit nie...



Oh the life of a queen!



CHEERS!



Kan sy hoor of ek gister 'n stuk 'chocolate' koek geëet het.....

Uuuhh, Suster, eks nie eintlik 'n bang ou nie, maar ek voel nie meer so lekker nie!!!!



Ek wonder hoekom voel ek so senuweagtig?

Mmmm, liewe Kersvader, ek soek 'n vakansiehuis in "Plet" en 'n rooi sportsmotor en 'n diamanttring en 'n nuwe klerekas en....en...en...



Tot 'n volgende aksiebelaaide Nutcracker groot ek julle met 'n snik!!! Hope u had fun with the photo's, because I surely did!!!



INSULIN RESISTANCE

By Ronel Breet



I love deadlines. I especially love the whooshing sound they make as they fly by.

—Douglas Adams Author, *Hitchhiker's Guide to the Galaxy*.

What is insulin resistance?

Insulin resistance (IR) is an impaired biological response/cellular resistance to either exogenous or endogenous insulin. Insulin stimulates glucose uptake into tissues, and its ability to do so varies greatly among individual persons. In insulin resistance, tissues have a diminished ability to respond to the action of insulin. To compensate for resistance, the pancreas secretes more insulin. Insulin-resistant persons, therefore, have high insulin levels.

The syndrome can be defined as a cluster of abnormalities, including obesity, hypertension, dyslipidemia and type 2 diabetes, that are associated with insulin resistance and compensatory hyperinsulinemia.

How is it diagnosed?

Factors associated with increased likelihood of IR.

- Strong family history of diabetes.
- History of gestational diabetes.
- Polycystic ovary syndrome.
- Impaired glucose metabolism: fasting glucose level between 6.1 – 7.1 mmol/L or impaired glucose tolerance, with a 2h post-75-g glucose load level between 7.8-11.1 mmol/L.
- Obesity: Body mass index of 30 kg/m² or more.
- Increased waist-to-hip ratio: 1.0 in men and 0.8 in women.
- Acanthosis nigricans (characterized by hyperpigmented, velvet plaques of body folds, though involvement in other areas occurs as well) is caused by hyperinsulinemia, a consequence of IR that occurs associated with obesity.

Once you can accept the universe is matter expanding into nothing that is something, wearing stripes with plaid comes easy.

—Albert Einstein



What can I do about it?

Non pharmacological approach to insulin resistance syndrome.

- Exercise: 30 minutes of moderate-intensity physical activity most or, preferably, all – days of the week.
- Weight reduction: dietary modification to attain a healthy body weight.
- Dietary fiber: include foods high in fiber in their diet (fruit, vegetables and whole grains).



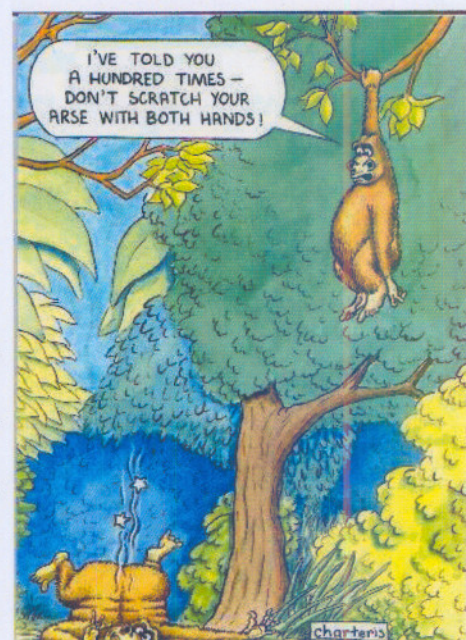
Things to remember

By:
Janine Mukuddem-Petersen

- The additional points for your respective energy intakes are not optional but included in calculating your various energy intakes.
- It is vital for the success of this study that no food is wasted and all additional points utilized.
- Ensure that your forms are completed according to the correct menu day as it appears in the dining hall.
- Forms are to be placed in the red box daily.
- Tuesdays and Fridays you will be weighed prior to having lunch.
- Individual changes can be made to meals if really necessary and upon request.
- To make the lunch time meal more delightful, you are welcome to bring a companion to join you- please inform us at least 2 days in advance.

Women and cats will do as they please, and men and dogs should relax and get used to the idea.

—Robert A. Heinlein



Honest criticism is hard to take, particularly from a relative, a friend, an acquaintance, or a stranger

—Franklin P. Jones

RESEPTE



Mexikaanse Quiche

Totale aantal porsies: 6

Porsie grootte: 260g

*One has fear in front of a goat, in
back of a mule, and on every side of
a fool*

—Edgar Watson Howe

Zelda Pieterse



Skondeeg

Betanddele	Metode
3 ml kookolie	Smeer pasteiskottel.
50g margarien	Sny in klein stukkes.
175 koekmeel	Sif meel, bakpoeier en sout saam. Plaas in die mengbak van die menger.
12.5g bakpoeier	
1.5g (2ml) sout	
100ml melk	Voeg melk by meel- mengsel en klits tot goed deurgemeng. Ver- meerder melk indien nodig as deeg te styf is.
125 ml koekmeel	Bestrooi werksopperv- lakte met meel en rol deeg 2cm dik uit.
½ eier	Klits eier goed saam met die 5ml melk. Bestryk die deeg met die eier en melkmengsel
5 ml melk	
Giet die vulsel vir die Mexikaanse Quiche in	

Vulsel

Betanddele	Metode
350g skondeeg	Voer pasteibak uit met skon- deeg
12.5ml olie	Braai uie in warm olie vir 10min of tot deurskynend
175g uie, gekap	
1x425g blikkie pilchards in ta- matiesous	Meng uie, vis en blatjang lig- gies. Skep in uitgevoerde pasteibak
37.5g blatjang	
100g kaas, gerasper	Strooi oor vis
175ml melk	Meng melk, eiers, sout en peper goed en giet oor vis in pastei- bak
1 eier	
Sout & peper na smaak	
Bak by 200°C vir 20min of totdat die mengsel gestol het en bruin is	



*Never tell your
mom her diet's
not working.
—Joel, 14 Ad-
vice from Kids*



**A great team
can reach the unreachable...**



**Teamwork by:
Janine Mukuddem-Petersen**

Information for Authors

THE AMERICAN JOURNAL OF CLINICAL NUTRITION

Revised January 2004

The purpose of *The American Journal of Clinical Nutrition* (*AJCN*) is to publish original research studies relevant to human and clinical nutrition. Case studies, untested hypotheses, and geographically restricted surveys are not considered suitable for the *AJCN*. Well-controlled clinical studies that describe scientific mechanisms, efficacy, and safety of dietary interventions in the context of disease prevention or a health benefit will be considered. Epidemiologic studies relevant to clinical nutrition will also be considered. Solicited editorials, book reviews, solicited or unsolicited review articles, invited controversy position papers, and letters to the Editor that relate to prior *AJCN* articles are essential components of the *AJCN*. All submitted material with scientific content will undergo peer review by the Editors or their designees before acceptance for publication.

Symposia or workshop articles may be published as supplements to the *AJCN* and are funded by their sponsors at a special page charge. The *AJCN* welcomes queries about the publication of supplements. The *AJCN* uses a 2-part acceptance process for supplements. The first step involves editorial acceptance of the topic and content as provided by the symposium organizer; the second step involves anonymous peer review of the individual articles. To be considered for publication, supplement articles must be received within 3 mo of each symposium or workshop. Each manuscript should not exceed 15 text pages, exclusive of tables, figures, and references; must adhere to *AJCN* style and format; and will be reviewed according to the same scientific standards used to evaluate original research articles.

All material to be considered for publication in a regular issue should be submitted electronically at the following website: <http://www.rapidreview.com/ASCN2/CALogon.jsp>. See "Tips for authors submitting manuscripts to the *AJCN*" below for helpful advice regarding electronic submission.

All material to be considered for publication in a supplement issue should be sent to the following address: Editor's Office, *The American Journal of Clinical Nutrition*, 3247 Meyer Hall, University of California, One Shields Avenue, Davis, CA 95616-8790. Phone: (530) 752-8363; fax: (530) 752-8371; e-mail: ajcn@ucdavis.edu.

Original manuscripts will be considered with the understanding that no part has been published, simultaneously submitted, or already accepted for publication elsewhere, other than in abstract form.

Material published in the *AJCN* is copyrighted by The American Society for Clinical Nutrition, Inc (ASCN), and may not be reproduced without permission from the Managing Editor, The American Society for Clinical Nutrition, 9650 Rockville Pike, Bethesda, MD 20814-3998. E-mail: journal@ascn.faseb.org. If the original source is cited, the abstract appearing at the beginning of each article may be reproduced without permission.

SUMMARY OF REQUIREMENTS

Each manuscript component should begin on a new page in the following sequence:

- Title page
- Abstract and key words
- Text
- Acknowledgments
- References
- Tables: each table on a separate page, complete with title and footnotes
- Legends for figures
- Figures

Identify on the title page the author who will be responsible for correspondence regarding the manuscript. The signed Authors' Agreement form and copies of any documents granting permission needed to reproduce material in print and electronic form or to use illustrations of identifiable subjects must be sent to the Editorial Office. Authors should keep copies of all submitted material.

The *AJCN* encourages authors to provide the names, fields of interest, addresses, telephone and fax numbers, and e-mail addresses of **4–6 unbiased and qualified potential expert reviewers** from outside the authors' institutions.

MAJOR SECTIONS OF THE *AJCN*

- Editorials
- Review Articles
- Special Invited Articles, including Controversies and Perspectives
- Original Research Communications
- Letters to the Editor
- Book Reviews
- Books Received
- ASCN Announcements
- Calendar of Events

The "Information for Authors" is found in each January issue and is also available on the *AJCN*'s website (www.ajcn.org/misc/ifora.shtml).

Letters to the Editor that refer to a recent *AJCN* article must be received within 12 wk of the article's publication. Letters must be typewritten (double-spaced), should include a title page, should have no more than 10 references, and **should not exceed 1000 words**. All letters will be subjected to editorial review and decision before acceptance. The *AJCN* does not accept letters that are unrelated to a specific, recently published article; that contain unpublished data; or that engage in personal slander or invective. Letters may be submitted by mail or online at <http://www.rapidreview.com/ASCN2/CALogon.jsp>.

CRITERIA FOR MANUSCRIPT ACCEPTANCE

The *AJCN* can publish only about 30% of the more than 800 original submissions received per year. Submitted manuscripts may be rejected without detailed comments after initial review by at least 2 *AJCN* editors if the manuscripts are considered inappropriate or of insufficient scientific priority for publication in the *AJCN*. All other manuscripts undergo a complete review by at least 2 consulting editors or other selected experts. Criteria for acceptance by the *AJCN* include originality, validity of data, clarity of writing, strength of the conclusions, and potential importance of the work to the field of clinical nutrition. Submitted manuscripts will not be reviewed if they do not conform to standard English usage and to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Internet: <http://www.icmje.org/>; also available free of charge from the Secretariat Office, *Annals of Internal Medicine*, American College of Physicians, Independence Mall West, Sixth Street at Race, Philadelphia, PA 19106-1572).

FORMAT AND STYLE REQUIREMENTS

Articles are copyedited according to *AJCN* style policy, the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals," and the style manual of the Council of Science Editors (Scientific style and format: the CBE manual for authors, editors, and publishers. 6th ed. Cambridge, United Kingdom: Cambridge University Press, 1994).

Instructions for manuscript preparation

The manuscript should be formatted as follows: 216 × 279 mm (8½ × 11 in) or ISO A4 (212 × 297 mm), with margins of at least 2.5 cm; use double-spacing and 12-point type throughout. Do not justify the right margin. **The abstract and text pages should have line numbers in the left margin.** Number pages consecutively in the upper right-hand corner of each page, beginning with the title page. Foreign authors are advised to have their manuscripts reviewed by a scientific colleague who is fluent in English so that the manuscripts will conform to US English usage and grammar.

Title page

The title page should contain 1) the title of the article, beginning with a key word if possible, with only the first letter of the first word capitalized; 2) the first name, middle initial, and last name of each author; 3) the affiliation of each author at the time the work was done, with the use of author initials in parentheses to designate which affiliation corresponds to which author; 4) disclaimers, if any; 5) the name, mailing address, telephone and fax numbers, and e-mail address of the author responsible for correspondence about the manuscript; 6) the name and mailing and e-mail addresses of the author to whom requests for reprints should be addressed or a statement that reprints will not be available from the author; 7) sources of support, including grants, fellowships, and gifts of materials (eg, chemicals, experimental diets); and 8) a short running head of **not more than 50 characters** (count letters and spaces).

Authorship

Each author must have participated sufficiently, intellectually or practically, in the work to take public responsibility for the content of the article, including the conception, design, and conduct of the experiment, and for the data interpretation. An

article with corporate (collective) authorship must specify the key persons responsible for the article; others contributing to the work should be recognized separately. A description of the contribution of each author must be provided in the Acknowledgment section. The Editors may require authors to justify the assignment of authorship. Authors must disclose in the Acknowledgment section any advisory board affiliations with and financial or personal interests in any organization sponsoring the research at the time the research was done. All authors must sign a statement agreeing to these requirements for authorship with the transfer of copyright (www.ajcn.org/misc/agree.pdf).

Abstract and key words

A properly constructed and informative abstract is helpful for the initial editorial review of the submitted manuscript. Original research articles must include a structured abstract that contains no more than 250 words, is written in **complete sentences**, and includes the following headings:

Background: Provide 1 or 2 sentences that explain the context of the study.

Objective: State the precise objective, the specific hypothesis to be tested, or both.

Design: Describe the study design, including the use of cells, animal models, or human subjects. Identify the control group. Identify specific methods and procedures. Describe interventions, if used.

Results: Report the most important findings, including results of statistical analyses.

Conclusions: Summarize in 1 or 2 sentences the primary outcomes of the study, including their potential clinical importance, if relevant (avoid generalizations).

Review articles, special articles, and reports should include an unstructured abstract (no more than 250 words) that states the purpose of the article and emphasizes the major concepts and conclusions.

Below the abstract, provide and identify as such at least 6 key words or short phrases, including the subject group, that will assist indexers in cross-indexing your article; do not use adjectives. Use terms from the medical subject headings list from *Index Medicus* whenever possible.

Text

Use active voice whenever possible. Use past tense when describing and discussing the experimental work on which the article is based. Reserve present tense for reference to existing knowledge or prevailing concepts and for stating conclusions from the experimental work. Clearly differentiate previous knowledge and new contributions. Do not use *level* when referring to a concentration. Use metric units of measure; SI units are no longer required. Use *triacylglycerol* instead of *triglyceride*.

The text of observational and experimental articles should be divided into sections with the following headings: Introduction, Subjects (or Materials, for cell or animal studies) and Methods, Results, and Discussion. Long articles may require subheadings within some sections. Authors should consult recent issues of the *AJCN* for guidance on the formatting of other types of articles, book reviews, and editorials.

Introduction

Clearly state the purpose of the article. Summarize the rationale and background for the study or observation, giving only strictly pertinent references. Do not include methods, data, results, or conclusions from the work being reported. The Introduction should be limited to 1.5 typewritten pages.

Subjects (or Materials) and methods

Describe clearly your selection of the experimental and control subjects and provide eligibility and exclusion criteria and details of randomization. Describe the methods for, and success of, any masking (blinding) of observations. Report any complications of experimental treatments. Identify the methods, apparatus (manufacturer's name and location in parentheses), and procedures in sufficient detail to allow other researchers to reproduce the results. When referring to centrifugation, give force $\times$ g, duration, and temperature. Do not use trademark names, such as Teflon, as generic terms. Give references for established methods, including statistical methods; provide references and brief descriptions of methods that have been published but are not well known; and describe new or substantially modified methods, giving reasons for using them and evaluating their limitations. Identify precisely all drugs and chemicals used, including generic names, dosages, and routes of administration. If trade names for drugs and chemicals are included, give the manufacturer's name and location.

Ethics. When reporting experiments on human subjects, indicate that the procedures followed were in accordance with the ethical standards of the responsible institutional or regional committee on human experimentation or in accordance with the Helsinki Declaration of 1975 as revised in 1983. Do not use patients' names, initials, or hospital identification numbers. When reporting experiments on animals, indicate approval by the institution's animal welfare committee and state whether the National Research Council's guide for the care and use of laboratory animals was followed.

Statistics. Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (eg, CIs, SDs, or SEs), even for differences that were not significant. Report the numbers of observations. When appropriate, explain the choice of sample size in terms of the power to detect effects. References for study design and statistical methods should be to standard works when possible rather than to articles in which designs or methods were originally reported. Specify any general-use computer programs used, including the version number and the manufacturer's name and location. Include general descriptions of statistical methods in the Subjects (or Materials) and Methods section and specific descriptions in each table and figure legend. Indicate whether variables were transformed for analysis. Provide details about what hypotheses were tested, what statistical tests were used, and what the outcome and explanatory variables were (where appropriate). Indicate the level of significance used in tests.

If the study design involves direct control of more than one factor or measurement of factors that are reasonably uncorrelated with each other (such as sex and age), the statistical analysis should consider all factors simultaneously. For example, if serum HDL was measured in both men and women in 3 age groups, the analysis should be a two-factor analysis of

variance with interaction, including sex and age group as main effects, rather than 2 separate one-factor analyses. Within-group analyses should be done only if significant interactions have been shown. For example, 1) if subjects in 2 treatment groups were measured at 2 or more time points, the change over time within each group should not be assessed unless there was a significant group $\times$ time interaction; 2) if subjects from 2 ethnic groups were randomly assigned to treatment groups, comparisons between treatments should not be done within an ethnic group unless there was a significant ethnicity $\times$ treatment group interaction. For post hoc comparisons of means, control for overall type I error by using Tukey's, Dunnett's, or Scheffe's test.

When data are summarized in the Results section, specify the statistical methods used to analyze them. Avoid nontechnical uses of technical statistical terms, such as *random* (which implies a randomizing device), *normal*, *significant*, *correlation*, *sample*, and *parameter*. Avoid value judgments about the results of statistical analyses with phrases like "nearly reached significance" or statements that means or proportions were similar when they were merely not significantly different. Do not report *P* values in the text if already given in the tables or figures. Define statistical terms, abbreviations, and symbols not listed under "Commonly used approved abbreviations." Detailed statistical analyses, mathematical derivations, and the like may sometimes be suitably presented as one or more appendixes.

Results

Present your results in a logical sequence in the text, tables, and figures. Do not present specifics of data more than once and do not duplicate data from tables or figures in the text; emphasize or summarize only important observations. Do not present data from individual subjects except for very compelling reasons. Report losses to observation (such as dropouts from a clinical trial). Use boldface for the first mention of each table or figure.

Discussion

The Discussion should not exceed 4 typewritten pages except in unusual circumstances as approved by the Editor. Emphasize concisely the novel and important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given in the Introduction or Results. Include the implications of the findings and their limitations and relate the observations to other relevant studies. Link conclusions with the goals of the study and avoid unqualified statements and conclusions that are not completely supported by the data. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses and recommendations when warranted by the results and label them clearly as such.

Acknowledgments

Acknowledge only persons who have made substantive contributions to the study. Authors are responsible for obtaining written permission from everyone acknowledged by name and for providing to the Editor a copy of the permission, if requested. Each author is required to list his or her contribution to the work (such as design of the experiment, collection of data, analysis of data, writing of the manuscript, or provision of

significant advice or consultation) and to disclose any financial or personal relationships with the company or organization sponsoring the research at the time the research was done. Such relationships may include employment, sharing in a patent, serving on an advisory board or speakers' panel, or owning shares in the company. The source of support for the research reported in the paper should be listed on the title page, not as an acknowledgement.

References

Number references consecutively in the order in which they are first mentioned in the text. Identify references by Arabic numerals in parentheses. References cited in tables or in legends to figures should be numbered according to the first citation of the table or figure in the text. Appendixes should have a separate reference section.

It is rarely necessary to cite more than 50 references in an original research article. Try to avoid citing published abstracts as references [if a published abstract is cited, include "(abstr)" at the end of the reference]. Abstracts from scientific meetings not published in peer-reviewed journals may not be used as references. Unpublished observations and personal communications (written, not oral) may not be used as references but may be inserted in parentheses with the names of the responsible researchers and the year of the observation or communication. Authors are responsible for obtaining written permission from everyone so cited and for providing to the Editor a copy of the permission, if requested. Doctoral dissertations may be used as references. Include manuscripts accepted but not yet published; designate journal name followed by "(in press)." Report foreign titles in the original language, identify the language, and provide the English translation in parentheses. The references must be verified by the author against the original documents.

Journals

1) Standard journal article: list all authors when 6 or fewer; when 7 or more, list only the first 3 and add "et al." Abbreviate journal titles according to *Index Medicus* style, which is used in MEDLINE citations.

Jeffery RW, Wing RR, Sherwood NE, Tate DF. Physical activity and weight loss: does prescribing higher physical activity goals improve outcome? *Am J Clin Nutr* 2003;78:684-9.

2) Corporate author

National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). 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) final report. *Circulation* 2002;106:3143-421.

Books and other monographs

3) Personal authors

Shils M, Shike M, Olson J, Ross AC. *Modern nutrition in health and disease*. 9th ed. Baltimore: Lippincott Williams & Wilkins, 1998.

4) Committee report or corporate author

National Research Council. *Recommended dietary allowances*. 10th ed. Washington, DC: National Academy Press, 1989.

Food and Nutrition Board, Institute of Medicine. *Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoids*. Washington, DC: National Academy Press, 2000.

5) Chapter in book

Young VR, El-Khoury AE. The notion of nutritional essentiality of amino acids, revisited, with a note on the indispensable amino acid requirements in adults. In: Cynober L, ed. *Amino acids in health and disease*. Boca Raton, FL: CRC Press, 1995:191-232.

6) Agency publication

US Department of Agriculture, US Department of Health and Human Services. *Nutrition and your health: dietary guidelines for Americans*. Washington, DC: US Government Printing Office, 2000. [USDA Home and Garden Bulletin no. 232.]

Internet references

7) Website

National Center for Health Statistics. National Health and Nutrition Examination Survey. Version current 1 October 2003. Internet: <http://www.cdc.gov/nchs/nhanes.htm> (accessed 13 October 2003).

8) Online journal article

Sinha A, Madden J, Ross-Degnan D, Soumerai S, Platt R. Reduced risk of neonatal respiratory infections among breast-fed girls but not boys. *Pediatrics* [serial online] 2003;112:c303. Internet: <http://pediatrics.aappublications.org/cgi/content/full/112/4/e303> (accessed 14 October 2003).

Tables

Each table should appear on a separate page; remember to use **double-spacing**. Number tables consecutively with Arabic numerals (do not use 1A, 1B, etc) and supply a brief descriptive title for each. Give each column a short or abbreviated heading. Place explanatory matter in footnotes, not in the heading or table title. Each table should contain enough detail (including statistics) that the table is intelligible without reference to the text. Explain in footnotes all nonstandard abbreviations that are used in the table. Commonly used approved abbreviations (*see* the section of the same name below) may be used without explanation. Additionally, explanations are not needed for ANOVA, BMI, F (females), and M (males). For footnotes, use superscript Arabic numerals. For reporting results of statistical analyses, superscript letters can be used if explaining the results in the usual manner would be too complicated (*see* a recent issue of the *AJCN* for examples). The first appearance in a horizontal row determines the order of the footnotes. Identify statistical measures of variation, such as SD and SE. **Omit internal horizontal and vertical rules**. Cite each table in the text in consecutive order. Use boldface for the first mention of each table. If you use data from another published source, acknowledge the source fully. Number references in tables according to the location of the first citation of each table in the text.

Figures

Cite each figure in consecutive order in the text. Use boldface for the first mention of each figure. Spell out the word "Figure"; do not use "Fig." If a figure has been published, acknowledge the original source and submit written permission from the copyright holder to reproduce the material in print and electronic format. Except for documents in the public domain,

permission is required from the copyright holder, regardless of authorship or publisher.

Legends for all figures should be typed with double-spacing on a separate page (not on the figures themselves). Each legend should contain enough detail, including statistics, to make the figure intelligible without reference to the text. Explain all nonstandard abbreviations used in the figure (see below for list of standard abbreviations under "Units and abbreviations"). When symbols, arrows, numbers, or letters are used to identify parts of the figures, identify and explain each one clearly in the legend. Explain internal scale and identify the method of staining in photomicrographs.

Figures must be good-quality, unmounted glossy prints or high-quality laser-printed copies, usually 127×173 mm but no larger than 203×254 mm. Lettering and symbols must be large enough to be readable when reduced to 1 column width (<8.5 cm) or, in rare cases, to 2 column widths. The use of color will be evaluated for each figure on an as-needed basis, and the author must pay an extra charge if color is used. Reprints of articles with color figures will be billed at a higher charge because of the additional costs of printing color. Do not use 3-dimensional figures unless necessary. When labeling axes, capitalize only the first word and proper nouns; use lowercase letters for the remaining words and put units in parentheses.

UNITS AND ABBREVIATIONS

Use only standard abbreviations. Consult the following sources for standard abbreviations: *Scientific Style and Format and Standard for Use of the International System of Units (SI): the Modern Metric System* (American Society for Testing and Materials. IEEE/ASTM SI 10-1997. West Conshohocken, PA: ASTM, 1997). Avoid abbreviations in the title, and avoid the use of abbreviations for single words. The complete phrase or expression should precede the first use of an abbreviation in the text unless it is a standard unit of measurement, chemical compound preceded by a digit, or one of the following standard abbreviations: ADP, AIDS, AMP, ATP, DMEM, DNA, EDTA, eg, EGTA, GDP, GTP, HCl, HDL, HEPES, HIV, HPLC, ie, LDL, In, MEM, MOPS, NAD, NADH, NADP, NADPH, RNA, RPMI, tris, and VLDL. (See also the standard abbreviations for statistical terms below.)

Metric units are required and the use of the International System of Units (SI units) is optional. For a comprehensive listing of SI conversion factors, consult *SI Units for Clinical Measurement* (Young DS, Huth EJ. Philadelphia: American College of Physicians, 1998) or *Am J Clin Nutr* 1998;67:166–81. Dosage forms and dietary ingredients may be expressed in gram or mole quantities. Energy may be expressed in kilocalories or joules; the conversion factor for converting kilocalories to kilojoules is 4.184. Do not report energy in Calories with a capital C; use kcal, MJ, or kJ instead. Temperatures should be reported in degrees Celsius. Blood pressures should be reported in millimeters of mercury. Use of katal to report enzyme activity is optional.

The *AJCN* follows the vitamin nomenclature policy of the IUPAC-IUB Joint Commission on Biochemical Nomenclature (Biochemical nomenclature and related documents. A compendium. 2nd ed. London: Portland Press, 1992). Authors are responsible for ensuring that their terminology conforms with this policy.

Commonly used approved abbreviations

Standard units of measurement

ampere, A	liter, L
becquerel, Bq	meter, m
coulomb, C	minute, min
curie, Ci	mole, mol
day, d	month, mo
degree Celsius, °C	ohm, Ω
farad, F	pascal, Pa
gram, g	second, s
hertz, Hz	sievert, Sv
hour, h	volt, V
joule, J	watt, W
katal, kat	week, wk
kelvin, K	year, y
kilocaloric, kcal	

Usage

length: m, mm, μm (not acceptable: in, ft, yd, Å, m μ)

area: m^2 , mm^2 , μm^2 (not acceptable: sq in, in ^{2} , μ^2)

volume: L, mL, μL , pL (not acceptable: pint, gallon, cc, ccm, λ , $\mu\mu\text{L}$)

mass: kg, g, mg, μg , ng, pg (not acceptable: oz, lb, gr, gm, gms, mgm, mgms, mgs)

mass concentration: kg/L, g/L, mg/L, $\mu\text{g/L}$ (not acceptable: mg %)

substance concentration: mol/L, mmol/L, $\mu\text{mol/L}$, nmol/L (not acceptable: M, N)

Combining prefixes

tera- (10^{12}), T	micro- (10^{-6}), μ
giga- (10^9), G	nano- (10^{-9}), n
mega- (10^6), M	pico- (10^{-12}), p
kilo- (10^3), k	femto- (10^{-15}), f
milli- (10^{-3}), m	atto- (10^{-18}), a

Statistical terms

coefficient of correlation, sample, r
 coefficient of multiple correlation, R
 coefficient of variation, CV
 confidence interval, CI
 degrees of freedom, df
 mean, $\bar{x}$
 not significant, NS
 number of observations, n
 probability, P
 standard deviation, SD
 standard error of the estimate, SEE
 standard error of the mean, SEM
 variance ratio, F

CORRECTING PROOF

Contact the Production Office with any change in e-mail address from the time the manuscript was submitted. When page proofs are available, the corresponding author will be notified via an e-mail that includes instructions on how to download the proofs from a specified URL address. These proofs are to be checked carefully, and any changes are to be indicated clearly in the margins of the page proof. Changes made by technical editors for style, grammar, and readability **are**

not to be altered by authors unless a scientific error has been introduced. The page proofs should be returned to the *AJCN* Production Office. If changes are received after the deadline, the article will be printed as set.

CHARGES TO AUTHORS

Submission

The responsible author must agree to pay page charges when the manuscript is submitted for publication. Requests for waivers of the charge must be submitted to the Editor-in-Chief with the original manuscript. After publication of their articles, responsible authors will be billed \$75 per printed journal page (3 double-spaced manuscript pages equal $\approx$ 1 printed journal page) for the first 8 pages and \$120 for each additional page (except for reviews, for which the page charges are \$75 per page for all pages). This charge does not apply to reports of ASCN meetings, official ASCN statements, letters to the Editor, book reviews, or articles invited by the Editor-in-Chief. All checks should be drawn on a US bank; there will be a \$20 charge for insufficient funds. Payment may also be made with VISA, MasterCard, or American Express. Bank transfers are accepted; there is a \$20 charge per transfer. Please contact the Production Office for transfer information.

Page proofs

The cost of changes for correction of composition, typographic, editorial, and minor author errors will be absorbed by the *AJCN*. Major author changes will be billed to authors at the rate of \$10 per occurrence.

Reprints

Order forms for reprints are sent with page proofs. Please follow the instructions on the form to order reprints.

Supplements

After a supplement has been found appropriate for publication, the guest editor must contact the *AJCN* to make financial arrangements. Write to the Managing Editor, *The American Journal of Clinical Nutrition*, 9650 Rockville Pike, Bethesda, MD 20814-3998. E-mail: journal@ascn.fascb.org.

SUPPLEMENTAL MATERIAL

Supplemental material may be attached to the published article on the *AJCN* website. This material should be included with the manuscript when it is submitted electronically.

Tips for authors submitting manuscripts to the *AJCN*

To submit your manuscript online, please access the following website: <http://www.rapidreview.com/ASCN2/CALogon.jsp>.

To find out if you already have an account, you may send an e-mail to ajcn@ucdavis.edu. If you have an account, we can give you your username and password information.

Your manuscript must adhere to the following format. If not, we will insist that you make the corrections and re-upload your manuscript files before the manuscript is passed on to the Editor-in-Chief.

- Manuscript must be double-spaced throughout
- Manuscript must have line numbers in the left margin of abstract and text pages
- Manuscript must have the proper items in the Acknowledgment section (ie, conflict of interest statement and description of the contributions of the authors)
- Manuscript must have references cited in numerical order

When submitting a new manuscript, fill in all relevant text boxes and then **be sure** to click "Save but do not submit" at the bottom of the page. This will save your work and assign your manuscript a temporary number. You may then finish your submission by uploading your manuscript files.

If you encounter any problems while submitting online, please contact us by phone [(530) 752-8363] or e-mail (ajcn@ucdavis.edu) and describe the problem. We will answer your message promptly and help you finish the submission process.

The American Society for Clinical Nutrition, Inc

The American Journal of Clinical Nutrition

Authors' Agreement

1. This publishing agreement ("Agreement") is between the undersigned author ("Author") and The American Society for Clinical Nutrition, Inc, a nonprofit corporation with offices at 9650 Rockville Pike, Bethesda, Maryland 20814-3998 USA ("ASCN"), regarding publication of the manuscript identified below (the "Article") in *The American Journal of Clinical Nutrition*. This Agreement is effective on the date last signed.

Manuscript Number: _____

Article Title: _____

2. The Author represents and warrants that to the best of his or her knowledge (a) the scientific findings described in the Article are original and do not infringe on the intellectual property rights of any third party; (b) the Article and its scientific findings have not been previously published nor submitted for publication elsewhere except in abstract form, and there is no offer or agreement for publication pending; (c) the Article and this Agreement identify all co-authors who have substantially contributed to the concept, data collection and analysis, or preparation of the manuscript and therefore who may have intellectual property claims to the content, and do not include as an author anyone who does not have an intellectual property claim, and each Author's contribution to this work has been identified in the Acknowledgments section of the Article; (d) publication of the Article will not violate the terms of any funding agreement, employment agreement, or other contractual arrangement, or the privacy rights of the authors; (e) the Author has identified in a footnote to the title all sources of funding relating to the research represented in the Article; (f) the Author has no undisclosed financial or personal interest in any company or organization connected in any way with the research represented in the Article, including serving as an expert witness or public advocate, grantee, shareholder, option holder, advisor, consultant, employee, or officer, and that any such real or potential conflict of financial or personal interest is disclosed in the Acknowledgments section of the article; (g) the publication of this Article does not require the permission of any other person; (h) the Author has the legal power to enter into this Agreement and grant the rights set forth below (for US government authors, the provision applies only to the extent to which the copyright is transferable); (i) the facts, conclusions, and opinions stated in the Article represent the Author's research, conclusions, and opinions and are believed to be substantiated, accurate, valid, and reliable; (j) the Author has reviewed and approved for publication the version of the Article submitted to *The American Journal of Clinical Nutrition* for publication, and the Author will review for accuracy the final version before publication.

3. For good and valid consideration, receipt of which is hereby acknowledged, the Author hereby grants ASCN the perpetual, paid-up, irrevocable, transferable, and worldwide, sublicensable right to reproduce, publish, adapt, create derivative work, electronically post and disseminate, and publicly display copies of the Article in all media now known or later developed. This grant of right shall be exclusive.

4. The Author is free to use in other works the ideas, facts, and data appearing in the Article. The Author is free to reproduce the Article for purposes such as teaching, research, and scholarship, provided that ASCN is informed of such use and is identified as the publisher of the Article.

5. This Agreement contains the entire understanding of the parties with respect to the stated subject matter and can be modified only by a signed, written agreement. This Agreement shall be construed in accordance with the laws of the State of Maryland and the US copyright laws. This Agreement is binding upon the Author's heirs and personal representatives. If any term of this Agreement shall be found invalid by any court of competent jurisdiction, such provision shall be enforced to the fullest extent that it is valid and enforceable under applicable law, and all other provisions of this Agreement shall remain in full force and effect.

Author's Agreement**Manuscript Number:**

AGREED AND ACCEPTED:

Signature: _____

Print name: _____

Title: _____

Institution: _____

Date: _____

Signature: _____

Print name: _____

Title: _____

Institution: _____

Date: _____

Signature: _____

Print name: _____

Title: _____

Institution: _____

Date: _____

Signature: _____

Print name: _____

Title: _____

Institution: _____

Date: _____

Signature: _____

Print name: _____

Title: _____

Institution: _____

Date: _____

Signature: _____

Print name: _____

Title: _____

Institution: _____

Date: _____

Signature: _____

Print name: _____

Title: _____

Institution: _____

Date: _____

FOR OFFICIAL USE ONLY

The American Society for Clinical Nutrition, Inc

Signature: _____

Print name: _____

Title: _____

Institution: _____

Date: _____