IMPACT OF VITAMINS B12, B6 AND FOLATE SUPPLEMENTATION ON CARDIOVASCULAR RISK MARKERS IN AN ELDERLY COMMUNITY OF SHARPEVILLE

Submitted in fulfilment of the requirements of the degree of Doctor of Technology: Health Sciences in the Faculty of Health Sciences at the Durban University of Technology

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SEPTEMBER 2015

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ABSTRACT

Background: In a vulnerable low-income group with a confirmed high risk of cardiovascular disease, like the elderly in the Sharpeville care centre, an acute intervention is needed in order to improve their health profile. Previous studies suggested homocysteine lowering by vitamin B12, B6 and folate supplementation. The effect of vitamin B12, B6 and folate supplementation on the inflammatory response, thrombotic risk, lipid profile, hypertension, risk of metabolic syndrome and homocysteine metabolism in an elderly, black South African population has never been reported.

Objectives: The main aim of this interventional study was to assess the effect of vitamins B12, B6 and folate supplementation at 200% RDA for six months on cardiovascular risk markers of an elderly semi-urbanised black South African community.

Design: This study was an experimental intervention non-equivalent control group study design in 104 purposively selected samples of all the elderly attending the day-care centre.

Setting and participants: A homogeneous group of respondents was included in the study. All subjects were equivalent in age (>60 years), race (black), unemployed/pensioners (socio-demographic) and 60 years and older attending a day care centre in Sharpeville, situated in the Vaal region, Gauteng, SA.

Measurements: The distinctiveness of this study lies in the broad panel of parameters evaluating the CVR in correlation with the increased nutritional intake of vitamin B6, B12 and folate. These included: weight, height, waist, serum cholesterol, high density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, blood pressure, fibrinogen, high-sensitivity C-reactive protein (HS–CRP), homocysteine, vitamin B12, folate, glucose, insulin, adiponectin and fibronectin.

Results: A very high incidence (66.36%) of hyperhomocysteinaemia is present in the sample. The mean serum homocysteine level in hyperhomocysteinaemic individuals decreased statistically significantly from 25.00±8.00 umol/l to 18.80±12.00 umol/l after the intervention. The number of respondents with an increased homocysteine level decreased from 100% (baseline) to 67% (follow-up). The supplementation was beneficial (statistically significant changes) to the glucose levels, fibrinolytic status, vitamin B6 serum levels,
fibronectin levels and haemopoeiesis (decreased macrocytosis) of all the individuals (regardless of their homocysteine status).

**Conclusion:** It is concluded that supplementation of vitamins B6, B12 and folate at 200% RDA for six months is an effective homocysteine-lowering approach as a strategy to reduce hyperhomocysteinaemia in an elderly population and thereby reduce cardiovascular risk (CVR). The supplementation intervention mentioned is not an effective multifactorial strategy to decrease CVR although beneficial effects were found with other CVR markers independent of homocysteine status.
DEDICATION

I dedicate this thesis to my loving and supportive family - Marko, Amé, Pa and Ousus.
ACKNOWLEDGEMENTS

I hereby express my sincere appreciation to the Almighty for providing me with the opportunity not only to successfully complete the requirements for the qualification, but also for the privilege of this life journey. The completion of this thesis would not have been possible without the help, assistance, guidance and support of various individuals. I would like to convey my gratitude for their contribution to the following people:

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<tr>
<td>ABEI</td>
<td>Amino-Butyl-Ethyl Isoluminol</td>
</tr>
<tr>
<td>ADA</td>
<td>American Diabetes association</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>AVERT</td>
<td>Adverting HIV and AIDS</td>
</tr>
<tr>
<td>AI</td>
<td>Adequate intake</td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency Syndrome</td>
</tr>
<tr>
<td>ARV</td>
<td>Antiretroviral therapy</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>C</td>
<td>Carbon</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>Calcium</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CDL</td>
<td>Chronic disease of lifestyle</td>
</tr>
<tr>
<td>CH</td>
<td>Hydrocarbons</td>
</tr>
<tr>
<td>CH₂</td>
<td>Methylene group</td>
</tr>
<tr>
<td>CH₃</td>
<td>Methyl group</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>Cis</td>
<td>On the same side (geometric isomerism)</td>
</tr>
<tr>
<td>CLIA</td>
<td>Chemiluminescence Immunoassay</td>
</tr>
<tr>
<td>CN⁻</td>
<td>Cyanide</td>
</tr>
<tr>
<td>Co</td>
<td>Cobalt</td>
</tr>
<tr>
<td>CoA</td>
<td>Coenzyme A</td>
</tr>
<tr>
<td>Co Ltd</td>
<td>Company limited</td>
</tr>
<tr>
<td>CRC</td>
<td>Chemical Rubber Company</td>
</tr>
<tr>
<td>CRP</td>
<td>Collagen-related peptide</td>
</tr>
<tr>
<td>CRP-HS</td>
<td>C-Reactive Protein High Sensitivity</td>
</tr>
<tr>
<td>COOH</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficience of Variance</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>CVR</td>
<td>Cardiovascular Risk</td>
</tr>
<tr>
<td>dATP</td>
<td>2’-deoxyadenosin triphosphate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>dCTP</td>
<td>Deoxycytidine triphosphate</td>
</tr>
<tr>
<td>dCTP</td>
<td>Deoxyguanosin triphosphate</td>
</tr>
<tr>
<td>DDS</td>
<td>Dietary diversity score</td>
</tr>
<tr>
<td>DHA</td>
<td>Docosahexaenoic acid</td>
</tr>
<tr>
<td>DHF</td>
<td>Dihydrofolate</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes Mellitus</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DRI</td>
<td>Dietary Reference Intakes</td>
</tr>
<tr>
<td>DSA</td>
<td>Development Southern Africa</td>
</tr>
<tr>
<td>dTDP</td>
<td>Deoxythymidine diphosphate</td>
</tr>
<tr>
<td>dTMP</td>
<td>Deoxythymidine monophosphate</td>
</tr>
<tr>
<td>dUMP</td>
<td>2'-Deoxyuridine-5 monophosphate</td>
</tr>
<tr>
<td>DUT</td>
<td>Durban University of Technology</td>
</tr>
<tr>
<td>EAR</td>
<td>Estimated average requirement</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>EER</td>
<td>Estimated energy requirements</td>
</tr>
<tr>
<td>Ed</td>
<td>Edition</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assays</td>
</tr>
<tr>
<td>EPA</td>
<td>Eicosapentaenoic acid</td>
</tr>
<tr>
<td>EPI</td>
<td>Expanded Programme for Immunization</td>
</tr>
<tr>
<td>Et al.</td>
<td>Et alii (masculine plural), Et aliae (feminine plural) – and others</td>
</tr>
<tr>
<td>ex.</td>
<td>Example</td>
</tr>
<tr>
<td>FA</td>
<td>Folic Acid</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
</tr>
<tr>
<td>FBDG</td>
<td>Food-based dietary guidelines</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acids</td>
</tr>
<tr>
<td>FFQ</td>
<td>Food frequency questionnaire</td>
</tr>
<tr>
<td>FG</td>
<td>Fibrinogen</td>
</tr>
<tr>
<td>FGDS</td>
<td>Food Group Diversity Score</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
</tr>
<tr>
<td>fl</td>
<td>Femtoliter</td>
</tr>
<tr>
<td>FVS</td>
<td>Food Variety Score</td>
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<tr>
<td>Gamma GT</td>
<td>Gamma-glutamyltranserase</td>
</tr>
</tbody>
</table>

xix
g/day: Gram per day

G/dl: Gram per decilitre

G/kg: Gram per kilogram

G/l: Gram per litre

G/ml: Gram per millilitre

GmbH: Gesellschaft mit beschränkter Haftung (German: Limited Liability Company)

GOD: Glucose oxidase

GOD-POD: Glucose Oxidase-Peroxidase

GP: Glycoprotein

GPA: Gauteng provincial administration

H: Hydrogen

h: Hour

H2O: Water / Aqua

Hb: Haemoglobin

Hct: Haematocrit

HDL: High-density lipoprotein

HIPOP-OHP: The High-risk and Population Strategy for Occupational Health Promotion

HIV: Human Immunodeficiency Virus

HMG-CoA: β-hydroxy-β-methylglutaryl-coenzyme A

HMWK: High molecular weight kininogen

HQ: Head Quaters

HPLC: High Performance Liquid Chromatography

HRP: Horseradish Peroxidase

HS-CRP: High-sensitivity C-reactive protein

IBL: Immuno - Biological Laboratories Co. Ltd.

IDL: Intermediate-density lipoprotein

IF: Intrinsic factor

IFCC: International Federation of Clinical Chemistry

IL: Interleukin

IANA: International Academy on Nutrition and Aging

iNOS: Inducible nitric acid synthase

INP: Integrated Nutrition Programme
JNC7 Joint National Committee 7
Kg Kilogram
Kcal/day Kilocalories per day
l litre
LDL Low-density lipoprotein
LSSA Lipid and Atherosclerosis Society of South Africa
m Metre
MBP Mannose-binding protein
MCH Mean cell haemoglobin
MCHC Mean cell haemoglobin concentration
MCP-1 Monocyte chemoattractant protein
MCV Mean cell corpuscular volume
mg Milligram
mg/day Milligram per day
mg/dl Milligram per decilitre
mg/l Milligram per litre
mmHg Millimetre mercury
mmol/l Millimoles per litre
MI Myocardial infarction
MNA Mini-Nutritional Assessment
MPV Mean platelet volume
MRC Medical Research Council
MSC Master of Science
MTHFR Methylene tetrahydrofolate reductase
MUFA Monounsaturated fatty acids
n number
N Nitrogen
NAD Nicotinamide adenine dinucleotide (oxidized)
NADH Nicotinamide adenine dinucleotide (reduced)
NCEP National Cholesterol Education Program
ng/ml Nanogram per millilitre
NIH National Institute of Health
nmol/l Nanomoles per litre
NRF National Research Foundation
Oxygen
OH
Hydroxyl
Oy
Osakeyhtiö (Finish: limited company)
P
Phosphorous
PAI-1
Plasminogen activator inhibitor -1
Pct
Plateletcrit
PDW
Platelet distribution width
PECAM-1
Platelet-endothelial-cell-adhesion-molecule-1
PEG
Polyethylene glycol
pg/ml
Pictogram per millilitre
PLP
Coenzyme pyridoxal-5’-phosphate
Plt
Platelet count
POD
Peroxidase
PROVE IT-TIMI
Pravastatin or atorvastatin evaluation and infection trial-22
PUFA
Polyunsaturated fatty acids
Rand
RBC
Red cell count
RDA
Recommended Daily Allowance
RDW
Red cell distribution width
RLU
Relative luminescence units
RSA
Republic of South Africa
SA
South Africa
SAA
Serum amyloid A
SAH
S-adenosylhomocysteine
SA Heart
South African Heart Association
SAM
S-adenosylmethionine
SAMRC
South African Medical Research Council
SANAS
South African National Accreditation System
SBP
Systolic blood pressure
sCAL
Serum Calibrator
SFA
Saturated fatty acids
SPIDIO
Société de pharmacologie et d’Immunologie-BIO
SPSS
Statistical Product and Service Solutions
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>SSA</td>
<td>Sub-Saharan Africa</td>
</tr>
<tr>
<td>TC</td>
<td>Total cholesterol</td>
</tr>
<tr>
<td>TFA</td>
<td>Trans-fatty acids</td>
</tr>
<tr>
<td>TFPI</td>
<td>Tissue factor pathway inhibitor</td>
</tr>
<tr>
<td>TG</td>
<td>Triglycerides</td>
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<tr>
<td>tHcy</td>
<td>Plasma homocysteine levels</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofolate</td>
</tr>
<tr>
<td>THR</td>
<td>Thrombin</td>
</tr>
<tr>
<td>THR</td>
<td>Triglyceride HDL-C ratio</td>
</tr>
<tr>
<td>THUSA</td>
<td>Transition and Health during Urbanization in South Africa</td>
</tr>
<tr>
<td>TM</td>
<td>Trademark</td>
</tr>
<tr>
<td>TMB</td>
<td>Tetra Methyl Benzidine</td>
</tr>
<tr>
<td>TNFα</td>
<td>Tumour necrosis factor alpha</td>
</tr>
<tr>
<td>TT</td>
<td>Thrombin time</td>
</tr>
<tr>
<td>TXA2</td>
<td>Thromboxane A₂</td>
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<td>Trans</td>
<td>On the other side (geometric isomerism)</td>
</tr>
<tr>
<td>μg/day</td>
<td>Microgram per day</td>
</tr>
<tr>
<td>μg/l</td>
<td>Microgram per litre</td>
</tr>
<tr>
<td>U.K</td>
<td>United Kingdom</td>
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<tr>
<td>μmol/l</td>
<td>Micromoles per litre</td>
</tr>
<tr>
<td>μm</td>
<td>micrometer</td>
</tr>
<tr>
<td>UNPD</td>
<td>United Nations Population Division</td>
</tr>
<tr>
<td>U.S.</td>
<td>United States</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>μU/l</td>
<td>Micro Unit per litre</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very low-density lipoprotein</td>
</tr>
<tr>
<td>VSMC</td>
<td>Vascular smooth muscle cell</td>
</tr>
<tr>
<td>VUT</td>
<td>Vaal University of Technology</td>
</tr>
<tr>
<td>VWF</td>
<td>von Willebrand factor</td>
</tr>
<tr>
<td>WBC</td>
<td>White blood cell count</td>
</tr>
<tr>
<td>WC</td>
<td>Waist circumference</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WHTR</td>
<td>Waist-to-height ratios</td>
</tr>
</tbody>
</table>
LIST OF SYMBOLS

±  Plus, minus
-  Till
%  Percentage
/  or
+  Plus or more than
<  Smaller than
>  Greater than
÷  Divided by
≤  Smaller than or equal
≥  Greater than or equal
α  Alpha
LIST OF ANNEXURES

Annexure A  Ethical approval University of the Witwatersrand
Annexure B  Ethical approval Durban University of Technology (DUT)
Annexure C  Letter of information and consent - individual
Annexure D  Field workers control list
Annexure E  Health, medical and behavioural questionnaire
Annexure F  Dietary intake (24-h recall)
Annexure G  Food consumption patterns questionnaires (FFQ)
Annexure H  Socio-demographic questionnaires
Annexure I  Clinical signs
Annexure J  VITAFORCE product insert
CHAPTER 1

THE PROBLEM AND ITS SETTING

1.1 INTRODUCTION

Cardiovascular disease (CVD) exists in epidemic proportions in developed countries and is an increasing problem in the developing world (Yusuf, Reddy, Ounpuu and Anand 2001:2864). Black elderly people in South Africa live in poor living conditions and have limited resources to maintain health and well-being (Du Rand and Engelbrecht 2001:15). A previous study conducted in the same community indicated the presence of a number of risk markers for cardiovascular disease, namely a mean fibrinogen level of 5.3±2.2 g/l, a 68% prevalence of hypertension (≥140/95 mmHg) and a deficient intake of vitamin B12 (91.1% for women and 66.7% for men) (Oldewage-Theron, Salami, Zotor and Venter 2008(a):7). Correlations between vitamins B12, B6 and folate status and cardiovascular disease have been demonstrated by numerous studies (Dhonukshe-Rutten, De Vries, De Bree, Van Der Put, Van Starveren and De Groot 2009:18). None of these studies, however, evaluated these phenomena in the African context. What is unique to this study is the extensive panel of cardiovascular risk markers measured.

1.2 CONTEXT OF THE RESEARCH

1.2.1 Global geriatric profile

Globally, the definition of “elderly” varies; the chronological and current definition used refers to people older than 60 years (Charlton, Ferreira and Du Plessis 2008:549), as it does in this study. The African definition varies from the Western definition in that old age may be defined according to the functionality, appearance and social role of the individual (Kinsella and Phillips 2005:38).
Globally, with the expansion of the section of the population older than 60 years, populations are ageing (Charlton et al. 2008:548). The demographic transition is created by the fact that fewer babies are being born and that people are living longer. Although this phenomenon occurs globally, in Sub-Saharan Africa (SSA) the high mortality associated with acquired immunodeficiency syndrome (AIDS) and other diseases has decreased the pace of transition, limiting the transition effect in Africa (Charlton et al. 2008:548). Despite this limitation on transition, a steady growth in the elderly population is nevertheless projected over the next two decades (Joubert and Bradshaw 2006:206).

1.2.2 South African geriatric profile

In South Africa (SA), 4.2 million, or 8% of the total population, were reported to be 60 years and older in 2011 and it is projected that by 2050 the number will have grown to 19% (United Nations Population Division 2006). According to the statistics obtained during the Census of 2011, Black Africans make up 65.3% of the population older than 60 years of age (Statistics South Africa Census in brief 2011). The projected growth in the number of the elderly in the next twenty years indicates that females will be more affected than males (Joubert and Bradshaw 2006:208). In spite of wider distribution of social and older persons’ grants currently, chronic poverty is still prevalent (Barrientos, Ferreira, Gorman, Heslop, Legido-Quigley and Lloyd-Sherlock 2003; Møller and Ferreira 2003). Studies have shown that the pension benefits of Black African elderly persons are commonly utilised to sustain family members by paying for food, rent, utilities and, often, the education of grandchildren (Barrientos et al 2003; Møller and Ferreira 2003). Charlton et al (2008:552) found that Black African households headed by an elderly person had the highest risk of food poverty across all ethnic groups. Black elderly persons depending on their grant as a source of income can be defined as a low-income group, for whom household food insecurity and malnutrition are a common problem. Malnutrition has negative effects on the health status of the elderly, including chronic diseases of lifestyle (Lesourd 2006:324; Charlton et al 2008:559, 569). Older people are the most frequent users of the health care services, and improving the health status of the elderly should,
therefore, have a positive effect on the health care budget of the country (Charlton et al 2008:554).

Even though the Human Immunodeficiency Virus (HIV) infection rate in the elderly is as low as 2–3% (Averting HIV and Aids (AVERT) 2008), its secondary effects are multifaceted, as 60% of AIDS orphans in South Africa are estimated to live with their grandparents (Monash and Boerma 2004:560). Because of lack of formal support, the elderly caregivers of the orphans (mainly grandmothers) are faced with financial, health and emotional challenges (Maitse and Majake 2005; Joubert and Bradshaw 2006:206).

1.2.3 Geriatric profile in Sharpeville

Oldewage-Theron and co-workers have studied the same elderly community in Sharpeville since 2004 and have observed the following (Oldewage-Theron et al 2008(a):7; Oldewage-Theron, Samuel, Grobler and Egal 2008(b):25; Oldewage-Theron, Samuel and Venter 2008(c):571; Medoua, Egal and Oldewage-Theron 2009: 260; Oldewage-Theron and Kruger 2009:306:

- The elderly in Sharpeville have a low literacy level. Only five percent of the aged in Sharpeville live alone; the majority live with other family members (the average number of persons per household is 4.9). The majority live in brick houses with access to safe water, electricity and sanitary and waste removal services.

- The majority of the respondents receive a state pension and have a household income of between R501 and R1000 per month, with food expenses of less than R200 per week. Food insecurity and poor food choices and variety were identified. This is a major contributory factor to nutritional and health status.

- The major health complaints were eye and upper respiratory tract infections. A very low activity level was identified. Identified as health risk markers were the 68% prevalence of hypertension (≥140/95 mmHg) and obesity (29.5% overweight: Body Mass Index (BMI) 25–29), 27.9% obese: BMI 30–34.5 and 26.2% very obese: BMI 35+). Biochemical blood markers indicated that 41.8% of respondents had a cholesterol level of 5.5–6.2 mmol/l (borderline risk of cardiovascular
disease), 22% of respondents had an increased blood glucose level (>5.9 mmol/l), 73.1% of the respondents had a decreased zinc level (<50μg/l) and 20.9% of respondents had a low serum iron level (<9 μmol/l for female and 11.6 μmol/l for males). Also observed were a very high risk of cardiovascular disease (mean fibrinogen level was 5.3±2.2 g/l, whereas the normal range is 1.8–3.5 g/l), and a deficient intake of vitamin B12 (91.1% for women and 66.7% for men).

It is clear from these studies that these elderly communities live in poverty, experience food insecurity, are malnourished, and that CVD risk factors are present among them.

The nutrition transition indicates a modification of the dietary patterns and nutrient intake after the alteration of circumstances or environment, and is marked by demographic transition and epidemiological transition (Vorster and Bourne 2008:234). Vorster and Bourne (2008:235) indicate that the epidemiological transition is a change from an environment where malnutrition-related infectious diseases are highly prevalent to an environment where there is an increase in chronic diseases of lifestyle. The nutrition transition, prevalent in industrial countries (Pekka, Pirjo and Ulla 2002:250), also exists in developing countries (Puoane, Steyn, Bradshaw, Laubscher, Fourie, Lambert and Mbananga 2002:1038). The results obtained from Sharpeville confirm that the double burden of disease (both over nutrition and under nutrition) is present in this elderly community.

1.2.4 Policy approaches to the ageing in South Africa

The South African Health policy focuses on children, youth and maternal care. The health needs of the elderly are marginalised and, therefore, not addressed (Charlton et al. 2008:575). Prior to 1991 (formulation of policies in the United Nations Principles of Older Persons1), approaches to the elderly were humanitarian or welfare-orientated (Charlton et al 2008:549). Latest approaches are considering the elderly as contributors to family,

---

1 Adopted by General Assembly resolution 46/91 of 16 December1991, http://www2.ohchr.org/English/law/olderpersons.htm
community and society, and therefore, participants in the social, economic and political environment (Charlton et al 2008:549).

In 2002, the *Madrid International Plan of Action on Ageing* called for a plan to ensure adequate food and nutrition for the elderly. An integration of policies, approaches and responses to critical areas has been suggested (Charlton et al 2008:550). However, in SA this has not yet materialised.

### 1.2.5 Nutrition-related health problems in the elderly

Malnutrition is defined as under nutrition (if the diet contains insufficient energy, protein, vitamins and minerals) and over nutrition (if too much energy is consumed) (Kuzwayo 2008:566). The nutritional status of the aged has been identified as the major determining factor of their cognitive and physical functionality (Charlton et al 2008:554). Malnutrition among the elderly is negatively associated with health status, resulting in increased infection rate, hospitalisation, morbidity and mortality (Charlton, Kolbe-Alexander and Nel 2007:534). Globally, a direct relation exists between health and disease patterns within a population and the ageing of the population (Joubert and Bradshaw 2006:210). In order to understand nutritional problems in the elderly it is important to understand the physiological changes that contribute to poor nutrition.

The elderly are at risk of under nutrition. Factors that contribute to inadequate nutritional intake are: social factors (poverty, isolation, difficulty in preparing food, needing assistance with feeding), physical factors (difficulty in chewing and swallowing, decrease in appetite and food intake, poor dentition, diminished sense of taste and smell, interaction of medication, constipation, decline in lactose production, liver function, insulin function, kidney function, lung function and vision), and psychological/emotional factors (depression, dementia, anorexia, fear of choking) (Wardlaw and Kessel 2002:722; Charlton et al 2008:559). Insufficient nutrition results in the loss of physiological function and enhances the onset and progression of disease (Bates, Benton, Biesalski, Staehelin, van Staveren, Stehle, Suter, Wolfram 2002:103). Wardlaw and Kessel (2002:722) indicated
that approximately 30% of elderly persons in America have lost their teeth, and dentures may complicate chewing. Charlton et al (2008:555) indicated that the Black African elderly in South Africa have an insufficient micronutrient intake, with a dietary reference intake (DRI) of <67%, and vitamins A, B6, C, D, E, K, folate, biotin and minerals such as calcium, selenium, magnesium and copper identified as inadequate. Oldewage-Theron and Kruger (2008:128) found similar results in a group of elderly in Sharpeville, Gauteng, South Africa, and identified an insufficient intake of energy, vitamins (A, B2, B6, C, D, E, folate and biotin) and calcium, selenium and magnesium. Biochemical tests indicated a zinc deficiency.

As indicated in 1.2.3, the double burden of disease (presence of both under nutrition and over nutrition) exists also among the elderly. The most prevalent/common nutritional problems experienced by the elderly are anaemia, impaired cognitive function, unhealthy bones (osteoporosis, falls, fractures), and chronic diseases of lifestyle (obesity, hypertension).

**1.2.5.1 Anaemia**

Anaemia is defined by the World Health Organisation (WHO) as haemoglobin (Hb) <13 g/dl (males) and <12 g/dl (females) (Morley and Thomas 2007:488). Anaemia causes fatigue and cardiovascular complications because of the decrease in oxygen transported as a result of the decrease in haemoglobin (Charlton et al 2008:570). Causes of anaemia are as follow: 1) Failure of the bone marrow to produce red blood cells due to inadequate nutrients (vitamin B12, folate, vitamin B6 or iron), 2) rapid loss of red cells (haemorrhage), and 3) increased destruction (haemolysis) of erythrocytes. Charlton, Kolbe-Alexander and Nel (2005:477) found that the prevalence of anaemia is higher in older South Africans than their counterparts in Europe and United States.
1.2.5.2 Impaired cognitive function

Malnutrition has been identified as the cause of cognitive impairment and mental illness in older people (Charlton et al 2008:550). Epidemiological studies have indicated that certain macronutrients and micronutrients (vitamin C, vitamin E, flavonoids, unsaturated fatty acids, vitamin B12 and folate) have protective effects against cognitive decline (Gillette-Guyonnet, Abellan van Kan, Andrieu, Barberger Gateau, Berr, Bonnefoy, Dartigues, de Groot, Ferry, Galan, Hercberg, Jeandel, Morris, Nourhashemi, Payette, Poulain, Portet, Rousel, Ritz, Rolland and Vellas 2007:150). Sufficient intakes of these nutrients in the elderly are therefore important.

1.2.5.3 Impaired immune function

The elderly are at risk of infection owing to compromised immune function caused by immune senescence and malnutrition (Charlton et al 2008:550). A healthy nutritional status is required in order to maintain the immune system (Morley and Thomas 2007:488). Recurrent infections and reduced wound healing are indicators of a deprived immune system; a contributing factor is insufficient protein, vitamins (particularly vitamin E and B6) and zinc (Wardlaw and Kessel 2002:722). Micronutrient supplementation was found to have beneficial effects on the immune response of the elderly (Charlton et al 2008:550).

1.2.5.4 Osteoporosis, falls, fractures and bone health

Skeletal conditions characterised by decreased bone density, reduced bone strength and deteriorating microscopic composition of the bone (osteoporosis) cause falls and fractures in the elderly. Bone loss with age is genetically controlled (peak bone mass in mid-20s) but varies among individuals. The risk of osteoporosis can be modified by environmental factors such as physical activity, nutrition (adequate calcium and vitamin D is needed), medication and disease (Charlton et al 2008:574).
1.2.5.5 Obesity, hypertension and chronic diseases of lifestyle (CDL)

Obesity is considered a global epidemic, with 1.6 billion adults overweight and 400 million obese, while numbers are still increasing (Kastorini, Milionis, Goudevenos and Panagiotakos 2010:537). As per definition, obesity is a BMI ≥30, overweight is a BMI of 25.0–29.9, healthy weight a BMI of 18.5–24.9 and underweight a BMI of <18.5 (BMI = \( \frac{\text{weight (kg)}}{\text{height}^2 \text{(m)}} \) (WHO 1997; Charlton et al 2008:574). The distribution of fat is a more critical factor as a predictor of CVD than the total amount of fat. Central obesity or upper body fat is an increased amount of fat stored around the abdominal area of the body, which holds an increased risk of the development of cardiovascular disease compared with lower body fat (as indicated in figure 1) (Geissler and Powers 2006:364; Whitney and Rolfes 2008:263).

![FIGURE 1 COMPARISON BETWEEN UPPER BODY FAT AND LOWER BODY FAT](adapted from Whitney and Rolfes 2008:263)

For every 1% increase above ideal BMI, the cardiovascular risk (CVR) increases by 3.3% for females and 3.6% for males (Kastorini et al 2010:537). In old age, changes in body composition are evident in increased body fat, redistribution of body fat to the abdomen
and loss of muscle mass (sarcopenia) (Wittert 2007:46). As lean tissue decreases it is replaced by fat, which increases with increased fat intake and decreased activity (Wardlaw and Kessel 2002:722).

The elderly are predisposed to fat accumulation and fat redistribution as a result of decreased physical activity (Morley and Thomas 2007:46). Weight gain and increased abdominal or central obesity, as stated, are common problems in the elderly and are linked to an increased risk of cardiovascular disease, hypertension and diabetes (Charlton et al 2008:569). The elderly most at risk of mortality are those who are both sarcopenic (decrease in muscle) and obese (increase in fat) (Morley and Thomas 2007:47). Obesity can also aggravate other chronic conditions such as respiratory diseases, arthritis and impaired physical mobility. A cross-sectional study among peri-urban, free-living black elderly people in Cape Town, SA, indicated that 65% of women were obese and 20% overweight. Underweight was noticed in twenty percent of the males and only in 2.2% of the women (Charlton et al 2007:540). Results obtained in a peri-urban community in Sharpeville showed that 4.5% of males and 26.4% of females were very obese (BMI >35), 13.6% of males and 27.7% of females were obese (BMI 30-34.9), and that 18.2% of males and 29.7% of females were overweight (BMI 25-29.9), while, in contrast, 9.1% of males and 0.00% of females were underweight (Oldewage-Theron et al 2008(b):566). These results correspond to results obtained from studies performed globally (Wittert 2007:46).

Obesity increases the risk of chronic diseases of lifestyle (CDL) (discussed in chapter 2.2) and therefore decreases life expectancy (Kastorini et al 2010: 537) but can be counteracted by increased physical activity (Wardlaw and Kessel 2002:725). Lifestyle interventions that include diet and initiation of physical activity have a clinically significant effect of weight loss and a decrease in cardiovascular risk factors (Goodpaster, Delany, Otto, Kuller, Vockley, South-Paul, Thomas, Brown, McTigue, Hames, Lang and Jakicic 2010:1795).

Chronic diseases of lifestyle contribute to 84% of mortalities in South Africa (Joubert and Bradshaw 2006:210). The Department of Health (2002) has indicated that more than 50%
of South Africans aged 60 years and older are hypertensive (BP ≥140/90 mmHg). Hypertension is directly related to cardiovascular disease in the elderly as well as in other population groups, with devastating health effects (Morley and Thomas 2007:51) (discussed in 2.2).

Diabetes mellitus is the most common but also the most complex CDL. All nations and generations are affected by diabetes with devastating effects. Globally, 20% of the elderly suffer from diabetes (Morley and Thomas 2007:430). The growing rate of diabetes and the mortality and morbidity associated with it indicate that an interdisciplinary approach is needed to address this disease in the older person (Morley and Thomas 2007:438) (discussed in 2.4.9).

Cardiovascular disease as a CDL for the purpose of this study will be discussed in Chapter 2.

1.2.6 Background of this study

This study forms part of a multi-micronutrient supplementation programme to address malnutrition among the elderly attending a day care centre. The day care centre was established in 2004 in Sharpeville and offers skills training and religious activities aimed at low-income elderly persons (aged ≥60 years), and provides breakfast and lunch on the days the elderly voluntarily attend the centre. Sharpeville is situated in the Vaal region (see figure 2), Gauteng, SA. The Vaal region is approximately 70 km south of Johannesburg and is a highly industrialised and polluted region (Medoua et al 2009:260). Sharpeville has a population of 45 000 people, of which an estimated 7.3% (3285) are aged 60 years and older. Forty-six percent of the Vaal region’s households live in poverty (McIlrath and Slabbert 2003). This is also true for the elderly community. Their only means of income is the social pension. Møller and Ferreira (2003) indicated that elders share this limited resource with family members. Poor social status is a contributor to malnutrition and health risk, which this programme aims to address.
The programme was ethically approved by the Medical Ethical Committee for Research on Human Beings of the University of the Witwatersrand, Johannesburg (M070126), funded by the National Research Foundation (NRF) and the Vaal University of Technology (VUT), and managed by Prof. W.H Oldewage-Theron. In 2004-2005 zinc and iron deficiencies were diagnosed; Prof. W.H Oldewage-Theron conducted a multi-micronutrient (zinc and iron) supplementation study in 2007-2008. This study is a follow-up, as vitamins B6, B12 and folate deficiencies were still prevalent in these elderly people after the zinc and iron supplementation study ended, together with an increase in fibrinogen and blood pressure. The researcher is responsible for the current study that forms part of the bigger programme.

FIGURE 2 SCHEMATIC ILLUSTRATION OF THE VAAL REGION, GAUTENG, SOUTH AFRICA
1.3 MOTIVATION

Positive correlations between vitamin B12, B6 and folate status and cardiovascular disease have been demonstrated by numerous international studies (Dhonukshe-Rutten et al 2009:18). However, none of these studies has evaluated such correlations in the African context. Contradictory results were found in previous studies and a possible reason was found to be an insufficient concentration of supplementation (Morley and Thomas 2007:160; Acikel, Dagon and Akedemir 2009:327; Dhonukshe-Rutten et al 2009:18). Lowering of homocysteine was found in studies using between 100% and 200% of the Recommended Daily Allowance (RDA) of vitamin B6, B12 and folate (van der Griend, Biesma, Haas, Faber, Duran, Meuwissen and Banga 2000:225; Verhoef and de Groot 2005:119; Morley and Thomas 2007:160; Acikel et al 2009:327). To the researcher’s knowledge, this study is novel because no study of this kind has been conducted internationally in an elderly community. The novelty of this study is also extended by the variety of interactive parameters of cardiovascular risk markers measured (as indicated in the conceptual framework, figure 3) and the supplementation of \( >100\% \) RDA of vitamin B6, B12 and folate for a six-month period.

The researcher decided on a six-month follow-up study, as other studies varied from three months to 24 months, but none after six months. A difference was observed in most studies (Kaul, Zadeh and Shah: 2006:915; Acikel et al 2009:327; Dhonukshe-Rutten et al 2009:18). This extensive panel of interactive markers will give a clear indication of the mechanism by which the supplementation of vitamins B12, B6 and folate affect the biochemical predictors of risk of cardiovascular disease, and will thus meaningfully contribute to knowledge of geriatric treatment that may be useful for health care workers.

1.4 AIM

The main aim of this study was to evaluate the effect of vitamins B12, B6 and folate supplementation at \( >100\% \) RDA for six months in terms of a panel of specialised
cardiovascular risk markers in a random sample of elderly men and women of low socio-economic status (n=200) with identified high cardiovascular risk. No side effects have been reported for the time frame and concentration of supplementation used, according to the supplier (see annexure L).

1.5 OBJECTIVES

The specific objectives of this study were to:

- Determine the correlation between cardiovascular risk markers (lipogram, fibrinogen, high sensitive C-reactive protein (HS-CRP) and the homocysteine metabolism (vitamin B12, folate and homocysteine) in an elderly community.
- Evaluate the effect of vitamins B12, B6 and folate supplementation at >100% RDA for six months on the homocysteine metabolism in an elderly community.
- Determine the prevalence of metabolic syndrome as cardiovascular risk marker in an elderly community.
- Assess the correlation between dietary intake and cardiovascular risk markers.
- Assess whether vitamin B12, B6 and folate supplementation at >100% RDA for six months has an effect on the coagulation status (fibrinogen and plasminogen activator inhibitor -1 (PAI-1) as cardiovascular risk marker.
- Assess the effect of vitamin B12, B6 and folate supplementation at >100% RDA on the metabolic syndrome status of an elderly community.
- Assess the effect of vitamin B12, B6 and folate supplementation at >100% RDA on tissue factors (adiponectin and fibronectin) as cardiovascular risk markers.
- Assess the effect of vitamin B12, B6 and folate supplementation at >100% RDA on the lipid profile of the subjects (cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides) as cardiovascular risk marker.
- Assess the effect of vitamin B12, B6 and folate supplementation at >100% RDA on inflammatory marker HS-CRP, as cardiovascular risk markers.

A conceptual framework (figure 3) was developed by the researcher, and represents the objectives of the study.
FIGURE 3  CONCEPTUAL FRAMEWORK OF THE OBJECTIVES OF THE STUDY

The question marks (?) in figure 3 represent the interactions that were measured in this study.
1.6 RELEVANCE

Notably, limited studies have been done in this vulnerable population, who’s nutritional, socio-economic and cultural lifestyles are substantially different from those of developed countries (Oldewage-Theron et al 2008(a): 23).

As indicated in section 1.2.4, there is no policy in South Africa addressing the needs of the elderly. The Department of Health adopted the Integrated Nutrition Programme (INP) in 2004. The aim of the INP is to facilitate a multi-sectorial approach to address nutritional challenges in South Africa. Although the INP does not specify nutritional challenges of the elderly as an entity, this study does adhere to the INP framework on multiple levels (refer to framework as indicated in figure 4). This study assesses needs, implements action and analyses results on community level, which may suggest action that could be implemented on a national level. This study will also address multiple objectives of the INP (figure 4). It will: a) investigate the effect of supplementation on household food insecurity b) investigate the effect of supplementation on micronutrient and malnutrition control, and c) measure disease-specific nutritional support, all of these forming part of the INP strategies to address malnutrition in all South Africans throughout the life cycle.

The World Bank (2006) has confirmed that malnutrition undermines economic growth and sustains poverty. The Copenhagen Consensus (2004) concluded that, of 17 potential developmental investments, nutrition interventions generate the fifth highest returns. Malnutrition slows economic growth through three routes: 1) direct loss in productivity from poor physical status, 2) direct loss due to impaired cognitive function and 3) losses due to increased health care costs.

Addressing nutrition-related health challenges brings greater returns in the form of economic benefits than addressing welfare and social issues or protection of human rights; this study should thus indirectly result in economic benefit, as a healthier elderly community will ease the burden on the health care system and be more productive.
1.7 OUTLINE OF THE THESIS

This thesis will be divided into seven chapters. Chapter one provides information on the background and framework of the study, stipulating the problem and its setting. An extensive literature review is reported in chapter two (cardiovascular risk markers and homocysteine-lowering mechanisms) and chapter three (strategies to address health problems in the elderly). The objectives, methodology followed, as well as results and discussion on the baseline survey are reported in chapter four. In chapter five, the objectives, methodology, and results of the intervention study are reported. The overall conclusions, discussions and recommendations relating to the study are included in
Chapter six. Chapter seven is a reference list compiled using the Harvard style of referencing, as prescribed by the guidelines of the Durban University of Technology (DUT).

A systemic flow diagram representing the planning and administration process of the research project is given in figure 5.

FIGURE 5 PLANING, ADMINISTRATION and REPORTING OF THE RESEARCH PROJECT
CHAPTER 2

LITERATURE REVIEW – CARDIOVASCULAR RISK FACTORS FOR THE ELDERLY

2.1 INTRODUCTION

Cardiovascular disease (CVD) as discussed in this study refers to a group of conditions affecting the heart – referred to as coronary heart disease (CHD) – as well as the vascular system. The pathophysiology is caused by atherosclerosis that narrows the small blood vessels that decrease the blood supply and consequently the oxygen supply to the tissues. Atherosclerosis is enhanced by certain contributory factors referred to as cardiovascular risk markers.

It is predicted that by the year 2020 CHD and stroke will still be the first and second leading causes of death globally (Murray and Lopez 1996:325-396). Although cardiovascular disease exists in epidemic proportions in developed countries it is also an increasing problem in the developing world (Yusuf et al 2001:2855-2864). Even in low- to middle-income countries such as SA cardiovascular disease is already responsible for about one tenth of healthy years lost second only to HIV and AIDS and still rising (WHO 2002; Pieters and Vorster 2008:171).

2.2 CHRONIC DISEASE OF LIFESTYLE (CDL)

South Africa suffers from a quadruple burden of disease (poverty-related diseases CDL HIV/AIDS and the effect of social instability caused by crime and violence) which has a severe effect on the prevention of and cost-effective health care management of chronic disease (Steyn 2006:1). The continuous urbanisation and westernisation of the Black African population of SA is marked not only by demographic transition but also by health transition resulting in an increased prevalence of CDL (Pieters and Vorster 2008:171). The demands made by the burden of disease on the limited resources of the health care service of SA exceed the demands experienced in the developed countries (Steyn 2006:1).
Chronic diseases of lifestyle are a group of diseases such as obesity, hypertension, diabetes and ultimately cardiovascular diseases and cancer for which unhealthy lifestyle elements (smoking lack of exercise malnutrition) are predisposing factors (Steyn 2006:1) (refer to section1.2.5.5). The conceptual framework of the interrelationship between risk behaviour and the development of CDL has been indicated in figure 6 (adapted from Steyn 2006:1).

- Cigarette smoking may lead to tobacco addiction resulting in an increase in smoking. Smoking as discussed (in 2.4.3) is an independent risk marker of CVD but also a cause of obstructive lung disease and an inducer of lung cancer.
- A poor diet of inadequate fibre, vitamins and minerals and/or excess saturated fat and cholesterol can cause dyslipidaemia hypertension and obesity which are also known risk markers of CVD (discussed in 2.4.1 2.4.5 and 2.4.6).
- Excess salt and alcohol are contributory factors to hypertension which as mentioned above is an independent risk marker of CVD.
- Excess alcohol can also contribute to the risk of diabetes, hypertension, renal disease and nutrition-induced cancers.
- Obesity and diabetes are CVD risk markers (discussed in 2.4.1 and 2.4.9), that are caused by excessive kilojoule intake and insufficient exercise.

Chronic diseases of lifestyle are therefore preventable conditions that are caused by modifiable factors (Steyn, Blaauw, Lombard and Wolmarans 2008:699). Joubert and Bradshaw (2006:216) have highlighted the importance of addressing the high rate of morbidity and mortality as an effect of CDL on the South African elderly community by implementing preventative strategies. Stroke rather than ischaemic heart disease is prominent in black South Africans; however with the health transition associated with urbanisation ischaemic heart disease is on the increase (Vorster, Kruger, Venter, Margetts and Macintyre 2007:321; Pieters and Vorster 2008:164).

The focus of this project is the risk of CVD; therefore the rest of the chapter will focus on CVD in the context of CDL.
2.3 CARDIOVASCULAR DISEASE

Cardiovascular disease is a result of impaired blood flow and is caused by atherosclerosis which involves structural and compositional changes in the *Tunica intima* layer of the arteries. Atherosclerosis of the cardiac arteries causes myocardial infarction or angina of cerebral
arteries. It causes a stroke or if the atherosclerosis occurs in the peripheral circulation it causes intermittent claudication and gangrene (Krummel 2008:835; Mudau, Genis, Lochner, Strijdom 2012:222; World Heart Federation 2012). The main clinical conditions that can be induced by atherosclerosis are angina pectoris (pain in the chest activated by stress or exertion) and coronary thrombosis (myocardial infarction and cerebral thrombosis) (Mudau et al 2012:222; World Heart Federation 2012). In the elderly the cardiac output is decreased, atherosclerotic plaque accumulates that reduces the vascular blood flow (Torzewski and Bhakdi 2013:22). Additionally age has been recognised at a contributing factor to endothelial dysfunction and thereby contributing to CVD (Mudau et al 2012:225).

2.3.1 Atherosclerosis

Atherosclerosis is a process of plaque formation in which soft fatty strips accumulate on the inner wall of the artery (especially in the area of branches). The fatty strips then become hardened by the secondary accumulation of lipoproteins, cholesterol, triglycerides, platelets monocytes, calcium and fibrous connective tissue, a process that causes an inflammatory response (Burtis 2008:618; Cross 2012:249). Plaque formation is initiated as a response to endothelium wall injury (Rubin, Strayer and Rubin 2011:445). The atherosclerotic process is a confirmed immune-mediated inflammatory response (Nyström 2007:79; Burtis 2008:618). The process of atherosclerosis (as indicated in figure 7) narrows and thickens the artery and also narrows the passage (Pazos, Mongrain and Tardif 2010:1520). Plaque formation develops at any age (beginning in childhood) and in any blood vessel but affects mainly the coronary arteries and can be well developed at an age of 30 years (Moore and Tabas 2011:341; Torzewski and Bhakdi 2013:22). The causes of endothelial injury are hypertension, cigarette smoking, diabetes, homocysteine, hypercholesterolemia, oxidised low-density lipoprotein (LDL) and obesity. The pathophysiological process of atherosclerosis is divided into five phases (Moore and Tabas 2011:341):

- **Phase 1:** In response to chronic arterial endothelium injury an accumulation of fatty streaks occurs. Fatty streaks are lipid-filled cells containing macrophages and smooth muscle cells. They are non-obstructive and do not always progress to advanced lesions.
- **Phase 2:** Plaque which contains a high content of lipid (derived from plasma LDL) is very unstable and is prone to rupture.
- **Phase 3:** Plaque becomes a complicated lesion with a non-occlusive thrombus.
- **Phase 4:** Plaque becomes an occlusive thrombus and this phase is associated with MI angina.
- **Phase 5:** Additionally plaque becomes fibrotic with the same clinical outcome as phase 4.

The enhancement of the plaque formation is a result of the conversion of macrophages to foam cells by oxidised LDL. In contrast HDL decreases the macrophage activity (Newsholme and Leech 2009:511; Torre 2009:148; Rubin, Strayer and Rubin 2011:445. Limiting the plaque build-up by a diet lower in cholesterol (with desirable LDL: HDL) and consuming sufficient vitamin B12 B6 and folate will reduce the risk of cardiovascular disease (Bhargava, Bhargava, Manocha, Kankra, Das and Srivastava 2011:225). The instability of atherosclerotic plaque (vulnerability of plaque to rupture) increases proportionally with plaque volume and with elevated macrophages and LDL-cholesterol content (Leone, Landini and Picano 2010:2504).

**FIGURE 7  SCHEMATIC DIAGRAM OF THE ATHEROSCLEROTIC PROCESS**
(Faqs.org n.d.; Medimagery.com n.d.)
2.4 CARDIOVASCULAR RISK MARKERS

Geissler and Powers (2006:364) have indicated that earlier studies in the 1970s focused on the correlation between CHD and dietary intake and paved the way for epidemiological studies that investigated risk factors for CHD. Risk markers for cardiovascular disease have been categorised as: irreversible potentially reversible physiological and geographical factors as indicated in table 1.

**TABLE 1 RISK FACTORS FOR CORONARY HEART DISEASE** (Geissler and Powers 2006:364; World Heart Federation 2012).

![Table 1: Risk Factors for Coronary Heart Disease](image)
2.4.1 Obesity and inactivity

As described in chapter 1 (section 1.2.5.5) and chapter 3 (section 3.2.1) obesity is an independent risk factor for cardiovascular disease; together with inactivity it will increase the risk of cardiovascular disease (World Heart Federation 2012).

2.4.2 Age, gender and family history

With advancing age the risk of CVD increases with a steady progression of atherosclerosis (Mudau et al 2012:222; World Heart Federation 2012). The history of CVD of an individual is directly proportional to the risk of CVD (the earlier the age of onset and the more family members affected the greater is the risk of CVD) (Whitney and Rolfes 2008:628). It is confirmed that men are at greater risk of developing CVD than women even though the reason for the difference is not completely understood (Whitney and Rolfes 2008:628; World Heart Federation 2012). Estrogen has an inhibiting effect on LDL oxidation and increases the production of large very low-density lipoprotein (VLDL) and therefore has a protective effect against atherogenesis (Novella, Laguma-Fernández, Lázaro-Franco, Sobrino, Bueno-Beti, Tarin, Monsalve, Sanchis and Hermenegilo 2013:12).

2.4.3 Cigarette smoking

Leone et al (2010:2504) defined smoking as an addictive chemical toxicosis with acute or chronically harmful effects on the cardiovascular and respiratory systems and the epithelial gland target organs. Cigarette smoking doubles the risk of coronary artery disease and contributes seven-fold to the increase in risk for peripheral arterial disease (Price, Mowbray, Lee, Rumley, Lowe and Fowkes 1999:345). Cigarette smoking increases the blood pressure and increases the heart’s workload; it deprives the heart muscle of oxygen and damages the platelets that increase coagulation and clot formation. Toxins in cigarettes damage the blood vessels and increase atherosclerosis (Mudau et al 2012:222; World Heart Federation 2012). The exact pathophysiological mechanism by which smoking contributes to the development of atherothrombosis is not clear but the suggested atherogenic mechanisms include: alteration in the
prothrombotic and antithrombotic factors: arterial stiffness, inflammation, changes in blood flow dynamics, endothelial interference, alteration in lipid metabolism by increasing lipolysis, insulin resistance tissue, lipotoxicity and lower antioxidant availability (Price et al 1999:344; Leone et al 2010:2504). In a systematic review Critchley and Campbell (2003:491) indicated that persons with known CVD who stopped smoking had a 30% lower risk of myocardial infarction (MI) during the following three to seven years. Cessation of smoking reverses platelet activation and decreases coronary heart spasm as well as ventricular arrhythmias (Chow, Jolly, Rao-Melacini, Fox, Anand and Yusuf 2011:756). Tamura, Tanaka, Okamura, Kadowaki, Yamato, Tanaka, Nakamura, Okayama, Ueshima and Yamagata (2010:12) concluded that in spite of the common occurrence of weight gain after cessation of smoking, the risk of CVD nevertheless decreased markedly. Cigarette smoking is consequently the largest preventable cause of death and morbidity globally (Leone et al 2010:2505).

### 2.4.4 Diet

Diet as a risk factor for cardiovascular disease is based on the fact that a diet high in saturated fatty acids, trans-fatty acids and cholesterol, and low in fruit and vegetables, elevates the LDL cholesterol and increases atherosclerosis. In contrast a diet rich in fruit, vegetables, omega-3 fatty acids, omega-6 fatty acids and antioxidants lowers the risk of CVD (Steyn et al 2008:698; Bhupathiraju and Tucker 2011:1494). Studies evaluating the effect of dietary patterns on cardiovascular risk found that a healthy eating pattern (including high fibre, oily fish, fruit, vegetables, low intake of red meat fats and alcohol) was inversely associated with inflammatory markers (Hamer and Mishra 2010:496; Oliveira, Rodriguez-Artalejo, Gaio, Santos, Ramos and Lopes 2011:246). Convincing evidence exists indicating that moderate alcohol intake increases HDL and thereby decreases the risk of CVD in contrast to unfiltered coffee which increases LDL and thereby increases the risk of CVD (Geissler and Powers 2006:371; Krummel 2008:855; Bhupathiraju and Tucker 2011:1494).
2.4.4.1 Fatty acids

Fatty acids are organic acids (carbon atom chain with hydrogen attached) with an acid group (COOH) and methyl groups (CH₃) at the ends (FAO 2008:22; Vannice and Rasmussen 2014:136). Natural fatty acids contain an even number of carbons up to a chain length of 24 carbons. Variation in chain length depends on the source: meats, fish and vegetable oils have long-chain fatty acids (12–24 carbons) whereas dairy products have short-chain (<6 carbons) and medium-chain fatty acids (6–10 carbons) (Whitney and Rolfes 2008:140).

Saturated fatty acid is a chain where all four carbon binding sites are linked either to the next carbon or to a hydrogen molecule (stearic acid) (FAO 2008:22; Siri-Tarino, Sun, Hu and Krauss 2010:385; Vannice and Rasmussen 2014:136). When one pair of hydrogen atoms (monounsaturated) or more pairs (polyunsaturated) have been removed a double bond is formed between the adjoining carbons. The location of the double bond of polyunsaturated fatty acids is identified as an omega number (e.g. in the omega-3 fatty acid the first double bond occurs on the third carbon from the methyl group whereas in omega-6 the first double bond occurs on the sixth carbon from the methyl group) (FAO 2008:22; Krummel 2008:857; Whitney and Rolfes 2008:142; Baum, Kris-Etherton, Willett, Lichtenstein, Rudel, Maki, Whelan, Ramsden and Block 2012:217; Vannice and Rasmussen 2014:136). Saturated fatty acids (SFA) increase LDL and therefore increase the risk of CVD (FAO 2008:22; Baum et al 2012:216; Vannice and Rasmussen 2014:136). The main sources of saturated fatty acids are identified as dairy products (whole milk cream cheese and butter) animal fats and vegetable fats (coconut and palm) (Krummel 2008:855; Whitney and Rolfes 2008:157). It is suggested that both saturated and trans-fatty acids should be replaced in the diet by monounsaturated and polyunsaturated fats in order to have optimal prevention against CVD (FAO 2008:22; Steyn et al 2008: 720; Baum et al 2012:216; Vannice and Rasmussen 2014:136).

2.4.4.2 Trans-fatty acids (TFA)

The double bond in the natural form of unsaturated fatty acids is in a cis-isomer form (hydrogen next to the double bond is on the same side of the carbon chain) but in some situations the two
hydrogen atoms adjacent to the double bond are on opposite sides of the chain (trans-fatty acids) (Gallagher 2008:53; Brouwer, Wanders and Katan 2013:541). Whitney and Rolfes (2008:157) and Steyn et al (2008:723) summarised the sources of trans-fatty acids as deep-fried foods, snack chips, margarine, some cheeses, meat, dairy products, cakes, cookies, pastry and crackers. The flexibility of a membrane depends on the configuration of the fatty acids; cis-double bonds form links and result in a less compact arrangement than the trans-fatty acids that pack tightly resulting in a stiff and rigid membrane with limited flexibility (Gallagher 2008:53). Studies suggest that trans-fatty acids have a HDL-lowering effect and increase LDL and therefore increase the risk of CVD (Micha and Mozaffarin 2008:147; Whitney and Rolfes 2008:157). Mozaffarin (2010:14) summarised the contribution of TFA to CVD as a multiple pathway mechanism (figure 8) involving lipid metabolism increased inflammatory response and adiposity and decreased endothelial function and insulin sensitivity.

FIGURE 8 EFFECT OF TRANS-FATTY ACIDS ON CVD RISK MARKERS
2.4.4.3 Essential fatty acids

Essential fatty acids refer to the pair of fatty acids, linolenic acid and linoleic acid that cannot be physiologically produced and therefore need to be supplied by food sources. In order to obtain an optimal ratio between omega-6 and omega-3 (2:1 or 3:1) more fish and less meat should be consumed (Gallagher 2008:53).

a) Omega-3 fatty acids

Linolenic acid (18-carbon omega-3 fatty acid) the primary member of the omega-3 fatty acid is obtained from soybeans butternuts walnuts flaxseeds Canola oil and fish (Steyn et al 2008:723; Bhupathiraju and Tucker 2011:1499; Vannice and Rasmussen 2014:139). Small amounts of 20-carbon [eicosapentaenoic acid (EPA)] and 22-carbon [docosahexaenoic acid (DHA)] can be produced by the body but dietary sources can be found in human milk and fish. EPA and DHA are vital for growth and development (brain and eyes) and for immune response. A deficiency is associated with depression (Steyn et al 2008:722; Whitney and Rolfes 2008:154; Vannice and Rasmussen 2014:139). Omega-3 decreases the risk of CVD by preventing thrombus formation lowering blood pressure and protecting against irregular heart beat (Steyn et al 2008:723; Vannice and Rasmussen 2014:139). In contrast an excessive intake of omega-3 may interfere with wound healing inhibit immune function and decrease bleeding time (Whitney and Rolfes 2008:159).

b) Omega-6 fatty acids

Linoleic acid (18-carbon omega-6 fatty acid) is obtained from poultry fat nuts seeds and vegetable oils (sunflower safflower corn soybean and cottonseed) meat and eggs (Steyn et al. 2008:723; Bhupathiraju and Tucker 2011:1499; Vannice and Rasmussen 2014:139). Replacing dietary carbohydrates and saturated fatty acids (SFA) by an increased intake of omega-6 lowers LDL and increases HDL (Geissler and Powers 2006:369; Krummel 2008:857; Siri-Tarino et al 2010:502).
2.4.4.4 Fibre

The structural part of the plant is called dietary fibre and therefore vegetables legumes whole grain and fruit all contribute to the fibre intake. Fibre is a polysaccharide but cannot be broken down by the digestive enzymes and therefore contributes no energy (provides no monosaccharide to the body) (Phillips 2013:4). Fibre is differentiated into soluble (dissolves in water and forms a gel) and insoluble fibres. **Soluble fibre** is classically found in oats citrus fruit barley and legumes. It lowers cholesterol and glucose levels and therefore has a protective effect against CVD and diabetes (Steyn et al 2008:725). Lowering of cholesterol is achieved by the binding of fibre to bile acids thereby escalating its excretion. This inhibits the production of cholesterol by the liver resulting in lower blood cholesterol (Steyn et al 2008:725; Bhupathiraju and Tucker 2011:1504; Hermsdorff, Barbosa, Volp, Puchau, Bressan, Zulet and Martínez 2012:1119; Yanai, Katsuyama, Hamasaki, Abe, Tada and Sako 2014:321). **Insoluble fibre** is found in whole grain and vegetables. It cannot be fermented and promotes bowel movement and alleviates constipation (Kurniawan and Simadibrata 2011:196).

2.4.4.5 Antioxidants

Free radicals (molecules with one or more unpaired electrons) oxidise LDL which oxidises the membrane’s polyunsaturated fatty acids and thereby accelerates atherosclerosis. Antioxidants (vitamin E and C) protect against the described oxidative stress decrease inflammatory response and arterial damage and prevent thrombosis. Vitamin C also lowers blood cholesterol and increases HDL (Steyn et al 2008:725; Hermsdorff et al 2012:1119).

2.4.5 Lipids

Lipids present in the blood (cholesterol triglycerides and phospholipids) are water insoluble and therefore are transported bound to proteins (lipoproteins). They vary in size and density (Beckett, Walker, Rae and Ashby 2008:164). Lipids act as energy sources (triglycerides) are a crucial
component to maintain cell structure (cholesterol and phospholipids) and have certain specialised functions in the endocrine system (adrenal and sex hormones) (Beckett et al 2008:164).

A hydrophobic lipid (cholesterol esters triglycerides) forms the core of a lipoprotein which is enclosed by non-esterified cholesterol and hydrophilic phospholipids with Apo lipoproteins on the surface that regulate the lipoprotein metabolism (Hughes and Jefferson 2008:151). Lipoproteins are classified as: Chylomicrons, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) (Beckett et al 2008:165; Zhang, Song, Cavigiolio, Ishida, Zhang, Kane, Weisgraber, Oda, Rye, Pownall, and Ren 2011:175; Remaley, Rifai and Warnick 2015:398).

Lipids are metabolised by two pathways: the exogenous (dietary) pathway or the endogenous pathway (reverse cholesterol transport) as indicated in figure 9 (Peckett, Wright and Riddell 2011:1500).

A) Exogenous pathway:

1. Dietary cholesterol and fatty acids are absorbed in the small intestine where fatty acids intracellularly (intestinal mucosal cells) bind to glycerol to form triglycerides – cholesterol that has been esterified.
2. Chylomicrons are formed as a result of the combining of lipids with the Apo lipoproteins.
3. Chylomicrons are then released into the circulation.
4. Triglycerides are hydrolysed by lipoprotein lipase in the capillaries and release free fatty acids (utilised as energy or stored as adipose tissue).
5. Chylomicron remnants are reabsorbed by the liver. (Cohn, Kamili, Wat, Chung and Tandy 2010:45).
B) **Endogenous pathway:**

1. VLDL (consisting mainly of triglycerides and small amounts of unesterified cholesterol) synthesised by the liver is released into the circulation.
2. VLDL is then hydrolysed by lipoprotein lipase depleting triglycerides and forming IDL.
3. IDL is removed from circulation either by LDL receptors or converted by hepatic lipase to LDL.
4. LDL is absorbed in the liver via LDL receptors on the surface of the hepatocytes incorporated into the cell (by engulfing of the cell membrane) broken down by lipase and secreted.
5. LDL can also be absorbed by non-hepatic cells where steroid hormones are produced or it can be stored as cholesterol esters.
6. HDL is produced in the liver and intestinal mucosal cells from Apo lipoproteins and phospholipids and converts chylomicron remnants and IDL to Apo lipoproteins cholesterol and phospholipids. HDL also removes cholesterol from intracellular sources (e.g. atherogenic foam cells) and therefore facilitates reverse cholesterol and has an anti-atherogenic effect.

(Chatterjee and Sparks 2011:1429; Remaley et al 2015:401)

A lipoprotein profile includes: total cholesterol (TC) LDL-cholesterol (LDL) HDL-cholesterol (HDL) and triglycerides (TG) determined on a fasting serum sample (Persson 2011:1)). LDL can be 1) quantified by direct measurement or 2) calculated by the Friedewald formula (LDL = (TC) - (HDL) – (TG/5) (Anwar, Khan and Khan 2014:8). The Friedewald formula is commonly used to calculate an estimated LDL. The Friedewald formula however has its limitations as comparative studies have indicated, but it remained in use (Contois, Warnick and Sniderman 2011:264; Martin, Blaha, Elshazly, Brinton, Toth, McEvoy, Joshi, Kulkarni, Mize, Kwiterovich, DeFilippis, Blumenthal and Jones 2013:733; Anwar et al 2014:8). The Friedewald formula becomes invalid when triglyceride values rise above 4.52 mmol/l (Anwar et al 2014:8).
2.4.5.1 Cholesterol

Cholesterol is a steroid produced mainly by the liver and small intestines (although all nucleated cells have the ability to synthesise cholesterol) under the control of β-hydroxy-β-methylglutaryl-coenzyme A (HMG-CoA) reductase but is also present in the diet (Beckett et al 2008:165). The cholesterol dietary intake goal for prevention of chronic diseases as indicated by the WHO/Food and Agriculture Organization (FAO) expert consultation is 0-300 mg/day (Nishida, Uauy, Kumanyika and Shetty 2004:245). Cholesterol intake (meats, eggs and milk) increases blood cholesterol level and thereby increases the risk of CVD (Steyn et al 2008:724). Cells other than
hepatic cells do not have the ability to oxidise cholesterol. High levels of unesterified cholesterol have a harmful effect on cells (Jiménez-López, Ríos-Marco, Marco, Segovia and Carrasco 2010:6). Total cholesterol measurements in the plasma include all lipoprotein fractions (60–70% LDL 20–30% HDL 10-15% VLDL) and have a positive correlation with CHD (Krummel 2008:844; Di Angelantonio, Sarwar, Perry, Kaptoge, Ray, Thompson, Wood, Lewington, Sattar, Packard, Collins, Thompson and Danesh 2009:1993).

2.4.5.2 Chylomicrons

Chylomicrons have a density of <0.96 g/ml. They are composed of 90% triglycerides 5% cholesterol 3% phospholipids and 2% proteins. The metabolic function of chylomicrons is to transport ingested dietary fat to the liver. In the periphery lipoprotein lipase (LPL) hydrolyses the triglycerides in the chylomicrons and the liver metabolises the chylomicron remnants and delivers cholesterol back to the periphery (contributes to atherogenesis). Increased consumption of fat meals increases chylomicrons and remnants and therefore contributes to atherogenesis (Perssson 2011:2; James and Mamo 2012:7).

FIGURE 10  CHEMICAL STRUCTURE OF THE CHOLESTEROL MOLECULE
(Dawnmeisch.files.wordpress.com  2008)
2.4.5.3 VLDL and IDL

Very-low-density lipoprotein (VLDL) has a density of 0.95–1.006 g/ml. It is composed of 60% triglycerides 10% cholesterol 18% phospholipids and 10% protein whereas IDL has a density of 1.006–1.019 g/ml and is composed of 40% triglycerides 30% cholesterol 20% phospholipids and 10% protein (Liu, Shu, Xie, Lai, Liao, Su, Lin, Chen, Lin, Chong and Liu 2012:16401; Lehti, Käkelä, Hörkkö, Kummu, Helske-Suikko, Kupari, Werkkala, Kovanen, and Öörni 2013:2; Remaley et al 2015:398). The production of VLDL takes place in the liver and supply peripheral tissue with triglycerides (Persson 2011:2). The larger molecules of VLDL are not atherogenic but the VLDL remnants and the IDL are absorbed by hepatic receptors and converted to LDL. VLDL and IDL can be determined only by ultracentrifugation and are therefore not routinely measured (Maki, Dicklin, Davidson, Mize and Kulkarni 2012:31).

2.4.5.4 LDL

The density of LDL is 1.019–1.063 g/ml and the composition is 10% triglycerides 50% cholesterol 15% phospholipids and 25% protein (Huang, Hu, Lin, Lin and Sun 2010:962; Younis, Soran, Sharma, Charlton-Menys, Greenstein, Elsewiedy and Durrington 2010:290). LDL is the major cholesterol carrier (70%) in the blood and is thus directly proportional to the total cholesterol concentration (Chen, Ma, Liang, Peng and Zuo 2011:64 Persson 2011:3). Low-density lipoprotein (LDL) is synthesised during VLDL catabolism in the liver, adrenals and gonads via the LDL receptors. LDL has a half-life of 2-3 days, during which it is oxidised and absorbed by the macrophages and endothelial cells of the arterial wall where it contributes to atherogenesis (Persson 2011:3). The strong association between increased serum LDL levels and increased risk for CHD has been repeatedly confirmed by epidemiological studies (Sniderman, McQueen, Contois, Williams and Furberg 2010:152; Persson 2011:3; Sone, Tanaka, Tanaka, Iimuro, Oida, Yamasaki, Oikawa, Ishibashi, Katayama, Ohashi, Akanuma and Yamada 2011:3451).
2.4.5.5 HDL

The density of HDL is 1.063–1.210 g/ml. It is composed of 5% triglycerides 20% cholesterol 25% phospholipids and 50% protein (Tsompanidi, Brinkmeier, Fotiadou, Giakoumi and Kypreos 2010:4). The high protein component of HDL accounts for its metabolic function of removing cholesterol from tissue back to the liver, and considered as an important anti-atherogenic pathway and it modulates inflammation (Navab, Reddy, Van Lenten and Fogelman 2011:222; Persson 2011:3). The inverse correlation between serum HDL and CVR is well known and widely accepted (Rifai et al 2008:415; Navab et al 2011:222; Persson 2011:3). Studies involved in improving poor lifestyle habits have been known to have a positive effect on the HDL levels (Rouvre, Vol, Gusto, Born, Lantieri, Tichet and Lecomte 2011:118). The presence of additional CVD risk markers (hypertension obesity diabetes smoking and inactive lifestyle) in individuals with low HDL has also been noted (Rouvre et al 2011:118; Hughes and Jefferson 2008:151).

2.4.5.6 Triglycerides

Triglycerides are fatty acid esters of glycerol (Beckett et al 2008:165). The lipid fractions triglycerides are both synthesized intrinsically in the liver and obtained externally by the absorption in the intestine (Sarwar, Danesh, Eiriksdottir, Sigurdsson, Wareham, Bringham, Boekholdt, Khaw and Gudnason 2007:450). Triglycerides in vivo are formed by binding three fatty acids to a glycerol side chain. This inhibits the danger of cell damage by the carboxylic acid (CooH) head of the fatty acids. A water molecule is released at each side where ester (-O-) bonding is produced (figure 10). Triglyceride is water insoluble (hydrophobic) because it contains no free hydroxyl groups. Thus lipids can be transported in blood and stored in adipocytes as an energy source (Gallagher 2008:56).
In spite of the latest conclusion by the American National Cholesterol Education Program-Adult Treatment Panel (NCEP-ATP III) that insufficient evidence exist confirming that triglyceride concentration is an independent CVR marker, numerous epidemiological and meta-analytical studies on large sample population confirmed a direct correlation between cardiovascular risk markers and serum triglyceride level (Sarwar et al 2007:451; Leone et al 2010:2504).

2.4.5.7 Lipoprotein profile

Dyslipidaemia is the alteration in circulating lipids and lipoproteins and is regarded as an independent cardiovascular risk marker (Butler 2010:26). Serum lipid profiles (cholesterol, HDL, LDL and triglycerides) are used to classify the cardiovascular risk of an individual (table 2) and are used to determine treatment. The South African Heart and Atherosclerosis Society adopted the revised guidelines (as reported in table 2) of the European Society of Cardiology and the European Atherosclerosis Society (Reiner, Catapano De Backer, Graham, Taskinen, Wiklund, Agewall, Alegria, Chapman, Durrington, Erdine, Halcox, Hobbs, Kjekshus, Filardi, Riccardi, Storey, Wood, Bax, Vahanian, Auricchio, Baumgartner, Ceconi, Dean, Deaton, Fagard, Filippatos, Funck-
The ratio of triglyceride level to HDL (THR) is a reliable predictor of the risk of CVD (Gambardella, Blair, McKinley, Baker and Harkin 2011:249).

### 2.4.5.8 Managing dyslipidaemia

The requirements for a diet managing dyslipidaemia should include:

- Sufficient energy intake to maintain normal body weight (2403 kcal/day minus 10 kcal/day for each year above 19 years for males and minus 7 kcal/day for each year above 19 years for females) (Institute of Medicine 2002);
- A variety of foods to ensure sufficient macronutrient and micronutrient intake (Steyn et al 2008:724);
- A diet low in saturated fatty acids (SFA) (0–10% of total energy) and trans-fatty acids (<1% of total energy) (Nishida et al 2004:247; FAO/WHO Expert Consultation 2008:3);
- Consumption of monounsaturated fatty acids (MUFA) (calculated: total fat – (SFA+PUFA+TFA) and polyunsaturated fatty acids (PUFA) (6–11% of total energy) (Nishida et al 2004:247; FAO/WHO Expert Consultation 2008:3; South African Heart Association (SA Heart) and Lipid and Atherosclerosis Society of South Africa (LASSA) 2012:187);
- Sufficient dietary fibre (27-40 g/day) and foods high in soluble fibre (pectin, oat, bran, dry, beans, guar gum and psyllium) (Steyn et al 2008:724; SA Heart and LASSA 2012:187);
- Enough protein to build and maintain body tissues (10–15% of total energy) (Nishida et al 2004:247);
- Moderate alcohol consumption (not more than two drinks for males and one drink for females per day) but individuals with hypertriglyceridemia should avoid alcohol (SA Heart and LASSA 2012:187);
• Low sodium consumption (5g salt per day) (Na 2000 mg although the 2010 American food-based dietary guidelines (FBDG) recommended for ages 51 to 70 years:1,300 mg/ day; for ages 71 years and older:1,200 mg/day) (Steyn et al 2008:724; U.S. Department of Agriculture and U.S. Department of Health and Human Services 2010:21; SA Heart and LASSA 2012:187).

**TABLE 2  CLASSIFICATION OF CARDIOVASCULAR RISK ACCORDING TO THE SERUM LIPID PROFILE**

<table>
<thead>
<tr>
<th>LEVEL</th>
<th>Cholesterol mmol/l</th>
<th>HDL-C mmol/l</th>
<th>LDL mmol/l</th>
<th>Triglyserides mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desirable</td>
<td>&lt;5.2</td>
<td>&gt;1.5</td>
<td>&lt;1.8</td>
<td>&lt;1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.6-3.3</td>
<td></td>
</tr>
<tr>
<td>Borderline</td>
<td>5.2-6.2</td>
<td>1.3-1.5</td>
<td>3.4-4.1</td>
<td>1.7-2.2</td>
</tr>
<tr>
<td>High Risk</td>
<td>&gt;6.2</td>
<td>&lt;1 (male)</td>
<td>4.1-4.9</td>
<td>2.3-5.6</td>
</tr>
<tr>
<td></td>
<td>&lt;1.3 (female)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very high</td>
<td></td>
<td>Above 4.9</td>
<td>Above 5.6</td>
<td></td>
</tr>
</tbody>
</table>

The prevalence of dyslipidaemia varies across the regions in SSA due to increased urbanisation and changing of lifestyle factors (epidemiological transition) (Belue, Okoror, Iwelunmor, Taylor, Degboe, Agyemang and Ogedegbe 2009:4). Similar variation was observed in SA where a significant difference in the prevalence of dyslipidaemia occurs in different ethnic groups. (Sliwa, Lyons, Carrington, Lecour, Marais, Raal and Stewart 2012:391). The use of antiretroviral therapy
(ARV) also leads to an increase in dyslipidaemia, SA has an increase HIV rate and, therefore, an increase ARV treatment (largest programme internationally) and thus also an increase in prevalence of dyslipidaemia (Levitt, Steyn, Dave and Bradshaw 2011:1692S). Sliwa et al (2012:391) reported that the ethnic group with the highest prevalence of dyslipidaemia (cholesterol) is the Indian group (70%), followed by the Mixed Ancestry group (60%), then the White European group (54%) and lastly the group from African descent (39%). Similar patterns were reported for abnormal HDL and triglyceride levels but the incidence of elevated LDL differs among the people from the white European group (66%), those from African descent (63%), the Indian group (62%) and the Mixed Ancestry group (57%) (Silwa et al 2011:391). Similar results were obtained by other studies indicating that the prevalence of dyslipidaemia amongst black South Africans (independent of rural or urban) is about 30% (Raal, Blom, Naidoo, Bramlage and Brudi 2013:330; Oldewage-Theron and Egal 2013:25; Sengwayo, Moraba and Motaung 2012:43).

2.4.5.9 Effect of vitamins B12, B6 and Folate supplementation on lipid profile

Vitamin B12 are inversely associated with triglyceride and VLDL and positively associated with HDL. Increasing vitamin B12 levels will therefore decrease the VLDL and triglycerides levels and increase the HDL levels (Mahalle, Kulkarni, Garg and Naik 2013:293).

A decrease (10%) in the total cholesterol and a 36.6 mg/dl reduction of triglycerides in hypertriglyceridemia patients were observed after 6 weeks of vitamin B6 (50 mg/day) supplementation (Hlias, Reslan, Sarieddine, Nasreddine, Taan Azar, and Obeid 2012:1674). Similar results were reported on animal models where the total cholesterol levels and the serum triglyceride levels of albino rats were significantly reduced after vitamin B6 supplementation (Adekunle and Adedeji 2011:352).

Higher levels of folate were associated with lower levels of LDL and higher levels of HDL and, therefore, higher levels of folate are associated with a favourable lipid profile (Wahab, Zafreen, Siddique, Akter, Parveen, Chowdhury, Parveen and Arslan 2009:21; Semmler, Moskau, Grigull, Farmand, Klockgether, Smulders, Blom, Zur, Stoffel-Wagner and Linnebank 2010:1; Ganeshan, Karthikumar, Viswanath, Renjith and Alin 2014:1141).
2.4.6 Hypertension

Hypertension (blood pressure (BP) level of >140/95 mmHg) is a multi-factorial disease that involves pathological changes in the neuronal renal hormonal and vascular control mechanisms (Hynynen and Khalil 2006:95). Hypertension is a major risk factor for CVD and renal disease (Lin, Pao, Wu, Lin, Chien, Hung, Chen, Liu, Tsai, Gau, Wu and Hwang 2007:231). The two determinants of BP are the cardiac output (contraction of the heart muscle that pumps the blood away from the heart) and the peripheral pressure (resistance of the blood flows in the arterioles). The cardiac output (systolic blood pressure (SBP)) is directly proportional to the increase in heart rate or increase in blood volume whereas the peripheral pressure (diastolic blood pressure (DBP)) is influenced by the arterial diameter (Whitney and Rolfes 2008:634). Blood pressure is used to classify hypertension as indicated in table 3. It is defined as normal pre-hypertension stage 1 hypertension and stage 2 hypertension. Classification is indicative of treatment to be implemented (Joint National Committee 7 (JNC7) Express 2010:3).

The risk factors for hypertension are ageing, genetics, obesity, salt sensitivity and alcohol intake (Zheng, Sun, Zhang, Xu, Li, Hu, and Sun 2010:221). Hypertension is a major public health concern seeing that more than six million South Africans have a blood pressure >140/95 mmHg and more than three million a BP level of >160/95 mmHg (Van Rooyen, Kruger, Huisman, Wissing, Margetts, Venter and Vorster 2000:779). Oldeyage-Theron et al (2008(a):7) reported that in the Sharpeville elderly community 68% of the respondents were hypertensive (>160/95 mmHg) but only 36.8% of these were using prescribed hypertensive medication.
TABLE 3 THE CLASSIFICATION OF BLOOD PRESSURE FOR ADULTS ACCORDING TO THE SOUTH AFRICAN GUIDELINES 2011 (Seedat and Rayner 2012:62)

<table>
<thead>
<tr>
<th>BP classification</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>120-129</td>
<td>80-84</td>
</tr>
<tr>
<td>Prehypertension</td>
<td>130-139</td>
<td>85-89</td>
</tr>
<tr>
<td>Stage 1 hypertension</td>
<td>140-159</td>
<td>90-99</td>
</tr>
<tr>
<td>Stage 2 hypertension</td>
<td>160-179</td>
<td>100-109</td>
</tr>
<tr>
<td>Stage 3 hypertension</td>
<td>≥180</td>
<td>≥110</td>
</tr>
</tbody>
</table>

**2.4.7 Inflammatory Markers**

The inflammatory response aims to recruit immunologically active cells and factors to the site of injury to aid in the removal of pathogens or damaged cells either localised or systemic (Wood 2006:26). An inflammatory response is initiated by damage to the vascular cell lining (figure 12) resulting in a series of mechanisms (acute-phase response) including haemodynamic (vasodilatation) activation of endothelial cells (increased adhesion molecule expression) increased permeability (enhanced protein movement) and an increase in acute-phase proteins (Wood 2006:32; Beckett et al 2008:95). Acute-phase proteins are: fibrinogen (coagulation factor I); haptoglobin (binds to haemoglobin thereby reducing iron concentration that is required for bacterial metabolism); complement factor C3 (cleaved to C3a (activates mast cells) and C3b opsonisation); mannose-binding protein (MBP) (binds to mannose-containing sugars on the pathogen’s surface and thereby helps with recognition); serum amyloid A (SAA) (inhibits fever and platelet activation); C-reactive protein (CRP) (binds to phosphorylcholine on the surface of pathogens and contributes to opsonisation) (Wood 2006:35).
The acute-phase response is initiated by the macrophages which recognise the injury and release Interleukin-1 (IL-1) Interleukin-6 (IL-6) and Tumour necrosis factor-α (TNF-α) (Wood 2006:31). The cytokine IL-1 has a direct action on the brain resulting in somnolence (drowsiness reduces physical activity and conserves energy) fever (protects against infection and replication of pathogens) and loss of appetite (conserves energy) (Wood 2006:35; Beckett et al 2008:94). Interleukin-6 (IL-6) stimulates the hepatocytes to produce acute-phase proteins resulting in a marked increase of plasma levels of these proteins (Wood 2006:35). Exposed injured endothelial cells cause the release of vaso-active substances together with TNF-α which increases the vascular permeability while it also activates the haemostatic response and the complement system (Beckett et al 2008:94).

Vessel injury can also be caused by high LDL-cholesterol hypertension cigarette toxins and elevated homocysteine. During the inflammatory response that aims to repair the damage to the artery wall LDL-cholesterol becomes trapped in the lesion that is engulfed by the macrophages and the free radicals oxidise the LDL trapped in the macrophage and eventually become plaque (Whitney and Rolfes 2008:627). C-reactive protein (CRP) is a β-globulin which is bound strongly to phospholipids and increases twentyfold to thirtyfold during an infectious or inflammatory response and is therefore considered a credible marker for systemic inflammation (Beckett et al 2008:95). Interleukin-6 (IL-6) and TNF-α are pro-inflammatory cytokines which stimulate the production of CRP in the liver. Elevated CRP IL-6 and TNF-α are strong independent predictors of risk of future cardiovascular events (Hynynen and Khalil 2006:95; Nyström 2007:79) but also of cartilage degeneration (Stannus, Jones, Cicuttini, Parmeswaran, Quinn, Burgess, and Ding 2010:1441) and heart failure (Kalogeropoulus, Georgiopoulou, Psaty, Rodondi, Smith, Harrison, Liu, Hoffmann, Bauer, Newman, Kritchevsky, Harris and Butler 2010:2129) in older people.
2.4.7.1 Effect of vitamins B12, B6 and Folate supplementation on inflammatory response

Although an indirect mechanism of the interaction between vitamin B12 and inflammatory response via the homocysteine lowering pathway could be present, no studies could be found evaluating the effect of vitamin B12 supplementation on the inflammatory markers. This study will thus be the first study to investigate this relationship, specifically in black elderly respondents.

Vitamin B6, at a concentration of 100 mg/day, was found to effectively suppressed pro-inflammatory cytokines (IL-6 and TNF-α) (Huang, Wei, Wu and Huang 2010:1007). Levels of PLP have been inversely associated with HS-CRP (Abbenhardt, Miller, Song, Brown, Cheng, Wener, Zheng, Toriola, Neuhouser, Beresford, Makar, Bailly, Maneval, Green, Manson, Van Horn and Ulrich 2014:718). Ulvik, Midttun, Pedersen, Nygård and Ueland (2012:1072) suggested that the acute phase and activated cellular immunity are associated with increased cellular uptake and catabolism of vitamin B6.
Dietary folate intake was significantly associated with HS-CRP, IL-6, IL-8 and monocyte chemoattractant protein-1 and TNF-α (Ho, Xue, Cushman, McKeown-Eyssen, Sandler, Ahnen, Barry, Saibil, Bresalier, Rohan and Baron 2009:1650; Chung, Kim, Lee, Do, Kim, Oh, Kang, Shin 2011:62; Kolb and Petrie 2013:164; Abbenhart et al 2014:715).

2.4.8 Diabetes Mellitus (DM)

2.4.8.1 Aetiology

As defined by the American Diabetes association (ADA) diabetes is a collection of various metabolic disorders including elevated blood glucose levels decreased concentration of insulin and the defective action of insulin (Steyn et al 2008:700). Diabetes mellitus is the sixth highest cause of death globally. In addition to being a primary cause of death, diabetes is also an underlying condition accompanying other CDL (CVD, stroke and kidney disease) (Whitney and Rolfes 2008:636). It was estimated that by 2010 more than 200 million people globally would be suffering from DM (Steyn et al 2008:700). In SSA the prevalence of DM is rising fast. Rapid uncontrolled urbanisation and lifestyle changes have been identified as the possible driving force (Mbanya, Motala, Sobngwi, Assah and Enoru 2010:2254). Prevalence of DM in SA (aged 20-79 years) in 2010 has been reported as 4.5% of the population and it predicted to rise to 5.6% in 2030 (Shaw, Sicree and Zimmet 2010:7). A study conducted in Bellville, Cape Town, reported the prevalence of DM amongst the coloured community to be 28%, compared to 4.4% for impaired glycaemia and 15% for impaired glucose tolerance. The prevalence of metabolic syndrome varied from 55.4% to 62%, depending on the definition (Erasmus, Soita, Hassa, Blanco-Blanco, Vergotine, Kengne and Matsha 2012:841). A study conducted amongst 1099 urban dwellers in Cape Town indicated that the prevalence of DM is 13%, with 11.2% impaired glucose tolerance and 1.2% impaired fasting glycaemia (Peer, Steyn, Lombard, Lambert, Vythilingum and Levitt 2012:1).

Owing to the lack of insulin activity, glucose is unable to enter the cells and accumulates in the plasma (hyperglycaemia). The pathophysiological action of chronically elevated glucose levels is as follows:
• Cells start to convert glucose to sugar alcohols that cause distension of cells (cells in the lenses of the eye causing blurry vision) and have a toxic effect on all cells in the body.
• Some cells produce glycoprotein (attach glucose to amino acids) resulting in an ineffective protein.
• Failure of nerve function and poor circulation are caused by damage to the blood vessels and nerves.
• Poor circulation together with hyperglycaemia increases risk of infection.

(Sacks and Path 2015:617)

2.4.8.2 Classification

a) Type I Diabetes

Type 1 Diabetes is an autoimmune reaction affecting the pancreatic islet beta cells resulting in impaired insulin secretion. The rate of β-cell destruction varies. In some individuals the destruction is rapid and in others the destruction and therefore the onset of diabetes is slower (Reagan 2012: 72; Rewers 2012:91; Wu, Ding, Gao, Tanaka and Zhang 2013:667). This form of diabetes is the least common (only 5–10%). It is usually diagnosed during childhood or young adult life but can occur at any age. Daily insulin replacement is needed (Steyn et al 2008:700; Rewers, Pihoker, Donaghue, Hanas, Swift and Klingensmith 2009:72). Insulin is required for the cells to absorb glucose. Without insulin a possible life-threatening condition might arise (Alian, Hashemipour, Dehkordi, Hovsepian, Amini, Moadab and Javanmard 2012:12). Insulin is a protein that is destroyed by gastro-intestinal tract enzymes and therefore needs to be injected (Niu, Lu, Hovgard and Wu 2011:1155). Patients are generally slender although rare cases of obesity may occur. Symptoms include hyperglycaemia, sudden weight loss and the presence of ketones in the urine (Mbanya et al 2010:2257).
b) **Type 2 Diabetes**

This is a lifestyle-related condition (resulting from inactivity and unhealthy eating habits) associated with insulin resistance and a reduction in muscle adipose and hepatic cells and sensitivity to insulin (American Diabetes Association (ADA) 2007; Rowan, Jamnik and Riddell 2010:72). In order to compensate for the reduction in insulin activity the pancreas increases insulin production resulting in increased plasma insulin levels (hyperinsulinaemia). Over time the over-production of insulin becomes less effective until the β cells become exhausted and ultimately impaired insulin production and results in hyperglycaemia (Motta, Lima, Arsa, Russo, Sales, Moreira, Morais, Almeida, Araujo, Moraes, Pesquero, Simões and Campbell 2010:364). Type 2 diabetes affects older obese and inactive people with gradual onset with both insulin resistance and insulin deficiency being present (Taube, Schlich, Sell, Eckardt and Eckel 2012:H2149). Although the cause of type 2 diabetes is unknown the risk is substantially increased by inherited factors ageing inactivity and abdominal obesity (ADA 2007; Rejeski, Ip, Bertoni, Bray, Evans, Gregg, and Zhang 2012:1210). Hyperglycaemia affects multiple organs and can lead to arterial hypertension (Motta et al 2010:364). It is estimated that the cause of death in 80% of individuals suffering from type 2 diabetes will be due to thrombotic complications of which 75% will result from a cardiovascular event (Bongdanov and Østerud 2010:112).

c) **Insulin Resistance / Glucose Intolerance / Metabolic Syndrome**

This is a pre-diabetic condition with fasting glucose levels between 5.0 mmol/l and 7.0 mmol/l and glucose tolerance test levels between 7.8 mmol/l and 11.1 mmol/l (Steyn et al 2008:700). Metabolic syndrome has been confirmed by numerous studies and in spite of differences in the classification, the syndrome has been defined by the WHO as the presence of insulin resistance (hyperinsulinaemia >17 μU/l) together with at least two of the following symptoms:

- Hypertension
- Glucose intolerance
- Dyslipidaemia (High triglycerides and low HDL)
- Obesity

The role of overweight in the development of metabolic syndrome is not clearly understood but the secretion of hormones by the adipose tissue has been identified as a main reason. The development of insulin resistance in the muscle tissue appears to be facilitated by increased fatty acids reduced secretion of adiponectin and secretion of cytokines (inflammatory response) (Taube et al :H2148). The prevalence of metabolic syndrome is very high and as much as a quarter of the American adult population has metabolic syndrome (Richard, Couture, Desroches, Charest and Lamarche 2010:1). A 10% reduction in body weight improves all the metabolic syndrome parameters (Richard et al 2010:6).

d) Gestational Diabetes

This is a condition of increased blood glucose levels during pregnancy (ADA 2007).

2.4.8.3 Acute complications

Frequently elevated blood glucose levels cause secondary diseases that include cardiovascular disease, blindness, kidney failure and chronic infections. Acute complications of DM are hypoglycaemia and ketoacidosis. Hypoglycaemia (blood glucose levels <4.0 mmol/l) is a condition caused by insufficient food intake or an excess of insulin and leads to the loss of consciousness. Diabetic Ketoacidosis (presence of ketones in the urine) is a severe and acute condition resulting from a lack of insulin and lack of dietary control (Steyn et al 2008:702). In figure 13 it is indicated that the metabolic complications of diabetes type 1 and 2 differ. The absence of insulin in type 1 diabetes prevents glucose from entering the cells and the cell needs to catabolise protein and fat for energy leading to weight loss (hunger leads to overeating) and ketoacidosis (acetone breath, ketoaemia, ketonuria) and a possible fatal diabetic coma. Type 2 diabetes in contrast has limited glucose entering the cells resulting in hunger and overeating and weight gain. In both type 1 and 2 diabetes an osmotic movement into the blood as a result of hyperglycaemia (which can also spill over into urine (glycosuria)) can result in polyuria causing an electrolyte imbalance (because of dehydration) and excessive thirst leading to a hyperosmolar hyperglycaemic state or a possible fatal coma (Reece, Leguizamón and Wiznitzer 2009:1789).
FIGURE 13 METABOLIC COMPLICATIONS OF UNTREATED DIABETES (Whitney and Rolfes 2008:639)
2.4.8.4 Long-term complications

The long-term complications of DM can be divided into macrovascular complications (coronary artery disease cerebrovascular disease peripheral vascular disease) and microvascular disease (retinopathy nephropathy and neuropathy) and are complicated by the duration of the condition (Mbanya et al 2010:2256; Reagan 2012). Macrovascular complications are a result of atherosclerosis (accounts for 80% of mortalities in DM) and caused by the presence of dyslipidaemia (Steyn et al 2008:716).

2.4.8.5 Management

Diabetes is a chronic disease that requires continuing medical care and patient education to manage acute complications and lifestyle adjustments (Al-Arouj, Assaad-Khalil, Buse, Fahdil, Fahmy, Hafez, Hassanein, Ibrahim, Kendall, Al-Madani, Nakhi, Tayeb, and Thomas 2010:1900). Burr et al (2010:72) indicated that lifestyle changes such as physical activity and a balanced diet are as effective as drug treatment in the prevention of type 2 diabetes. Nutritional intervention is the basis of diabetes management and should aim to maintain euglycaemia promote healthy body weight improve lipid profile maintain healthy blood pressure and encourage healthy eating habits and physical activity. Dietary recommendations can be summarised as follows: energy intake should be individualised to achieve healthy body weight i.e. carbohydrates should contribute 45–65% of total energy intake proteins 10–20% of total energy intake and fats <30% of total energy intake (Dworatzek, Arcudi, Gougeon, Husein, Sievenpiper, Williams and Canadian Diabetes Association Clinical Practice Guidelines Expert Committee 2013:S46-S48).

Meal distribution should be standardised daily in order to have uniformity from day to day in consideration of insulin administration in order to maintain euglycaemia. A fast-release carbohydrate together with a low glycaemic index food should be consumed immediately in a hypoglycaemic condition. During ketoacidosis small doses of rapid insulin should be administered hourly and dehydration should be prevented (Sivanandan, Sinha, Jain and Lodha 2011:574).
2.4.8.6 Effect of vitamins B12, B6 and Folate supplementation on metabolic syndrome and diabetes

Metformin reduces the levels of folate and vitamin B12 and thereby increasing the serum homocysteine levels (Aghamohammadi, Gargari and Aliasgharzadeh 2011:210). Increased plasma homocysteine, triglyceride, waist circumference and decreased vitamin B12, vitamin B6 and folic acid have been observed in type 2 diabetes elderly patients. Incipient neuropathy was associated with more distinct alteration in the B vitamin metabolism (Ebesunun and Obajobi 2012:48, Nix, Zirwes, Bangert, Kaiser, Schilling, Hostalek and Obeid 2014:158). The vitamin B status has also been inversely associated with metabolic syndrome in Chinese adults (Bian, Gao, Zhang, Wang, Liu, Zhang and Huang 2013:106). High doses of Vitamin B12, B6 and folate (1 mg/day, 25 mg/day and 2.5 mg/day) resulted in a decrease in glomerular filtration rate and an increase in vascular event in patients with diabetic nephropathy (House, Eliasziw, Cattran, Churchill, Oliver, Fine, Dresser and Spence 2010:1603).

Folic acid supplementation lowered plasma homocysteine, serum malondialdehyde levels, glycemic control, insulin resistance and improved serum folate and vitamin B12 levels in patients with type 2 diabetes (Aghamohammadi et al 2011:210; Gargari, Aghamohammadi and Aliasgharzadeh 2011:33). Furthermore, folic acid supplementation improved the endothelial function (measured by the adhesion molecules) in respondents with Type 1 diabetes (Alian, et al 2012:12).

2.4.9 Haemostasis

The development of coronary artery disease and myocardial infarction has both atheromatous and thrombotic components. Evidence exists implicating the involvement of a number of coagulation and fibrinolytic proteins (Skurk et al 2001:1336). Normal response to vascular damage depends on closely linked interaction between the blood vessel wall circulating platelets and blood coagulation factors in order to stop bleeding but this response must be controlled to prevent extensive clot formation. Haemostasis is thus a finely balanced system of clot formation and fibrinolysis (Faxalv 2009; Hoffbrand and Moss 2011:264; Russo 2012:2). The haemostatic
response as represented in figure 14 (Faxalv 2009; Hoffbrand and Moss 2011:264) is activated by the damaged vascular area resulting in the following rapid well-balanced multifactorial mechanisms:

1. **Vasoconstriction** of the injured area (damaged vessel and response constriction of adjacent vascular system) slows the blood flow and prevents exsanguination enhances contact activation of thrombocytes and the activation of the coagulation cascade. In addition the thromboxane A$_2$ and amines (released from platelet activation) and fibrinopeptides (released during fibrin formation) which are vasoactive agents contribute to vasoconstriction.

2. The endothelial cell damage (collagen exposure) activates the **adhesion of thrombocytes** to the connective tissue facilitated by von Willebrand factor (VWF). Adhered platelets release their granule content (as a result of stimulation by collagen exposure and thrombin synthesised at the site of injury) which activates the platelet prostaglandin production that stimulates the thromboxane A$_2$.

3. The release of adenosine diphosphate (ADP) prompts the thrombocytes to swell and **aggregate**. Circulating platelets are attracted to the area of injury and aggregates contributing to the formation of the haemostatic plug covering the exposed connective tissue.

4. **The coagulation cascade** is initiated by VIIa tissue factor platelets and Ca$^{2+}$ formed as a result of the vascular injury. Membrane phospholipids are released by platelet aggregation and accelerate coagulation.

5. The haemostatic plug is **stabilised** by fibrin (produced by the coagulation cascade) and by the platelet-induced clot retraction/compaction. Thrombin synthesised at the site of injury converts plasma fibrinogen to fibrin stimulates platelet aggregation and secretion and activates factors XI XIII V and VIII. A solid mass of fibrin network transforms the haemostatic plug into a solid mass.

6. **Physiological limitation of coagulation** begins simultaneously with clot formation in order to prevent unregulated coagulation that leads to intravascular occlusion. This is achieved by coagulation factor inhibitors blood circulation and fibrinolysis.
2.4.9.1 Platelet / Thrombocytes

a) Production

Watson and Harrison (2011:775) have described the production of thrombocytes in the bone marrow under the regulation of thrombopoietin (protein produced in the liver) as a 10-day differentiation process of megakaryocytic cytoplasmic fragmentation. The megakaryoblast arises from the haemopoietic stem cell and through a process of differentiation develops into a megakaryocyte. The megakaryocyte matures by replicating DNA without nuclear or cytoplasmic division (endomitotic synchronous replication) resulting in an increase of cytoplasmic volume and nuclear material. Large mature megakaryocytes undergo fragmentation and give rise to 1000–5000 platelets. The normal platelet count is 150–400 X 10^9/l with a lifespan of 7–10 days (McPherson and Pincus 2011:790; Watson and Harrison 2011:773).

FIGURE 14  HAEMOSTATIC RESPONSES ON VESSEL INJURY (Gomez, Tuddenham and McVey 2011:753)
b) Structure

Platelets are discoid cells with a diameter of 3.0 X 0.5 μm and a mean cell volume of 7–11 fL. The ultrastructure of the platelets as indicated in figure 15 consists of the following:

- **Glycoproteins (GP)** play an important role in the adhesion and aggregation of platelets (Glycoprotein Ia facilitates adhesion to collagen; Ib and IIb/IIIa attach platelets to VWF; IIb/IIa are also receptors for fibrinogen).

- **The plasma membrane** forms a canalicular system by invagination into the platelet interior resulting in an extended reactive area for the absorption of plasma coagulation proteins. Plasma membrane phospholipids facilitate the conversion of factor II (prothrombin) to IIa (thrombin) and factor X to Xa.

- **Thrombocyte storage granules** are discharged into the open canalicular system during the release and amplification stage. They are divided into three types:
  1. **α-granules**: Contain fibrinogen VWF heparin antagonist platelet-derived growth factor β-thromboglobulin and other coagulation factors.
  2. **Dense granules**: Less prominent but contain ADP adenosine triphosphate (ATP) 5-hydroxytryptamine and calcium.
  3. **Lysosomes**: Contain hydrolytic enzymes and peroxisomes (containing catalase).

- **Cytoskeletal proteins** play an important role in the activation of platelets after vessel injury but have been found to have antigenic properties that are linked to auto-immune diseases.

As indicated in figure 16 the functions of platelets in the immune system are multi-fold: tissue repair and development maintaining and regulating vascular flow and inflammatory response; however the main function is the formation of a stable haemostatic plug after vessel damage (Hoffbrand, Catovsky, Tuddenham and Green 2011:775; McPherson and Pincus 2011:790).

Nuyttens, Thijs, Deckmyn and Broos (2011:S26) described the function of the platelets as follows:

- **Platelet adhesion**
  Subsequent to vascular injury the GPIb-XI-V complex on the platelets binds to VWF and adheres to the exposed sub-endothelial matrix proteins resulting in the activation of GPIIb/IIIa. Collagen also binds to GPIA/IIA. Stronger interaction is established by the
additional bonding of GPIIb/IIIa with VWF and GPVI integrin α1/β2 with collagen and other sub-endothelial matrix components. This receptor binding activates platelets by a signalling cascade.

**FIGURE 16 FUNCTIONAL CHARACTERISTICS OF THE PLATELETS (Hoffbrand and Moss 2011:775; McPherson and Pincus 2011:790)**

- Platelet activation
  Activated thrombocytes become more spherical and extrude long pseudopodia (figure 17) that improve platelet–platelet and platelet–vessel wall interaction. The actin cytoskeleton
brings about the flattening of the platelets with granules and organelles centred (like a fried egg). Granules are then secreted. Activation of platelets increases the production of GPIIb/IIa molecules that enhance aggregation.

![Morphological Changes of Washed Platelets Exposed (per second) to Activator](image)

FG = fibrinogen; CRP = Collagen-related peptide; THR = thrombin

**FIGURE 17 MORPHOLOGICAL CHANGES OF WASHED PLATELETS EXPOSED (per second) TO ACTIVATOR (Hoffbrand et al 2011:778).**

**Platelet aggregation** is achieved by cross-linking of active GPIIb/IIa and fibrinogen resulting in a strong connection that further activates thrombocytes. A large platelet plug (degranulated platelets adhering to each other) which closes the area of vessel injury is formed by continuous thrombocyte activation (by ADP and Thromboxane A2 (TXA2)).

- **Platelet release reaction and amplification**
  Activation of the platelets induces intracellular signalling that leads to the release of α- and δ-granules active in the formation and stabilisation of the platelets. Adenosine diphosphate (ADP) is released from the dense granules and is functional in the positive feedback during platelet activation. Thromboxane A2 (TXA2) is formed as a result of the activation of cytosolic phospholipase A2 which releases arachidonic acid from the membrane phospholipids and metabolised cyclo-oxygenase to TXA2. The function of TXA2 is to: 1)
decrease cyclic adenosine monophosphate (cAMP) levels in the platelet; 2) initiate the release reaction; 3) activate vasoconstriction and 4) supply positive feedback (figure 18).

- **Platelet pro-coagulant activity**
  The aggregation and release of platelets and the exposed membrane phospholipids with calcium have an activating effect on the tenase (activates factor IXa VIIa and X in order to form factor Xa) and the prothrombinase (formation of thrombin from the interaction of factors Xa Va and prothrombin (II)).

- **Growth factors** present in the platelet granules enhance vascular healing by stimulating the multiplication of muscular smooth muscle cells.

- **Natural inhibitors of platelet function**
  The increase of cAMP also achieved by prostaglandin and prostacyclin in the platelet (figure 15) decreases the concentration of free calcium ions and thereby inhibits the aggregation and adhesion action of the platelets and prevents deposition on unaffected vascular endothelium. Retraction of the platelet clot is achieved by the mediation of GPIIb/IIa receptors that link cytoplasmic actin filaments and the surface bound fibrin polymers. Endothelial cells release nitric oxide and prostacyclin which inhibits platelet activation and promotes vasodilatation. Endothelial cells also express the transmembrane protein PECAM-1 which inhibits platelet activation.
FIGURE 18  SYNTHESSES OF PROSTACYCLIN AND THROMBOXANE (Hoffbrand and Moss 2011:267; McPherson and Pincus 2011:790)
2.4.9.2 Coagulation

Blood coagulation is a sequential proteolytic activation of circulating proteins (table 4) resulting in the formation of thrombin which converts soluble fibrinogen into fibrin (Hoffbrand and Moss 2011:267). Fibrin entangles the aggregated platelet and converts the unstable platelet plug into a stable haemostatic plug (Tanaka, Key and Levy 2009:1434).

Fibrinogen is recognised as an independent risk marker of CVD. Fibrinogen because of its mass also has a direct effect on the blood viscosity and a physical functional effect on platelet aggregation (The Fibrinogen Studies Collaboration 2007:867). Studies have indicated an increased level of plasma fibrinogen in black South Africans (Pieters and Vorster 2008:171). The Fibrinogen Studies Collaboration (2007:867) concluded that an increase of 1 g/L in plasma fibrinogen doubles the risk of CVD. The production of fibrinogen is stimulated by inflammatory reaction (IL-6 key regulator) infection neoplasia and tissue damage (Carty, Heagerty, Heckbert, Jarvik, Lange, Cushman, Tracy and Reiner 2010:1).


<table>
<thead>
<tr>
<th>FACTOR</th>
<th>DESCRIPTIVE NAME</th>
<th>PLASMA CONCENTRATION (mg/l)</th>
<th>PLASMA HALF-LIFE (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Fibrinogen*</td>
<td>3000</td>
<td>90</td>
</tr>
<tr>
<td>II</td>
<td>Prothrombin*</td>
<td>65</td>
<td>100</td>
</tr>
<tr>
<td>III</td>
<td>Tissue factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Labile factor*</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>VII</td>
<td>Proconvertin*</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td>VIII</td>
<td>Antihaemophilic factor*</td>
<td>10</td>
<td>0.1</td>
</tr>
<tr>
<td>IX</td>
<td>Christmas factor</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>X</td>
<td>Stuart-Power factor*</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>XI</td>
<td>Plasma thromboplastin antecedent</td>
<td>45</td>
<td>5</td>
</tr>
<tr>
<td>XII</td>
<td>Hageman(contact) factor</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>XIII</td>
<td>Fibrin-stabilising factor / preklikrein (Fletcher factor) / Fitzgerald factor (HMWK, High molecular weight kininogen)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The prothrombin (thrombin interacts with them) group is vitamin K-dependent and requires Ca$^{2+}$ for activation. These factors are increased during pregnancy inflammation and the use of oral contraceptives (Tanaka et al 2009:1434; Hoffbrand et al 2011:758).
The coagulation cascade (figure 19) is activated either by the extrinsic pathway (also called the tissue factor pathway) or the intrinsic pathway (also known as the contact activation pathway. The extrinsic pathway is activated by the interaction of tissue factor (exposed by injured vascular endothelial cells) and circulating factor VII. Factor VIIIa then binds to tissue factor in the presence of the platelet phospholipids and calcium activates factor X and factor IX (Faxalv 2009; Tanaka et al 2009:1434; Batty and Smith 2010:532; Hoffbrand et al 2011:748; Russo 2012:2).
A small amount of Factor Xa activates prothrombin to form thrombin but the majority activates the common pathway: factor V platelets and in the presence of Ca$^{2+}$ activates prothrombin to form thrombin. Thrombin activates fibrinogen to form fibrin. The intrinsic pathway is activated by direct contact when prekallikrein converts to Kallikrein in the presence of HMWK. HMWK activates factor XII which activates factor XI in the presence of Ca$^{2+}$. Factor IX then in the presence of Ca$^{2+}$ platelets and factor VIIa (activated by extrinsic pathway) also activates the common pathway (Faxalv 2009; Tanaka et al 2009:1434; Batty and Smith 2010:532; Hoffbrand et al 2011:748; Russo 2012:2).

2.4.9.3 Fibrinolysis and inhibition of blood coagulation

The effect of thrombin is limited by some inhibiting factors: 1) tissue factor pathway inhibitor (TFPI) is produced by endothelial cells and is present in plasma and platelets accumulates at the site of injury where it inhibits factor Xa, VIIa and tissue factor; 2) direct serine protease inactivation of thrombin by antithrombin; 3) heparin and heparin co-factor II; 4) other proteins that also contribute to the inhibition of thrombin are $\alpha_2$-macroglobulins $\alpha_2$-antiplasmin $C_1$-esterase inhibitor and $\alpha_2$-antitrypsin (Batty and Smith 2010:533; Krone, Allen and McCrae 2010:55; Hoffbrand and Moss 2011:274).

Protein C and Protein S are vitamin K-dependent serine proteases and are produced by the endothelial cell (after thrombin binds to the receptor thrombomodulin). They inhibit coagulation co-factors V and VII (Batty and Smith 2010:533; Krone, Allen and McCrae 2010:55; Hoffbrand and Moss 2011:274).

Vasodilatation with the increased blood flow has a diluting effect on coagulation factors thereby inhibiting coagulation. Activated coagulation factors are removed from circulation by the liver and reticuloendothelial cells (Batty and Smith 2010:533; Krone, Allen and McCrae 2010:55; Hoffbrand and Moss 2011:274).

Fibrinolysis is an essential part of haemostasis. PAI-1 is the main inhibitor of the fibrinolytic system; elevated PAI-1 concentration was found to be associated with an increased incidence of
coronary heart disease deep-vein thrombosis and thrombotic events in malignancies (Skurk et al 2001:1336). Skurk et al (2001:1336) concluded that angiotensin II promotes PAI-1 production and release by human fat cells. PAI-1 levels of urbanised black South Africans are still within normal levels although higher than those of rural communities (Pieters and Vorster 2008:171). PAI-1 is a single-chain glycoprotein with a molecular weight of 47 kilodaltons which inhibits the activators of plasminogen in the plasma resulting in inhibition of fibrinolysis (IBL international 2008:3).

2.4.9.4 Fibrin network

A variation in the structure of the fibrin network has an additional contribution to CVD risk. Thinner fibres with more ramification and smaller intrinsic pores are dense and more resistant to lysis (increased atherothrombotic risks) compared with shorter thicker fibres. Because the fibrin network structure is complex and is influenced by fibrinogen concentration clearly much more research is needed (Pieters and Vorster 2008:171).

Pieters and Vorster (2008:171) have indicated that limited information is available on the haemostatic profile of black South Africans.

2.4.9.5 Effect of vitamins B12, B6 and Folate supplementation on haemostasis

Several mechanisms have been proposed to clarify the link between homocysteine and prothrombotic state. The oxidative damage to the endothelium, combined with inhibition of the vasculo-protective function of nitric oxide, enhances thrombosis (Antoniades, Antonopoulos, Tousoulis, Marinou and Stefanadis 2009:9).

A study evaluating the effect of vitamin B12 (500 μg), vitamin B6 (25 mg) and folate (2.5 mg) supplementation for three months, found no significant effect on fibrinogen or von Willebrand factor in elderly patients (Stott, MacIntosh, Lowe, Rumley, McMahon, Langhorne, Tait, O’Reilly, Spilg, MacDonald, MacFarlane and Westendorp 2005:1320).
Inconsistent results were reported on the effect of folate supplementation on fibrinogen concentration (Mierzecki, Kloda, Jastrzębska, Chelstowski, Honczarenko, Kozłowska-Wojciechowska and Naruszewicz 2012:697). Studies reported a beneficial anticoagulative outcome of folic acid by reducing plasma fibrinogen levels and increasing PAI-1 (Mayer, Simon, Rosolova, Hromádka, Subrt and Vobrubova 2002:1). Liem, Reyynierse-Buitenwerf, Zwinderman, Jukema and Van Veldhuisen (2003:2105) stated that no significant correlation could be found between folic acid supplementation and fibrinogen concentration.

2.4.10 Fibronectin

Fibronectin is a universal and vital component in the extracellular matrix (ECM), which is secreted and assembled by mesenchymal cells. Fibronectin has a dual function in the ECM, both as a regulator of cellular processes and as a support protein to maintain and direct tissue organization. Plasma fibronectin is also present and is synthesized by the hepatocytes (To and Midwood 2011:1; Hubmacher, Sabatier, Annis, Mosher and Reinhardt 2011:5322).

The modular protein fibronectin is composed of three domains (I, II and III). Domain I and II are each stabilised by two intermolecular disulphide bonds where domain III lacks the disulphide bond. Homocysteine is able to reorganize the disulphide bond patterns in proteins (including fibronectin). Altered disulphide bonds consecutively influence the stability and function of the protein (Hubmacher et al 2011:5322).

The role of fibronectin in thrombus formation, atherosclerosis and cardiac repair following a myocardial infarction is independent of haemostasis and therefore, been identified as a therapeutic target in prevention of thrombotic disease (Maurer, Tomasini-Johansson and Mosher 2010:287).

Although the interaction between fibronectin and homocysteine is well described no studies could be found reporting on the effect of homocysteine lowering supplementation on the fibronectin levels. This study will therefore contribute valuable novice information on the effect of vitamin B12, B6 and folate supplementation of fibronectin levels.
2.4.11 Adiponectin

Adipocytes produce the protein adiponectin that is present in high concentrations in the peripheral circulation (Ai, Otokozawa, Asztalos, White, Cuppies, Nakajima, Lamon-Fava, Wilson, Matsuzawa and Schaefer 2011:346). The functions of adiponectin in the cardiovascular system include an anti-inflammatory, anti-apoptotic and anti-hypertrophic effect and it controls monocyte adhesion to the vascular endothelium. Additionally adiponectin is an important regulator of endothelial nitric oxide synthase. Serum adiponectin levels are reduced in obese people and patients with DM and coronary arterial disease (Okamoto 2011:1; Wilson, Sabatine, Wiviott, Ray, De Lemos, Zhou, Rifai, Cannon and Morrow 2011:1147; Taube et al 2013:485; Gustafsson, Lind, Söderberg, Zilmer, Hulte and Ingelsson 2013:1467; Stoner, Lucero, Palmer, Jones, Young and Faulkner 2013:1359). Ai et al (2011:346) confirmed in the Framingham offspring study that adiponectin is an independent risk factor for cardiovascular disease due to its inhibition of the expression of adhesion molecules on the endothelial cells.

Bełtowski and Tokarzewska (2009:7) indicated in a review article that studies demonstrated the disrupting effect of homocysteine on adipokine (adiponectin included) production. In spite of the confirmed interaction between adiponectin and homocysteine no study reporting on the effect of vitamin B12, B6 and folate supplementation (homocysteine lowering agents) was found and will this study contribute significantly to the knowledge gap.

2.4.12 Homocysteine

2.4.12.1 Chemical structure of homocysteine

Homocysteine exists as a zwitterion with a molecular formula of \( \text{C}_4\text{H}_9\text{NO}_2\text{S} \) at neutral pH with a molecular mass of 135.18 g/mol (Karolczak and Olas 2009:624) (figure 20).
Homocysteine is a sulphur-containing amino acid produced in the metabolism of the essential amino acid methionine. Plasma homocysteine occurs in three different forms: 70% occurs as albumin-bound, 30% is bound to thiols in a disulfide linkage and only 1% is present as free homocysteine (Sawula, Banecka-majkutewica, Kadzinski, Jakobkiewicz-Banecka, Wegrzyn, Nyka and Banecki 2009:445).

2.4.12.2 Homocysteine metabolism

Homocysteine is metabolised by a) the trans-sulphuration pathway which results in the production of cystathionine a process that requires vitamin B6 and the main route of metabolism is via a methionine-conserving pathway a process that requires methyltetrahydrofolate (from folic acid) and vitamin B12 as co-factor or alternatively b) by the remethylation pathway taking place in the kidney and liver (where betaine is utilised instead of folate) (Kaul et al 2006:914; Wierzbicki 2007:143; Sawula et al 2009:445) (figure 21). The two pathways are synchronised by S-adenosylmethionine (SAM) the source of all methyl groups for methylation reactions within the cell (Selhub 1999:217). The by-product S-adenosylhomocysteine (SAH) is then rehydrolysed to form homocysteine (Wierzbicki 2007:144). Homocysteine therefore reduces the methylation potential whereas vitamin B12 and folate increase the potential (Wierzbicki 2007:144).
2.4.12.3 Plasma homocysteine

Plasma homocysteine levels (tHcy) include free bound and oxidised homocysteine. Normal values range from 5–15 μmol/l (Kaul et al 2006:914; Wierzbicki 2007:143). Elevated values are 16–30 μmol/l (mild), 31–100 μmol/l (moderate) and 31–100 μmol/l (severe) (Kaul et al 2006:914). Optimal serum homocysteine levels are obtained if measured on a fasted sample although if blood is collected after a methionine load a more representative value can be obtained to determine disturbances in the metabolism of homocysteine (Kaul et al 2006:914). Kaul et al (2006:915) have indicated that the cause of hyperhomocysteinemia can be multifactorial with two-thirds of cases caused by a dietary deficiency of vitamin B12, folate and vitamin B6. Other causes are genetic enzyme polymorphism (MTHFR) methionine synthase, cystathionine B synthases, genetics, gender (influenced by body size and oestrogen levels), lifestyle factors (alcohol, coffee intake and smoking), chronic diseases (renal failure, end-stage diabetes, systemic lupus erythematosus and hyperproliferative disorders), medication (methotrexate, sulphonamides, antacids) and age. In the elderly elevated homocysteine levels could result from glomerular filtration rate together with decreased vitamin B12 and folate absorption (Kaul et al 2006:914; Acikel et al 2009:327; Sawula et al 2009:445).

Therapeutic strategies in lowering plasma homocysteine levels will be discussed in chapter 3 (3.4).

2.4.12.4 Homocysteine as a CVD risk marker

McCully proposed an association between elevated plasma homocysteine and the development of atherosclerosis (McCully 1969:126). This theory was later confirmed by multiple studies. Ntaios et al (2008:4) suggested a causal relationship between hyperhomocysteinemia and cardiovascular complications such as ischaemic heart disease. Studies in animal models have shown that elevated homocysteine promoted atherosclerosis by increased oxidative stress impaired endothelial function and increased thrombogenicity (Kanani, Sinkey and Browning 1999:1167; Hoffmann, Lalla and Lu 2001:682; Lentz 2005:653; Kaul et al 2006:914; Wierzbicki 2007:144).
FIGURE 21 PATHWAYS INVOLVED IN HOMOCYSTEINE METABOLISM (Wierzbicki 2007:144).

Epidemiological retrospective and prospective clinical studies have complemented these findings, and established homocysteine as a potent independent risk factor for atherothrombotic vascular disease (Verhoef and Kok 1998:435; Kaul et al 2006:923; Antoniades et al 2009:15; Siri.).
Kaul et al (2006:917) concluded that homocysteine is related to multiple pathophysiological mechanisms involved in atherothrombosis and summarised these as follows:

a) Atherogenesis:
   - Expression of TNF-α and iNOS (inducible nitric acid synthase) induces vascular inflammation.
   - Oxidative stress is augmented.
   - The oxidation of low-density lipoprotein is increased.
   - DNA hypomethylation and gene expression for cell growth and differentiation is stimulated.
   - The macrophage uptake of lipoproteins is enhanced.
   - Oxidative stress induces endothelial dysfunction asymmetric dimethylarginine amplified inflammation and reduces the bioavailability of nitric oxide.
   - It induces 3-hydroxy-3-methylglutaryl-CoA which increases the accumulation of lipids.
   - Vascular smooth muscle cell (VSMC) DNA synthesis and proliferation is enhanced.
   - Endothelial cell toxicity is amplified.

b) Thrombogenesis:
   - Tissue factor activity is enhanced.
   - Increased expression of monocyte chemo attractant protein (MCP-1) and IL-8 resulting in an increased leukocyte-endothelial interaction.
   - Increased endothelial-cell-factor V activity.
   - Activates protein C and thereby inhibits the factor Va inactivation.
   - Reduces the antithrombin III binding to endothelium.
   - Inhibits the tissue plasminogen activator binding to the endothelial cells
   - Augments the fibrin lipoprotein (a) binding.
   - Decreases the activation of cell surface thrombomodulin and protein C
   - Increases thrombocyte aggregation.
2.5 CONCLUSION

As is evident in the literature that elevated homocysteine levels are directly proportional to cardiovascular risk. The cause of elevation of homocysteine levels is multifactorial. In a homocysteine-lowering approach to decrease cardiovascular risk all the factors which influence homocysteine levels need to be considered in order to determine an effective mechanism. Furthermore, inconsistent information is available on the influence of vitamin B12, B6 and folate on these risk factors and this study thus aimed to clarify this in the elderly.
CHAPTER 3

LITERATURE REVIEW – STRATEGIES TO ADDRESS HEALTH PROBLEMS IN THE ELDERLY

3.1 INTRODUCTION

There is a global epidemiological transition, in which there is growth not only in the number of older people, but also in their proportion in relation to other age groups; this is due to the change in morbidity dominated by infectious diseases, chronic diseases of lifestyle (CDL) and disabilities (Pulisetty and Morley 2007:1). The elderly are at nutritional risk globally, but particularly in developing countries, where they have been exposed to a lifetime of limited access to health care and a healthy diet (Claussen, Charlton, Gobotswang and Holmboe-Ottesen 2005:88). Macro-economic conditions are directly bringing about changes in diet and lifestyle, as seen in the effect of urbanisation on nutritional status (the nutrition transition) globally (Valdés-Ramos and Solomons 2002:146). Malnutrition resulting from poor dietary diversification (as described in 1.2.5) is often present among the elderly living in poverty (Oldewage-Theron and Kruger 2008:101).

Charlton et al (2007:534) have indicated that malnutrition among community-dwelling older persons is largely undetected. Appropriate screening and interventions addressing malnutrition among the elderly have been reported to improve outcomes significantly (Charlton et al 2007:534). Oldewage-Theron and Kruger (2008:126) have found that the elderly community of Sharpeville are poor (income of R501–1000) and that their carbohydrate-based diet, with its insufficient intake of fruit and vegetables, nutrient adequacy ratio of <0.6, dietary diversity score of 3.41 and food variety score of 4.77, is a health risk.

Because the majority of diseases prominent in the aged population are nutrition-related, nutritional intervention is indicated to mitigate the burden of disease in the elderly, improve their quality of life and have a beneficial effect on health care budgets (Pulisetty and Morley 2007:1).
3.2 GLOBAL FOOD-BASED APPROACHES TO ADDRESS MALNUTRITION

Food-based strategies to overcome malnutrition include dietary diversification and food fortification (Kruger, Hendricks and Puoane 2008:682). Food-based strategies are implemented to address micronutrient deficiency. However, because an unhealthy diet is the primary causative factor in CDL, food-based strategies will also have a preventative effect on CDL (Steyn et al 2008:697).

3.2.1 Fortification

3.2.1.1 Definition

Food fortification is the addition of nutrients at constant quantities (higher levels than present in food) to food products that are widely consumed by the community (Kruger et al 2008:683).

3.2.1.2 Aim

The aim of food fortification is to improve the quality of the diet and to address micronutrient deficiencies present in communities due to insufficient intake (Kruger et al 2008:683).

3.2.1.3 Advantages

Globally, fortification with micronutrients has been used to reduce nutrient deficiencies (e.g. of iodine, vitamin D, thiamine, niacin, iron and vitamin A) and has proved to be of great benefit to public health (Refsum and Smith 2008:253). Fortification has been identified as a sustainable, cost-effective medium- to long-term strategy in addressing micronutrient deficiencies in developing countries (Kruger et al 2008:683). In SA, all maize meal and bread flour (white and brown) have been fortified with six vitamins, iron and zinc since 7 October 2003 (INP 2004:10). Wardlaw and Kessel (2002:730) have indicated that consuming fortified foods is necessary for the elderly to meet their folate and vitamin B12 requirements.
3.2.1.4 Disadvantages

With the nutritional status varying from country to country, it is important to optimise the fortification dosage, since high dosages of certain micronutrients (e.g. Vitamin A and folic acid) can be harmful (Refsum and Smith 2008:253). In the choice of vehicle for fortification, the following must be borne in mind: a) it should be consumed by the majority of the population, b) it must retain appropriate levels of the micronutrients during processing, storage and preparing of the food, and c) fortification should not affect the acceptability (colour, taste or appearance) of the product (Kruger et al 2008:683).

3.2.1.5 Successes

Kruger et al (2008:682) summarised the effectiveness of global fortification programmes as follows:

- Vitamin A fortification of sugar in Central America significantly reduced deficiency in pre-school children.
- Improved vitamin A status in children in the Philippines has been obtained through fortification of margarine and wheat flour.
- Iron fortification is a technical challenge but success has been achieved using the ferric form to fortify wheat flour, cereals, milk powder, infant formula, maize mail and sugar.
- Iodisation of salt has been successful because of its wide consumption, acceptability, low cost and simple technology.
- Zinc fortification is very costly but milk powder, wheat flour and maize mail have successfully been fortified to address deficiencies in high-risk groups (infants and young children).

Refsum and Smith (2008:253) have shown that bread fortified with a low concentration of vitamin B12 improved serum vitamin levels and had a beneficial effect on functional markers (homocysteine, holotranscobalamin, methylmalonic acid).
3.2.2 Food diversification

3.2.2.1 Definition

Dietary diversification is the quantification of the number of nutritious food groups compared to the number of food items from each group (Oldewage-Theron and Kruger 2009:300). Dietary diversification includes the production and consumption of nutrient-rich foods (Kruger et al 2008:682). The production of nutrient-rich foods is achieved through small animal, poultry and fish production as well as through vegetable gardens (home, community or school) (Kruger et al 2008:682). Lack of nutrition knowledge is a factor that contributes to making unsuitable food choices. Nutrition education therefore has a beneficial effect on dietary diversification (Wenhold, Kruger and Muehlhoff 2008:460).

3.2.2.2 Aim

Multiple nutrient deficiencies are caused by a lack of food or of certain food products. Food diversification addresses this problem holistically (Kruger et al 2008:665).

3.2.2.3 Advantages

Food diversification is the most sustainable tool for addressing malnutrition because several nutrient deficiencies are addressed (Kruger et al 2008:682). Through dietary diversification, multiple deficiencies are addressed simultaneously (Kruger et al 2008:682).

3.2.2.4 Disadvantages

Food diversification is a long-term strategy that does not address acute deficiencies (Kruger et al 2008:682). Dodd and Bayerl (2008:314) concluded that, in spite of nutritional guidelines, very few people consume the prescribed five fruits or vegetables a day. Oldewage-Theron and Kruger (2009:300) concluded that, in an elderly low-income community studied, the prevalence of low
dietary diversity is a result of low income. These findings are comparable to those of studies in Botswana and other regions of SA (Claussen et al 2005:90).

### 3.2.2.5 Successes

Community gardens, school gardens and small farming (cattle, sheep and poultry) can produce micronutrient-rich food (Kruger et al 2008:682). The resources required to sustain successful food production are water, seeds, capital, training, a labour force, fencing, fertiliser and pesticides (Kruger et al 2008:682). The dietary diversification approach should be accompanied by a nutrition education programme to optimise healthy food choices (Kruger et al 2008:682).

### 3.2.3 Nutrition education

#### 3.2.3.1 Definition

Nutrition education is the communication of educational messages to optimise nutrition behaviour verbally or via print or electronic media (Kruger et al 2008:680). Information can be disseminated through schools, community organisations or the health services (Kruger et al 2008:680). The main objective of the South African food-based dietary guidelines is to promote cautious, balanced and healthy food choices by diverse South African population groups, which are at different stages of the nutrition transition (Mbhenyane, Makuse, Ntuli, Mbatsani and Sayed 2008:226). The South African food-based dietary guidelines can be applied effectively in a nutrition education programme educating/treating individuals suffering from CDL (Steyn et al 2008:697).

#### 3.2.3.2 Aim

The message conveyed by a nutrition education programme is aimed either to influence general feeding behaviour or to provide specific information to facilitate healthier choices (Kruger et al 2008:680). Sahyoun, Pratt and Anderson (2004:58) confirmed the need to educate the elderly to enable them to adjust their dietary patterns.
3.2.3.3 Advantages

Conteno (2007:17) points out that a meta-analysis has confirmed that nutrition education increased knowledge by 33 percentiles, attitudes by 14 percentiles and behaviour by 19 percentiles. A review article examining results of nutritional educational programmes for the elderly from 1990 till 2003 found that positive outcomes were reached where simple and direct messages were conveyed (e.g. lower sodium will reduce hypertension) (Sahyoun et al 2004:59). Behaviour change depends on the understanding of the principles of communication and of what encourages change in an individual (Sahyoun et al 2004:59). Nutrition education enhances the knowledge of an individual, resulting in an informed choice (Conteno 2007:336).

3.2.3.4 Disadvantages

Malnutrition is a multi-factorial condition and nutrition education should not be used in isolation but in combination with other intervention strategies (Behr and Ntsie 2008:315). The public health applications of nutritional education of the elderly are limited, because the message of change that is directed towards the individual has short-term effects and is not sustainable (Sahyoun et al 2004:59). The affordability and availability of healthier food choices directly influence the success of a nutritional educational programme in a low-income group (Conteno 2007:336).

3.2.3.5 Successes

Behr and Ntsie (2008:323) made the following recommendations for assuring an effective nutrition education programme:

- The programme should be part of a wider interventional approach to address health and nutritional challenges within socioeconomic development.
- Approaches, messages and support material should enhance awareness and increase knowledge and motivation for behavioural changes.
- Health communication and promotion models should be incorporated in developing nutrition education material, especially for members of food-insecure households.
• The community should be actively engaged in all stages of developing a programme.
• Methods should be appropriate for target groups.
• A multimedia approach should be employed.
• The capacity-building programme should include capacity building of the implementers.
• The commitment of political leaders, policy makers and resource providers is essential.
• An effective monitoring and evaluation plan needs to be in place.

Sahyoun et al (2004:59) suggested that nutrition education programmes for the elderly should be directed towards social and environmental change in order to adjust lifestyle choices.

3.3 SUPPLEMENTATION – NON-FOOD-BASED APPROACH

3.3.1 Definition

Supplementation is the intake of a commercially prepared product (capsule, pill, liquid solution or powder) to increase dietary intake of amino acids, vitamins, minerals and herbs. It is considered as complementary and alternative medicine. The supplementation industry has become a billion-dollar industry, where the healthy take supplementation to compensate for possible insufficiencies in food intake (Rogovik, Vohra and Goldman 2010:311; Kamangar and Emadi 2012:221).

3.3.2 Aim

The aims of supplementation are both to address possible nutrient deficiencies due to inadequate intake and to act as a preventative measure against disease (Kamangar and Emadi 2012:221; Kruger et al 2008:685). Supplementation is a temporary solution to address acute micronutrient deficiencies until more sustainable measures (such as a food-based approach) are effective (Kruger et al 2008:685).
3.3.3 Advantages

The main advantage of supplementation is to a) correct overt deficiencies, b) support increased nutrient requirements, c) improve nutritional status, d) improve the immune response, and e) reduce risk of disease (Whitney and Rolfes 2008:361; Kruger et al 2008:682).

a) Correct overt deficiencies

Supplementation increases nutrient intake in a population with physiological limitations by achieving the recommended nutrient intake (Sebastian, Cleveland, Goldman and Moshfegh 2007:1323).

b) Support increased nutrient requirements

The nutrients needed at different stages of life differ, and an increased nutrient need develops as physiological changes occur. For example, women of childbearing age need increased levels of iron due to menstrual blood loss; during pregnancy and lactation there is a higher demand for nutrients, particularly folate, to prevent neural tube defects; and a new born or infant needs an increased nutrient intake to address growth and immune demands, as do the aged with reduced food intake and absorption abilities (Du Plessis, Labuschagne and Naude 2008:354; Swart and Dhansay 2008:396; Charlton et al 2008:559).

c) Improve nutritional status

Subclinical deficiencies may occur in individuals (vegetarians, dieters, the elderly and patients with chronic disease) with insufficient intake. Substitutions may assist these individuals in reaching the required intake (Sebastian et al 2007:1323).
d) *Improve immune response*

In conditions where the body is under severe stress (after major surgery, during treatment of alcohol or other drug addiction), or after prolonged illness and extensive injury, supplementation is indicated and has improved the immune response (Bendich and Zilberboim 2010:669).

e) *Reduce risk of disease*

Increased intake of vitamins and minerals has been indicated as a preventative measure for heart disease, cancer and osteoporosis, and for this reason, supplementation is prescribed (Soni, Thurmond, Miller, Spriggs, Bendich and Omaye 2010:349).

3.3.4 Disadvantages

The risk of overconsumption of nutrients might arise with extensive use of supplements (Sebastian et al 2007:1323). Food rarely causes nutrient imbalances or toxicities, but this may occur with supplementation (Soni et al 2010:348). A misplaced sense of security might arise when people are consuming multivitamins and therefore neglect to pay attention to their eating patterns, or there could be a misunderstanding that high dosages of multivitamins might cure chronic diseases (e.g. Cancer or HIV/AIDS) and no treatment is needed (Whitney and Rolfes 2008:361; Rogovik et al 2010:311 ).

Thomson (2008:478) reported that the most vulnerable (elderly) are the least likely to take supplementation tablet, therefore indicative of poor compliance.

3.3.5 Successes

Sebastian et al (2007:1326) showed that supplementation doubled the vitamin B12 intake in a study with 1777 daily supplement users.
In SA (since 2002), as part of the Expanded Programme for Immunisation (EPI) high-dose vitamin A is given to mothers (6–8 weeks post-partum) and to babies (6–60 months) and to those diagnosed with malaria, diarrhoea, acute respiratory infection, measles, severe under nutrition and xerophthalmia (Kruger et al 2008:685). The combination of iron and vitamin-A supplementation has been found to be more effective in the prevention of iron deficiency than iron alone (Kruger et al 2008:686).

3.4 STRATEGIES FOR LOWERING HOMOCYSTEINE BY ADDRESSING VITAMIN B12, FOLATE and B6 DEFICIENCY

Ever since McCully (1969), 42 years ago, proposed the direct relation between elevated plasma homocysteine levels and cardiovascular disease, and the benefit of high doses of vitamin B12, B6 and folate treatment on the homocysteine levels of adolescents with homocysteinuria (Jamison, Hartigan, Kaufman, Goldfarb, Warren, Guarino and Gaziano 2007:1163), homocysteine as a cardiovascular risk marker and strategies to lower plasma homocysteine levels have been an ongoing topic of investigation. Vitamin B12, B6 and folate are directly involved in the homocysteine metabolism (Wierzbicki 2007:143). In spite of confirmation from epidemiological studies indicating the association between cardiovascular risk and elevated homocysteine, homocysteine-lowering intervention studies have not been consistent in their findings (Jamison et al 2007:1163; Albert, Cook, Gaziano, Zaharris, MacFayden, Danielson, Buring and Manson 2008:2027; Kaul et al 2006:914). In a review article, Kaul et al (2006:917) concluded from existing evidence that the impact of homocysteine-lowering strategies using vitamin B6, B12 and Folate can be divided into the following: 1) Impact on replacement outcomes (preventing post-prandial endothelial function, reducing incidence of positive stress electrocardiograms, significant regression in carotid plaque area; however, impact on inflammatory markers has not been found); 2) Impact on clinical outcomes; 3) Reducing angiographic restenosis. Kaul et al (2006:915) summarised the therapeutic options in lowering homocysteine as:

500–5000 mg folic acid, 10–500 mg vitamin B6, 1000–3000 mg vitamin B12, 500–900 mg trimethylglycine, 205–3000 mg choline, 205–1000 mg inositol, 30–90 mg zinc, 200–800 mg S-adenosyl-methionine.
All grains in SA are fortified with folic acid (7.15 g/kg) and vitamin B6 (16.24 g/kg) but not with vitamin B12, as per regulations of the foodstuff, cosmetics and disinfectant act (54/1972).

3.5 VITAMIN B12

3.5.1 Characteristics of vitamin B12

Cobalamin (vitamin B12) is a multifaceted molecule consisting of a corrin ring (tetrapyrrol ring structure with a metal ion cobalt molecule in the centre) with Aminopropanol bridge that connects the ring to ribonucleotide (figure 22) (Chatthanawaree 2011:227; Jelkmann 2012:2). *The arrow indicates that the spare electron pairs on the nitrogen attract them to the cobalt

The differentiation is a result of different groups chelated to the cobalt atom (CN\(^-\) - cyancobalamin (figure 22), OH\(^-\) - hydroxocobalamine, H\(_2\)O – aquocobalamin, CH\(_3\) – methylcobalamin, 5’-deoxy-5’adenosine – adenosylcobalamin) (Geissler and Powers 2006:202, Jelkmann 2012:2). The main physiological forms of vitamin B12 are methylcobalamin and 5-deoxyadenosylcobalamin (Hoffbrand et al. 2011:68).

### 3.5.2 Metabolism of vitamin B12

The absorption of vitamin B12 takes place in the distal ileum, when a complex of vitamin B12 and intrinsic factor (IF) bind to the surface receptor cubilin and then to a second protein amnionless, which directs endocytes of the complex. Vitamin B12 is then absorbed into portal blood and IF is destroyed. In the portal blood, vitamin B12 attaches to transcobalamin, a plasma-binding protein that delivers vitamin B12 to the bone marrow and into cells (figure 23) (Hoffbrand and Moss 2011:46; Burtis and Bruns 2015:473, Jelkmann 2012:2).

### 3.5.3 Function of vitamin B12

The cofactor cobalamin is required for the function of the enzymes methionine synthase and L-methylmalonyl-CoA mutase (Riedel 2011:1784; Lieberman and Marks 2013:752, Jelkmann 2012:2). During methionine synthase (figure 24) homocysteine is converted to methionine, when the methyl group is transferred from 5-methylene tetrahydrofolate to cobalamin to form methylcobalamin and tetrahydrofolate while methylcobalamin donates its methyl group that binds to homocysteine to form methionine (required for the synthesis of S-adenosylmethionine (SAM)) (Hoffbrand and Moss 2011:46, Jelkmann 2012:3). S-adenosylmethionine (SAM) is required in many cellular methylation reactions (including the methylation of RNA and DNA) (Newsholme and Leech 2009:511; Pepper and Black 2011:619). Vitamin B12 is also the coenzyme required to remove the methyl group from folate, thereby activating folate (Das and Kaul 2008:141; Pepper and Black 2011:619). Vitamin B12 and folate are also required to maintain the nerve fibres of the surrounding sheath and promote their normal growth, as well as bone cell activity and metabolism (Whitney and Rolfes 2008:343; Manolis, Manolis, Poulidakis and Melita 2013:51).
Leucine aminomutase is a vitamin B12-dependent enzyme used to catalyse the interconversion of β-leucine (metabolyte from intestinal bacteria) and leucine. Vitamin B12 (as co-enzyme 5-deoxyadenosylcobalamin) synthesises succinyl-CoA from L-methylmalonyl-CoA by L-methylmalonyl-CoA mutase as well as valine and cholesterol (Geissler and Powers 2006:202; Riedel et al. 2011:1784; Lieberman and Marks 2013:752). Succinyl-CoA is necessary for haemoglobin synthesis and energy production in the Krebs cycle (Hoffbrand and Moss 2011:46, Jelkmann 2012:3).
3.5.4 Dietary sources of vitamin B12

Dietary sources of vitamin B12 are animal products (meat, fish, chicken, milk and cheese) and are rarely found in plants or yeast (Burtis and Bruns 2015:473). The RDA for vitamin B12 for the elderly is 2.4 μg/day (Institute of Medicine, Food and Nutrition Board. 1998; Manolis et al 2013:51).

![Diagram of Biochemical Functional Characteristics of Vitamin B12](image)

**FIGURE 24** BIOCHEMICAL FUNCTIONAL CHARACTERISTICS OF VITAMIN B12 (Hoffbrand and Moss 2011:46)

3.5.5 Status of vitamin B12

In a consensus statement (Bates et al 2002:106), it was concluded that vitamin B12 and folate are high-risk micronutrients in the elderly population and that a higher RDA is needed owing to low bioavailability. Charlton et al (2007:541) detected in a black elderly free-living community in Cape Town, SA, that 11.3% of the population had a decreased concentration of serum vitamin B12 (<200 pg/ml). Oldewage-Theron et al (2008(b):22) reported that in the elderly Sharpeville...
community, 29.2% of the male respondents and 10.5% of the female respondents were very often identified as having decreased serum vitamin B12.

3.5.6 Deficiencies of vitamin B12

Vitamin B12 is stored in large quantities in the liver and a deficiency is developed over years (Newsholme and Leech 2009:511). Vitamin B12 deficiencies in the elderly are mainly due to malabsorption (Hajjar and Nahhas 2007:161; Mirkazemi, Peterson, Tenni and Jackson 2012:277) which is caused by any of the following:

- Atrophic gastritis, which may result in a decreased acid secretion resulting in decreased vitamin B12 absorption; a similar effect is observed with prolonged histamine (H₂) receptor antagonists or proton pump inhibitors;
- Bacterial overgrowth in the small intestine, which causes vitamin B12 deficiency because of the over-absorption of the vitamin by the bacteria;
- Degradation of R-protein due to pancreatic insufficiency;
- Ileal resection or bypass, or total gastrectomy;
- Crohn’s disease;
- Tropical and celiac spree;
- Malignancy.

A contributing factor to deficiency is drug–nutrient interaction, where Cimetidine (treatment for ulcers), Colchicine (treatment for gout) and antacids reduce the absorption of vitamin B12 (Wardlaw and Kessel 2002:727). Epidemiological studies from different countries reported a decline in vitamin B12 status of the elderly due to low bioavailability (Morley and Thomas 2007:160).

3.5.7 Intervention strategies

The format of vitamin B12 used in fortification and supplementation is cyanocobalamin, which is readily converted to 5-deoxyadenosylcobalamin and methylcobalamin (Hajjar and Nahhas 2007:161). Thomas (2007:120) recommended that the daily allowance (2.4 μg) in the elderly
should be obtained via supplementation or fortification, because of the high prevalence of atrophic gastritis. Atrophic gastritis causes a decrease in acid-pepsin secretion by the gastric mucosa, lessening the release of vitamin B12 from food protein (Thomas 2007:118). Bacterial overgrowth in the small intestine and stomach also occur in atrophic gastritis (due to hypochlorhydria), and the bacteria bind to vitamin B12. The traditional treatment for vitamin B12 deficiency has been intramuscular injections of cobalamin; more recently, supplementation has been preferred in spite of age-associated decreased absorption (Hajjar and Nahhas 2007:163, Jelkmann 2012:3). The absorption of protein-bound vitamin B12 decreases with age, in contrast with the absorption of crystalline vitamin B12 (used in fortification and supplementation), which does not decrease with age (Thomas 2007:118).

The percentage of individual older adults meeting the estimated average requirement (EAR) with use of vitamin B12 supplementation was statistically significantly different when compared with nonusers, and it was found that supplementation doubled the intake of vitamin B12 and could therefore compensate for decreased absorption (Sebastian et al 2007:1326).

3.5.8 Vitamin B12 and relationship with CVD and CVR markers

Reduction of methionine as a result of insufficient cobalamin result in increased homocysteine levels (Moreira, Brasch and Yun 2011: 876). Elevated serum homocysteine levels have been elucidated as an independent cardiovascular risk marker since McCully’s finding in 1968 (Jamison et al 2007:1163). Additionally homocysteine increase superoxide (O$_2^-$) levels resulting in increased oxidative stress, causing an inflammatory state and increase atherosclerosis and ischemia reperfusion. Additionally oxidative stress in return inhibits the cobalamin metabolism and enhances the cycle (Moreira et al 2011: 876).

Hyperhomocysteinaemia are also associated with arterial endothelial dysfunction and impaired vascular function, early marker of atherosclerosis. Intact endothelium maintains vascular integrity and regulates vasomotor tone (Bleie, Strand, Ueland, Vollset, Refsum, Igland, Nordrehaug and Nygård 2011:270).
In was found that vitamin B12 supplementation enhanced arterial endothelial function (Kwok, Chook, Qiao, Tam, Poon, Ahuja, Woo, Celermajer and Woo 2012:571). Vitamin B12 supplementation was found to increase heart variability, suggestive of sympathetic involvement, in healthy elderly (Sucharita, Thomas, Antony and Vaz 2012:70).

3.6 VITAMIN B6

3.6.1 Characteristics of vitamin B6

Vitamin B6 refers to three structures (pyridoxine, pyridoxal and pyridoxamine) that are related to pyridine and their derivatives (Cellini, Montioli, Oppici, Astegno and Voltattorni 2014:158). As indicated in figure 25, all three forms can be converted to the coenzyme pyridoxal-5’-phosphate (PLP) (Hellmann and Mooney 2010:443; Cellini et al 2014:158). The three vitamers are metabolically interchangeable and their physiological activity is alike (Geissler and Powers 2006:194; Hellmann and Mooney 2010:443). They are involved in diverse metabolic reactions and are therefore the most important coenzyme (Cellini et al 2014:159). Pyridoxal-5’-phosphate (PLP) transfers NH₂ (amino groups) from amino acids to keto acid and is therefore required in urea and protein metabolism (Di Salvo, Budisa, Contestabile and Safo 2011:1597).

3.6.2 Metabolism of vitamin B6

Pyridoxal, pyridoxine and pyridoxamine are hydrolysed by alkaline phosphatase and absorbed in the lumen of the upper small intestine by passive diffusion, enhanced by an acidic environment (Geissler and Powers 2006:194; Hajjar and Nahhas 2007:137; Cellini et al 2014:159). Pyridoxal phosphate and pyridoxal are transported from the liver, being bound to albumin and haemoglobin. Extracellular alkaline phosphatase hydrolysates pyridoxal phosphatease to pyridoxal followed by phosphorylation, which thereby clasps the vitamin in the cells (Di Salvo et al 2011:1598).

Excess vitamin B6 is oxidised to 4-pyridoxic acid and excreted (Geissler and Powers 2006:194). Frequent intake is required (Bender 2011:29). However, unlike other water-soluble vitamins, vitamin B6 is stored in abundance in muscle tissue, bound to glycogen phosphorylase. Vitamin B6
is released from the muscle during starvation as the muscle glycogen is depleted and redistributed (Geissler and Powers 2006:194; Whitney and Rolfes 2008:336).

**FIGURE 25  VITAMIN B6 CHEMICAL STRUCTURES**  (adapted from Bender 2010:30; Di Salvo et al 2011; Cellini et al 2014:159).

### 3.6.3 Function of vitamin B6

Pyridoxal-5′-phosphate (PLP) is involved in almost all amino acid metabolism, including:

- Synthesis of niacin or the neurotransmitter serotonin from the amino acid tryptophan. (Ye, Maras, Bakun and Tucker 2010:1660; Dhallal, et al 2012:535).
- Production of aminolevulinic acid (a haem precursor) (Ye et al 2010:1660; Wachowska, Muchowicz, Firczuk, Gabrysiak, Winiarska, Wańczyk, Bojarczuk and Golab 2011:4143).
• Production of sphingolipids that are required in the myelin sheath (surrounding the nerve cells) (Lowther, Yard, Johnson, Carter, Bhat, Raman, Clarke, Ramakers, McMahon, Naismith and Campopiano 2010:1683).

• Pyridoxal phosphate terminates the steroid hormone nuclear action by facilitating the release of the hormone from the DNA-binding receptor complex in order to terminate the nuclear action of the hormone (Bender 2011:32).

3.6.4 Dietary sources of vitamin B6

Dietary sources include meat, fish, potatoes and bananas which are good sources. However, it is also present in nuts, whole grain, fortified cereal and leafy vegetables, chicken, legumes, non-citrus fruit, liver and soy products (Dhalla et al 2012:535; Cellini et al 2014:159). The bioavailability differs according to food type, with pyridoxine glycoside as the least bioavailable. Vitamin B6 (5–75%) obtained from plant sources is in the form of glycosylated pyridoxine (Geissler and Powers 2006:194; Kim and Cho 2014:688). The RDA for adults is 1.3 mg/day (aged 19–50 years) with a maximum of 100 mg/day (Bender 2011:29). For the elderly male the RDA is 1.7 mg/day, and for the elderly female, 1.5 mg/day (Gallagher 2008:99).

3.6.5 Status of vitamin B6

Owing to the abundance of vitamin B6 in a variety of food sources, deficiency is not very common. Marginal levels, however, are commonly known to affect steroid hormone responsiveness and amino acid metabolism (Bender 2011:35).

3.6.6 Deficiencies of vitamin B6

Vitamin B6 deficiency in the elderly often occurs in conjunction with other nutritional disorders. Symptoms accompanying vitamin B6 deficiency are weakness, microcytic anaemia, scaly dermatitis, angular stomatitis, cheilosis, glossitis, peripheral neuropathy, irritability, depression, confusion and convulsions. Vitamin B6 deficiency is caused by insufficient intake, malabsorption, alcoholism, liver cirrhosis and dialysis. Certain medications (isoniazid, hydralazine, penicillamine,

### 3.6.7 Intervention strategies

Supplementation of vitamin B6 (50-200mg/day) is widely used for conditions like premenstrual stress, depression, hypertension, morning sickness during pregnancy, carpal tunnel syndrome, and for the prevention of protein non-enzymatic glycation in diabetic patients (Hellmann and Mooney 2010:448,452; Yamagishi 2011:218). During a prolonged intake of isoniazid, supplementation of vitamin B6 is recommended (Asif 2013:114). Sebastian et al (2007:1326) found a statistically significant difference in the EAR of elderly persons supplementing their diet with vitamin B6 compared with those who did not supplement. Vitamin B6 deficiency is associated with increased homocysteine levels and it has been demonstrated that supplementation of vitamin B6 (above RDA) has a preventative effect on cardiovascular disease (Sawula et al 2009:445).

### 3.6.8 Vitamin B6 and relationship with CVD and CVR markers

The beneficial effect of vitamin B6, in reducing the adverse effect of uncontrolled diabetes by reducing glycated haemoglobin, has been reported. The amino acids of protein reacts with pyridoxal or pyridoxal phosphate instead of been glycated (Bender 2011:33).

As mentioned vitamin B6 act as coenzyme in the irreversible transsulfuration of homocysteine to cysteine. Higher vitamin B6 level is associated with lower homocysteine levels (Ye et al 2010:1660). Hyperhomocysteinaemia promote oxidative damage, inflammation and endothelial dysfunction and are therefore an independent cardiovascular risk factor (Adekunle and Adedeji 2011:352).

Animal models indicated that a decrease in blood pressure was observed after supplementation with vitamin B6. This could be due to improvement in the central nervous system, or interaction between pyridoxal phosphate and membrane calcium channels (Bender 2011:33; Dhallal et al 2012:538).
Fat metabolism requires carnitine, obtained either directly through diet or via synthesis requiring lysine and vitamin B6. Vitamin B6 supplementation in hypertriglyceridemia men reduced plasma cholesterol and HDL concentration (Hlias, Reslan, Sarieddine, Nasreddine, Taan, Azar and Obeid 2012:1674). Vitamin B6 deficiency was also found to be associated with decreased in plasma PUFA (n-6) and (n-3) which may be associated with elevated cardiovascular risk and a contributing factor to anti-inflammatory response (Adekunie and Adedeji 2011:352; Zhao, Lamers, Ralat, Coats, Chi, Muller, Bain, Shankar, Newgard, Stacpoole and Gregory 2012:1791).

Low circulating vitamin B6 have been inversely related to inflammatory markers (Hs-CRP, fibrinogen, IL-6 and TNF-α) and related to the occurrence of inflammatory diseases (rheumatoid arthritis, cardiovascular disease, chronic inflammatory bowel disease and diabetes) (Huang, Wei, Wu and Huang 2010:1007; Shen, Lai, Mattei, Ordovas and Tucker 2010:337; Lotto, Choi and Friso 2011:186).

3.7 FOLATE

3.7.1 Characteristics of folate

The folates are a group of compounds that derive from Folic acid [pteroylglutamic (PGA)]. The pteroylglutamic acid structure (figure 26) varies in dietary folates:

- An additional hydrogen atom is present at position 7 and 8 (dihydrofolate) or at 5, 6, 7 and 8 (tetrahydrofolate).
- An additional formyl group is present at position 5 or 10 and a methyl group at position 5. The 5-formyl-tetrahydrofolate is the most resistant to atmospheric oxidation and most common format used in pharmaceutical products.
- An additional glutamate moiety is attached to γ-carboxyl group.

(Hoffbrand and Moss 2011:47).
3.7.2 Metabolism of folate

A zinc-dependent enzyme (conjugate) hydrolyses folate in the intestinal lumen (Zhao, Diop-Bove, Visentin and Goldman 2011:9). Folate is absorbed by passive and active mechanisms in the jejunum (Ohrvik and Witthoft 2011:477; Nazki, Sameer and Ganaie 2014:13; Visentin, Diop-Bove, Zhao and Goldman 2014:252). Folate is transported as loosely bonded to albumin (as 5-methyltetrahydrofolate) and absorbed by cells via a high-affinity folate receptor (Ponziani, Cazzato, Danese, Fagiuoli, Gionchetti, Annicchiarico, D’aversa and Gasbarrini 2012:379; Visentin et al 2014:252) (figure 25). The methyl group needs to be enzymatically removed with the assistance of vitamin B12 in order for the folate coenzyme to become active (Stover and Field 2011:329). Excess folate is secreted by the liver into bile and transported to the gallbladder, where it is reabsorbed and re-circulated (Ponziani et al 2012:379). Unabsorbed folate is excreted in urine after enterohepatic recirculation; insignificant amounts are stored (McPartlin 2009:215). Gastrointestinal (GI) tract injury results in a decrease in reabsorption of folate and an increase in excretion (Zhao et al 2011:9).
3.7.3 Function of folate

The physiological involvement of folate is centred mainly on the transfer of single-carbon units (Kirsch, Hermann, Eckert, Geisel and Obeid 2013:497). During the metabolism of amino acids and nucleic acids, the folate coenzyme accepts and donates one-carbon groups (Burdge and Lillycrop 2012:1924; Abbenhardt et al 2014:715). The methylation of RNA and DNA, as in many other biological reactions, requires methyl, which is donated by the S-adenosylmethionine synthesised in the presence of folate (Glier, Green and Devlin 2013:2; Abbenhardt et al 2014:714). Folate acts as a coenzyme (5, 10-methylene tetrahydrofolate (THF) polyglutamate) in the synthesis of thymine monophosphate from its precursor deoxyuridine monophosphate (figure 27) (Hoffbrand and Moss 2011:47, Glier et al 2013:2; Kirsch et al 2014:497).

3.7.4 Dietary sources of folate

Because folate cannot be physiologically synthesised, concentration depends on consumption (Hoffbrand and Moss 2011:47). Folate is omnipresent in nature but is very temperature sensitive and it is lost by excessive cooking and canning (Hajjar and Nahhas 2007:158). Green leafy vegetables, citrus fruit, legumes, yeast, liver and organ meats contain the highest concentration of folate (Burtis, Ashwood and Fowler 2008:492; Koike, Hama, Kawagashira, Hashimoto, Tomita, Lijima, Sobue 2012:821). Heat and oxidation during food preparation and storage have a destructive effect on folate, destroying up to 50% of the original concentration (Bassett and Sammán 2010:298). The RDA for folate is 400 μg/day with an upper limit of 1000 μg/day for adults (older than 70 years included) (Antoniades et al 2009:12; Crider, Bailey and Berry 2011:373).
3.7.5 Status of folate

Normal serum folate level is 3–17 ng/ml or 6.0-28 nmol/l (Lin et al 2007:232; Burtis et al 2008:848). Folate deficiency is relatively common (8–10%) (Geissler and Powers 2006:194). It is estimated that 2.5–34% of the elderly experience folate deficiency (Hajjar and Nahhas 2007:159, Koike et al 2012:821). In a peri-urban black elderly population in Cape Town, SA, the prevalence
of low red cell folate concentration (<111.6 ng/ml) was 12.2% in male respondents and 19.8% among the female respondents (Charlton et al 2007:541). In results obtained from a peri-urban community in Sharpeville, Oldewage-Theron et al (2008(b):26) reported that 20.8% of males and 32.5% of females had a serum folate level lower than 9 nmol/l.

### 3.7.6 Deficiencies of folate

Folate deficiency is caused by malnutrition, impaired absorption, lactation, genetic defects, smoking, inflammatory bowel disease, medication (triamterene, anti-epileptic drugs, chemotherapy and methotrexate), extreme alcohol intake, bariatric surgery and atrophic gastritis (Koike et al 2012:821). Folate deficiency symptoms are megaloblastic anaemia, smooth red tongue (“beefy tongue”), mental confusion, weakness, fatigue, irritability, headache, shortness of breath and elevated homocysteine as well as neural tube syndrome in new born (Koike et al 2012:821; Kirsch et al 2013:498). Folate as previously mentioned is required for the synthesis of dTMP from dUMP. Deficiency of folate leads to increased dUMP, resulting in increased integration of uracil into the DNA instead of thymine. Excessive uracil in DNA leads to: point mutation; generation of single and double stranded DNA breaks; chromosome breakage and micronucleus formation (Fenech 2012:22).

### 3.7.7 Intervention strategies

The pharmacologic form of folate (namely folic acid) used in supplementation and fortified foods is more stable than folate present in natural food (Burdge and Lillycrop 2012:1924; Kirsch et al 2013:497). Folic acid has a higher bioavailability compared to folate 1ug folate = 0.6 ug folic acid (Borradale and Kimlin 2012:413)

Epidemiological studies have indicated the benefit of folic acid supplementation because of the high prevalence of folate deficiency in the elderly (Kirsch et al 2013:498). The Food and Nutrition Board of the Institute of Medicine has concluded that the total intake of folate should not exceed 1000 μg daily (Crider et al 2011:373). Folate is required for the remethylation of homocysteine into methionine. Folate deficiency, therefore, results in the accumulation of homocysteine and increased cardiovascular risk (Shane 2011:5S).
3.7.8 Folic acid and relationship with CVD and CVR markers

It is projected that supplementation with folic acid can decrease the risk for ischaemic heart disease by 16%, deep vein thrombosis by 25% and stroke by 24% (Scorsatto, Uehara, Luiz, De Oliveira and Rosa 2011:889). It is indicated that low serum folate levels is a cardiovascular risk marker independently from homocysteine level (Imamura, Murakami, Takahashi, Cheng, Numaguchi, Murohara and Okumura 2010:728). Folate as a donor of one-carbon units is essential for methylation and affects numerous metabolisms involved in cardiovascular disease (Abbenhardt et al 2014:714).

Accurate replication of DNA and its repair is essential in healthy aging. If DNA repair capacity of the cell is exceeded by the rate of damage to the genome serious defects in cellular and tissue physiology occur, resulting in degenerative diseases including CVD (Fenech 2010:236).

Four mechanisms in reducing atherosclerosis have been identified by which folate is involved. They are: 1) Optimizing methylation cycle and thereby directly reducing the homocysteine levels; 2) Act directly as an antioxidant; 3) Interact with enzyme endothelial nitric oxide synthase; 4) Affect cofactor bioavailability of nitric oxide (Ganeshan, Kartihumar, Viswanath, Renjith and Alin 2014:1142).

Carotid intima-media thickness has been accepted as a reliable and premature marker for atherosclerosis. Folic acid supplementation (18 months) significantly reduced the carotid intima media and therefore reduced the cardiovascular risk (Ntaios, Savopoulos, Karamitsos, Economou, Destanis, Chryssogonidis, Pidonia, Zebekakis, Polatides, Sion, Grekas and Hatzitolios 2010:16). Individuals with lower folate levels have elevated vascular endothelial function (Imamura et al 2010:728).

Chronic inflammation is an important underlying pathogenic factor in degenerative diseases like cardiovascular disease. Increased inflammatory state has been linked with folate deficiency. Decrease in inflammatory markers (HS-CRP, IL-6 and TNF-α) were observed after folate
supplementation (Ho, Xue, Cushman, McKeown-Eyssen, Sandler, Ahnen, Barry, Saibil, Bresalier, Rohan and Baron 2009:1650; Kolb and Petrie 2013:164; Abbenhardt et al 2014:715).

Homocysteine a thiol-containing aminoacid is produced when the methionine (essential amino acid) is metabolised to cysteine. High levels of homocysteine induce injury of endothelial cells, and enhance vascular inflammation, atherogenesis and atherosclerotic plaque formation. Folic acid supplementation reduces homocysteine levels by about 25% (Liu, Tian, Zhang, Gao and Zhou 2014:31).

3.8 THERAPEUTIC EFFECT OF DIFFERENT DOSAGES OF HOMOCYSTEINE LOWERING VITAMINS IN COMBINATION AND INDIVIDUAL

Hyperhomocysteinaemia are positively associated with endothelial dysfunction, oxidation of low-density lipoprotein and monocyte adhesion (enhance atherosclerosis). B vitamins down regulates the homocysteine levels through the pyridoxal phosphate dependent pathway (vitamin B6 dependent) or the remethylation pathway (vitamin B12 and folate dependent) (Bertoia, Pai, Cooke, Joosten, Mittleman, Rimm and Mukamal 2014:94). A Large scale randomised controlled trial reported that a combination therapy had a beneficial effect than supplementation of a single B vitamin (eg. Folate) (Zhou, Tang, Wu, Lu, Wei, Qin, Wang, Xu, He 2011:1).

Studies that evaluated the effect of vitamin B12 supplementation on the homocysteine levels or cardiovascular risk, used variable concentrations, with a minimum 25 μg/day and a maximum of 1000 μg/day, without any adverse reaction (Deshmukh, Joglekar, Lubree, Ramdas, Bhat, Naik, Hardikar, Raut, Konde, Wills, Jackson, Refsum, Nanivadekar, Fall and Yainik 2010:495; O’Leary and Samman 2010:306; Dullemeijer, Souverein, Doets, Van Der Voet, Van Wijngaarden, De Boer, Plada, Dhonukshe-Rutten, In’t Veld, Cavelaars, De Groot and Van’t Veer 2013:394). A meta-analysis indicated that a median supplementation dose of 0.4 mg/day (range 0.02-1.0 mg/day) resulted in a 7% decrease in homocysteine (O’Leary and Samman 2010:306).

Supplementation levels of vitamin B6 between 25-500 mg/day of pyridoxine hydrochloride have been reported in numerous studies, single of in combination with folate and vitamin B12 with
positive effect on inflammatory markers serum homocysteine levels (Bender 2011:29; Huang et al 2010:1008; Ulvik et al 2012:1073). In rare genetic conditions, where individuals have a very low affinity of pyridoxal phosphate-dependent enzyme, supplementation of 200 to 1000 mg/day throughout life are required, no adverse effect have been reported even on these high dosages (Bender 2011:29).

Folic acid is considered safe with an intake level of up to one mg per day. The lowest adverse effect observed was 5 mg/day, although intakes of 15-100 mg/day was only associated with limited direct toxicity (Burdge and Lillycrop 2012:1925). In a meta-analysis conducted on randomised trials it was found that a range of folic acid supplementation levels were used, with a minimum concentration of 0.8 mg/day and a maximum of 40 mg/day, without any adverse reaction (Lee, Hong, Chang and Saver 2010:1207; Yang, Lee, Hong, Ovbiogele and Saver 2012:748; Qin, Huo, Xie, Hou, Xu and Wang 2013:724). Qin et al (2011:485) indicated that in a meta-analysis done including 3886 participants that folic acid (5-40 mg/day) supplementation reduced homocysteine with >20%.

The Vitamins Intervention for Stroke Prevention Trial - compared high dose (25 mg pyridoxine, 0.4 mg cobalamin and 2.5 mg folic acid) with low dose (200 µg pyridoxine, 6 µg cobalamin and 20 µg folic acid) B vitamin supplementation. A favourable 2 µmol/l reduction in the homocysteine levels were observed in the high dose group compared to the low dose group (VITATOPS Trial Study Group 2010:855). Skarupski, Li, Outang, Evans and Morris (2010:330) indicated that an additional 10 mg vitamin B6 and 10 µg vitamin B12 lower the odds by 2% of participant presenting with symptoms of depression. Supplementation of folic acid (5mg daily) and vitamin B12 (500 µg twice daily) in patients with acute ischemic stroke reduced the plasma asymmetric dimethylarginine and homocysteine levels within 12 weeks (Xia, Li, Wang, Ma and Wu 2014:1587). In contrast, other studies have not found any beneficial effect of the supplementation of 2.5-40 mg folic acid, 2-50 µg vitamin B12 and 20-100 mg vitamin B6 on the morbidity or the mortality of participants over a period of 3-5 years, despite the significantly reduced homocysteine levels (Marti-Carvajal, Solá, Lathyris, Karakitsiou and Simancas-Racines 2013:1; Nursalim, Siregar and Widyahening 2013:152).
3.9 CONCLUSION

Vitamin B12, B6 and folate have been studied as homocysteine-lowering therapy for preventing atherothrombotic events in high-risk populations without clear evidence and remain the subject of on-going investigation (Acikel et al 2009:327; Siri et al 1998:439). Prospective cohort studies projected that a reduction in serum homocysteine of 3 μmol/L would decrease the risk for CVD by 18% and stroke by 24% (Holmes, Newcombe, Hubacek, Sofat, Ricketts, Cooper, Breteler, Bautista, Sharma, Wittaker, SMEETH, Fowkes, Algra, Shmeleva, Szolnoki, Roest, Linnebank, Zacho, Nalls, Singleton, Ferrucci, Hardy, Worrall, Rich, Matarin, Norman, Flicker, Almeida, Van Bockxmeer, Shimokata, Khaw, Wareham, Bobak, Sterne, Smith, Talmud, Van Duijn, Humphries, Price, Ebrahim, Lawlor, Hankey, Meshia, Sandhu, Hingorani and Casas 2011:378).

Dhonukshe-Rutten et al (2009:18) has suggested that several approaches to increase the intake of vitamin B12, B6 and folate are possible. These are: 1) a food-based approach that includes a healthier diet rich in green leafy vegetables, 2) food fortification, and 3) supplementation. In the proposed sample population with poor living conditions and low literacy, a food-based approach is not affordable and sustainable. Furthermore, the long-term effect of a food-based approach may be less effective in the elderly due to their lowered absorption ability. Because the need for intervention in this group is acute, a supplementation approach is more advisable.

Dhonukshe-Rutten et al (2009:23) argue that controversial results in supplementation studies might be due to insufficient concentration of vitamin B12 and folate in the supplement, and suggest that doses >200% should be used. Kolb and Petrie (2013:164) argued that although numerous studies reported limited effect of vitamin B6, B12 and folate supplementation on morbidity and mortality, a life time of adequate B vitamin levels have a vascular protective effect. According to the literature, none of the above-mentioned studies, or any study, was carried out on an African population. The African context, where the nutritional, socio-economic and cultural lifestyles are substantially different from those of European populations, should thus be studied (Oldewage-Theron et al 2008:23). The scientific and epidemiological significance of this study is to achieve optimal reduction of the homocysteine levels, without any adverse reactions, by means
of a combination vitamin supplement (vitamins B12, B6 and folate) as this has not been studied before in a black elderly population of South Africa.
CHAPTER 4

BASELINE SURVEY – OBJECTIVES, METHODOLOGY, RESULTS AND DISCUSSION

4.1 INTRODUCTION

Notably, few studies have been done in the vulnerable elderly population in SA, where the nutritional, socio-economic and cultural lifestyles are substantially different from those of developed countries (Oldewage-Theron et al, 2008(b):23).

Cardiovascular disease exists in epidemic proportions in developed countries and is an increasing problem in the developing world. Black elderly people in SA live in poor living conditions often resulting in household food insecurity and malnutrition, which have negative effects on the health status of the elderly, including chronic diseases of lifestyle.

Hyperhomocysteinaemia has been identified as an independent risk factor for atherothrombotic vascular disease (Kaul et al 2006:923; Antoniades et al 2009:15; Siri et al 1998:435). Positive correlations between vitamins B12, B6 and folate status and cardiovascular disease have been demonstrated by numerous studies (Dhonukshe-Rutten et al 2009:18).

In this chapter, a baseline study conducted in an elderly population attending a care centre in Sharpeville is described and discussed.

4.2 OBJECTIVES

The main aim of the baseline study was to determine the pre-intervention nutritional, health and socio-economic status of the sample. The results reported in this chapter will be compared with the post-intervention results (chapter 5).
The specific objectives of the baseline study were to:

- Determine the socio-economic position and general health profile of the elderly population.
- Determine the food consumption patterns and nutrient intake of the elderly population.
- Determine the anthropometric indexes of the elderly population as markers for nutritional status and cardiovascular risk.
- Determine the presence of dyslipidaemia in the population as a cardiovascular risk marker.
- Determine the haemostatic status of the individuals (using fibrinogen and PAI-1 as indicators) as cardiovascular risk marker.
- Determine the prevalence of metabolic syndrome as a cardiovascular risk marker in an elderly community, using serum glucose, insulin, weight and blood pressure as indicators.
- Determine the presence of an inflammatory response, using HS-CRP as indicator.
- Determine the vitamin B12, folate, B6 status.
- Determine the baseline homocysteine, adiponectin and fibronectin levels as indicators of risk of CVD.

4.3 DESIGN

This was a cross-sectional survey (De Vos, Strydom, Fouche and Delport 2010: 137) of all the elders attending the day-care centre in Sharpeville.
4.4 ETHICAL CONSIDERATIONS

This study was ethically approved both by the ethics committee of the University of the Witwatersrand, Johannesburg (M070126) (Annexure A) and by the ethics committee of Durban University of Technology (DUT) (Annexure B). The South African Medical Research Council’s ethical guidelines for human research were applied during fieldwork. Because of the high rate of illiteracy among the elderly participants, the letter of information and consent (Annexure C) approved by the DUT was verbally explained to them by the researcher and translated into Sotho by a trained fieldworker. The letter was a brief introduction to the study, giving a short motivation and indicating the purpose of the study. The procedure of the study was described and confidentiality was assured. It was explained that the study was voluntary; although the participants did not receive any remuneration they were also not liable for any expenses. Participants who signed or gave verbal consent were used for this study.

Introductory visits were made to the day-care centre explaining the objectives of the project to the management and the attendees of the day-care centre in order to obtain approval from both parties. All the elderly attending the day-care centre (100%) completed and signed the consent forms (Annexure C) in order to agree to participate in the study. Notices were distributed two weeks before the fieldwork commenced. All the elderly attending on the day (87.5%) were included. Attendance at the day-care centre is voluntary. The following actions were followed in order to assure that this study was conducted with high ethical practice:

- Participation in this study was voluntary and autonomous. Before informed consent was given care was taken to ensure that the subject understood the aim, objectives and methodology of the study.
- Communication were done / translated to Sotho in order to ensure participants understanding.
- Participants were treated sensitively, helpfully and with respect to ensure their dignity.
• All actions were at all times non-maleficence.
• Confidentiality was assured at all times. Subject numbers were allocated on arrival. No names were linked to any of the information only the subject number.
• Fieldworkers were trained in order to act competent and professional.

4.5 SAMPLING STRATEGY AND SAMPLE SIZE

The study was carried out among the elderly people (60 years and older) attending a day-care centre in Sharpeville, Vaal region. It was a purposively selected sample (33%) of all the elders (n=110) attending the day-care centre in Sharpeville and signed the consent form (as discussed in 1.2.6). All subjects were equivalent in age (>60 years), race (black), resident in Sharpeville, Vaal region, unemployed / pensioner (socio-demographic).

To determine the number of subjects (n) needed to study homocysteine the following calculation was used:

\[ n = \frac{2 \times (u+v)^2 \times s^2}{E^2} \]

Where:
- \( u = 1.28 \), corresponding to a \( \beta \) for the test of 90% power
- \( E = 4.00 \), expected mean change in homocysteine (Kaul et al 2006:923)
- \( v = 1.96 \), 95% significance for two-tailed hypothesis
- \( s = 5.5 \), Standard deviation of the change in homocysteine levels (Kaul et al 2006:923)

A total of 40 subjects are therefore needed to obtain statistically representative data from this community. However, 110 of the elders gave consent to participate and all were included in the sample to ensure statistical significance should some of the elders drop out during the study.
4.5.1 Inclusion criteria

- Respondents 60 years and older
- Attending the day-care in Sharpeville
- Consent given

4.5.2 Exclusion criteria

Individuals were excluded that cannot make an informed consent due to conditions such as Alzheimer’s and Senile dementia. Due to the low prevalence (2-3%) of HIV amongst the specified population group it will not be considered as a possible confounding variable (AVERT 2008). No exclusion was made to the individuals to individuals suffering from any chronic condition.

4.6 MEASURING INSTRUMENTS

4.6.1 Methods to combat error

The quality of the study is determined by the validity and reliability of the data. Pietersen and Maree (2010:215) defined reliability as the reproducibility of a measuring instrument, validity is the degree to which the measuring instrument assesses accurately and precisely what it purports to measure. All questionnaires had been pre-tested for validity and reliability during previous studies in the same elderly community (Oldewage-Theron et al 2008(a):8). Each laboratory analysis was run in conjunction with a commercial validated control serum. No result was accepted unless the control values were within the 2SD range of the indicated control value.

Reliability (inter-item correlation) is determined by Cronbach’s alpha coefficient (0.90 – highly reliable; 0.80 – moderately reliable; <0.70 – low reliability) (Pietersen and Maree 2010:216). Coefficient of variance (CV) of the laboratory methods (reported in 4.8.1) were
Validity is differentiated in (Pietersen and Maree 2010:217):

- The extent to which the measuring instrument appears to be valid is called **face validity**. This is not quantifiable but care was taken through literature study that all measuring instrument (laboratory procedures) used in this study have a high degree of face validity.
- The degree by which the instrument covers the absolute content of the meticulous concept is determined by **content validity**. In this study content validity was insured by using laboratory procedures that were confirmed by literature to cover all possibilities needed to be included in order to achieve accurate measurements.
- The presence of rational relationship between variables needs to be covered by measuring instruments in order to achieve **construct validity**. A conceptual framework (see figure 3) was compiled from literature in order to identify and measure all variable that could have an influence on the results.
- **Criterion validity** quantitatively measures the degree by which an instrument measures what it is expected to measure. Criterion validity of the laboratory procedures have been monitored by measuring a control serum continuously while running the method.

Pietersen and Maree (2010:216) identified the following factors as possible threads to the validity of a measuring instrument:

- The reliability of the measuring instrument.
- Bias might arise when answering questionnaires where respondents might tend to answer yes to all questions. Questionnaire should be formulated to have both affirmative and negative answers.
- Respondents might answer in a way that they think might be socially acceptable. This was overcome by using well trained fieldworkers and pre-tested questionnaires.
- Due to cultural or language differences some groups might score certain items
higher than others.

### 4.6.2 Recruitment and training of fieldworkers

A group of multi-disciplinary field workers were recruited in order to assist the researcher in conducting the fieldwork. Sotho speaking (to prevent translation bias) post graduate students of the Hospitality Management and Tourism department at the Vaal University of Technology were recruited to assist in the completion of consent forms and questionnaires. A detailed information session was given to all the fieldworkers informing them of the aim, objectives, the relevance and the study procedure in order to ensure better understanding of the context and their role in the study.

The recruited fieldworkers were trained to complete the quantitative food frequency questionnaires (QFFQ’s), the use of food models and the 24-hour recall questionnaires. During the training session emphasis has been placed on techniques to prevent interviewer bias, asking leading questions. The field worker also participated in the validation of the questionnaires in order to standardize the process. Ten volunteers from Vaal University of Technology staff members were involved in a pilot study to determine proficiency of the field workers in completing the questionnaires and to address any shortcomings (time frame needed for the completion of questionnaire, communication and translation skills).

Anthropometric measurements (weight, height and waist circumference (WC)) were taken by trained dietician (SA) and a public health nutritionist (USA).

A registered nursing sister was recruited for the blood collection and the blood pressure measurements. Three B Tech Biomedical Technology students (HPCSA registered) were recruited to assist the researcher (HPCSA registered biomedical technologist) with blood analysis.
4.6.3 Data Collections – Fieldwork control and process

The baseline measurements were taken from everyone that consented to participate. High ethical consideration (as described in 4.5) was given during the execution of the following procedures:

1) **Station 1** - On arrival a subject number was allocated to the participant. A file containing field workers control list (see Annexure D) was labelled with the participant’s subject number. All questionnaires were labelled with the subject number and placed inside the file. A Ziploc bag containing the blood tubes labelled with the subject’s number was handed to the participant after confirmation that the subject had been fasting. The subject was then asked to continue to the next station.

2) **Station 2** - Blood pressure and clinical signs (see questionnaire Annexure I) were taken by the phlebotomist (also qualified nursing sister) before blood collection and noted on a fieldworkers control list (see Annexure D). Blood was collected from the fasted subject by using a vacutainer system from the vena cephalica of the fasted elder. Two 7ml clotted blood tubes as well as, a 3ml Glucose tube, 5ml EDTA tube and 5 ml sodium citrate blood tubes were collected from each subject. Blood was placed in a cooler box (8°C), and protected against direct sunlight. Breakfast (soft porridge with milk or peanut butter sandwich with tea) was served after blood collection.

3) **Station 3** - Anthropometric measurements (weight, height and WC), and the completion of a health, medical and behavioural questionnaire (Annexure E) were completed by a registered dietician. After completion the controllers list were signed and the participant requested to continue to station 4.

4) **Station 4** - The fieldworker completed the dietary intake (24-h recall) (Annexure F) and food frequency questionnaires (FFQ) (Annexure G) as well as the socio-demographic questionnaires (Annexure H) in a one-on-one interview with each
participant, demonstrating food types and portion sizes using food models. Probing for answers assured that accurate and complete data were collected. The questions were explained in a language preferred by the respondents with caution to prevent interviewer’s bias, a process Babbie and Mouton (2001:249) endorsed for a sample with high illiteracy level. The field worker assured that all the questionnaires was completed and replaced in the file before they signed acknowledgement on the controllers list and the participant was requested to continue to the next station.

5) The subject was requested to return to the reception desk (station 1) where files were handed in and control list verified to assure that all the data collection processes were completed.

4.6.4 Socio-demographic questionnaire

a) Description

The population was defined by information gather by the socio-demographic questionnaire (Annexure H). Measurements included type of house; number of people in the household, household’s economic status (monthly income).

b) Reliability

The same questionnaire developed, validated and standardized by Oldewage-Theron et al (2005:13-26) was used. The same ten elders completed the questionnaire weekly for four consecutive weeks. Answers were compared and high correlation (r>0.6; p≤0.05) confirmed reproducibility.
c) **Data collection procedure**

Trained Sotho speaking fieldworkers completed the questionnaires in a one to one interview with the elder. Questions were asked in Sotho and questionnaires were completed in English. Care was taken to avoid interviewer’s bias.

d) **Statistical analyses**

Data were captured on Microsoft Office Excel and transported to Statistical Package for Social Science (SPSS), version 22. Descriptive statistical analyses were done; means and standard deviations (SD) were computed for each variable.

### 4.6.5 24-hour recall questionnaire

a) **Description**

Daily quantitative food consumption patterns were determined by a one-day recall or record method (24-hour recall questionnaire). Due to the short attention span of the elderly, and because the 24-hour recall questionnaire is fast and easy to administer, it was decided to utilize this questionnaire to measure actual dietary food item intake and to determine the top 20 most consumed food items. Subjects were asked by a trained fieldworker to recall their exact food intake during the previous 24h period. Walsh and Joubert (2007:294-296) concluded that a 24-hour recall questionnaire (Annexure F) is a fast and simple instrument in determining the dietary intake of an individual, providing that the fieldworkers are well trained to assist the participant in recalling and reporting information.

b) **Reliability**

The 24-hour recall questionnaire developed by Oldewage-Theron et al (2005:13-26) was used. Dietary intake were determined using the 24-h recall questionnaires in different
populations (n=722 Vaal and 395 Qwa-Qwa) by a registered dietician using a program based on the South African local food consumption tables (SAMRC FoodFinder® program). The recommended standard for estimating the prevalence of inadequate intakes within a group was determined by the EAR, and the adequate intake (AI) levels used for those nutrients without an EAR. The results of both (24-hour recall and FFQ) questionnaires were compared against each other to test for validity of food intakes.

c) Data collection procedure

A validated procedure (Gibson 2005:42) was used for completion of the 24-hour recall in a one-on-one interview situation between the fieldworker and subject to gather information needed: **Step 1**- A complete list of all foods and beverages consumed the previous day is recorded; **Step 2** – Standardized probe questions were used to collect detailed information (eg. cooking method and brand names); **Step 3** – Amount of each item consumed are obtained (often in household quantities) recorded, by using measuring cups, spoons rulers and food models as memory aids and; **Step 4** – The questionnaire is reviewed to insure that all items have been recorded (including vitamin and mineral supplements. The 24-hr recall questionnaire was completed for three days, one weekend and two weekdays and the average / mean of the three questionnaires was used for comparing with EAR.

d) Statistical analyses

The dietary intake and food consumption patterns (24-hr recall) were analyzed by a registered dietician on the Food Finder program, which is based on the South African food composition tables (Langenhoven, Kruger, Gouws and Faber 1991). Data were captured on Microsoft Office Excel and transported to SPSS, version 22. Descriptive statistical analyses were done; means and standard deviations were computed and compared with the EAR for elderly ≥70 years. Energy was compared to the estimated energy requirements (EER) as calculated using the revised Harris-Benedict equation that is more accurate for obese subjects (mean sample age, weight and height were used) (Roza and Shizgal.
4.6.6 Food frequency questionnaire (FFQ)

a) Description

Dietary variety was determined by using an adapted Food Frequency questionnaire (Matla 2008). It has been found that dietary diversity, specifically (Food Variety Score) FVS and (Food Group Diversity Score) FGDS, are good indicators of dietary adequacy in the elderly and can therefore be used as reference measurement for dietary intake assessments (Oldewage-Theron and Kruger, 2009:9; Claussen et al 2005). The food frequency questionnaire was used to calculate the dietary variety and is not time-consuming, thus resulting in a reduced burden for the respondent. It is inexpensive, reasonably accurate, valid and quick to administer (Joubert and Ehrlich 2007:295). The FFQ consisted of a list of foods categorized according to the FAO nine nutritious food groups, namely cereal, flesh, legume, vitamin A-rich vegetables and fruit, other vegetables, other fruit, dairy, fat and egg groups.

b) Reliability

The FFQ (Annexure G) used was a questionnaire standardized by Oldewage-Theron and Kruger (2009:306) of the population of the Vaal Region, where food items corresponding the dietary patterns were listed. Focus group discussions were held to include most popular food items to be included in the FFQ. The original standardized questionnaire (Matla 2008) was adapted to include the food items mentioned in the FFQ as well as the local names of the food items.

c) Data collection procedure

The trained field worker completed the questionnaires by requesting the elder, in a one on one interview, to reflect on the food items consumed during the past seven days, and ticked
it from a list. Food models were used to correctly identify the food items consumed.

\[ d) \text{ Statistical analyses} \]

The data were captured on a Microsoft Office Excel spread sheet by the researcher and analyzed on the SPSS, version 22 programme for frequencies, means and SDs. The different dietary diversity measures were calculated as follows: 1) the overall variety score (simple count of food items); 2) a variety score across all nine food groups; and 3) a variety score within every food group (Hatloy, Torheim and Oshaug 1998). These scores were calculated for a reference period (Ruel 2003) of seven days. The dietary diversity score (DDS) consisted of a simple count of single foods and food groups, similar to scores used in previous studies in developing countries (Clausen et al 2005). The nine nutritious food groups recommended by the United Nations Food and Agricultural Organization (FAO) were used to classify food intakes categorically. Fewer than 30 foods consumed in the period of seven days indicated low food variety, 30 to 60 foods indicated medium variety, and more than 60 foods, high variety. One to three food groups, 4-5 food groups and 6-9 groups indicated low, medium and high FGDS (Matla 2008). All the dietary diversity scores (FVS, FGDS and DDS) were calculated from the seven-day FFQ.

\[ 4.6.7 \text{ Health questionnaire} \]

\[ a) \text{ Description} \]

Health assessment gives a short insight into the health status of the elderly at present and assist in the evaluating health needs. Questions asked assessed the family history of diseases, current illness (duration, severity and nature), variation in appetite weight loss, allergy, alcohol intake, smoking and medication used and preferred health care facilities used.

\[ b) \text{ Reliability} \]
An adapted questionnaire developed and validated by the Gauteng provincial administration (GPA) was used. The same questionnaire was used in the same elderly population previously and proved to be reliable (Oldewage-Theron et al. 2008(a):13-26).

c) Data collection procedure

The validated questionnaire was completed in a one on one interview by the trained Sotho speaking fieldworker.

d) Statistical Analyses

Data were captured on Microsoft Office Excel and transported to SPSS, version 22. Descriptive statistical analyses were done; Frequencies were computed.

4.7 OPERATIONAL PROCEDURE

4.7.1 Anthropometric measurements

a) Description

Anthropometric measurements (weight, height and WC) were measured and recorded. Body mass index (BMI) was calculated as weight (kg) divided by height (m²).

b) Reliability

Standardized measuring procedures were performed with respondents wearing light clothing and bare foot (Lee and Nieman 2007:170).

c) Data collection procedure
Weight was measured in duplicate on an electronic Phillips (model HF350) bathroom scale and average was reported. A scales 2000 portable stadiometer was used to measure the height. The stadiometer was placed upright against a perpendicular wall. WC was measured at the area halfway between lower rib and iliac crest with a Seca non-stretchable measure tape in a horizontal position around the body.

d) **Statistical analyses**

Weight, height and WC measurements were captured on Microsoft Office Excel and transported to SPSS, version 22. Descriptive statistical analyses were done; means and standard deviations were computed for each variable. Furthermore, the body mass index (BMI) were calculated and used to determine the frequencies of underweight (<18.5 kg/m\(^2\)), normal weight (≥18.5<25 kg/m\(^2\)), over weight (≥25<30 kg/m\(^2\)) and obesity (≥30 kg/m\(^2\)) (WHO 1995:271). Waist-to-height ratios (WHTR) were calculated as it has been found that BMI does not identify higher risk respondents within normal weight range and that WHTR is a better predictor for multiple coronary risk factors than BMI and can be an independent predictor for metabolic risk regardless of gender. Means and SD of the WHTR was calculated as well as the frequencies of those subjects at risk (>0.5 cut-off) (Lee, Song and Sung 2008:837; Hsieh, Muto, Yoshinaga, Tsuji, Arimoto, Miyagawa, Hoshihara and Hara 2006:187; Maffeis, Banzato and Talamini 2008:210).

4.7.2 **Blood pressure and clinical signs**

a) **Description**

Blood pressure measurements were taken by Tensoval Hartmann® duo control monitor. This monitor, in contrast with similar electronic blood pressure instruments, uses two methods (Korotkoff and Oscillometric methods) to determine and report the blood pressure (Hartmann in South Africa 2013). The Korotkoff method is the most accurate and reliable of the method, where the Korotkoff sound is detected to determine the blood pressure (similar to the stethoscope). The microphone detecting the sound is place in the device and not in the cuff in order to reduce malfunction due to incorrect handling (Hartmann in South
Africa 2013). The second method is the oscillometric method, that detects the pulse waves (caused by the arterial pulsation) and not the sound is used to indirectly algorithmically calculating the blood pressure (Hartmann in South Africa 2013). Although the oscillometric method is less accurate than the Korotkoff method it is suitable for people with low pulse sounds. A combination of the two methods is therefore more accurate. The South African hypertension guidelines 2011 (Seedat and Rayner, 2012:62), as indicated in table 3, was used as cut off values to stratify the risk for hypertension.

The physical signs of malnutrition as summarized in table 5 can be detected by looking at the eyes, hair, skin, lips, oral mucosa, tongue, nails, muscular extremities and neurological reactions.

b) Data collection procedure

The qualified nursing sister took the blood pressure in duplicate on the left arm of the seated responded after a two to three minute resting time.

Individuals were assessed for clinical signs of malnutrition as indicated in table 5 by the nursing sister and reported on fieldworkers control list.

c) Reliability

It is recommended by the European Union and International hypertension organizations that all blood pressure measuring instruments should be independently validated. Hartmann® adhered to the validation protocols as described by: Advancement of Medical Instrumentation; British Hypertension Society; European Society of Hypertension to validate the Tensoval blood pressure instrument (Hartmann in South Africa 2013).

As reported in literature and summarized in Table 5, observations of malnutrition by physical signs is according to face validation.
**TABLE 5  CLINICAL SIGNS FOR MALNUTRITION** (Charlton et al 2008:567; Hoffbrand and Moss 2011:50)

<table>
<thead>
<tr>
<th>CLINICAL SIGNS</th>
<th>POSSIBLE NUTRITIONAL RELATED ABNORMALITIES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hair:</strong></td>
<td></td>
</tr>
<tr>
<td>Brittle and breakable</td>
<td>Protein deficiency</td>
</tr>
<tr>
<td><strong>Eyes:</strong></td>
<td></td>
</tr>
<tr>
<td>Pale eye membrane</td>
<td>Anaemia (iron, vitamin B12, folate deficiency)</td>
</tr>
<tr>
<td>White growths on cornea</td>
<td>Hypertipaeoma</td>
</tr>
<tr>
<td><strong>Skin:</strong></td>
<td></td>
</tr>
<tr>
<td>Pallor and mild icterus</td>
<td>Anaemia (iron, vitamin B12, folate deficiency)</td>
</tr>
<tr>
<td>Xerosis</td>
<td>Essential fatty acids deficiency</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>Niacin deficiency</td>
</tr>
<tr>
<td>Flaky dermatitis</td>
<td>Protein deficiency</td>
</tr>
<tr>
<td>Poor tissue turgor</td>
<td>Dehydration</td>
</tr>
<tr>
<td>Oedema</td>
<td>Protein and thiamine deficiency</td>
</tr>
<tr>
<td>Purpura, Perifollicular haemorrhage</td>
<td>Vitamin C, B12, K vitamin and folate deficiency</td>
</tr>
<tr>
<td>Pressure source</td>
<td>Protein deficiency</td>
</tr>
<tr>
<td>Poor wound healing</td>
<td>Protein, zinc, vitamin C, iron</td>
</tr>
<tr>
<td><strong>Oral cavity:</strong></td>
<td></td>
</tr>
<tr>
<td>-Lips + mucosa</td>
<td></td>
</tr>
<tr>
<td>Angular cheilosis, stomatitis</td>
<td>Iron, protein, riboflavin, niacin, vitamin B12 and folate deficiency</td>
</tr>
<tr>
<td>Swollen bleeding gums</td>
<td>Vitamin C deficiency</td>
</tr>
<tr>
<td><strong>Tongue:</strong></td>
<td></td>
</tr>
<tr>
<td>Magenta tongue</td>
<td>Riboflavin deficiency</td>
</tr>
<tr>
<td>Cracked, raw</td>
<td>Niacin deficiency</td>
</tr>
<tr>
<td>Glossitis: beefy red painful</td>
<td>Vitamin B12, folate, pyridoxine and iron deficiency</td>
</tr>
<tr>
<td>Atrophic papillae</td>
<td>Riboflavin, niacin and iron deficiency</td>
</tr>
<tr>
<td><strong>Nails:</strong></td>
<td></td>
</tr>
<tr>
<td>Spoon shaped nails</td>
<td>Iron and chromium deficiency</td>
</tr>
<tr>
<td><strong>Muscular extremities:</strong></td>
<td></td>
</tr>
<tr>
<td>Muscular pains</td>
<td>Biotin and selenium deficiency</td>
</tr>
<tr>
<td>Muscle twitching</td>
<td>Pyridoxine deficiency</td>
</tr>
<tr>
<td>Muscle weakness</td>
<td>Sodium and chloride deficiency</td>
</tr>
<tr>
<td>Pain in calves, weak thighs</td>
<td>Thiamine deficiency</td>
</tr>
<tr>
<td>Muscle cramps</td>
<td>Sodium and chloride deficiency</td>
</tr>
<tr>
<td><strong>Neurological:</strong></td>
<td></td>
</tr>
<tr>
<td>Disorientation</td>
<td>Dehydration, Thiamin and sodium deficiency</td>
</tr>
<tr>
<td>Decreased vibratory sense, ataxia, optic neuritis</td>
<td>Vitamin B12 and folate deficiency</td>
</tr>
<tr>
<td>Weakness, paraesthesia of legs</td>
<td>Thiamine, pyridoxine, pantothenic acid, vitamin B12</td>
</tr>
<tr>
<td>and folate deficiency</td>
<td></td>
</tr>
<tr>
<td>Mental disturbances, psychosis</td>
<td>Niacin, magnesium, vitamin B12 and folate deficiency</td>
</tr>
</tbody>
</table>

---

**d) Analysing**

Diastolic and systolic measurements were captured on Microsoft Office Excel and transported to SPSS, version 22. Descriptive statistical analyses were done; means and standard deviations were computed for each variable. Blood pressure measurements were
used to classify hypertension with reference to the South African hypertension guidelines 2011 (Seedat and Rayner 2012:62) as indicated in Table 3.

Frequency (%) of clinical signs of malnutrition was calculated and reported.

4.7.3 Biochemical analysis

Blood was collected by a qualified phlebotomist using a vacutainer system from the vena cephalica of the fasted elder. Blood was collected between 07:00 and 10:00 to avoid dural variation.

Samples collected included: Two 7ml clotted blood, the 3ml glucose tube, and 5 ml sodium citrate blood were separated within two hours of collection and stored at -80ºC until analyzed. The EDTA collected blood was used for full blood count that was completed within four hours after collection. Blood parameters were performed according to standard laboratory protocol in order to comply with SANAS accreditation requirement, by the principle investigator (a registered Medical Technologist) with the assistance of B Tech Biomedical Technology students, supervised by the principle investigator.
• **THE LABORATORY EQUIPMENTS USED TO PERFORM THE ANALYSES WERE:**

1) *High performance liquid chromatography (HPLC) method*

Vitamin B₆ was determined using Agilent 1260 Infinity HPLC system. The system consists of a quaternary pump, ultra-low carryover autosampler and thermostatic column compartment. The analyte was separated with Cline® analytical column for B₁, B₂ and B₆ (special reversed-phase column) with a Cline® Guard column connected at the inlet. Behind the column, was a T-Union (T-connector) connected to a ClinLab® Peristaltic pump used to mix the mobile phase with reagent N prior to detection. This enhanced the fluorescence yield, resulting in improved detection sensitivity (Recipe® 2010).

2) *Sysmex CA500 instrument*

Fibrinogen levels were determined by means of a fully automated Sysmex CA 500 instrument measuring the clotting time. Four detectors are used by the instrument to determine light scattering at 660 nm caused by the clot formation.

3) *Automated Konelab™ instrument.*

Konelab 20i random access automated clinical chemistry system was used for the analyses of: cholesterol, HDL, triglycerides, γGT, glucose, homocysteine, HS-CRP. The system uses a single channel interference filter photometer with beam splitting reference, measuring the reaction in the cells in sequence. The measuring principles are colorimetric and turbudimetric with a spectral range of 340-800 nm (Konelab 1999:12-3).
4) **Maglumi 1000 immuno analyser**

Chemiluminescence Immunoassay (CLIA) system (Maglumi 1000) were used to determine the vitamin B12, folate, insulin and ferritin levels. Two technologies are utilized by this instrument namely a) Labeling technology (determining the reactive mode) and b) separation technology (determining the sensitivity, accuracy and precision of reagents). Labeling is performed by a small non-enzyme molecule with an acid and alkaline buffer stability. Nano magnetic micro beads are used in the separation technology to: a) enlarge the reaction area of antigens and antibodies and thereby shorten the reaction time; b) improve the “capture” between antigen and antibody and thereby enhance sensitivity; c) mix liquid reagent thoroughly to limit inter-intra-assay discrepancy; d) better accuracy is achieved by the absorption of antigen or antibody by a chemical reaction to the micro bead (Shenzhen New Industries Biomedical Engineering Co., Ltd 2012:2 (a)).

5) **Beckman Coulter™ AC.T™ 5diff Haematology analyser**

This is a fully automated haematology analyzer providing 26 parameters including a complete five-part differential white blood cell count. Twenty parameters include White blood cell count (WBC), red cell count (RBC), haemoglobin (Hb), haematocrit (Hct), mean cell (corpuscular) volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count (plt), mean platelet volume (MPV), neutrophil count (% and #), eosinophil count (% and #), basophil count (% and #), lymphocyte count (% and #) and monocyte count(% and #). Additional six non-essential parameters are also reported, they are: plateletcrit (Pct), platelet distribution width (PDW), immature leukocytes (% and #) and atypical lymphocytes (% and #). (Beckman Coulter 2000:2-1).
6) *Enzyme Linked Immunosorbent Assays (ELISA).*

Immuno - Biological Laboratories Co. Ltd. (IBL) international standardized kids was used to quantitatively determine the concentration of PAI-1 and fibronectin, additionally a BioVendor Laboratomi medicína standardized kids were used to determine adiponectin levels all by means of an ELISA method. As illustrated in figure 28 a micro well coated with Anti-human antigen (adiponectin, PAI-1 or fibronectin) was used. Sample or standard were added to the micro well where the specific protein (adiponectin, PAI-1 or fibronectin) in plasma serum bond to the Anti-human antibody, a biotin-conjugate anti-human antibody (against adiponectin, PAI-1 or fibronectin) was then added to form a sandwich binding with the complex already formed. After incubation all the unbounded proteins are washed away and Streptavidin-Horseradish Peroxidase (HRP) was added that also bounded to the complex, after incubation another washing process followed to wash away all unbounded Streptavidin-HRP. Tetra Methyl Benzidine (TMB) a substrate solution reactive to HRP was used as a colouring agent (chromagen). The colour reaction directly proportional to the quantity of the protein present develops. The reaction is stopped by the addition of 1M Phosphoric acid. Absorbance was measured at 450 nm.

FIGURE 28  SCHEMATIC REPRESENTATION OF THE PRINCIPLE OF ELISA METHOD
ELISA assays were performed manually using calibrated pipettes. The absorption was read on a Rayto RT-6100 micro plate reader.

**PRINCIPLE, PROCEDURE AND QUALITY ASSURANCE OF EACH TEST ARE AS FOLLOW:**

**4.7.3.1 Adiponectin**

- **Description**

A solid phase sandwich ELISA method in micro plates pre-coated with recombinant human adiponectin in combination with anti-human adiponectin was used to determine the adiponectin concentration.

- **Validation**

Seven standards with variable concentrations (10 µg/ml, 5 µg/ml, 2 µg/ml, 1 µg/ml, 0.5 µg/ml, 0.2 µg/ml, 0.1 µg/ml and blank) were done in duplication in order to compile a standard curve from which the sample concentration were determines (BioVendor Laboratomi medicína a.s. 2009:5).

- **Procedure**

ELISA was performed manually using calibrated pipettes, using Biovendor research and diagnostic product kit. The absorption was read on a Rayto RT-6100micro plate reader.

- **Analysing**

Means of the serum Adiponectin levels were compared with the reference values of 7.6-15.2 µg/ml (BioVendor Laboratomi medicína a.s. 2009:16).
4.7.3.2 Cholesterol

\textit{a) Description}

As described by Allain, Poon, Chan, Richmond and Fu (1974:475) cholesterol esters are enzymatically hydrolyzed to cholesterol and fatty acids. Cholesterol oxidase oxidized the free cholesterol to cholest-4-en-3-one and hydrogen peroxide. A quinoneimine dye (chromophore) that’s measurable at 500-550 nm, are formed when the hydrogen peroxide combines with hydroxybenzoic acid and 4-aminoanipyrine (Thermo Fisher Scientific Oy 2007 (a)).

\textit{b) Validation}

Cholesterol parameter was calibrated with a commercial Konelab™ calibrator (sCAL) before the start of analysis. Control serums (Lipotrol, Nortrol and Abtrol) were run daily and documented to assure accuracy and precision.

\textit{c) Procedure}

An automated method on the Konelab™ has been used in the quantification of the serum cholesterol in this study.

\textit{d) Analysing}

Means of the serum cholesterol levels were compared with the reference values of : <5.2 mmol/l considered as desirable, 5.2-6.2 mmol/l borderline and >6.2 mmol/l high risk. (NCEP 2002:II-5).
4.7.3.3 Fibrinogen

a) Description

The method used is a modification of the Clauss method, where citrated plasma is mixed with excess thrombin resulting coagulation. The clotting time is directly related to the fibrinogen concentration in the plasma (Dade Behring Marburg GmbH 2003:H 1).

b) Validation

This method is validated by Dade Behring with a coefficient of variance of 2.9% in normal control plasma (N) and 7.2% in the abnormal control plasma (P). The coefficient of variance from day to day was 1.6% (control plasma N) and 3.4% (control plasma P) (Dade Behring 2003). An internal quality control was performed after calibration as well as after every eight hours while running the samples. Commercially prepared normal control plasma (N) and pathological range control plasma (P) were run as samples and results were documented and used as the internal quality control.

c) Procedure

Quantitative plasma fibrinogen levels were measured on an automated Sysmex CA500 instrument, using Dade Behring Multifibren U reagents.

d) Analysing

Means of the plasma fibrinogen levels were compared with the reference values of 1.8-3.5 g/L (Dade Behring Marburg GmbH 2003:H 1)
4.7.3.4 Fibronectin

b) Description

A solid phase sandwich ELISA method in micro plates pre-coated with anti-human fibronectin antibody was used to determine the fibronectin concentration determines (Immuno-Biological Laboratories Co.Ltd. (IBL) international 2009:3)

d) Validation

Seven standards with variable concentrations (20 ng/ml, 10 ng/ml , 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.63 ng/ml, 0.31 ng/ml and blank) were done in duplication in order to compile a standard curve from which the sample concentration were determines Immuno-Biological Laboratories Co.Ltd. (IBL International GmbH 2009:16)

e) Procedure

ELISA was performed manually using calibrated pipettes, using Biovendor research and diagnostic product kit. The absorption was read on a Rayto RT-6100 micro plate reader.

f) Analysing

Means of the serum Fibronectin levels were compared with the reference values of 12-124 μg/ml IBL International GmbH 2009:30).

4.7.3.5 Folate

a) Description

A competitive immunoluminometric assay (CLIA) was used to determine the concentration of folic acid present in the serum. Amino-Butyl-Ethyl Isoluminol (ABEI)
labeled by a purified Folic Acid antigen and uses a folic acid-binding protein to label Fluorescein isothiocyanate (FITC). Serum, ABEI label, FITC label and magnetic beads were mixed and incubated at 37°C to form an antigen-antibody complex. Sedimentation of the complex is allowed in a magnetic field, supernatant then gets removed, and a starter reagent then was added after washing. The starter reagent initiates the flash chemiluminescent reaction; the light signal is measured by a photomultiplier as relative luminescence units (RLU) within 3 seconds and is directly proportional to the concentration folic acid present in the serum (Shenzhen New Industries Biomedical Engineering Co., Ltd 2012:1(a)).

\[ b) \text{ Validation} \]

Accuracy was determined by diluting a high concentration calibrator by a ratio of 1:2, and the concentration of the diluted sample measured for ten times. The recovery of the measured concentration was 90-100% of the expected value. Precision Inter and intra-assay coefficient of variance was ≤15% (Shenzhen New Industries Biomedical Engineering Co., Ltd 2012:2 (a)).

\[ c) \text{ Procedure} \]

Folate was determined by a CLIA method on an automated Maglumi 1000 system.

\[ d) \text{ Analysing} \]

Normal serum folate level is 3–17 ng/ml or 6.0-28 nmol/l (Lin et al 2007:232; Burtis et al 2008:848). Means of the serum folate levels in this study were compared with the reference values of 5.21-20 ng/ml (Shenzhen New Industries Biomedical Engineering Co., Ltd 2012:2 (a)).
4.7.3.6 Full Blood Count

a) Description

Full blood count consists of a panel of indexes counting the number of blood cells (red cell (erythrocyte), platelets (thrombocyte), total white cell (leukocyte) count, as well as, a differential leukocyte count. The haemoglobin concentration and the volume of the red cells and the platelets are also included. These parameters are determined by absorbance, focused flow impedance and cytochemistry methods (Beckman Coulter™ 2000:2-1).

b) Validation

The instrument used was validated by determining the reproducibility, linearity, accuracy by Beckman Coulter. Prescribed maintenance was adhered to in order to assure a well functional instrument. Instrument was calibrated with a commercial Beckman Coulter™ calibrator before the start of analysis. Control samples (low, normal and high) were run daily and documented to assure accuracy and precision.

c) Procedure

The full blood count analyses were done on an automated Beckman Coulter™ AC.T™ 5diff Haematology analyser.

d) Analysing

Means of the blood parameters were compared with the reference values (Hoffbrand and Moss 2011:425):
TABLE 6  FULL BLOOD COUNT REFERENCE VALUES

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>REFERENCE RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MALE</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>13.5-17.5</td>
</tr>
<tr>
<td>Red cell count (x 10^12/L)</td>
<td>4.5-6.5</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>40-52</td>
</tr>
<tr>
<td>MCV (FL)</td>
<td>80-95</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>27-34</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>20-35</td>
</tr>
<tr>
<td>Platelet count (x 10^9/L)</td>
<td>150-400</td>
</tr>
<tr>
<td>Total white cell count (x 10^9/L)</td>
<td>4.0-11.0</td>
</tr>
<tr>
<td>Neutrophil (x 10^9/L)</td>
<td>2.5-7.5</td>
</tr>
<tr>
<td>Eosinophil (x 10^9/L)</td>
<td>0.04-0.44</td>
</tr>
<tr>
<td>Basophil (x 10^9/L)</td>
<td>0.01-0.1</td>
</tr>
<tr>
<td>Monocyte (x 10^9/L)</td>
<td>0.2-0.8</td>
</tr>
<tr>
<td>Lymphocyte (x 10^9/L)</td>
<td>1.5-3.5</td>
</tr>
</tbody>
</table>

4.7.3.7 Gamma-GT

a) Description

The transfer of glutamic acid to acceptors (glycylglycine) is catalyzed by Gamma-GT and releases 5-amino-2-nitrobenzoate, which absorbs light at 405 nm (Burtis, Ashwood and Fowler 2008:371). The absorbance increase is directly proportional to Gamma-GT activity (Thermo Fisher Scientific Oy 2007 (b)).

b) Validation

Calibration was done by measuring the response (dA/min) and then converting the results by a calculation factor. Control serums (Nortrol and Abtrol) were run daily and documented to assure accuracy and precision.
c) Procedure

Gamma-GT in serum was quantified by measuring the activity by an automated enzymatic on the Konelab™.

d) Analysing

Means of the Gamma-GT were compared with the reference values of <55 U/L (male) and 38 U/L (female) (Thermo Fisher Scientific Oy 2007 (b)).

4.7.3.8 Glucose

a) Description

The principle of the method used is based on glucose oxidase (GOD) that’s modified by Trinder colour reaction, and catalysed by enzyme peroxidase (POD). D-gluconate and hydrogen peroxide is formed during GOD reaction (Thermo Fisher Scientific Oy 2008 (a)). Hydrogen peroxide, 4-aminoantipyrine and phenol react to form a quinoneimine dye, with an absorbance measurable at 510 nm (Burtis et al 2008:474).

b) Validation

The glucose parameter was calibrated with a commercial Konelab™ calibrator (sCAL) before the start of analysis. Control serums (Nortrol and Abtrol) were run daily and documented to assure accuracy and precision.

c) Procedure

Serum glucose concentrations were determined by an automated method on the Konelab™.


d) Analysing

Means of the blood parameters were compared with the reference values of 4.1-5.9 mmol/l (Burtis et al 2008:849)

4.7.3.9 HDL

a) Description

The test is a homogeneous enzymatic colorimetric test, where magnesium sulfate in the presence of dextran sulfate forms water-soluble complexes with VLDL, LDL and chylomicrons (resistant to PEG modified enzymes) (Burtis et al 2008:384). The cholesterol levels of the solution with the HDL remaining are determined enzymatically by cholesterol oxidase coupled with peg to the amino group (Thermo Fisher Scientific Oy 2007(c)).

b) Validation

HDL parameter was calibrated with a commercial Konelab™ calibrator (HDL/LDL calibrator) before the start of analysis. Control serums (Lipotrol) was run daily and documented to assure accuracy and precision.

c) Procedure

An automated method on the Konelab™ has been used to measure HDL in serum.

d) Analysing

Means of the HDL were compared with the reference values of: >1.5 mmol/l considered as desirable, 1.3-1.5 mmol/l borderline and <1.3 mmol/l high risk (NCEP 2002:II-10).
4.7.3.10 Homocysteine

a) Description

Oxidised homocysteine is reduced to free homocysteine that reacts with S-adenosylmethionine (SAM) and catalysed by a homocysteine S-methyltransferase to form methionine and S-adenosylhomocysteine (SAH). Enzymatic reactions including; adenosine deaminase, SAH hydrolase and glutamate dehydrogenase hydrolysed SAH into adenosine and homocysteine by SAH hydrolase. The formed homocysteine is a result of the co-substrate SAM is cycled into the homocysteine conversion reaction by the homocysteine S-methyltransferase. The formed adenosine is hydrolysed into inosine and ammonia that reacts with glutamate dehydrogenase with concomitant conversions of NADH to NAD\(^+\). Serum homocysteine concentration is directly proportional to the amount of NADH converted to NAD\(^+\) (Demeditec Diagnostic GmbH. 2009:1).

b) Validation

The supplier determined the accuracy by running 66 serum samples in comparison with an existing commercial method. Correlation coefficient \((r^2=0.976, \text{ slope of } 0.98 \text{ and } y \text{ intercept of } 0.87)\) was determined by Linear regression\(^+\) (Demeditec Diagnostic GmbH. 2009:2).

c) Procedure

A Demeditec Homocysteine Enzymatic Assay was used to determine the serum homocysteine concentration on an automated Konelab\(^\text{TM}\) analyser.

d) Analysing

Means of the serum homocysteine concentration was compared with the reference values of \(<15 \text{ μmol/l}\) (Kaul et al 2006:914).
4.7.3.11 HS-CRP

a) Description

An immunoprecipitation method was used and increase in absorbance was measured at 540 nm (Thermo Fisher Scientific Oy 2007 (d)). Serum is mixed with micro-particles that are coated with anti-human CRP, resulting in precipitation of the immune complex. The change in absorbance is directly proportional to the concentration CRP present in serum (Burtis et al 2008:333).

b) Validation

HS-CRP parameter was calibrated with a commercial Konelab™ calibrator included in the reagent kit before the start of analysis. CRP High Sensitivity Control was run daily and documented to assure accuracy and precision (Thermo Fisher Scientific Oy 2007 (d)).

c) Procedure

Low concentration of CRP in serum was quantified with an automated method on the Konelab™.

d) Analysing

Means of the serum HS-CRP were compared with the reference values of <3 mg/dl (Pearson, Mensah, Alexander, Anderson, Cannon, Criqui, Fadl, Fortman, Hong, Myers, Rifai, Smith, Taubert, Trancy and Vinicor 2003:508).
4.7.3.12 Insulin

a) Description

A sandwich immunoluminometric assay was used to determine the serum insulin level. An anti-insulin monoclonal antibody is used to label the ABEI and another to label the FITC. Serum, ABEI label, FITC label and nano magnetic micro beads coated with sheep anti FITC are mixed and incubated at 37°C to form a sandwich complex. Sedimentation of the complex is allowed in a magnetic field, supernatant then gets removed, and a starter reagent then was added after washing. The starter reagent initiates the flash chemiluminescent reaction; the light signal is measured by a photomultiplier as RLU within 3 seconds and is directly proportional to the concentration folic acid present in the serum (Shenzhen New Industries Biomedical Engineering Co., Ltd 2012:1 (b)).

b) Validation

Accuracy was determined by diluting a high concentration calibrator by a ratio of 1:2, and the concentration of the diluted sample measured for ten times. The recovery of the measured concentration was 90-100% of the expected value. Precision Inter and intra-assay coefficient of variance was ≤15% (Shenzhen New Industries Biomedical Engineering Co., Ltd 2012:2 (b)).

c) Procedure

Insulin concentration was determined by a sandwich-immunoluminometric assay on an automated Maglumi 1000 system.

d) Analysing

Means of the serum insulin concentrations were compared with the reference values of 4.03-23.46 μIU/ml (Shenzhen New Industries Biomedical Engineering Co., Ltd 2012:2)
4.7.3.13 LDL

a) Description

The Friedewald formula was used to calculate the LDL value: LDL= Total cholesterol - HDL - (triglyceride/5) (Martin et al 2013:733).

b) Validation

Validated total cholesterol, HDL and triglyceride values were used in calculations. GGT was determined to exclude possible liver disease that would affect the validation of the results.

c) Analysing

Means of the LDL values were compared with the reference values of: 2.6-3.3 mmol/l for individuals with no known risk was considered as desirable, 3.4-4.1 mmol/l borderline, 4.1-4.9 mmol/l high risk and >4.9 mmol/l considered as very high risk (NCEP 2002:II-5).

4.7.3.14 PAI-1

c) Description

A solid phase sandwich ELISA method with two different high specific antibodies was used to determine the PAI-1.

g) Validation

Seven standards with variable concentrations (5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625
pg/ml, 313 pg/ml, 156 pg/ml, 78 pg/ml and blank) were done in duplication in order to compile a standard curve from which the sample concentration were determines (Gibson (2005:42) (IBL international GmbH 2008:4).

\[ ... \]

**h) Procedure**

ELISA was performed manually using calibrated pipettes, using IBL kit. The absorption was read on a Rayto RT-6100 micro plate reader.

\[ ... \]

**i) Analysing**

Means of the serum PAI-1 levels were compared with the reference values of 1.2-286 ng/ml (IBL international GmbH 2008:29).

**4.7.3.15 Triglycerides**

**a) Description**

The principle of the test is based on the fact that triglycerides are hydrolyzed to glycerol and fatty acids by lipase. The glycerol then undergoes phosphorylation and forms glycerol-3-phosphate, which are oxidized to dihydroxyacetone phosphate and hydrogen peroxide (Thermo Fisher Scientific Oy 2008 (b)). A reaction between the hydrogen peroxide, 4-aminoantipyrine and 4-chlorophenol forms a quinoneimine dye, with an absorbance measurable at 510 nm (Burtis et al 2008:474).

**b) Validation**

Triglyceride parameter was calibrated with a commercial Konelab™ calibrator (sCAL) before the start of analysis. Control serums (Lipotrol, Nortrol and Abtrol) were run daily and documented to assure accuracy and precision (Thermo Fisher Scientific Oy 2008 (b)).
c) Procedure

To quantify serum triglyceride levels an automated method on the Konelab™ has been used.

d) Analysing

Means of the triglyceride values were compared with the reference values of: <1.7 mmol/l considered as desirable, 1.7-2.2 mmol/l borderlines, 2.3-5.6 mmol/l high risk and >5.6 mmo/l considered as very high risk (NCEP 2002:II-7).

4.7.3.16 Vitamin B6

a) Description

The preferred technique of quantifying vitamins is HPLC, because of the high selectivity of this technique. A complete kit from Recipe® containing: mobile phase, standard solution, serum calibrator, sample preparation vials, P precipitant and Reagent N was used. Agilent 1260 Infinity fluorescence detector was used following reaction with reagent N. HPLC conditions were as follows; injection volume: 50µl, injection interval: 10.0 min, mobile phase: commercial mobile phase, flow rate: 1.0 ml/min, column temperature: 35°C, ClinLab® Peristaltic pump flow rate: 0.2 ml/min, detector wavelengths: 370 nm excitation and 470 nm emission. Run time: 4 minutes.

b) Validation

System suitability test (SST) of the developed and optimized method is determined by repeatability, linearity assay. ClinTest® vitamin B₆ standard solution repeated six times was used to evaluate repeatability and precision of the method using relative standard
deviation. Linear correlation coefficient of the curve used for determination of linearity, correlation coefficient (r) of 0.999.

c) Procedure

Agilent 1260 Infinity HPLC system was used to determine the vitamin B6 values.

d) Analysing

Levels I, II and III serum controls from Recipe® were analyzed with the samples. According to manufacturer’s instruction serum controls ranges are 9.04-13.6 µg/l, 16.9-25.3 µg/l and 24.7-37.1 µg/l for level I, II and III respectively. Normal range of plasma vitamin B6 is 8.6-27.2 µg/L (Turgut, Kaya, Arslan, Demir, Güler, & Kaya 2010:134).

4.7.3.17 Vitamin B12

a) Description

A CLIA was used to determine the concentration of vitamin B12 present in the serum. ABEI labeled by a purified vitamin B12 antigen and uses a vitamin B12-binding protein to label FITC. Serum, ABEI label, FITC label and magnetic beads were mixed and incubated at 37°C to form an antigen-antibody complex. Sedimentation of the complex is allowed in a magnetic field, supernatant then gets removed, and a starter reagent then was added after washing. The starter reagent initiates the flash chemiluminescent reaction; the light signal is measured by a photomultiplier as RLU within 3 seconds and is directly proportional to the concentration vitamin B12 present in the serum (Shenzhen New Industries Biomedical Engineering Co., Ltd 2012:1 (c)).
b) **Validation**

Accuracy was determined by diluting a high concentration calibrator by a ratio of 1:2, and the concentration of the diluted sample measured for ten times. The recovery of the measured concentration was 90-100% of the expected value. Precision Inter and intra-assay coefficient of variance was ≤15% (Shenzhen New Industries Biomedical Engineering Co., Ltd 2012:1 (c)).

c) **Procedure**

Vitamin B12 was determined by a CLIA method on an automated Maglumi 1000 system.

d) **Analysing**

Means of the serum vitamin B12 concentration were compared with the reference values of 200-1100 pg/ml (Bain, Bates, Laffan and Lewis 2012:202).

### 4.8 DATA ANALYSIS

All the data were captured in Microsoft Office Excel and transported to SPSS, version 22. Descriptive statistical analyses were done:

- Means and standard deviations were computed for each variable (age, weight, height, WC, BMI, DBP, SBP, glucose, insulin, cholesterol, HDL, LDL, triglyceride, homocysteine, vitamin B6, vitamin B12, folate, fibrinogen, HS-CRP and dietary intakes of: energy, total protein, total fat, cholesterol, SFA, PUFA, MUFA, fibre, carbohydrates, sugars, sodium, vitamin B6 folate and vitamin B12).
- Correlation coefficients were used to examine the association between the study variables (p≤0.05).
- Frequencies were used to determine the percentage of participants with abnormal results. The nutrient intakes were compared with the Dietary Reference Intakes.
(DRI) (Institute of Medicine 2003), specifically, the Estimated Average Requirement (EAR) values, blood parameters were compared with recommended reference values.

All statistical analysis was executed under the supervision and the assistance of a Biostatistician.

4.9 RESULTS

4.9.1 Sampling

As indicated in the aim (1.4) it was planned to study 200 of the elderly men and women attending the day-care centre. However, only 110 gave consent to participate. Due to the elders’ attention span and not wanting to tire them during the measurements, measurements were taken on different days. Attendances are voluntary and not everybody attends the centre every day. This resulted in some of the participants being absent on some of the measurement days Therefore, only subjects with a complete database (completed questionnaires, anthropometry, blood pressure and blood results) were included in the data analyses. This resulted in the sample size being 104. As indicated with the power calculation (4.5) a sample size of 40 is needed in order to have statistically relevant results.

4.9.2 Socio-economical profile of the sample

The sample was 100% black, 70.4% widowed with a mean age of 73±9 years (aged between 60-110 years), 15% were male and 85% females. Only 5% lived alone, 95% shared their houses with family (average household size of 4.9 persons). Majority are staying in brick houses (99%) where 29.4 % has ≤ two rooms, 40.5% three to four rooms and 30.1% has houses with >four rooms (figure 29). All the respondents have access to safe water, electricity, sanitary facilities and waste removal.
The respondents have a very low literacy (only 24% received secondary or tertiary education). All the subjects participating in the study receive a state pension, only 4.2% of the respondent’s partners are employed. The monthly income distributions of the households are between R0 and ≥3000, with the majority of the households living on pension of the elderly as the main income.

In 54.7% of the households the grandmother are considered as the head of the household and in 47% of the households the grandmother was also responsible for the wellbeing of the children in the household.

A chronic money shortage to buy food was reported: always (7.4%), often (47.9%) and sometimes (25.1%), thus indicating household food insecurity in the majority of the household (figure 30).
4.9.3 Nutrient intake and food consumption patterns of the sample

While a low mean energy intake was observed in the majority (87% females, 81% males) of the participants, the mean total protein intake was sufficient, although 35% and 63% of the women and men respectively did not meet the EAR for protein. Most of the protein sources (table 8) that contributed to this were mainly chicken, milk and starchy foods, all with relatively low cholesterol content. This is reflected in the total fat and dietary cholesterol intakes that were below the recommended levels. However, a minority of the participants had high intakes, 28% and 18% respectively. The respondents had a very low fibre intake. 92% of women and 100% of men did not meet the DRI of fibre intake.

The total fat intake contributed 30.1% of the total energy intake, meeting the recommended guidelines for total fat intake. The mean fatty acid intakes for monounsaturated fatty acids (MUFA), and TFA were within the recommendations for a healthy diet (table 7). The SFA
intake was marginally higher than recommended. This may be as a result of the adequate chicken daily consumption.

The mean carbohydrate (COH) intake showed sufficient intakes compared to EAR, although 28% of the respondents did not meet the EAR for COH. The total sugar intake was within the recommended guideline.

Additionally the sample had a low mean intake of vitamin B6 and folate. Respectively 91% of the respondents did not meet the DRI for vitamin B6, 95% folate and 60% for vitamin B12, despite an adequate mean intake for vitamin B12.

The results in Table 7 showed that the elderly in this study consumed a mainly carbohydrate-rich diet as ten of the Top 20 most commonly consumed food items are carbohydrate-rich foods. These include maltabella (sorghum porridge) in the second place with a per capita intake of 100 g per day, followed by soft maize meal porridge 5th (76 g), stiff maize meal porridge 6th (74 g), rice 7th (71 g), bread 8th (71 g), samp or maize rice 12th (19 g), potatoes 13th (18 g), sugar 15th (15 g), scones 16th (14 g) and oats 18th (13 g). The results further found that according to the top 20 list (table 8) of commonly consumed food items by the study population only two vegetables and one fruit item (sources of antioxidants) appeared, and those were consumed by a small number of the respondents thus very small per capita food portion sizes of vegetables and fruit were consumed. No food item rich in omega 3 and 6 appeared on this list. Although red meat appeared twice, first as beef mince (no 14 on top 20 list) and then beef (no 17), small per capita portion sizes were consumed, except for chicken with a per capita intake of 92 g per day.

Although 88 different food items were mentioned by all the respondents, this was not a mean intake value of all the respondents, but meant that different combinations of the individual food items were consumed by the respondents. The individual food items consumed by one specific person in the seven-day period ranged from 4 to 77 food items.
The mean FVS±SD was 28.79±13.69, indicating a low food variety (<30 food items) (Matla 2008). The cereal group showed the highest mean FVS±SD of 7.91±3.53, followed by the vegetable and vitamin A-rich groups with 5.03±3.03 and 3.90±1.55 respectively. However, the cereal and vegetable food groups showed the most variety in terms of individual food items (n=16), followed by the flesh and other fruit groups with 15 individual items each (table 9). These results confirm the findings of the Top 20 most commonly consumed food items (24-hour recall Table 7) as the cereal group showed the most variety, however, contradictory results were found for the vegetable and fruit group as only two vegetables and one fruit item appeared on the Top 20 list.

The cereal group showed the highest mean FVS±SD of 7.91±3.53, followed by the vegetable and vitamin A-rich groups with 5.03±3.03 and 3.90±1.55 respectively. However, the cereal and vegetable food groups showed the most variety in terms of individual food items (n=16), followed by the flesh and other fruit groups with 15 individual items each (table 9).

The mean individual FGDS±SD for the total group was 8.15±1.33 and the total range of food groups used during the seven-day data collection period was 1-9. The majority of respondents (n=94, 95.9%) could be classified with a high FGDS (6-9 food groups) (Matla 2008), with only 2.1% (n=2) with medium FGDS (4-5 food groups) and low (0-3 groups) dietary diversity respectively FGDS (Matla 2008). Although the results indicated that most of the elderly consumed between 7 and 9 of the nutritious food groups, only six nutritious food groups were represented in the Top 20 most commonly consumed food items as measured by actual intakes (24 hour recall, table 7).
## TABLE 7  NUTRIENT INTAKE

<table>
<thead>
<tr>
<th>Variable/ nutrient</th>
<th>Recommended intake (EAR)</th>
<th>Actual intake (mean ±SD)</th>
<th>% of energy intake (%E)</th>
<th>% of respondents &lt; 100% EAR</th>
<th>% of respondents &gt; 100% EAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>8198(w)³(EER)²</td>
<td>5215±2435</td>
<td>87(w)</td>
<td>81(m)</td>
<td></td>
</tr>
<tr>
<td>Total protein (g)</td>
<td>46(w)a</td>
<td>65±37</td>
<td>35(w)</td>
<td>63(m)</td>
<td></td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>20-35%Eb</td>
<td>46±33</td>
<td>30.1</td>
<td>20</td>
<td>28</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>&lt;300a</td>
<td>187±134</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saturated fatty acids (g)</td>
<td>10%Eb</td>
<td>16±13</td>
<td>11.45</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Polyunsaturated fatty acids (g)</td>
<td>6-11%Eb</td>
<td>8±6</td>
<td>5.73</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>Trans fatty acids (g)</td>
<td>&lt;1%Eb</td>
<td>2±3</td>
<td>0.91</td>
<td>74</td>
<td>7</td>
</tr>
<tr>
<td>Monounsaturated fatty acids (g)</td>
<td>0-13%Ec</td>
<td>18±14</td>
<td>13.0</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Total dietary fibre (g)</td>
<td>21(w)a</td>
<td>9±7</td>
<td>92(w)</td>
<td>100(m)</td>
<td></td>
</tr>
<tr>
<td>Total Carbohydrates (g)</td>
<td>100a</td>
<td>134±59</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total sugars (g)</td>
<td>&lt;10%</td>
<td>24±17</td>
<td>8</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>&lt;2500a</td>
<td>727.18±615.55</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B6 (mg)</td>
<td>1.4a</td>
<td>0.74±0.47</td>
<td>91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folate (μg)</td>
<td>320a</td>
<td>109.64±100.36</td>
<td>95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B12 (μg)</td>
<td>2a</td>
<td>3.00±4.34</td>
<td>60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Dietary reference intakes according to the Institute of Medicine
b Dietary reference intakes according to FAO and WHO recommendation
c MUFA = Total fat –PUFA – SFA – TFA
d Estimated energy requirements (EER) for women were calculated based on mean ±SD age for the women (72 years), mean ± SD height and weight 1.55±0.07 m and 72.0±15 kg respectively and low activity levels. The EER of 8198 kJ was thus calculated as 448 - (7.95 x age [72]) + Physical Activity Level (PAL) for low active [1.5] x (11.4 x weight [72] x height [1.55]) x 4.18 kJ. Estimated energy requirements (EER) for men were calculated based on mean ±SD age for the men (73 years), mean ±SD height and weight 1.67±0.1 m and 69.4±17 kg respectively and low activity levels.
activity levels. The EER of 8228 kJ was thus calculated as 448 - (7.95 x age [73]) + PAL for low active [1.5] x (11.4 x weight [69] x height [1.67]) x 4.18 kJ.

**TABLE 8  TOP 20 FOOD ITEMS CONSUMED BY THE RESPONDENTS (n=190)**

<table>
<thead>
<tr>
<th>Rank</th>
<th>Food item</th>
<th>Total daily intake</th>
<th>Mean ± SD daily intake</th>
<th>Number of respondents consumed (%)</th>
<th>Per Capita</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tea</td>
<td>55760</td>
<td>369±168</td>
<td>79</td>
<td>293</td>
</tr>
<tr>
<td>2</td>
<td>Maltabella</td>
<td>19060</td>
<td>293±96</td>
<td>34</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>Milk</td>
<td>17600</td>
<td>113±108</td>
<td>92</td>
<td>93</td>
</tr>
<tr>
<td>4</td>
<td>Chicken</td>
<td>17560</td>
<td>185±101</td>
<td>50</td>
<td>92</td>
</tr>
<tr>
<td>5</td>
<td>Maize meal (soft)</td>
<td>14420</td>
<td>258±84</td>
<td>29</td>
<td>76</td>
</tr>
<tr>
<td>6</td>
<td>Maize meal (stiff)</td>
<td>14100</td>
<td>164±81</td>
<td>45</td>
<td>74</td>
</tr>
<tr>
<td>7</td>
<td>Rice</td>
<td>13448</td>
<td>160±75</td>
<td>44</td>
<td>71</td>
</tr>
<tr>
<td>8</td>
<td>Bread</td>
<td>13409</td>
<td>129±62</td>
<td>54</td>
<td>71</td>
</tr>
<tr>
<td>9</td>
<td>Pumpkin</td>
<td>5190</td>
<td>87±34</td>
<td>32</td>
<td>27</td>
</tr>
<tr>
<td>10</td>
<td>Coffee</td>
<td>4515</td>
<td>282±33</td>
<td>8</td>
<td>23</td>
</tr>
<tr>
<td>11</td>
<td>Cabbage</td>
<td>3870</td>
<td>77±33</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>12</td>
<td>Samp / maize rice</td>
<td>3560</td>
<td>187±76</td>
<td>6</td>
<td>19</td>
</tr>
<tr>
<td>13</td>
<td>Potato</td>
<td>3340</td>
<td>78±32</td>
<td>23</td>
<td>18</td>
</tr>
<tr>
<td>14</td>
<td>Beef (mince)</td>
<td>3160</td>
<td>186±84</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>15</td>
<td>Sugar</td>
<td>2856</td>
<td>15±11</td>
<td>99</td>
<td>15</td>
</tr>
<tr>
<td>16</td>
<td>Scone</td>
<td>2733</td>
<td>130±77</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>17</td>
<td>Beef</td>
<td>2570</td>
<td>161±70</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>18</td>
<td>Oats</td>
<td>2520</td>
<td>252±19</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>19</td>
<td>Stew (chicken)</td>
<td>2490</td>
<td>138±37</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>20</td>
<td>Fruit Juice</td>
<td>2145</td>
<td>214±152</td>
<td>5</td>
<td>11</td>
</tr>
</tbody>
</table>
### TABLE 9 SUMMARY OF VARIETY OF FOOD ITEMS CONSUMED WITHIN THE NUTRITIOUS FOOD GROUPS (N=98)

<table>
<thead>
<tr>
<th>Food group</th>
<th>Mean</th>
<th>SD</th>
<th>Range of individual food items consumed per group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereals, roots and tubers</td>
<td>7.91</td>
<td>3.53</td>
<td>1 – 16</td>
</tr>
<tr>
<td>Other vegetables</td>
<td>5.03</td>
<td>3.03</td>
<td>0 – 16</td>
</tr>
<tr>
<td>Vitamin A-rich fruit and vegetables</td>
<td>3.90</td>
<td>1.55</td>
<td>0 – 8</td>
</tr>
<tr>
<td>Flesh foods (meat, poultry, fish)</td>
<td>3.88</td>
<td>2.57</td>
<td>0 – 15</td>
</tr>
<tr>
<td>Fats and oils</td>
<td>2.22</td>
<td>0.79</td>
<td>0 – 4</td>
</tr>
<tr>
<td>Dairy</td>
<td>2.54</td>
<td>1.60</td>
<td>0 – 8</td>
</tr>
<tr>
<td>Other fruit</td>
<td>2.99</td>
<td>3.36</td>
<td>0 – 15</td>
</tr>
<tr>
<td>Legumes and nuts</td>
<td>1.86</td>
<td>1.35</td>
<td>0 – 5</td>
</tr>
<tr>
<td>Eggs</td>
<td>1.00</td>
<td>0.00</td>
<td>0 – 1</td>
</tr>
<tr>
<td>Total food variety (FVS)</td>
<td>28.79</td>
<td>13.69</td>
<td>4 – 77</td>
</tr>
</tbody>
</table>

### TABLE 10 SUMMARY OF FOOD GROUP DIVERSITY (DDS) (N=98)

<table>
<thead>
<tr>
<th>Number of food groups consumed (n=9)</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>10.2</td>
</tr>
<tr>
<td>8</td>
<td>36</td>
<td>36.7</td>
</tr>
<tr>
<td>9</td>
<td>47</td>
<td>48.0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>98</td>
<td>100.0</td>
</tr>
</tbody>
</table>
4.9.4 General health profile

Self-reported health problems experienced by the subjects were: mainly eye infections (14.3%), upper respiratory infections (33.3%), painful joints (9.5%) and chronic headaches (42.9%) and heart problems (36.5%). The usage of chronic medication was reported as mainly treatment for hypertension (29.6%), diabetes (18%), asthma (3%), and arthritis (4%), the subjects reported to have taken these medication for more than five years. None of the respondents reported the usage of lipid lowering therapy. The majority of respondents (63.6%) visited the local clinic when medical help was needed, and 64.3% had to reach the clinic on foot. The local clinics usually do not screen for CVR factors and thus no lipid lowering therapy is provided. Only blood pressure and capillary glucose levels (finger pricks) are standard operational procedure at the local clinics.

The respondents reported that 88.3% never smoked but many reported to snuff tobacco. The mean gamma GT level of the sample (33.51±23.35 U/L) was below the upper limit of which 27% was increased indicating liver damage due to alcohol abuse.

4.9.5 Anthropometric and weight indexes of the sample

The mean weight (kg) of the sample was 71.84±15.13 (ranged from 39–127). As indicated in table 11 the mean weight for men was 68.53±15.92 and for women 72.55±14.96.

The mean WC (cm) was 93.4±11.79. For men (table 11) the mean WC was above the ideal recommendation of 88 cm (Gibson 2005:285), 56% was increased and thus indicating an increased CVR. A total of 85% female respondents had a WC above the recommended value of 80 cm.

The mean BMI for the group was 29.1±5.82, indicating overweight (WHO 1997). The BMI results (see table 9) indicated that overweight and obesity were more prevalent than underweight and normal weight in this elderly sample, 0% males and 17% females were very obese (BMI >35), 11% males and 32% females were obese (BMI 30-34.9), 50%
males and 31% females were overweight (BMI 25-29.9) in contrast 11% males and 0% females were under weight.

The waist: height ratio for both men and women (table 9) was above the recommended value of 0.5 (Lee et al 2008:837; Hsieh et al 2006:187; Maffeis et al 2008:210) and therefore indicate an additional risk for CVD.

**TABLE 11 ANTHROPOMETRIC INDEXES OF SUBJECTS**

<table>
<thead>
<tr>
<th>Anthropometric parameter</th>
<th>Mean(±SD)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>WC (cm)</td>
<td>92.17±14.00</td>
<td>93.66±11.54</td>
<td></td>
</tr>
<tr>
<td>Height(m)</td>
<td>1.66±0.11</td>
<td>1.55±0.07</td>
<td></td>
</tr>
<tr>
<td>Weight(kg)</td>
<td>68.53±15.92</td>
<td>72.55±14.96</td>
<td></td>
</tr>
<tr>
<td>Waist: Height</td>
<td>0.56±0.08</td>
<td>0.60±0.07</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>24.92±4.88</td>
<td>30.00±5.63</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BMI classification(%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Under weight (BMI=&lt;18.5)</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Normal weight (BMI&gt;18.5≤24.9)</td>
<td>28</td>
<td>20</td>
</tr>
<tr>
<td>Overweight (BMI≥25≤29.9)</td>
<td>50</td>
<td>31</td>
</tr>
<tr>
<td>Obese (BMI≥30≤4.9)</td>
<td>11</td>
<td>32</td>
</tr>
<tr>
<td>Very obese (BMI≥35)</td>
<td>0</td>
<td>17</td>
</tr>
</tbody>
</table>

**4.9.6 Blood pressure results**

Blood pressure is used to classify hypertension, as indicated in table 12. It is defined as normal, pre-hypertension, stage 1 hypertension, stage 2 hypertension and stage 3 hypertension (Joint National Committee 7 (JNC7) Express 2010:3). Results obtained indicated that the mean blood pressure was 139/82 for the total group of elderly men and women. Only 36% of the sample had normal blood pressure with 46% being classified as hypertensive, ranging from 22% stage 1, 14% stage 2 and 10% stage 3 hypertensive.
TABLE 12  HYPERTENSION CLASSIFICATIONS

<table>
<thead>
<tr>
<th></th>
<th>Criteria*</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systolic blood pressure (mmHg)</td>
<td>Diastolic blood pressure (mmHg)</td>
</tr>
<tr>
<td>Normal</td>
<td>120–129</td>
<td>80–84</td>
</tr>
<tr>
<td>Pre-hypertensive</td>
<td>130–139</td>
<td>85–89</td>
</tr>
<tr>
<td>Stage 1 hypertension</td>
<td>140–159</td>
<td>90–99</td>
</tr>
<tr>
<td>Stage 2 hypertension</td>
<td>160–179</td>
<td>100–109</td>
</tr>
<tr>
<td>Stage 3 hypertension</td>
<td>≥ 180</td>
<td>≥ 110</td>
</tr>
</tbody>
</table>

* Criteria as specified by the Joint National Committee 7 Express (2010:3).

4.9.7  Quality control results of laboratory tests

Accuracy was measured by calculating the CV of the control values performed during the analyses and reported in table 13. The analysis can be accepted as accurate seeing that none of the CV results was higher that 15%.

The precision was monitored by the SD. No control value higher that 2SD was accepted. 1SD values for each test was calculated and reported in table 13.

TABLE 13  LABORATORY METHODS USED IN BLOOD ANALYSES

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Instrument</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum vitamin B6</td>
<td>HPLC</td>
<td>Agilent Infinity HPLC system</td>
<td>0.26</td>
<td>3.48</td>
</tr>
<tr>
<td>Serum vitamin B12</td>
<td>immunoenzymometric assay method</td>
<td>Maglumi 1000 immuno analyser</td>
<td>27.70</td>
<td>4.80</td>
</tr>
<tr>
<td>Serum folate</td>
<td>immunoenzymometric assay method</td>
<td>Maglumi 1000 immuno analyser</td>
<td>0.38</td>
<td>4.17</td>
</tr>
<tr>
<td>Parameter</td>
<td>Method</td>
<td>Instrument</td>
<td>SD</td>
<td>CV</td>
</tr>
<tr>
<td>-------------------</td>
<td>---------------------------------</td>
<td>----------------------</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Insulin</td>
<td>immunoenzymometric assay method</td>
<td>Maglumi 1000 immuno analyser</td>
<td>6.08</td>
<td>5.41</td>
</tr>
<tr>
<td>Plasma Fibrinogen</td>
<td>modified Clauss method (multifibrin U)</td>
<td>Dade Behring on an automated Sysmex CA500</td>
<td>0.08</td>
<td>2.01</td>
</tr>
<tr>
<td>Plasma PAI-1</td>
<td>ELISA</td>
<td>Rayto RT-6100 micro plate reader</td>
<td>2.37</td>
<td>4.70</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>ELISA</td>
<td>Rayto RT-6100 micro plate reader</td>
<td>0.98</td>
<td>7.30</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>ELISA</td>
<td>Rayto RT-6100 micro plate reader</td>
<td>1.92</td>
<td>5.00</td>
</tr>
<tr>
<td>γGT</td>
<td>Colorimetric method</td>
<td>Konelab™</td>
<td>1.70</td>
<td>1.67</td>
</tr>
<tr>
<td>Glucose</td>
<td>Colorimetric method</td>
<td>Konelab™</td>
<td>0.11</td>
<td>1.1</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Colorimetric method</td>
<td>Konelab™</td>
<td>0.14</td>
<td>3.20</td>
</tr>
<tr>
<td>HDL</td>
<td>Colorimetric method</td>
<td>Konelab™</td>
<td>0.03</td>
<td>2.30</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>Colorimetric method</td>
<td>Konelab™</td>
<td>0.03</td>
<td>2.50</td>
</tr>
<tr>
<td>Hs-CRP</td>
<td>Colorimetric method</td>
<td>Konelab™</td>
<td>0.03</td>
<td>1.03</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>Colorimetric method</td>
<td>Konelab™</td>
<td>1.42</td>
<td>2.33</td>
</tr>
</tbody>
</table>

### 4.9.8 The lipid profile of the sample

The mean serum cholesterol (mmol/l) was 4.62±1.69, HDL (mmol/l) 0.81±0.31, LDL (mmol/l) 3.53±1.63 and triglyceride (mmol/l) 1.57±1.09. The mean serum cholesterol and triglyceride levels of the sample was within the normal values of <5.2 mmol/l and <1.7mmol/l respectively. In contrast the mean serum HDL was below the normal value (>1.5 mmol/l) and the mean LDL levels of the sample was above the normal values (<1.8 mmol/l). As indicated in table 14, although the majority of subjects have desirable mean total serum total cholesterol (72.11%), triglycerides levels (66.34%) and LDL levels (52.88%), 6.62%, 17.3% and 23.16% presented with a high risk respectively. The opposite was found for HDL levels where the minority of respondents have a desirable HDL (1.92%) and 82.69% at high risk. The mean Triglyceride: HDL ratio (THR) was 2.42 mmol/l ± 2.24, 19% of respondents have a THR of higher than 3.5 mmol/l indicating an
increased risk for CVD. Gambardella et al (2011:249) indicated that from all the lipid ratio’s, THR is the most reliable predictor of the risk of CVD.

TABLE 14  LIPIDPROFILE OF THE SAMPLE

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Cholesterol</th>
<th>HDL</th>
<th>LDL</th>
<th>Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subjects (%)</td>
<td>Criteria (mmo/l)</td>
<td>Subjects (%)</td>
<td>Criteria (mmol/l)</td>
</tr>
<tr>
<td>Desirable</td>
<td>72.11</td>
<td>&lt;5.2</td>
<td>1.92</td>
<td>&gt;1.5</td>
</tr>
<tr>
<td>Borderline</td>
<td>18.27</td>
<td>5.2-6.2</td>
<td>15.39</td>
<td>1.3-1.5</td>
</tr>
<tr>
<td>High risk</td>
<td>6.62</td>
<td>&gt;6.2</td>
<td>82.69</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Very high risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Criteria as specified by the European Society of Cardiology, European atherosclerosis society (Reiner et al 2011:1780)

4.9.9  The haemostatic status of the sample

The mean fibrinogen level of the sample was 5.32±2.53 g/l, which was much higher than the recommended levels of (1.8-3.5 g/l). A total of 68% of the sample had a plasma fibrinogen level of higher than 3.5 g/l.

The sample had a mean PAI-1 level of 333±221 pg/ml, which is increased compared to the normal range of 1.2–286 pg/ml. A total of 54% of the subjects had an increased PAI-1 level.

4.9.10 Prevalence of metabolic syndrome in the sample

This study evaluated the presence of hyperinsulinaemia (serum insulin levels >17 μU/l) (58.65% of the sample) in combination with at least two of the following symptoms: hypertension (46% of the sample), increase plasma glucose levels (>6.1 mmol/l) (38.5% of respondents), dyslipidaemia (triglyceride ≥1.7 and HDL <1.3) (11.5% of the sample), elevated WC (>102 cm (men) (31% of men respondents) and >88 cm (women) (67% of men respondents).
women respondents)) as defined by WHO, to determine the prevalence of metabolic syndrome (Geissler and Powers 2006). Thus 43.27% could be classified as metabolic syndrome according to the mentioned criteria.

4.9.11 Inflammatory response in the sample

The mean HS-CRP of 6.28±4.33 mg/dl was above normal range (<3 mg/dl), 68.27% of respondents had an increased level.

4.9.12 The vitamin B12, folate, B6 status of the sample

The mean homocysteine levels for the sample as indicated in table 15 showed elevated levels, however only 66.36% of subjects had an increased homocysteine value (>15 umol/l). Despite a mean serum vitamin B12 and folate level for the total sample population that were within the normal range, 4.81% and 9.62% of the elderly had a vitamin B12 and folate deficiency respectively.

The mean vitamin B6 level for the respondents was extremely low 0.74±0.47, 98% (table 15) of respondents had a serum vitamin B6 level below the normal value of 8.6 μg/L. The red blood cell parameters of the respondents indicated that 8% of the respondents had a decreased red cell count (mean 4.56±0.54 x 10^{12}/L), 5% has a decreased haemoglobin level (mean 13.7±1.47 g/dl), 7 % had a decreased hematocrit level (mean 43±7%) and a total of 30% had an increased MCV indicating macrocytosis.

4.9.13 The adiponectin and fibronectin levels as indicators of risk for CVD.

Adiponectin and fibronectin as independent cardiovascular risk markers were measured. The mean adiponectin levels 7.53±7.82 μg/ml were below normal value (7.6–15.2 μg/ml), the mean fibronectin level of 72±12 ug/ml were within the normal range (12-124 μg/ml). A total of 29% of the respondents had a decreased adiponectin level.
### TABLE 15  HOMOCYSTEINE METABOLIC MARKERS OF THE SAMPLE

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean±SD</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine (umol/l)</td>
<td>19.79±9.40</td>
<td>&lt;15</td>
</tr>
<tr>
<td>Vitamin B12 (pg/ml)</td>
<td>678±342</td>
<td>200-1100</td>
</tr>
<tr>
<td>Folate (ng/ml)</td>
<td>13.59±9.20</td>
<td>5.21-20</td>
</tr>
<tr>
<td>Vitamin B6 (μg/L)</td>
<td>0.74±0.47</td>
<td>8.6 – 27.2</td>
</tr>
<tr>
<td>Red cell count (x 10¹²/L)</td>
<td>4.56±0.54</td>
<td>3.9 – 5.6</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>13.7±1.47</td>
<td>11.5 – 15.5</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>43±0.07</td>
<td>36 - 48</td>
</tr>
<tr>
<td>Mean cell volume (fl)</td>
<td>99.9±8.5</td>
<td>80 - 95</td>
</tr>
</tbody>
</table>

#### 4.10  INTERPRETATION AND DISCUSSION OF RESULTS

Studies from the early 1970’s focused on the relationship between CHD and dietary intake, and paved the way for epidemiological investigations. Risk markers for CVD have been categorized based on: irreversible (age, gender, genetics), potentially reversible (smoking, obesity, hypertension, physical inactivity, hyperglycaemia, hypercoagulability, dyslipidaemia, increased homocysteinaemia, acute inflammatory response), physiological (low socio-economic status, stressful environment, personality type) and geographical factors (seasonal influence, environmental pollution) (Geissler and Powers 2006:364).

Socio-demographic profile of the sample indicated that the group is characteristically homogenous in living in poverty and food insecurity with poor dietary intakes and subsequently malnutrition exists (both under - and over nutrition). This phenomenon is confirmed by the anthropometric results indicating the high prevalence of overweight and that obesity together with nutrient deficiency (vitamin B12 and folate) co-exists. Chronic medication is not considered as a confounding factor due to the low percentage of respondents taking the medication. Additionally the reported cases are taking the medication for more than five years and no new or resent prescriptions were reported.

Diet as a risk factor for CVD is based on the fact that a diet high in saturated fatty acids
(SFA), trans- fatty acids (TFA) and cholesterol, together with a diet low in fruit and vegetables elevates the LDL cholesterol and increases atherosclerosis (Lim and Choue 2013: 548; Bhupathiraju and Tuchker 2011: 1503). In this study, the TFA and cholesterol intakes were within the recommendations, however SFA were higher than the recommendations, with low fruit and vegetable intakes. Furthermore the LDL levels were elevated in 47.12% of the sample. In contrast, a diet rich in fruit, vegetables, omega-3 fatty acids, omega-6 fatty acids and antioxidants lowers the risk of CVD (Lim and Choue 2013: 548; Bhupathiraju and Tuchker 2011: 1503). Results from this study, the top 20 foods most commonly consumed indicated very limited intake of foods high in anti-oxidants. Studies evaluating the effect of dietary patterns on CVR found that a healthy eating pattern (including high fibre, oily fish, fruit and vegetables, low intake of red meat, fats and alcohol) was inversely associated with inflammatory markers (Hamer and Mishra 2010:491; Oliveira et al 2011:241). High intake of SFA and a low intake of fruit and vegetables (dietary fibre) and food sources rich in omega 3 and 6 was observed.

Data from this study showed low dietary variety as the mean food variety score (28.79) was low compared to a high mean individual FGDS±SD for the total group of 8.15±1.33 (Matla 2008). This shows contradictory results in that the FGDS indicated high dietary diversity and the FVS indicated low dietary diversity. This indicates that although most food groups were consumed by the elderly, only a few foods from each group were included. The cereal group showed the highest mean FVS±SD of 7.91±3.53, followed by the vegetable and vitamin A-rich groups with 5.03±3.03 and 3.90±1.55 respectively. However, the cereal and vegetable food groups showed the most variety in terms of individual food items (n=16), followed by the flesh and other fruit groups with 15 individual items each (table 9). Consuming one or two foods from each of the nine groups does not, therefore, constitute a varied intake. The food intakes of this group of elderly were thus not in line with the FBDG of “eat a variety of foods”. These findings were consistent with a previous study conducted in the same group of elderly (Oldewage-Theron and Kruger 2008:7), as well as a national study conducted in SA where low dietary diversity (mean FGDS of 4.02) was reported for adult South Africans. Furthermore, the national study found that the black ethnic group had the lowest mean FGDS of 3.63 and...
the highest percentage (50%) of people with low dietary diversity (Labadarios and Steyn 2011:8).

The elderly are predisposed to fat accumulation and fat redistribution as a result of decreased physical activity (Morley and Thomas 2007:146). Weight gain and increased abdominal or central obesity, as stated, are common problems in the elderly and are linked to an increased risk of cardiovascular disease, hypertension and diabetes (Charlton et al 2008:569). This was also observed in this elderly sample who reported light / low activity and the presence of abdominal or central obesity (waist: height increased in both men and women).

Hypertension is directly related to CVD in the elderly as well as in other population groups, with detrimental health effects (Morley and Thomas 2007:146). In SA the death rates from strokes among blacks are double those of whites, additionally hypertensive heart disease is ten times higher in the black community than in the white (Steyn 2005:6). In this black sample, 46% of hypertension was observed. SA also suffers from a quadruple burden of disease (poverty-related diseases, CDL, HIV/AIDS and the effect of social instability caused by crime and violence), which has a severe effect on the prevention of, and cost-effective health care management of, chronic disease (Steyn 2005:6).

Dyslipidaemia is regarded as an independent CVR marker (Whitney and Rolfes 2008:627). It is reported that in spite of the increased risk of CVD in the black SA, favorable lipid profiles are still present, with lower total cholesterol and higher HDL-cholesterol levels (Pieters and Vorster 2008:8). This study contradicts the reported findings as low HDL levels and high LDL levels were mainly observed in this elderly community.

The development of coronary artery disease and myocardial infarction has both atheromatous and thrombotic components. Evidence exists implicating the involvement of a number of coagulation and fibrinolytic proteins (Skurk et al 2001:1338). Fibrinogen is recognized as an independent risk marker of CVD (The fibrinogen studies collaboration...
Furthermore because of its mass; fibrinogen also has a direct effect on the blood viscosity and a physical functional effect on platelet aggregation (The fibrinogen studies collaboration group 2007). Studies have indicated an increased level of plasma fibrinogen in black South Africans (Pieters and Vorster 2008). The Fibrinogen Studies Collaboration (2007) also concluded that an increase of 1 g/L in plasma fibrinogen doubles the risk of CVD. Additionally this study found a decreased in fibrinolytic activity indicated by the increased PAI-1 levels. Results from this study indicated that this population has therefore a four times higher CVR, based on the high mean plasma fibrinogen and PAI-1 levels observed in the majority of the respondents.

All nations and generations are affected by diabetes with devastating effects. Globally, 20% of the elderly suffer from diabetes (Morley and Thomas 2007:145). Metabolic syndrome is defined as a cluster of CVR markers present in combination with hyperinsulinaemia (>17 μU/l) (Ma and Zhu 2013:518; Xun, Wu, He and He 2013: 1543). As reviewed by Ma and Zhu (2013:519), in spite of the differences in classifying this condition, it is confirmed by numerous studies as a valuable CVR marker.

C-reactive protein (CRP) is a β-globulin which is strongly bound to phospholipids. It increases twenty-fold to thirty-fold during an infectious or inflammatory response. Therefore, it is considered a credible marker for systemic inflammation (Beckett, Walker, Rae and Ashby, P 2008). Interleukin-6 (IL-6) and TNF-α are pro-inflammatory cytokines which stimulate the production of CRP in the liver. Elevated CRP, IL-6 and TNF-α are strong independent predictors of risk of future cardiovascular events (Hynynen and Khalil 2006:100). The prevalence of systemic inflammation in this elderly sample was confirmed in 68% of the respondents.

In this study 32% had hyperhomocysteinaemia which has been identified as an independent risk factor for atherothrombotic vascular disease (Siri et al 1998:435; Kaul et al 2006:923; Antoniades et al 2009:15). Dhonukshe-Rutten et al (2009:18) reported in a review that positive correlations between vitamins B12, B6 and folate status and CVD have been demonstrated in numerous studies, furthermore studies indicated that the cause
of hyperhomocysteinemia can be multifactorial, with two-thirds of cases caused by a dietary deficiency of vitamin B12, folate and vitamin B6 (Kaul et al 2006:923). Extremely low serum vitamin B6 was also observed.

In the study reported here irreversible (age related), potentially reversible and physiological (low income) risk markers were found to prevail, either singly or in combination with other markers. The elevated potential reversible risk markers present were hypertension, overweight, metabolic syndrome, hypercoagulability, haemocysteinaemia and acute inflammatory response. Statistical significant correlations were found between the lipogram and the fat intake as well as the homocysteine and the vitamin B6 intake. Therefore poor nutritional status was identified as a reversible risk factor. Correction of these unhealthy dietary intake components could reduce the cardiovascular risk of the sample.

4.11 CONCLUSION AND RECOMMENDATION

In this chapter the prevalence of elevated CVR factors were measured. The conceptual framework for this study as indicated in figure 3 indicated that the incidence of increased CVR factors needs to be determined before an intervention can be implemented.

A summary of the elevated cardiovascular risk markers in the sample are schematically presented in figure 31. Seeing that multiple elevated CVR markers were present in these elderly participants the results were divided in: 1) Inflammatory marker (HS-CRP); 2) Homeostasis (fibrinogen (measuring coagulability) and PAI-1 (measuring fibrinolysis)); 3) Homocysteine metabolism (macrocytosis, serum homocysteine, serum vitamin B6, B12 and folate); 4) Dietary intake (fibre, SFA, vitamin B6, folate and FVS); 5) Metabolic syndrome (hyperinsulinaemia, hypertension, increased blood glucose levels, dyslipidaemia and obesity); 6) Dyslipidaemia (hypercholesterolemia, decreased HDL, increased LDL, triglyceride and THR) and 7) Anthropometry (BMI, waist, adiponectin and W:H). All seven categories are classified as risk markers for CVD and each of these categories were further supported by the individual parameters contributing to these
categories. The effect of each of the factors on CVR is indicated by ↓ (decreased) or ↑ (increased) as well as the prevalence (%) of the elderly (as found in the results) showing an increased or decreased level for each of the parameters.

The homocysteine metabolic markers indicated that an increased serum homocysteine (66.36%), decreased serum vitamin B6 (98%), vitamin B12 (4.81%) and folate (9.62%) and macrocytosis (30%) were present. The homeostatic marker showed an increased fibrinogen level (68%) indicating hypercoagulability and increased PAI-1 (54%) indicating ineffective fibrinolysis. An increased serum HS-CRP (68.3%) indicating an inflammatory state. Dyslipidemia indicated a high risk for CVD by: increased serum cholesterol (6.62%), decreased HDL (82.69%), increased LDL (21.16%), increased triglyceride (17.31%) and an increased THR (19%).

The multifactorial metabolic syndrome was identified in 43.27% of the respondents. The individual markers for metabolic syndrome indicated 38.5% had an increased glucose, 58.65% increased serum insulin level, and 46% were hypertensive. Anthropometric measurements indicated that 49% were obese or overweight, 56% had an increased WC line and 29% had an increased adiponectin level.

It is concluded that this elderly sample was at high risk for cardiovascular disease. It is necessary to design an acute interventional approach to reduce the risk in this vulnerable population. Due to the multifactorial nature of hyperhomocysteinaemia (Kaul et al 2006:923) the researcher proposed that a homocysteine lowering strategy (vitamin B6, B12 and folate supplementation) should be implemented and evaluated for its effect on the homocysteine metabolism, coagulation, fibrinolysis, inflammatory response and hypertension. This was implemented and results are reported in the next chapter.
FIGURE 31 SCHEMATIC DIAGRAM OF CARDIOVASCULAR RISK MARKERS PRESENT IN SAMPLE
CHAPTER 5
VITAMIN B12, FOLATE, VITAMIN B6 INTERVENTIONAL STUDY– OBJECTIVES, METHODOLOGY, RESULT AND DISCUSSION

5.1 INTRODUCTION

In a vulnerable low income group, like the elderly in the care centre, with a confirmed high risk of cardiovascular disease (figure 31) an acute intervention is needed in order to improve their health profile. Previous studies suggested homocysteine lowering by vitamin B12, B6 and folate supplementation (Kaul et al 2006:917). The effect of vitamin B12, B6 and folate supplementation on the inflammatory response, thrombotic risk, lipid profile, hypertension, risk for metabolic syndrome and the homocysteine metabolism in an elderly, black South African population is discussed in this chapter.

5.2 OBJECTIVES

The main aim of the interventional study was to assess the effect of vitamins B12, B6 and folate supplementation at >100% RDA for six months on cardiovascular risk markers in the sample.

The specific objectives of this part of the study (intervention) were to:

- Determine the effect of the supplementation on the nutritional status of the group A (hyperhomocysteinaemia) compared to group B (normohomocysteinaemia).
- Evaluate the effect of the intervention on the homocysteine metabolism in the group A (hyperhomocysteinaemia) compared to the group B (normohomocysteinaemia).
- Assess if the intervention had an effect on the coagulation status fibrinogen and PAI-1 of the group A (hyperhomocysteinaemia) compared to the group B (normohomocysteinaemia).
• Analyse the effect the intervention had on the metabolic syndrome status of the group A (hyperhomocysteinaemia) compared to the group B (normohomocysteinaemia).
• Determine if the intervention had an effect on the lipid profile (cholesterol, HDL, LDL and triglycerides) of the group A (hyperhomocysteinaemia) compared to the group B (normohomocysteinaemia) as cardiovascular risk markers.
• Assess the effect of vitamins B12, B6 and folate supplementation at >100% RDA on inflammatory markers (HS-CRP) as cardiovascular risk markers.
• Analyse the effect the intervention had on adiponectin and fibronectin status of the group A (hyperhomocysteinaemia) compared to the group B (normohomocysteinaemia) as cardiovascular risk markers.

### 5.3 DESIGN

This study was an experimental intervention non-equivalent control group study design (Welman and Kruger 2001:79) in 104 purposively selected samples of all the elders attending the day-care centre.

### 5.4 METHODOLOGY

#### 5.4.1 Ethical consideration

As indicated in chapter 4(4.4.1) the ethical approval for this study was obtained from the ethical committee of the University of the Witwatersrand, Johannesburg (M070126) (Annexure A), and by the ethic committee of Durban University of Technology (DUT) (Annexure B). During field work the ethical guidelines for human research by The South African Medical Research Council were followed.
5.4.2 Recruitment and training of fieldworkers

The same fieldworkers recruited and trained for the baseline study (described in 4.4.2) were conducting the field work for the follow-up study as well. Anthropometric measurements (weight, height and waist circumference) were taken by the same trained dietician and a public health nutritionist as for the baseline study. The same registered nursing sisters collected and took the blood pressure measurements as in the baseline study.

5.4.3 Sampling strategy and Sample size

Sample recruited and data analysed for the baseline study (as described in 4.4.3) were used in the intervention study. All subjects were equivalent in age (>60 years), race (black), resident in Sharpeville, Vaal region, unemployed / pensioner (socio-demographic). Biochemical data obtained in the baseline study (4.9) were used to determine cardiovascular risk. The groups were selected from the baseline sample, namely: group A (n=61) were purposively selected subjects with elevated serum homocysteine levels (>15 umol/l), and group B (n=43) were purposively selected subjects with no increased homocysteine levels. The groups were thus non-equivalent according to size and homocysteine status. Therefore, for the rest of this chapter there will be referred to group A and group B respectively.

The baseline analysis identified the homocysteine levels of the respondents. These results were used to categorize respondents with hyperhomocysteinaemia and normal homocysteine levels, 61 elders with identified with elevated homocysteine levels and thus purposively allocated to group A; another 43 respondents without elevated homocysteine levels were purposively selected as group B. The high prevalence of hyperhomocysteinamia (66.35%) at baseline in the sample therefore resulted in the un-equivalent group sizes (group A (n=61); group B (n=43). However, the power calculation used to determine a statistical representative sample size to evaluate the effect on homocysteine as calculated and reported in chapter 4.5 indicated that a total of 40 subjects...
are needed to obtain statistically representative data from this community. To achieve the power of 90% and significance of 95%, both the groups thus had a sufficient number of respondents and the results can thus be used to draw conclusions.

5.4.4 Intervention study

The inserts of available products supplementing vitamin B12, B6 and folate were studied. It was planned to implement a supplementation of >200%. The VITAFORCE product was chosen because it contained the required >200% RDA of vitamins B12 and B6. Although the product did not meet 200% RDA (>70 years) for folate, this product had the highest available concentration of folate (Annexure J). Three quotations for this product were requested from local pharmacies and the cheapest supplier (DisChem) was chosen for the orders.

A six month supplementation program, with a commercially available product (VITAFORCE) was implemented as an intervention (see annexure J). The supplement contained >200% RDA of vitamins B12 (25 µg) (1250%) and B6 (50 mg) (3571%), and 100% RDA of folate (400 µg). Contradictory results were found in previous studies and a possible reason was found to be insufficient concentration of supplementation (Dhonukshe-Rutten et al 2009:18; Acikel et al 2009:327; Morley and Thomas 2007:160). Homocysteine lowering was found in these studies using between 100% – 200% RDA (Acikel et al 2009:327; Morley and Thomas, 2007:160; Verhoef and De Groot 2005:119; Van Der Griend et al 2000:225). The concentrations of vitamin B12, B6 and folate available in the VITAFORCE product did not exceed the concentrations reported to have an adverse reaction as reported in 3.8 and therefore considered safe.

The researcher decided on a six month follow-up study, as other studies varied from three months till 24 months, but after six month period a difference was observed in most studies (Dhonukshe-Rutten et al 2009:18, Acikel et al 2009:327; Kaul et al 2006:915).

Each subject received a 30-day supply of the chosen supplement monthly for a period of six months. A weekly follow-up visit was made to the day-care centre in order to improve
compliance and to receive feedback. Fieldworkers made random house-visits to encourage compliance; tablets were then counted in order to measure compliance in terms of tablets left over for the number of days needed.

5.4.5 Data collections, measuring instruments and analyses

Data collection procedures as described in chapter 4 (4.4.4) and the same measuring instrument as described in 4.4.5 were repeated after the six months supplementation program, with exception of the socio-demographic and health questionnaires.

5.4.6 Statistical analysis

All the data were captured in Microsoft Office Excel and transported to SPSS, version 22. The descriptive statistical analyses that were done are:

- Means and standard deviations were computed for each variable (age, weight, height, waist, WHTR, BMI, DBP, SBP, glucose, insulin, cholesterol, HDL, LDL, triglyceride, homocysteine, vitamin B6, vitamin B12, folate, fibrinogen, PAI-1, HS-CRP, adiponectin, fibronectin and dietary intakes of: energy, total protein, total fat, cholesterol, SFA, PUFA, MUFA, fibre, carbohydrates, sugars, sodium, vitamin B6 folate and vitamin B12).
- The nutrient intakes were compared with the Dietary Reference Intakes (DRI) (Institute of Medicine 2003), specifically, the Estimated Average Requirement (EAR) values, blood parameters were compared with recommended reference values.
- Frequencies were used to determine the percentage of participants with abnormal results.
- Paired t-tests were done to determine statistically significant differences before and after intervention within group A and group B respectively. Independent t-tests were used to determine the statistical significant change between the groups.
- The relationships between variables with significant results were further explored
by correlation coefficients ($p \leq 0.05$).

5.5 RESULTS

5.5.1 Dropouts

The dropout rate was less than 10% in both group A (1.65% dropout) and group B (4.65%) from baseline to follow-up measurements. It can therefore be concluded that the dropouts did not affect the outcome of this study.

5.5.2 Group profile of the sample

As indicated in table 16 the two groups were homogenous (no statistical significance between groups ($p > 0.05$)) in age, blood pressure (both systolic and diastolic), glucose, insulin, cholesterol, LDL, triglyceride, vitamin B12, fibrinogen and HS-CRP. The groups also were homogenous with regards to their nutrient intakes except for TFA ($p = 0.026$). Very low percentage of respondents reported the intake of prescribed chronic medication (reported 4.9.4), additionally the medication was taken for a long time before the interventional study already and are therefore not considered as a confounding factors.

Group A and group B were heterogeneous in the following characteristics: height ($p = 0.002$), weight ($p = 0.002$), BMI ($p = 0.049$), Waist: Height ($p = 0.003$), HDL (0.012), homocysteine ($p = 0.000$), folate ($p = 0.041$) and fibronectin ($p = 0.009$).

The mean of both group A and B was both above the reference values for the anthropometric parameters; BMI and Waist: Height. The mean systolic blood pressure for group A and B was above the normal value, where the mean diastolic blood pressure of group B was normal but group A was above normal. The mean insulin of both groups (A+B) was above the normal range. The mean glucose level for group A was above normal and group B was slightly below the upper limit of the normal value. In the lipid profile
both cholesterol and triglycerides was within the normal range for both groups but the HDL and LDL in both groups were abnormal.

The homosysteine levels in the two groups differ significantly (group A above normal and group B within the normal range), as that was the criteria for dividing the groups. In spite of that the mean of the other homocysteine metabolic markers, vitamin B12 and folate were within the normal range. The mean values for fibrinogen (haemostatic marker) and HS-CRP (inflammatory marker) were both above the normal values in group A and B.

As indicated above the mean dietary intakes of the two groups were homogenous, as no statistical significant differences were observed between the baseline nutrient intakes of the groups. The mean energy intake value in both the groups was below the recommended value. The protein, total fat and cholesterol intakes in both groups met the recommendation. The SFA and PUFA in both groups met the recommendation whereas the MUFA in both groups was below the recommendations (15%). The TFA differ in the two groups, group B met the recommendations but the mean intake of group A was above normal. The mean fibre intake in both the groups was very low. The mean carbohydrate, sugar, sodium and vitamin B12 in both groups were within the recommendations. The mean vitamin B6 and folate intake in both groups were below the recommendations.

Due to the fact that most of the variables measured showed homogeneity in the two groups, except for homocysteine, the dependant variable, it can be assumed that the significant changes observed after the intervention study, can thus be attributed to the vitamin B6, B12 and folate supplementation.
TABLE 16  DIFFERENCES IN CHARACTERISTICS BETWEEN GROUPS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference #</th>
<th>Group A</th>
<th>Group B</th>
<th>Statistical significance difference between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General characteristic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td>71.39±18.51</td>
<td>70.69±14.29</td>
<td>0.493</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td>73.1±14.9</td>
<td>65±3.57</td>
<td>0.034</td>
</tr>
<tr>
<td>Height (m)</td>
<td></td>
<td>1.56±0.76</td>
<td>1.49±0.42</td>
<td>0.002</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td></td>
<td>93.00±11.00</td>
<td>86.00±27.00</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Cardiovascular risk markers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>18-24</td>
<td>30.00±6.00</td>
<td>26.00±9.00</td>
<td>0.049</td>
</tr>
<tr>
<td>Waist : Height</td>
<td>&lt; 0.5</td>
<td>0.60±0.10</td>
<td>0.54±0.20</td>
<td>0.003</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>&lt;120</td>
<td>137±41</td>
<td>125±44</td>
<td>0.732</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>&lt;80</td>
<td>83±27</td>
<td>71±26</td>
<td>0.849</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.10-5.90</td>
<td>6.07±2.70</td>
<td>5.67±1.80</td>
<td>0.428</td>
</tr>
<tr>
<td>Insulin (uIU/ml)</td>
<td>4.03-23.46</td>
<td>24.40±26.90</td>
<td>37.50±31.70</td>
<td>0.136</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>&lt;5.20</td>
<td>4.66±1.98</td>
<td>4.63±1.17</td>
<td>0.082</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>&gt;1.50</td>
<td>0.82±0.23</td>
<td>0.79±0.40</td>
<td>0.012</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>&lt;1.80</td>
<td>3.50±1.90</td>
<td>3.56±1.16</td>
<td>0.087</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>&lt;1.70</td>
<td>1.67±1.17</td>
<td>1.43±0.96</td>
<td>0.243</td>
</tr>
<tr>
<td>Homocysteine (umol/l)</td>
<td>&lt;15</td>
<td>25.25±8.40</td>
<td>12.05±3.37</td>
<td>0.000</td>
</tr>
<tr>
<td>Vitamin B12 (pg/ml)</td>
<td>200-1100</td>
<td>676±338</td>
<td>681±351</td>
<td>0.653</td>
</tr>
<tr>
<td>Folate (ng/ml)</td>
<td>5.21-20.00</td>
<td>12.90±7.80</td>
<td>14.60±10.90</td>
<td>0.041</td>
</tr>
<tr>
<td>Vitamin B6 (µg/l)</td>
<td>8.60 – 27.2</td>
<td>1.30±0.69</td>
<td>1.40±0.81</td>
<td>0.736</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>1.80-3.50</td>
<td>5.40 ±2.50</td>
<td>5.30±2.50</td>
<td>0.706</td>
</tr>
<tr>
<td>HS-CRP (mg/dl)</td>
<td>&lt;3.00</td>
<td>6.07±4.13</td>
<td>6.57±4.64</td>
<td>0.081</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>7.60 – 15.20</td>
<td>7.08±4.68</td>
<td>8.16±10.36</td>
<td>0.490</td>
</tr>
<tr>
<td>Fibronecetin (µg/ml)</td>
<td>12 - 124</td>
<td>71.64±13.82</td>
<td>73.32±9.05</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>Dietary intake</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td></td>
<td>6182(w)</td>
<td>6809(m)</td>
<td>4807±2525</td>
</tr>
<tr>
<td>Total protein (g)</td>
<td></td>
<td>5513±2344</td>
<td>4807±2525</td>
<td></td>
</tr>
<tr>
<td>Total fat</td>
<td></td>
<td>46(w)</td>
<td>56(m)</td>
<td>809</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td></td>
<td>31±13</td>
<td>29±14</td>
<td>0.967</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td></td>
<td>185±121</td>
<td>190±151</td>
<td>0.105</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids</td>
<td></td>
<td>10±6.2</td>
<td>10±6.2</td>
<td>0.510</td>
</tr>
</tbody>
</table>

Chapter 5
5.5.3 The effect the intervention had on the nutritional intakes of the sample

As presented in table 17, at baseline both groups showed deficient intakes of total energy, dietary fibre, vitamin B6, B12 and folate. However, the groups did not show significantly different (p≤0.05) intakes of these nutrients. Although protein and carbohydrates showed mean intakes higher than the EAR respectively, 33% and 44% of the respondents had low protein intakes in groups A and B respectively compared to 23% and 35% for carbohydrates. Only 2% in both group A and group B respectively were above the recommended sodium and sugar intakes at baseline.

The mean total fat intake at baseline of both group A and B met the WHO guideline (20-35%E), however, 34% of the respondents in group A and 26% of group B had a total fat intake of above 35%E. The mean cholesterol intake of both group A and B is below 300 mg, although 16% of the respondents in group A and 26% of group B respondents were above the WHO guideline of 300 mg per day. Respectively 44% and 40% of the respondents in group A and B had a SFA intake of above 10%E. The mean SFA in group A was 11%E and group B 10%. The mean TFA in both group A and B was above the
### TABLE 17  THE EFFECT OF THE INTERVENTION ON THE DIETARY INTAKES OF THE SAMPLE

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>EAR&gt;70years</th>
<th>Baseline (Mean ±SD)</th>
<th>% Of Respondents with abnormal intakes</th>
<th>Follow-up (Mean ±SD)</th>
<th>% Of Respondents with abnormal intakes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>8198(w) (EER)</td>
<td>5513±2344*</td>
<td>4807±2525</td>
<td>64#</td>
<td>74#</td>
</tr>
<tr>
<td>Total protein (g)</td>
<td>46(w)</td>
<td>67±36*</td>
<td>60±38</td>
<td>33#</td>
<td>42#</td>
</tr>
<tr>
<td>Total fat</td>
<td>20-35%E</td>
<td>31±13*</td>
<td>29±14</td>
<td>34#</td>
<td>26#</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>&lt;300*</td>
<td>185±121</td>
<td>190±151</td>
<td>16*</td>
<td>26*</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td>10%E</td>
<td>11±6*</td>
<td>10 ±6</td>
<td>44*</td>
<td>40*</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids</td>
<td>6-11%E</td>
<td>5±3</td>
<td>5±3</td>
<td>77*</td>
<td>77*</td>
</tr>
<tr>
<td>Trans fatty acids</td>
<td>&lt;1%E</td>
<td>1.09±1.34*</td>
<td>0.66±1.03</td>
<td>34*</td>
<td>23*</td>
</tr>
<tr>
<td>Monounsaturated fatty acids</td>
<td>C</td>
<td>12±6*</td>
<td>11±6</td>
<td>7*</td>
<td>7*</td>
</tr>
<tr>
<td>Total dietary fibre (g)</td>
<td>21(w)30(m)</td>
<td>10±7</td>
<td>8±6</td>
<td>87#</td>
<td>100#</td>
</tr>
<tr>
<td>Total Carbohydrates (g)</td>
<td>100*</td>
<td>141±61</td>
<td>125±54</td>
<td>23#</td>
<td>35#</td>
</tr>
<tr>
<td>Total sugars (g)</td>
<td>&lt;10%</td>
<td>4.52.66*</td>
<td>3.88±3.03</td>
<td>2#</td>
<td>2#</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>&lt;2500*</td>
<td>772±600*</td>
<td>666±638</td>
<td>2*</td>
<td>2*</td>
</tr>
<tr>
<td>Vitamin B6 (mg)</td>
<td>1.4*</td>
<td>0.81±0.47</td>
<td>0.64±0.44</td>
<td>85#</td>
<td>95#</td>
</tr>
<tr>
<td>Folate (μg)</td>
<td>320*</td>
<td>113±79*</td>
<td>105±124</td>
<td>92#</td>
<td>98#</td>
</tr>
<tr>
<td>Vitamin B12 (μg)</td>
<td>2*</td>
<td>2.85±3.89</td>
<td>3.20±4.90*</td>
<td>59</td>
<td>58</td>
</tr>
</tbody>
</table>

* Deficient intake, * Increased intake, e, f and g refers to the statistical significant (p≤0.05) differences in the same row

- a  Dietary reference intakes according to the Institute of Medicine
- b  Guidelines according to FAO and WHO recommendation
- c  MUFA = Total fat –PUFA – SFA – TFA
Estimated energy requirements (EER) for women were calculated based on mean±SD age for the women (72 years), mean±SD height and weight 1.55±0.07 m and 72.0±15 kg respectively and low activity levels. The EER of 8198 kJ was thus calculated as $448 - (7.95 \times \text{age}[72]) + \text{Physical Activity Level (PAL) for low active}[1.5] \times (11.4 \times \text{weight}[72] \times \text{height}[1.55]) \times 4.18 \, \text{kJ}$. Estimated energy requirements (EER) for men were calculated based on mean±SD age for the men (73 years), mean±SD height and weight 1.67±0.1 m and 69.4±17 kg respectively and low activity levels. The EER of 8228 kJ was thus calculated as $448 - (7.95 \times \text{age}[73]) + \text{PAL for low active}[1.5] \times (11.4 \times \text{weight}[69] \times \text{height}[1.67]) \times 4.18 \, \text{kJ}$.

EAR of <1%, with 34% of respondents in group A and 23% in group B had an increased TFA intake. The mean MUFA intake in both the groups were within the EAR (15-20%E), 7% of the respondents in both group A and B had a MUFA intake of above 20%E. None of the baseline dietary fat intakes differ significantly between group A and B except for TFA.

In group A there was a statistically significant decrease in energy intake between the baseline and the intervention measurements, whereas in group B there was an increase in energy intake (not statistically significant). In group A the percentage of respondents not meeting the recommended energy intake increased from 64% (baseline) to 87% (follow-up), in contrast with group B where the respondents not meeting the requirements decreased from 74% (baseline) to 70% (follow-up). There was a statistical significant difference between group A and B in the follow up energy intake ($p=0.024$).

The mean total protein intake decreased in both groups from baseline to follow-up, however, significantly in group A and not significantly in group B. The respondents not meeting the EAR increased from 33% (baseline) to 44% (follow-up) in group A compared to a decrease from 42% (baseline) to 33% (follow-up) in group B.

Regarding the fat intake, the mean total fat intake as well as the SFA, TFA and MUFA in group A decreased significantly after the intervention. A very small but statistical significant change took place with the PUFA in both group A $(5\pm3; 5\pm2)$ and B $(5\pm3; 5\pm3)$. The changes within the group was statistical significant in both groups. In group A 77% of
the respondents did not meet the recommendation at baseline and it increased to 79% in the follow-up measurements. In contrast 77% of group B did not meet the recommendations at baseline but decreased to 74%. The differences between group A and B at the follow-up was significant. No significant changes were observed in other fat intakes of group B after the intervention.

The cholesterol intake decreased as reflected in the mean cholesterol intakes in both group A and B but was not statistically significant. The number of respondents not meeting the recommendations decreased from 16% (group A) and 26% (group B) to 8% and 14% respectively.

The very low mean dietary fibre intake reflected in the baseline measurements where 87% of respondents in group A and 100% in group B did not meet the recommendations and the follow measurements reflected that 97% of respondents in group A and 98% not meeting the recommendations. No significant changes in mean fibre intakes were observed in either of the groups. The same trend was observed for the mean carbohydrate intakes in both groups. However, the number of respondents not meeting the EAR for carbohydrates in group A decreased from 23% (baseline) to 21% (follow up) and in group B from 35% (baseline) to 23% (follow up). Although no significant difference in mean carbohydrate intake was observed between the groups at baseline, a significantly higher mean carbohydrate intake was observed in group B at follow-up. The opposite was observed at baseline where group A had a higher mean carbohydrate intake, however not significantly.

The sugar intake in group A decreased significantly from 4.05±2.66%E (baseline) to 3.06±2.95 %E (follow-up). The same trend was observed in group B, but it was not significant. Although no significant difference between the total sugar intake was observed between group A and B at baseline, group A showed a significantly lower total sugar intake at follow-up compared to group B.
The sodium intakes for both groups were much lower than the recommended cut-off point (<2500 mg) both at baseline and follow-up. The change in the mean sodium intake in group A from 772±600 (baseline) to 555±400 (follow up) was statistically significant with no statistically significant change observed in group B. The follow-up measurements reflected that the percentage of respondents with an increased sodium intake decreased in group A to 0% and stayed unchanged in group B.

In spite of the dramatic change in dietary intake of vitamin B6, B12 and folate during the intervention study, a significant increased intake of folate was observed in group A compared to a significant increase in vitamin B12 in group B after the intervention. No other significant differences were observed in vitamin intakes within and between the groups. However, the percentage of respondents not meeting the recommended intake in group A change from 85% (vitamin B6), 92% folate and 59% vitamin B12 at baseline to 0% after the intervention. In group B the percentage of respondents not meeting the recommended intake changed from 95% (vitamin B6), 98% folate and 58% vitamin B12 at baseline to 0% after the intervention.

5.5.4 The effect of the intervention on the anthropometric indexes (nutritional status) of the sample

The anthropometric measurements as reported in table 18 indicated that: The mean weight (kg) of group A at baseline was 73±15 and in group B 65±16, there was a statistical significant difference (p≤0.05) between the two groups. The weight decreased in group A and in group B after the intervention, the change was not statistically significant. Furthermore, no statistically significant differences between the groups were observed.

The mean height at baseline of group A differs significantly to the mean baseline height of group B. No changes occurred in the height measurements during the intervention period.

At baseline the mean waist circumference (cm) of group A (94±11) was significantly higher than in group B (86±13). The follow-up mean waist measurements decreased in
group A but remained unchanged in group B, this change was not significantly. No significant difference in waist circumference was observed between group A and B after the intervention.

**TABLE 18  ANTHROPOMETRIC INDEXES OF SUBJECTS AFTER THE INTERVENTION**

<table>
<thead>
<tr>
<th>Anthropometric parameter</th>
<th>Mean(±SD) baseline</th>
<th>Mean(±SD) follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>Height(m)</td>
<td>1.56±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.50±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weight(kg)</td>
<td>73.00±15.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.00±16.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>94.00±11.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.00±13.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>WHTR</td>
<td>0.60±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.58±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMI</td>
<td>30.15±6.00&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>25.00±6.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BMI classification</th>
<th>Baseline (%)</th>
<th>Follow-up (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>Under weight (BMI=&lt;18)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Normal weight (BMI=18-24.9)</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Overweight (BMI=25-29.9)</td>
<td>36</td>
<td>25</td>
</tr>
<tr>
<td>Obese (BMI=30-34.9)</td>
<td>28</td>
<td>23</td>
</tr>
<tr>
<td>Very obese (BMI=35+)</td>
<td>15</td>
<td>13</td>
</tr>
</tbody>
</table>

The mean WHTR at baseline for both groups was above the upper limit of 0.5. The difference between group A and B was not statistically significant. In group A the mean WHTR stayed constant at 0.60±0.10 (baseline) and 0.60±0.10 (follow up), with no statistical significant change. In group B the mean WHTR increased from 0.60±0.10 (baseline) to 0.61±0.07 (follow up) but the change was not statistically significant.

The difference of the mean WHTR at the follow-up between group A and B was not statistically significant.

The mean BMI for group A at baseline was 30±6, which differ significantly from the mean BMI of group B of 25±6. The mean BMI of group A decreased statistically significantly to 28.17±5 after
the intervention. The mean BMI in group B showed a slight increase (to 26±5) after the intervention but it was not statistically significant. The difference of the mean BMI at follow-up between group A and B was not statistically significant.

The BMI classification (table 18) indicated that in group A there was none of the respondents that were underweight, whereas in group B there was 5. In group A at baseline there was 16 respondents with normal weight compared to the 30 in group B. In both groups the baseline BMI values indicated that the majority of the respondents can be classified as overweight or obese, in group A 36% (overweight) and 28% (obese), and in group B 25% and 23% respectively. In group A 15% of respondents. After the intervention overweight and obese in both groups were still more prevalent than underweight and normal weight. The number of respondents classified as very obese (BMI >35) decreased slightly in group A from 15 % (baseline) to 14 % at (follow-up), in group B the number decreased from 13% (baseline) to 5% (follow-up). In group A the percentage of subjects classified as obese (BMI 30 – 34.9) decreased from 28% (baseline) to 25% (follow-up), the opposite occur in group B where an increased from 23% (baseline) to 26% (follow-up) was observed. During the intervention period an increased number of subjects classified as overweight (BMI 25.0-29.9) increased in both groups, group A it increased from 36% (baseline) to 41% (follow-up) and in group B it increased from 25% (baseline) to 34% (follow-up). In group A the subjects classified as normal weight (BMI 18-25) decreased from 16% (baseline) to 12% (follow-up). Similar observation was made in group B, where the number of respondents classified as normal weight decreased from 30% at baseline to 21% during the follow-up measurements. In group B the number of subjects underweight stayed unchanged (5%) during the intervention period, where the number of respondents classified as underweight in group A increased from 0% (baseline) to 2% (follow-up).

To summarize, it seems as some of the obese respondents moved into the overweight category in group A and group B after the intervention.
5.5.5 The effect of the intervention on Hypertension profile of the sample.

At baseline there was no statistical significant difference between the systolic and diastolic blood pressure of group A and B respectively. No statistical significant changes were observed before and after the intervention in both groups.

It is reported in table 19 that the percentage of respondents classified as having a normal blood pressure decreased during the intervention period in group A from 31% (baseline) to 29% (follow-up), in contrast it increased in group B from 35% (baseline) to 45% (follow-up).

**TABLE 19 HYPERTENSION CLASSIFICATIONS AFTER THE INTERVENTION STUDY**

<table>
<thead>
<tr>
<th>Blood pressure</th>
<th>Mean(±SD) baseline</th>
<th>Mean(±SD) follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>Mean systolic blood pressure (mmHg)</td>
<td>142±32</td>
<td>135±27</td>
</tr>
<tr>
<td>Mean diastolic blood pressure (mmHg)</td>
<td>86±22</td>
<td>76±18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Classification</th>
<th>Criteria</th>
<th>Baseline (%)</th>
<th>Follow-up (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBP (mmHg)</td>
<td>DBP (mmHg)</td>
<td>Group A</td>
</tr>
<tr>
<td>Normal</td>
<td>120–129</td>
<td>80–84</td>
<td>31</td>
</tr>
<tr>
<td>Pre-hypertensive</td>
<td>130–139</td>
<td>85–89</td>
<td>12</td>
</tr>
<tr>
<td>Stage 1 hypertension</td>
<td>140–159</td>
<td>90–99</td>
<td>22</td>
</tr>
<tr>
<td>Stage 2 hypertension</td>
<td>160–179</td>
<td>100–109</td>
<td>15</td>
</tr>
<tr>
<td>Stage 3 hypertension</td>
<td>≥180</td>
<td>≥110</td>
<td>17</td>
</tr>
</tbody>
</table>

There was a decrease in number of respondents classified as pre-hypertensive in both groups, in group A a decrease from 12% (baseline) to 7% (follow-up) and in group B a decrease from 20% (baseline) to 5% (follow-up). The number of respondents classified a stage 1 hypertensive increased from baseline to follow-up in both groups, from 22%
(baseline) to 27% (follow-up) in group A and from 23% (baseline) to 26% (follow-up) in group B. The number of respondents in group A classified as stage 2 hypertensive stayed unchanged at 15% after the intervention but in group B it increased from 8% (baseline) to 11% (follow-up). During the intervention period the number of respondents in group A classified as stage 3 hypertensive decreased from 17% (baseline) to 5% (follow-up), but in group B the number stayed unchanged at 8%.

### 5.5.6 The effect the intervention had on the lipid profile of the sample.

The mean total cholesterol in both group A and group B at baseline was below the recommended value of <5.10 mmol/l. The difference of the mean cholesterol levels between group A and B at baseline was not statistically significantly different. After the intervention and (follow-up) both groups’ mean cholesterol levels were still below the recommended value of <5.10 mmol/l. In group A the total cholesterol increased from 4.66±1.98 mmol/l to 5.10±1.01 mmol/l, in group B it increased from 4.63±1.17 mmol/l to 5.00±1.01 mmol/l, neither of the changes was significant. The percentage of subjects with total cholesterol above the upper limit of 5.10 mmol/l, increased in both group A and group B from 23% to 47% and from 42% to 46% respectively. The differences of the mean cholesterol levels of group A and B at follow-up was not statistical significant. (see table 20).

At baseline the HDL in both groups were lower than the recommended level of 1.50 mmol/l, 100% of the respondents in group A and 95% of the respondents in group B respectively had a decreased HDL level. The difference in the mean HDL levels of group A differed significantly from group B at baseline. Both groups showed a decrease after the intervention, however only group B showed a significant decreased HDL-level from 0.79±0.40 mmol/l to 0.64±0.20 mmol/l. In group A the number of the respondents that did not meet the normal HDL levels decreased after the intervention to 95%, whereas in group B the number increased to 100%. There was no statistical significance between group A and group B after the intervention.
### TABLE 20  THE EFFECT OF THE INTERVENTION ON THE LIPID PROFILE OF THE SAMPLE

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference Range</th>
<th>Baseline</th>
<th>Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean(±SD)</td>
<td>% abnormal values</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>&lt;5.20</td>
<td>4.66±1.98</td>
<td>23</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>&gt;1.50</td>
<td>0.81±0.22</td>
<td>100</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>&lt;1.80</td>
<td>3.50±1.90</td>
<td>92</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>&lt;1.70</td>
<td>1.67±1.20</td>
<td>36</td>
</tr>
<tr>
<td>THR</td>
<td>&lt;3.50</td>
<td>2.40±2.20</td>
<td>20</td>
</tr>
</tbody>
</table>

**Notes:**
- Value is significantly different from baseline in Group A.
- Value is significantly different from baseline in Group B.
The mean LDL at baseline in both group A (3.50±1.90 mmol/l) and group B (3.56±1.16 mmol/l) was increased. There was not a statistical significant difference between the mean LDL values of the two groups at baseline. After the intervention both groups showed an increase in mean LDL values, but neither of them was statistically significant. The mean LDL in group A increased to 4.00±1.03 mmol/l and group B to 4.03±1.05 mmol/l. There was no statistical significance between the mean LDL levels of the two groups at the follow-up. The percentage of subjects with elevated LDL (>1.80 mmol/l) increased in both groups during the intervention period in group A they increased from 92% (baseline) to 98% (follow-up) and in group B from 91% (baseline) to 100% (follow-up).

At baseline both the mean triglyceride levels for both group A (1.67±1.20 mmol/l) and group B (1.40±0.90 mmol/l) was within the normal range (<1.70 mmol/l), with no statistical difference between group A and B. No statistically significant changes between and within the groups were observed at follow-up. The number of respondents above the normal reference range (1.70 mmol/l) in group A decreased from 36% (baseline) to 33% (follow-up). In group B the opposite occur where there was an increased in respondents above the normal reference range from 30% (baseline) to 37% (follow-up).

At baseline there was no statistical significant difference between the mean THR of group A and B. The mean THR of both groups was normal (<3.50). No statistically significant changes were observed in group A and group B at follow-up. However, the mean THR in group A increased from the baseline measurements (2.40±2.20) to follow-up measurements (2.67±2.04) but the number of respondents above the reference range stayed unchanged at 20%. In group B the mean THR increased from 2.45±2.30 (baseline) to 3.05±2.04 (follow-up), and the percentage of respondents above the reference value from 19% (baseline) to 29% (follow-up).

The effect the intervention had on the lipid profile classification is reported in table 21. At baseline 77% of the respondents in group A had a desirable total cholesterol level, 12% was borderline and 11% indicated a high risk. This change to 58% desirable, 28% borderline and 13% at high risk at the follow-up measurements. In group B 63% of the respondents
respondents had desirable total cholesterol at baseline, 30% was borderline and 7% at high risk. After the intervention 65% was desirable, 23% borderline and the high risk group increased to 12%.

**TABLE 21  EFFECT OF THE SUPPLEMENTATION ON CLASSIFICATION OF LIPID PROFILE**

<table>
<thead>
<tr>
<th>Lipid Classification</th>
<th>Cholesterol</th>
<th>HDL</th>
<th>LDL</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Desirable</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference value</td>
<td>&lt;5.2</td>
<td>&gt;1.5</td>
<td>&lt;3.4</td>
<td>&lt;1.7</td>
</tr>
<tr>
<td>Baseline</td>
<td>Group A</td>
<td>77%</td>
<td>0%</td>
<td>64%</td>
</tr>
<tr>
<td></td>
<td>Group B</td>
<td>63%</td>
<td>5%</td>
<td>35%</td>
</tr>
<tr>
<td>Follow-up</td>
<td>Group A</td>
<td>58%</td>
<td>5%</td>
<td>27%</td>
</tr>
<tr>
<td></td>
<td>Group B</td>
<td>65%</td>
<td>0%</td>
<td>32%</td>
</tr>
<tr>
<td><strong>Borderline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference value</td>
<td>5.2-6.2</td>
<td>1.3-1.5</td>
<td>3.4-4.1</td>
<td>1.7-2.2</td>
</tr>
<tr>
<td>Baseline</td>
<td>Group A</td>
<td>12%</td>
<td>15%</td>
<td>16%</td>
</tr>
<tr>
<td></td>
<td>Group B</td>
<td>30%</td>
<td>16%</td>
<td>28%</td>
</tr>
<tr>
<td>Follow-up</td>
<td>Group A</td>
<td>28%</td>
<td>3%</td>
<td>33%</td>
</tr>
<tr>
<td></td>
<td>Group B</td>
<td>23%</td>
<td>7%</td>
<td>31%</td>
</tr>
<tr>
<td><strong>High risk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference value</td>
<td>&gt;6.2</td>
<td>&lt;1</td>
<td>4.1-4.9</td>
<td>2.3-5.6</td>
</tr>
<tr>
<td>Baseline</td>
<td>Group A</td>
<td>11%</td>
<td>85%</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>Group B</td>
<td>7%</td>
<td>79%</td>
<td>28%</td>
</tr>
<tr>
<td>Follow-up</td>
<td>Group A</td>
<td>13%</td>
<td>92%</td>
<td>23%</td>
</tr>
<tr>
<td></td>
<td>Group B</td>
<td>12%</td>
<td>93%</td>
<td>15%</td>
</tr>
<tr>
<td><strong>Very high risk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference value</td>
<td>&gt;4.9</td>
<td>&gt;5.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>Group A</td>
<td>15%</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Group B</td>
<td>9%</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>Follow-up</td>
<td>Group A</td>
<td>17%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Group B</td>
<td>22%</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>
At baseline 85% of group A respondents was classified as having a high risk HDL, 15% was borderline and 0% was desirable. In group B 79% was high risk, 16% borderline and only 5% had a desirable HDL. The follow-up measurements indicated that the number of respondents with high risk HDL increased in both group A and B to 92% and 93 % respectively. The percentage of respondents classified as borderline decreased to 3% (group A) and 7% (group B). In group A the respondents with a desirable HDL increased to 5%, whereas in group B they decreased to 0%.

The LDL classification in group A was 64% desirable, 16% borderline, 5% high risk and 15% very high risk at baseline. After the intervention the follow-up measurements indicated that only 27% of group A had a desirable LDL, 33% borderline, 23% high risk and the very high risk increased to 17%. A similar reaction was found in group B, where the number of respondents with a very high risk LDL increased from 9% to 22%, the high risk decreased from 28% to 15%, the borderline also decreased from 28% to 31% and the desirable group decreased from 35% to 33% from baseline to follow-up measurements respectively.

At baseline 64% of group A and 67% of group B subjects had a desirable triglyceride level, in group A the respondents increased to 67%, whereas in group B the number of respondents with a desirable triglyceride level decreased to 63% after the intervention. Both in group A and group B 15% of the subjects had a desirable triglyceride level. In group A this number stayed unchanged, but in group B it increased to 20% at follow-up measurements. The number of respondent with a high risk triglyceride level decreased from 21% to 18% in group A and from 15% they increased to 17% in group B. The number of respondents in both group A and group B decreased from 2% at baseline to 0% at follow-up measurements.

5.5.7 The effect of the intervention on the haemostatic status of the sample.

At baseline the mean fibrinogen level of both group A (5.37±2.59 g/l) and group B (5.25±2.48 g/l) was above the upper reference level of 3.50 g/l. The difference between
group A and group B at baseline was not statistically significant. The mean fibrinogen level of the respondents in group A increased statistical significantly (p=0.009) to 6.47±2.65 g/l after the intervention. In group B the mean fibrinogen level at baseline increased to 6.20±2.89 g/l after the intervention but was not statistical significant (p=0.066). The number of respondents with a fibrinogen level above normal in group A increased from 69% (baseline) to 95% (follow-up) and in group B a similar phenomenon occur where the mean fibrinogen level increased from 67% (baseline) to 85% (follow-up).

Baseline mean PAI-1 levels was increased (>286 pg/ml) for both group A (337±204 pg/ml) and group B (326±245 pg/ml). The difference between group A and group B not statistical significant (p=0.072). The follow-up mean PAI-1 level for group A decreased significantly (p=0.01) to 221±193 pg/ml. Group B follow-up mean PAI-1 levels also decreased to 246±166 pg/ml but was not statistical significant (p=0.071). The difference between group A and B at follow-up was not statistically significant. At baseline 59% of the respondents in group A had an increased PAI-1 level compared to the 15% in group B. After the intervention the number of respondents with an increased PAI-1 level decreased to 49% and 33% respectively.

5.5.8 The effect of the intervention on the prevalence of metabolic syndrome status in the sample.

The mean serum insulin in group A at baseline was 24.49±27.21 μU/l and in group B 37.54±31.70 μU/l, both above the upper reference level of 17.00 μU/l. The differences between group A and B at baseline was not statistically significant.

After the intervention the mean insulin levels in group A increased to 29.69±35.48 μU/l (not significant p=0.209) and in group B there was a decrease to 22.56±24.12 μU/l (p=0.001). The differences between the groups were not statistically significant. The percentage of respondents with a serum insulin level > 17.00 μU/l in group A showed a slight increase from 48% (baseline) to 50% (follow-up), in group B there was a significant decrease from 72% (baseline) to 49% (follow-up).
In group A the mean glucose level at baseline was 6.07±2.73 mmol/l, with 16% of the respondents with an increased (>5.90 mmol/l) level. In group B the mean glucose level at baseline was 5.67±1.80 mmol/l, with 40% of the respondents with an increased glucose level. The differences between group A and B at baseline was not statistically significant. After the intervention the mean glucose level of group A decreased to 5.90±2.80 mmol/l, with 16.47% of subjects with an increased glucose level. The change from baseline to follow-up in group A was not statistically significant. In group B the glucose level slightly increased to 6.06 mmol/l after the intervention with, with 35% of the respondents with an increased glucose level. The changes in group B were not statistically significant. The differences between group A and B at follow-up were also not significant.

The percentage of respondents classified as suffering from metabolic syndrome (criteria for classification 4.9.10) in group A increased from 40% (baseline) to 46% (follow-up), but in group B there was a deceased from 61% (baseline) to 49% (follow-up).

5.5.9 The effect of the intervention on the inflammatory markers of the sample.

In group A at baseline the mean HS-CRP was 6.07±4.13 mg/dl in group A. The percentage of respondents with an increased HS-CRP (>3.00 mg/dl) at baseline in group A was 67%. In group B the mean HS-CRP at baseline was 6.57±4.64 mg/dl, with 70% of the respondents with an increased HS-CRP level. The difference between group A and B at baseline was not statistically significant. In group A the mean HS-CRP increased to 7.04±3.97 mg/dl after the intervention, this was a statistically significant (p=0.045) change. The numbers of respondents with an increased HS-CRP in group A increased however to 82% after the intervention. In group B the mean the mean HS-CRP level and decreased to 5.57±4.37 mg/dl after the intervention. This was a statistical significant change. The number of respondents with an increased HS-CRP level decreased to 66% after the intervention. The differences between group A and B at the follow-up measurements was not statistically significant.
5.5.10 The effect of the intervention on the homocysteine metabolic markers of the sample

Results as indicated in table 22 demonstrated that the mean vitamin B12 results of group A at baseline was 677±345 pg/ml with only 7% of the respondents that was below the minimum reference value of 200 pg/ml. The mean vitamin B12 value of group B was 681±355 pg/ml, 2% of the respondents that was below the recommended reference value. There was no statistical significant difference between group A and B at baseline. The follow-up results indicated that in group A the mean vitamin B12 levels decreased statistically significantly to 567±311 pg/ml. In group B there was also a slight but insignificant decrease in the mean vitamin B12 levels to 671±321 pg/ml. The number of respondents in group A that had a decreased vitamin B12 level (<200 pg/ml) increased from 7% (baseline) to 12% (follow-up), the number of respondents in group B with a decreased vitamin B12 level stayed unchanged at 2%. The difference between group A and B at Follow-up was not significant.

The mean serum folate levels of group A at baseline (12.94±8.00 ng/ml) differ statistically significantly from group B (14.70±11.00 ng/ml). Both groups mean values are within the normal range (5.21 – 20.00 ng/ml). The percentage of respondents with a decreased serum folate level (<5.21 ng/ml) at baseline was 8% (group A) and 12% (group B). The follow-up folate measurements indicated that the mean of group A decreased statistically to 9.58±5.31 ng/ml, the same happened in group B where the mean folate values significantly decreased to 8.24±5.50 ng/ml. The number of respondents with a decreased folate level increased during the intervention period to 15% (group A), and to 27% (group B). There was no statistical significance between the groups after the intervention.

Extremely low serum vitamin B6 values were observed in both groups, 100% of the subjects in both group A and B were below the lower limit of the normal values of 8.60 μg/ml. Group A had a mean serum B6 value of 1.35±0.69 μg/ml and group B 1.40±0.80 μg/ml at baseline. The difference between group A and B was not statistically significant.
After the intervention the mean vitamin B6 levels in group A increased significantly to 5.03±2.49 μg/ml, the number of subjects below the minimum range decreased to 93%. In group B the mean serum vitamin B6 increased significantly to 5.72±6.15 μg/ml after the intervention. The number of subjects below the minimum reference limit decreased to 98%. The difference between group A and B after the intervention was not statistically significant.

At baseline 100% of subjects in group A had an increased (>15.00 umol/l) serum homocysteine level and 0% in group B as this was the selection criteria. The mean serum homocysteine level at baseline for group A was 25.00±8.00 umol/l and in group B 12.05±3.40 umol/l, the difference between the two groups was statistically significant. After the intervention the mean serum homocysteine levels in group A decreased statistically significantly to 18.80±12.00 umol/l. The number of respondents with an increased homocysteine level decreased from 100% (baseline) to 67% (follow-up). In group B 0% of the respondents had an increased serum homocysteine level at baseline, but this increased to 34% after the intervention. The mean homocysteine level in group B increased from (baseline) to 13.64±5.90 umol/l but this was not statistically significant. The difference in follow-up measurements between group A and group B was not statistically significant.

The red cell parameters of group A at baseline (as indicated in table 22) showed the mean RCC was 4.60±0.50 x 10^{12}/l, which did not differ significantly from the measurement of group B (4.45±0.60 x 10^{12}/l). Only 7% of the respondents in group A and 9% in group B respectively had a decrease RCC at baseline. A slight decrease in the mean RCC was observed in both the groups, group A decreased to 4.50±0.80 x 10^{12}/l and group B to 4.30±1.10 x 10^{12}/l. These changes was however not statistically significant as was the difference between the groups at the follow-up measurements. The number of respondents with a decreased red cell count (<3.90 x 10^{12}/l) decreased to 3% (follow-up) in group A and to 7% in group B after the intervention. The mean Hb of both group A (13.80±1.40
g/dl) and group B (13.50±1.60 g/dl) at baseline was within the normal reference range. There was no statistical significance between the two groups at baseline. The number of respondent with decreased Hb (<11.50 g/dl) increased in both group from baseline to follow-up measurements 3% to 11% (group A) and 7% to 15% (group B) respectively. The mean Hb levels in both groups also decreased significantly to 12.70±2.30 g/dl (group A), and to 12.20±3.10 g/dl (group B).

The difference between group A and B at follow-up was not statistically significant. The Hct showed similar effect with the mean baseline readings for group A (40±7%) and group B (44±1%) were within the normal range, an did not differ significantly from each other. The number of respondents with a decreased Hct (<36%) was 3% and 12% in group A and B respectively. After the intervention the mean Hct level for group A was 43±0.4%, this increased from baseline to follow-up was statistically significant. In group B the Hct increased to 47±6 % that was also significant. The difference between group A and B after the intervention was statistically significant. The number of respondents not meeting the minimum reference value in group A increased to 10% and in group B to 15.

The mean MCV as indicator for macrocytosis (>95 pg) at baseline was 91±10 pg (group A) and 93±6 pg (group B) respectively. There was no statistical significant difference between the groups at baseline. The number of respondents with macrocytosis was 31% and 23% in group A and B respectively. The follow-up measurements indicated that the MCV values decreased statistical significantly in both groups. After the intervention the MCV for group A decreased to 85±13 pg and in group B to 84±19 pg. The difference between the groups after the intervention was still not statistically significant. The number subjects with macrocytosis decreased to 7% and 5% in group A and B respectively.
### TABLE 22  THE EFFECT OF THE INTERVENTION ON HOMOCYSTEINE METABOLIC MARKERS OF THE SAMPLE

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference</th>
<th>Baseline</th>
<th></th>
<th>Follow-Up</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Group A</td>
<td>Group B</td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean±SD</td>
<td>Number of respondents abnormal value (%)</td>
<td>Mean±SD</td>
<td>Number of respondents abnormal value (%)</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>200-1100 pg/ml</td>
<td>677±345&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7</td>
<td>681±355</td>
<td>2</td>
</tr>
<tr>
<td>Folate</td>
<td>5.21-20 ng/ml</td>
<td>12.94±8.00&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>8</td>
<td>14.66±11.00&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>12</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>8.6-27.2 μg/ml</td>
<td>1.35 ±0.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100</td>
<td>1.40±0.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>&gt;15 μmol/l</td>
<td>25.30±8.00&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>100</td>
<td>12.04±3.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>3.9-5.6 x 10&lt;sup&gt;12&lt;/sup&gt;/l</td>
<td>4.60±0.50</td>
<td>7</td>
<td>4.45±0.60</td>
<td>9</td>
</tr>
<tr>
<td>RCC</td>
<td>11.5-15.5 g/dl</td>
<td>13.80±1.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3</td>
<td>13.50±1.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7</td>
</tr>
<tr>
<td>Hct</td>
<td>36-48%</td>
<td>40±7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12</td>
<td>44±1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12</td>
</tr>
<tr>
<td>MCV</td>
<td>90-95 pg</td>
<td>91±10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31</td>
<td>93±6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23</td>
</tr>
</tbody>
</table>
5.5.11 The effect of the intervention on Adiponectin and Fibronectin serum levels of the sample

At baseline the mean serum fibronectin levels of group A (71.64±13.81 μg/ml) was not statistically significantly different from the mean serum fibronectin levels of group B (73.32±9.05 μg/ml). There was no respondent with an increased fibronectin level at baseline. The follow-up results indicated that the mean serum fibronectin level of group A decreased significantly to 40.46±21.67 μg/ml. The mean serum fibronectin levels in group B also decreased significantly after the intervention to 47.30±21.14 μg/ml. There was no statistical significant difference between group A and B after the intervention.

The mean serum adiponectin levels of group A (7.08±4.68 μg/ml) was slightly below the lower reference level (7.53-7.82 μg/ml) at baseline. A percentage of 67% of the respondents in group A had an adiponectin level of below 7.53. At baseline the mean adiponectin level for group B was 8.17±10.36 μg/ml. In group B 50.82% of the respondents had a decreased adiponectin level. There was no statistical significant difference between group A and B at baseline. The follow-up mean adiponectin values indicated that both groups had an insignificant decrease in the serum adiponectin levels. Group A decreased to 7.03±4.24 μg/ml and group B to 7.60±5.67 μg/ml respectively. The difference between group A and B at follow-up was not statistically significant.

5.5.12 Correlation of variables in follow-up measurements

The relationships as reported in table 23 indicated that serum homocysteine had a positive relationship with: systolic blood pressure (r=0.229, p=0.019); diastolic blood pressure (r=0.207; p=0.035); WTHR (r=0.218, p=0.026); serum cholesterol (r=0.285, p=0.003); LDL (r=0.269; p=0.006); triglycerides (r=0.309, p=0.001); THR (r=0.345, p=0.000); glucose (r=0.291, p=0.003); RCC (r=0.305, p=0.002); Hb (r=0.294, p=0.002); MCV (r=0.250, p=0.011).
TABLE 23  SIGNIFICANT CORRELATIONS IN THE FOLLOW-UP MEASUREMENTS

<table>
<thead>
<tr>
<th>Serum homocysteine with:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure</td>
<td>0.229</td>
<td>0.019</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.207</td>
<td>0.035</td>
</tr>
<tr>
<td>WTHR</td>
<td>0.218</td>
<td>0.026</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>0.285</td>
<td>0.003</td>
</tr>
<tr>
<td>LDL</td>
<td>0.269</td>
<td>0.006</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.309</td>
<td>0.001</td>
</tr>
<tr>
<td>THR</td>
<td>0.345</td>
<td>0.000</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.291</td>
<td>0.003</td>
</tr>
<tr>
<td>RCC</td>
<td>0.305</td>
<td>0.002</td>
</tr>
<tr>
<td>Hb</td>
<td>0.294</td>
<td>0.002</td>
</tr>
<tr>
<td>MCV</td>
<td>0.250</td>
<td>0.011</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dietary vitamin B6 with:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>RCC</td>
<td>0.479</td>
<td>0.000</td>
</tr>
<tr>
<td>Hb</td>
<td>0.482</td>
<td>0.000</td>
</tr>
<tr>
<td>MCV</td>
<td>0.540</td>
<td>0.000</td>
</tr>
<tr>
<td>Dietary Folate</td>
<td>0.634</td>
<td>0.000</td>
</tr>
<tr>
<td>Dietary vitamin B12</td>
<td>0.906</td>
<td>0.000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dietary Folate with:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>RCC</td>
<td>0.297</td>
<td>0.002</td>
</tr>
<tr>
<td>Hb</td>
<td>0.330</td>
<td>0.001</td>
</tr>
<tr>
<td>MCV</td>
<td>0.444</td>
<td>0.000</td>
</tr>
<tr>
<td>Dietary B6</td>
<td>0.634</td>
<td>0.000</td>
</tr>
<tr>
<td>Dietary vitamin B12</td>
<td>0.656</td>
<td>0.000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dietary vitamin B12 with:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>RCC</td>
<td>0.391</td>
<td>0.000</td>
</tr>
<tr>
<td>Hb</td>
<td>0.399</td>
<td>0.000</td>
</tr>
<tr>
<td>MCV</td>
<td>0.454</td>
<td>0.000</td>
</tr>
<tr>
<td>Serum B6</td>
<td>0.226</td>
<td>0.023</td>
</tr>
<tr>
<td>Dietary B6</td>
<td>0.906</td>
<td>0.000</td>
</tr>
<tr>
<td>Dietary folate</td>
<td>0.656</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Statistical significance at P<0.05

The dietary intake of vitamin B6, vitamin B12 and folate showed similar relationships
with red cell parameters. As indicated in table 23 vitamin B6, vitamin B12 and folate correlated positively with RCC \((r=0.479, p=0.000); \ (r=0.391, p=0.000) \) and \(r(0.297 , p=0.002)\) respectively, and Hb with vitamin B6 \((r=0.482, p=0.000), \) vitamin B12 \((r=0.399, p=0.000) \) and folate \((r=0.330, p=0.001). \) Likewise vitamin B6 \((r=0.540, p=0.000), \) vitamin B12 \((r=0.454, p=0.000)\) and folate \((r=0.444, p=0.000)\) correlated with MCV.

The dietary markers also had a significant relationship with each other, vitamin B6 correlated with vitamin B12 \((r=0.906, p=0.000)\) and folate \((r=0.634, p=0.000). \) Vitamin B12 correlated with folate \((r=0.656, p=0.000).\)

### 5.6 INTERPRETATION AND DISCUSSION OF RESULTS


The characteristics of the hyperhomocysteinaemic and normohomocysteine groups indicated similarity in age, race, socioecomomical status blood pressure, glucose, insulin, cholesterol LDL triglyceride, vitamin B12, fibrinogen and HS-CRP and dietary intakes (except for TFA). The main difference between Group A and group B were obviously homocysteine used as the separation criteria, but also the anthropometric measurements (group A was higher than group B) HDL (group A was higher than group B) and serum folate levels (group A was higher than group B).
In this study a supplementation intervention approach instead of a food-based approach was followed as a homocysteine lowering strategy. In both groups, poor nutrient intakes, not meeting the EAR, were observed, except for total protein and carbohydrate intakes. No significant differences in dietary intakes were observed between the two groups at baseline, but at follow-up the hyperhomocysteinaemic consumed significantly less total fats, MUFA, total COH and sugars, as well as significantly more SFA than the normohomocysteine group. The supplementation did not result in significant changes in dietary intake from baseline to follow-up, except for significantly higher folate and vitamin B12 in the hyperhomocysteinaemic and normohomocysteine groups respectively. However, no significant relationships were observed between serum homocysteine and any of the dietary intake parameters and the changes in nutrient intakes could thus not have influenced the results. Significant positive relationships existed between the three supplementation vitamins, namely vitamin B6, vitamin B12 and folate, however. This confirms the known relationship between these vitamins.

Due to the decreased physical activity the aged are susceptible to weight gain and increased abdominal or central obesity (increased risk markers for CVD) (Morley and Thomas 2007:146; Charlton et al 2008:569). At baseline both groups had an increased waist circumference and a WHTR above 0.5 indicating that this population is at high risk for CVD. The intervention had no significant effect on the WHTR. In the BMI classification of group A there was a clear downwards trend, between the before and after measurements, in contrast with group B where there was an upward trend in the follow-up measurements compared to the baseline measurements. The slight but statistically insignificant change in weight and BMI that occurred theoretically in group A might have had an influence on the homocysteine levels. There was however, no significant correlation between BMI or weight and homocysteine levels or energy intakes. The decrease in the total energy intake was thus not correlated to the insignificantly lower BMI in group B. Because of no statistically significant correlations, no conclusive evidence exists that the weight loss had any effect on the results.
Results obtained indicated that the mean blood pressure at baseline for group A was 142/86 mmHg (stage 1) and for group B 137/76 mmHg (pre-hypertensive). The blood pressure stayed basically unchanged at the follow-up measurements where the blood pressure for group A was 142/88 mmHg and in group B was 137/79 mmHg. The prevalence of hypertension was evenly distributed over all the stages in both groups in both the groups. In group A there was a slight decrease in the prevalence of normal and pre-hypertensive subjects but an increase in stage 1 hypertension, in contrast there was a noteworthy decrease in the stage 3 hypertension. In group B there was a decrease in pre-hypertension stage but an increase in both normal and stage 1 hypertension. Hypertension is directly related to CVD in the elderly as well as in other population groups, with devastating health effects (Morley and Thomas 2007:51). This study confirmed the high prevalence of hypertension in the sample, but the homocysteine lowering intervention had very little effect on the blood pressure.

Dyslipidaemia is regarded as an independent cardiovascular risk marker (Whitney and Rolfes 2008:629). Serum lipid profiles (cholesterol, HDL, LDL and triglycerides) are used to classify the cardiovascular risk of an individual, and are used to determine treatment. This study indicated both groups had a mean cholesterol, triglyceride and THR that fell within the normal reference range both at baseline and at follow-up measurements with statistically insignificant changes. The mean HDL in both groups was below the normal range with a statistically significant higher level in the hyperhomocysteine group at baseline. In group B the HDL decreased significantly after the intervention and a decrease of 0.04 mmol/l was observed in the hyperhomocysteinaemic group, however, this was not significant. The reason for this was unclear as no significant correlation between any of the dietary and other variables with HDL levels were found. However, the HDL levels in both groups remained below the recommended level. The mean LDL level for group A and B was above the normal reference range and increased slightly (statistical not significant) in both groups after the intervention. The prevalence of dyslipidaemia according to the lipid classification showed there was a slight worsening in the classification of dyslipidaemia in both the groups when baseline results are compared with follow-up results. The
supplementation had therefore no effect on the lipid profile of the subjects, except for a significant decrease in HDL in the normohomocysteine group.

The homeostatic mechanism is a delicate balance between coagulation and fibrinolysis (Hoffbrand, Moss and Petit 2006:264). A haemostatic imbalance results in the development of CVD both due to atheromatous and thrombotic components (Skurk et al 2001:1336). Increased mean fibrinogen levels were present in both groups at baseline indicating that these elderly people were prone to hypercoagulability. There was an increase in the mean fibrinogen levels in both the groups at the follow-up measurements; therefore, the intervention did not decrease the coagulability of the elders. The PAI-1 levels in both groups were increased at baseline, and decreased (significantly in group A but not significant in group B) after the intervention. The follow-up PAI-1 measurements of both groups were below the upper limit of the normal reference range. The supplementation had therefore no effect on the coagulability of the respondents but increased the fibrinolytic activity.

Both groups at baseline presented with an increased mean insulin level. After the intervention the follow-up results indicated that the mean insulin levels in both groups were still elevated, in contrast group A increased (statistically not significant) and group B decreased. The mean glucose level of group A was above the upper limit of the reference range and decreased after the intervention, compared to group B that had a mean glucose level within the normal range at baseline and increased slightly after the intervention. The prevalence of metabolic syndrome in group A showed a slight increase after the intervention and in group B a slight decrease. It can be concluded that vitamin B6, B12 and folate supplementation did not decrease the prevalence of metabolic syndrome in the hyperhomocysteine population but had a positive effect on the metabolic syndrome status of the non-hyperhomocysteine population.

Interleukin-6 (IL-6) and TNF-α are pro-inflammatory cytokines which stimulate the production of CRP in the liver (Hynynen and Khalil 2006:95; Nyström 2007:80). Elevated HS-CRP, is a strong independent predictors of risk of future cardiovascular events.
(Hynynen and Khalil 2006:95; Nyström 2007:80). The respondents were in an acute inflammatory state with both the group presented with increased HS-CRP levels. The mean HS-CRP in group A increased not significantly after the follow-up measurements, in group B there was a statistical significant decreased in the mean HS-CRP observed. It can therefore be concluded that homocysteine lowering supplementation approach has a positive effect on the inflammatory state of people with normal homocysteine but not on hyperhomocystenaemic subjects.

Homocysteine as a cardiovascular risk marker and strategies to lower plasma homocysteine levels have been an on-going topic of investigation (Jamison et al 2007:1163). Association between cardiovascular risk and elevated homocysteine have been confirmed by epidemiological studies, but intervention studies with a homocysteine-lowering approach have not been consistent in their findings (Kaul et al 2006:914; Jamison et al 2007:1163; Albert et al 2008:2027). Vitamin B12, B6 and folate are directly involved in the homocysteine metabolism (Wierzbicki 2007:143). However, in this study no direct relationship was observed, a possible indirect relationship with haemopoeiesis was indicated. This study used serum homocysteine, vitamin B6, B12, folate as well as MCV (macrocytosis) as homocysteine metabolic markers. A very high incidence (66.36%) of hyperhomocysteinaemia is present in the sample, resulting in a much larger group A than group B. The mean serum homocysteine level in group A decreased statistical significantly, whereas the mean homocysteine level in group B increased. Serum vitamin B12 and folate levels in both groups fell within the normal reference range at baseline. Although a slight but statistical significant decrease of these two parameters occurs after intervention in both groups, the mean levels were still within the normal reference range. This phenomenon can be due to normal physiological action. Both vitamin B12 and folate are absorbed mainly by highly specific binding glycoprotein. The availability of the binding glycoproteins is indirectly proportional to the vitamin B12 and folate status (Gibson 2005: 617). It may thus be that because no vitamin B12 or folate deficiency were present in the sample at baseline or follow-up, no additional absorption took place in spite of excess availability. Very low serum vitamin B6 levels were observed in both groups and improved statistical significantly after the intervention, although in
both groups the serum levels did not reach the minimum reference value. The prevalence of macrocytosis (MCV > 95 pg) decreased significantly in both groups after the intervention.

No significant relationships were found between homocysteine and dietary vitamin B6, vitamin B12 and folate as suspected due to the intervention. Homocysteine, dietary vitamin B6, vitamin B12 and folate correlated however similarly with the red cell parameters. It can therefore be concluded that an indirect relation between homocysteine and dietary vitamin B6, vitamin B12 and folate. This can be explained by the biochemical interaction between vitamin B6, vitamin B12 and folate with homocysteine during haemopoeiesis (Hoffbrand et al 2011:46).

5.7 CONCLUSION AND RECOMMENDATIONS

It is concluded that a vitamins B12, B6 and folate supplementation at > 100% RDA for six months had a homocysteine lowering effect in hyperhomocysteinaemic individuals but not for normohomocystaemic individuals. The supplementation also seemed to have a greater benefit on the BMI of the hyperhomocysteinaemic individuals than on the BMI of the normohomocysteinaemic persons. The supplementation was beneficial to all the individuals (independently of their homocysteine status) on their glucose levels, their fibrinolytic status, vitamin B6 serum levels and on their haemopoeiesis (decrease macrocytosis).

The effect the supplementation had on cardiovascular risk markers is presented in figure 32. In this study irreversible (age related), potentially reversible and physiological (low income) risk markers were found to prevail, either singly or in combination with other markers (as indicated in chapter 4). The homocysteine-lowering-supplementation intervention affected the elevated potential reversible risk markers as follow: hypertension (no effect), overweight (decrease BMI), metabolic syndrome (no effect), lipogram (no effect), hypercoagulability (no effect on clot formation but increase fibrinolysis), homeocysteinaemia (decrease serum homocysteine levels and macrocytosis and increase
vitamin B6 levels), acute inflammatory response (no effect) and dietary intake (improve the vitamin B6, B12 and folate intake).

**FIGURE 32** CONCEPTUAL FRAMEWORK REPRESENTING THE EFFECT OF INTERVENTION ON CARDIOVASCULAR RISK MARKERS
CHAPTER 6
CONCLUSIONS AND RECOMMENDATIONS

6.1 INTRODUCTION

The major objectives of this study were to evaluate the effect of a six month, >100% RDA supplementation programme of Vitamins B6, B12 and folate on cardiovascular risk markers and their interaction. The research was conducted in a black, urbanized elderly South African community, living in poverty with confirmed increased cardiovascular risk.

This project was motivated by the high prevalence of hyperhomocysteinaemia together with hyperfibrinogenaemia, hypertension, obesity, hyperinflammatory state, dyslipidaemia (increased LDL levels, decreased HDL levels) and poor nutritional status in this elderly population.

6.2 RESEARCHER’S CONTRIBUTION

This study forms part of a multi-micronutrient programme to address malnutrition among the elderly attending a day care centre. This programme is coordinated by the Centre of Sustainable Livelihoods (CSL) at the Vaal University of Technology under the leadership of Prof W.H Oldewage-Theron. The studies are conducted by a multidisciplinary team consisting of administrator, nutritionists, a dietician, nursing sisters (phlebotomists), medical technologists and a statistician. Funding (from NRF and VUT) and ethical clearance (from WITS and DUT) were obtained before the implementation of the project.

Each team member took responsibilities for the section of the study that entails the individual’s specialty. The tasks were divided as follows:

- Copies, preparation of files (numbering and inclusion of all the questionnaires) and logistical arrangements beforehand are handled by the administrator. The day of the fieldwork the administrator man the reception desk (station 1).
- Compiling and verification testing of questionnaires, as well as the fieldworker training is done by the nutritionists and the HPCSA registered dietician.
• Anthropometric measurements are done by a nutritionists and the HPCSA registered dietician.
• The blood pressure measurements and the blood collection are done by the nursing sisters.
• Blood separation and analyses is done by HPCSA registered medical technologists.

The researcher is a HPCSA registered medical technologist and takes responsibility for all the planning of laboratory analyses and blood collection during fieldwork, oversee the blood separations process and is personally responsible for the blood analyses.

In this particular study the researcher designed and manages the project, by
• Project design and proposal writing.
• Being responsible for finding and comparing the different supplementation products and ultimately select and purchase the selected product.
• Coordinating all the fieldworkers and team members for data collection.
• The distribution and the overseeing of the compliance evaluation visits.
• The blood collection, separation and laboratory analyses.
• Statistical analyses in consultation with the statistician.
• Writing of thesis and articles / manuscripts

6.3 LIMITATIONS OF THE STUDY

The first limitation was that the sample population was purposively selected and not randomly. Due to the sample size (only 110 gave consent) and the number of elderlies attending the day care center (n=250) a purposive sample was used. However, the sample size was bigger than the required sample size according to the power calculation.

The second limitation was that the two groups were not evenly distributed. The purposively selected sample was divided into the two groups according to their homocysteine levels. Because of the prevalence of hyperhomomocysteine in the population it resulted in the two uneven groups (group A n = 61; group B n = 43). However, both groups met the minimum requirements with its number of respondents and statistical analysis could be performed.
The third limitation may be associated with the study design that there was not a true control receiving a placebo group and an experimental group receiving the vitamin B6, B12 and folate supplementation. Due to ethical consideration it was decided not to deprive any elderly included in this study from the possible health benefit of the supplement. Elderlies attending the day care centre but did not give consent to participate in the study also received the supplement not to exclude anyone.

The fourth limitation was the measuring of compliance. In spite of regular (once a week) visits made to the elderly’s homes and tablets were counted to ensure that the supplements were taken regularly, the actual intake of the tablet was not controlled and the researcher relied on the confirmation received from the subject that the tablets were consumed. The results confirmed that there was no reporting bias present.

The fifth limitation of this study was that the dietary intake was measured by a 24-hour recall only. The elderly tires very easily and could not be exhausted too much. Recall bias could have been present as a result of the poor memory identified in the elderly (Charlton et al 2007:540). However, 24-hour recall questionnaires were completed on three days (2 weekdays and a weekend day) to get a more reliable measurement. The FFQ were used as a reference measurement at baseline.

6.4 MAIN FINDINGS

The main findings of this study were as follows:

6.4.1 Literature study (Chapter 1-3)

- South Africa suffers from a quadruple burden of disease (poverty-related diseases, CDL, HIV/AIDS and the effect of social instability caused by crime and violence), which has a severe effect on the prevention of and cost-effective health care management of chronic disease (Steyn 2005: 1).
- In SA, 3.3 million, or 7.7% of the total population, were reported to be 60 years and older
in 2006 and it is projected that by 2050 the number will have grown to 19% (United Nations Population Division 2006).

- The elderly do not form part of any medical benefits from the Department of Health, they only receive social grants and are thus neglected by the health care system of SA (Charlton et al 2008:580).

- As indicated CVR markers can be categorized as: irreversible (age, gender, genetics), potentially reversible (smoking, obesity, hypertension, physical inactivity, hyperglycaemia, hypercoagulability, dyslipidaemia, increased homocysteinaemia, acute inflammatory response), psychosocial (low socio-economic status, stressful environment, personality type) and geographical factors (seasonal influence, environmental pollution) (Geissler and Powers 2006.).

- As is evident in the literature, elevated homocysteine levels are directly proportional to cardiovascular risk and the cause of the elevation is multifactorial.

6.4.2 Baseline study (Chapter 4)

- Baseline results indicated that the sample are living in poverty, and are food insecure. Poor dietary intakes and subsequently malnutrition exists (both under - and over nutrition), which was confirmed by the anthropometric results indicating the high prevalence of overweight and that obesity together with nutrient deficiency (vitamin B12 and folate) co-exists. High intake of SFA and a low intake of fruit and vegetables (dietary fibre) and food sources rich in omega 3 and 6 was observed, therefore an additional CVR marker prevalent in this vulnerable population.

- Increased CVR was confirmed in the sample with prevalence, hyperhomocysteinaemia together with hyperfibrinogenaemia, hypertension, obesity, hyperinflammatory state, dyslipidaemia (increased LDL levels, decreased HDL levels) and metabolic syndrome.

- Multiple correlations between different CVR markers were found, confirming the multifactorial course of CVD.
6.4.3 Intervention study (Chapter 5)

- This study confirmed that vitamins B6, B12 and folate supplementation at >100% RDA for six months had a lowering effect on the homocysteine metabolism of an elderly community. Serum homocysteine and macrocytosis were significantly reduced and serum vitamin B6 levels were significantly increased, therefore reduce the risk of CVD. The homocysteine lowering effect was observed in hyperhomocysteinaemic individuals but not in normohomocysteinaemic persons. The effect of the supplementation on the serum vitamin B6 levels and on the macrocystosis was beneficial independent of the subject’s homocysteine status.

- The supplementation of vitamins B6, B12 and folate supplementation at >100% RDA for six months did not reduce the prevalence of metabolic syndrome, as cardiovascular risk markers in the elderly community. The supplementation had however a reducing effect on the glucose levels in both the groups and are therefore beneficial independent of the homocysteine status.

- The homocysteine lowering strategy of this study did not reduce the waist, or WTHR, but a reducing effect on the BMI and weight was indicated in the hyperhomocysteinaemic group but not in the normohomocysteinaemic group.

- The results of this study indicated that the supplementation had no effect on the blood pressure of the subjects in either one of the groups.

- The intervention study did not have an effect in the dietary intake of the respondents apart for the significant increase of vitamins B6, B12 and folate intake in both groups.

- It was found that the supplementation of vitamins B6, B12 and folate at >100% RDA for six months did not have a positive effect on the coagulability (fibrinogen) but had a positive effect on the fibrinolysis (PAI-1) in both groups and thereby reduced the risk for CVD independently of homocysteine status.

- This study indicated that the supplementation had no lipid lowering effect on the sample the subjects in neither one of the groups.

- The results indicated that the homocysteine lowering intervention strategy of this study did not decrease the acute inflammatory state of the respondents.
• The results of this study indicated that the supplementation of vitamins B6, B12 and folate at >100% RDA for six months reduced the fibronectin levels in both groups and are therefore beneficial independent of homocysteine status.

• Although a direct relationship between the increased dietary intakes of vitamins, B6, B12 and folate could not be confirmed statistically an indirect relation was observed in the hyperhomocysteinaemic group via haemosynthesis (increased RCC and decrease in macrocystosis).

6.4.4 The significant findings of this study

Positive correlations between vitamin B6, B12 and folate status and cardiovascular disease have been demonstrated by numerous international studies as reported in a review by Dhonukshe-Rutten et al. (2009:18). Although this study could not find direct statistical correlations between the vitamin B6, B12 and folate intake and CVR markers, indirect relationships were demonstrated. The reported studies have not evaluated such correlations neither in the African context nor in an elderly population, this study is therefore the first to evaluate the effect of homocysteine lowering therapy in this population whose nutritional, socio-economic and cultural lifestyles are substantially different from those of developed countries (Oldewage-Theron et al 2008(a): 23).

Contradictory results were found in previous studies and a possible reason was found to be an insufficient concentration of supplementation (Morley and Thomas 2007: 160; Acikel et al 2009; Dhonukshe-Rutten et al 2009: 18). Lowering of homocysteine was found in studies using between 100% and 200% of the Recommended Daily Allowance (RDA) of vitamin B6, B12 and folate (Van Der Griend et al 2000:225; Verhoef and de Groot 2005: 119; Morley and Thomas 2007: 160; Acikel et al 2009). This study found a definite homocysteine lowering in hyperhomocysteinaemic subjects but not in individuals with normal homocysteine levels, with the supplementation of vitamins B6, B12 and folate at >100%RDA for 6 months. Kaul et al (2006:923) indicated the multifactorial nature of hyperhomocysteinaemia this study has found that although limited the homocysteine lowering strategy (vitamin B6, B12 and folate supplementation) had an additional effect on other CVR markers, where the glucose, fibronectin were decreased and fibrinolytic, serum vitamin B6 levels and haemosynthesis status improved in both groups. In the
hyperhomocysteinaemic group additional to the homocysteine levels the intervention also had a beneficial effect on the BMI.

6.5 CONCLUSIONS AND RECOMMENDATIONS

The following conclusions can be drawn from the results of this study:

- Black, South African elderly living in poverty is at high risk for CVD.
- A very high incidence of hyperhomocysteinaemia as an independent CVR marker prevalent in the elderly community.
- Supplementation of Vitamins B6, B12 and folate at >100% RDA for six months is an effective homocysteine lowering approach as a strategy to reduce hyperhomocysteinaemia in an elderly population, and thereby reduce the CVR.
- The intervention of vitamins B6, B12 and folate at >100% RDA for six months is not an effective multifactorial strategy to decrease CVR as indicated in figure 39, although limited effects were found with other CVR markers was the effect mainly on the homocysteine metabolism.
- The 90% power calculation was calculated on a change in homocysteine levels of 4 μmol/l, a change of 6 μmol/l (hyperhomocysteinaemic group) were observed during the homocysteine lowering intervention the results of this study can therefore be generalized to the black elderly population at large.

The results obtained from this study indicated that the following recommendations can be made to the:

6.5.1 Community:

In a poor black elderly community with an increased risk for CVD, vitamin B12, folate and B6 supplementation as a homocysteine lowering strategy can be implemented and therefore reduce the cardiovascular risk of the individual. The benefits are optimal in an individual with hyperhomocysteinaemia.
6.5.2 Policy makers:

Recommendations were made by Charlton et al (2008:575), that the marginalized geriatric health care provision needs urgent attention. This study recommends that reducing CVR in the elderly should be included in the health care plan, the following is proposed:

- An acute and drastic intervention is needed in order to reduce the high risk for CVD prevalent amongst the poor black elderlies, and to provide a better preventative health care to the aged in SA.
- Determining homocysteine levels as a screening test at primary health care level would be beneficial in the preventative treatment.
- Complete CVR prevention model should be included into the training curriculum of health care workers (as indicated in figure 31).
- Nutrition education addressing CVR prevention strategies should be implemented as a preventative strategy.
- The nutritional supplementation programme as part of the INP is not targeted for the older person. This study proposes that this programme should be revaluated and vitamin B6, B12 and folate supplementation as homocysteine lowering strategy should be included.
- FBDG adapted for the elderly in South African should be imposed. These guidelines should include CVR preventative strategies and specifically homocysteine lowering approaches.

6.5.3 Further research needed:

This study paves the way for further research in order to optimize homocysteine lowering therapy. Future studies should consider the following:

- The effect of vitamin B6, B12 folate should be studied using a case controlled design.
- Future studies should evaluate the effect of the homocysteine supplements (vitamin B6, vitamin B12 and folate) individually and not in combination.
- The interaction between the homocysteine lowering agents and the mechanism specific should be explored.
• The effect of genetic polymorphism of the homocysteine metabolites, present in the black African population as possible contributory factor to the high prevalence of hyperhomocysteinaemia should be studied.

6.6 PERSONAL REFLECTION ON THE STUDY

This study achieved the goals set for this research project and the benefits of the process included:

6.6.1 Reliability of the study

This study proved valuable in understanding the CVR profile of black, poor elderly of Sharpeville SA and offer reliable alternative strategies to be designed and implemented to reduce the risk of this unique population. Quality control standard of laboratory tests indicated that reliable and reproducible results were obtained. The validation and referencing procedure implemented to monitor the reliability of the questionnaires showed that valuable data was obtained. The fieldworkers were well monitored. Although compliance were only measured by observation (house to house visits) and reporting (verbal confirmation by respondents) results obtained confirmed that compliance report was trustworthy.

6.6.2 Data collection

Data collection for this study was well controlled. The field work and monitoring of compliance was well executed. The laboratory analysis offer the greatest challenge cost and time consuming wise. Continuous support from co-researchers, trained fieldworkers, and management of the day care centre as well as the respondents contributed to the success of the data collection process.
6.6.3 **Achievement of the objectives**

The study achieved its objectives firstly due to commitment and positivity participants. They tried their best to adhere to the prescription and process management as far as possible within their physical ability. “The psychological benefit” the supplementation had on the general feeling of wellbeing contributed to the willingness of the subjects to take the tablets as prescribed. Additionally the scientific contribution made by this study was achieved as result of extensive support system (VUT, CSL and research team members and technical support received from Replamed).

6.6.4 **Benefits of the study**

The benefits of this study are inter-, trans- and multi-disciplinary. The nutritional intake data obtained will benefit the nutritional scientific community in order to address nutritional challenges faced in caring for the elderly. The results could assist policy makers in planning a preventative as well as treatment strategy in improving health care to the elderly. Additionally this study is beneficial to the health care professionals in understanding the interaction of CVD and to design a more effective treatment strategy.

6.6.5 **Researcher’s personal gain**

To the researcher the journey of this study can be summarized in one word – GROWTH. The growth was on different levels of one’s being. Originally the challenge was to structure one’s idea / hypothesis suitable for a good study design (research methodological skills). The next challenge was to execute data collection in a reliable and valid manner (project management skills). Then the challenge was to obtain sufficient funding to perform the very expensive laboratory needed (financial management skills), additional to that was the time constrain of delivering the important reagents, additional to that was the physical time needed to be spend in the laboratory (time management skills). As a doctoral student the researcher had to acquire some much needed scholarly skills (ex. scientific writing, statistical analysis).
On a personal level the researcher was faced with the challenge of balancing the responsibility of family life with the challenges of a researcher. More often than not the balance yielded to the one side or the other, having a negative effect on the other.

During the process of the study challenges were encountered. The researcher had to learn the skill not to perceive the challenges as obstacles and allow them to hinder progress, but to stay confident in her own ability to find a solution.

In retrospect the personal benefit of this journey was far more than just obtaining an academic qualification, but much rather to be a scholar of life. I experienced great gratification in the meaningfulness of the study and to know that the work that’s been done was beneficial to the wellbeing of this vulnerable group.

### 6.7 SCOLARLY ACTIVITY RELATED TO THE STUDY

#### 6.7.1 Skilled based training

The researcher completed additional skills based training programmes in order to improve scholarly activity. The programmes are:

- Postgraduate student supervision at VUT (2011).
- Introduction to Stata - Summer School on Modern Methods in Biostatistics and Epidemiology – BiostatEpi, Italy (2011).
- Biostatistics I - Summer School on Modern Methods in Biostatistics and Epidemiology – BiostatEpi, Italy (2011)
- Epidemiology I - Summer School on Modern Methods in Biostatistics and Epidemiology – BiostatEpi, Italy (2011)
- Biostatistics II - Summer School on Modern Methods in Biostatistics and Epidemiology – BiostatEpi, Italy (2011)
- Epidemiology II - Summer School on Modern Methods in Biostatistics and Epidemiology – BiostatEpi, Italy (2011)
• Validation and Standardization of Laboratory Methods – Master Class (2012)
• Labquality days – International Federation of Clinical Chemistry, Helsinki (2013)
• Ethics Alive Symposium– University of Witwatersrand (Faculty of Health Science) (2013)
• Article writing workshop – South African Netherland research programme on alternative
development (DURBAN) (2013)
• Strengthening doctoral supervision – Netherlands organization for international
cooperation in higher education (VUT) (2013)
• Article writing for young authors – ASEV research development consultants (VUT) (2013)

6.7.2 Manuscripts prepared for publications in accredited journals

The following publications related to this study were prepared:

• **Grobler, C.**, Oldewage-Theron W. 2012. Cardiovascular risk of an elderly, black, South
African population, Sharpeville, South Africa. *Clinical Chemistry and Laboratory
Medicine*, 50(2):A41
• **Grobler, Christa.**, Oldewage-Theron, W.H. Association between cardiovascular risk
factors and nutritional status of a low income, urbanised, elderly, black, South African
community – currently assembled
• **Grobler, Christa.**, Oldewage-Theron, W.H. A conceptual framework for the evaluation of
homocysteine lowering therapy in decreasing cardiovascular risk. – currently assembled
• **Grobler, C.J.**, Oldewage-Theron, W.H; Napier C.E; Adam, J.K. The effect of vitamin
B12, folate and B6 supplementation on the cardiovascular risk factors and nutritional status
of low income, urbanised elderly black, South Africans – currently assembled
6.7.3 Papers delivered at international conferences

The following papers related to this study were delivered at international conference:

- **Advances and Controversies in B-Vitamins and Choline at the University of Leipzig (Germany) from 5–8 March 2012:**

- **Ninth Mediterranean Meeting on Hypertension and Atherosclerosis in Antalya, Turkey on March 14 - 18, 2012:**

- **World Congress of Clinical Lipidology in Budapest on 6-8 December 2012:**
  Grobler, C.J., Oldewage-Theron. Lipids and Homocysteine as Cardiovascular Risk Markers in Elderly Black Community, South Africa.

- **14th International Nutrition & Diagnostics Conference in Prague on 02-05 September 2014. Grobler, C.J; Oldewage-Theron.** Impact of vitamin B12, B6 and Folate supplementation on the cardiovascular risk markers in an elderly community of Sharpeville, South Africa

- **2nd World Congress of Clinical Lipidology conference in Vienna, Austria from the 5-7th December 2014. Christa Grobler, Anita Dukas, Wilna Oldewage-Theron.** The correlation between PCSK9 levels and metabolic syndrome in black elderly in South Africa.

- **2nd World Congress of Clinical Lipidology conference in Vienna, Austria from the 5-7th December 2014. Anita Dukas, Christa Grobler.** An evaluation of PCSK9 levels and an extended lipid profile in an elderly black community in South Africa.


Fenech, M. 2012. Folate (vitamin B9) and vitamin B12 and their function in the maintenance of nuclear and mitochondrial genome integrity. Mutation Research / Fundamentals and Molecular Mechanisms of Mutagenesis. 733:21-33.


Joint National Committee 7 Express. 2010. The seventh report of the joint national committee
on prevention, detection, evaluation and treatment of high blood pressure. *U.S Department of Health and Human Services.*


31:889-895.


Sciences. Cape Town : Oxford University Press


UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Oldewage-Theron

CLEARANCE CERTIFICATE

PROJECT

MULTI-MICRONUTRIENT SUPPLEMENTATION TO ADDRESS MALNUTRITION AMONGST THE ELDERLY ATTENDING THE SHARPEVILLE CARE OF THE AGED

PROTOCOL NUMBER M070126

INVESTIGATORS

Prof W Oldewage-Theron

DEPARTMENT

Inst. of Sustainable Livelihoods

DATE CONSIDERED

07.01.26

DECISION OF THE COMMITTEE

Approved Unconditionally (The Committee suggest-delay the quality of life information to the end of the study so that it does not confound findings)

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE

07.01.30

CHAIRPERSON

(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwwiss)

*Guidelines for written ‘informed consent’ attached where applicable

cc: Supervisor:

Prof W O-Theron

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10005, 10th Floor, Senate House, University.

I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. I agree to a completion of a yearly progress report.

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.
ANNEXURE B

Section C: Ethics

Note: Ethics requirements are faculty specific. Kindly ensure that you are aware of and have complied with the relevant ethics requirements.

Tick as appropriate:

<table>
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<th>Humans</th>
<th>Organisations</th>
<th>Animals</th>
<th>Environment</th>
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<td>Yes</td>
<td>No</td>
<td>Yes</td>
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<td>Yes</td>
<td>No√</td>
<td>Yes</td>
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</table>

Indicate Category (X)

1. Exempt from Ethics and Biosafety Research Committee Review (straightforward research without ethical problems)

2. Expedited review (minimal risk to humans, animals or environment) X

3. Full Ethics and Biosafety Research Committee review recommended (possible risk to humans, animals, environment, or a sensitive research area)

4. Full Ethics and Biosafety Research Committee review required (risk to humans, animals, environment, or a sensitive research area)

ETHICAL ISSUES CHECKLIST FOR RESEARCH APPROVAL

To be completed by all people wishing to conduct research under the auspices of Durban University of Technology.

1. Use the Durban University of Technology’s Research Ethics Policy and Guidelines to ensure that ethical issues have been identified and addressed in the most appropriate manner, before finalising and submitting your research proposal.

2. Please indicate [by a X as appropriate] which of the following ethical issues could impact on your research.

3. Please type the motivations/further explanations where required in the cell headed COMMENTS.

4. The highlighted response cells indicate those responses which are of particular interest to the Ethics Committee.
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<th>NO.</th>
<th>QUESTION</th>
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<td>1.</td>
<td><strong>DECEPTION</strong> Is deception of any kind to be used? and if so provide a motivation for acceptability.</td>
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<td>2.</td>
<td>Will the research involve the use of no-treatment or placebo control conditions? If yes, explain how subject’s interests will be protected.</td>
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<td>3.</td>
<td><strong>CONFIDENTIALITY</strong> Does the data collection process involve access to confidential personal data (including access to data for purposes other than this particular research project) without prior consent of subjects? If yes, motivate the necessity</td>
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<td>Will the data be collected and disseminated in a manner that will ensure confidentiality of the data and the identity of the participants? Explain your answer</td>
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<td>5.</td>
<td><strong>COMMENTS</strong> Subjects will receive a study number when consent is given to participate in the study. This number will serve as identification number on samples and questionnaires, that will be blinded in order to ensure confidentiality.</td>
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<td>6.</td>
<td>Will the materials obtained be stored and ultimately disposed of in a manner that will ensure confidentiality of the participants? If no, explain. If yes specify how long the confidential data will be retained after the study and how it will be disposed of.</td>
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<td>7.</td>
<td><strong>COMMENTS</strong> Data for this study will be stored for five years. Samples will be disposed by an accredited medical waste disposal company directly after analysis. At no stage will the identification of the participants be revealed. No samples will be stored for further investigations</td>
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<td>Will the research involve access to data banks that are subject to privacy legislation? If yes, specify and explain the necessity.</td>
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<td>Does recruitment involve direct personal approach from the researchers to the potential subjects? Explain the recruitment process</td>
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<td>Introductory visits will be made, explaining the objectives of the project and to obtain consent for the project from the elderly people who attend the day-care centre.</td>
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<td>Are participants linked to the researcher in a particular relationship, for example employees, students, family? If yes, specify how.</td>
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<td>If yes to 8, is there any pressure from researchers or others that might influence the potential subjects to enrol? Elaborate.</td>
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<td>Does recruitment involve the circulation/publication of an advertisement, circular, letter etc? Specify</td>
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<td>Will subjects receive any financial or other benefits as a result of participation? If yes, explain the nature of the reward, and safeguards</td>
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<tr>
<td>12</td>
<td>Is the research targeting any particular ethnic or community group? If yes, motivate why it is necessary/acceptable. If you have not consulted a representative of this group, give a reason. In addition explain any consultative processes, identifying participants. Should consultation not take place, give a motivation.</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>COMMENTS</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Only elders that will be attending the Sharpeville day care centre. This community is targeted because of the high risk for cardiovascular disease and the poor health status of this community</td>
<td></td>
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</tr>
<tr>
<td><strong>INFORMED CONSENT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Question</td>
<td>Comments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------</td>
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<td></td>
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</tr>
<tr>
<td>13</td>
<td>Does the research fulfill the criteria for informed consent? [See guidelines]. If yes, no further answer is needed. If no, please specify how and why.</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Does consent need to be obtained from special and vulnerable groups (see guidelines). If yes, describe the nature of the group and the procedures used to obtain permission.</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>COMMENTS</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Special care will be taken to ensure that the elders understand the consent form and agree.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Will a Subject Information Letter be provided and a written consent be obtained? If no, explain. If yes, attach copies to proposal. In the case of subjects who are not familiar with English (e.g. it is a second language), explain what arrangements will be made to ensure comprehension of the Subject Information Letter, Informed Consent Form and other questionnaires/documents.</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>COMMENTS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Multi-language Trained fieldworkers will be used as translators. Many of elders are illiterate and cannot read or write. Information letter and consent form will be translated into Sotho (dominant language)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Will results of the study be made available to those interested? If no, explain why. If yes, explain how</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>COMMENTS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Feedback sessions reporting results obtained will be given. Thesis will be available at the DUT library. Scientific findings will be published as articles in accredited journals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>RISKS TO SUBJECTS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Will participants be asked to perform any acts or make statements which might be expected to cause discomfort, compromise them, diminish self esteem or cause them to experience embarrassment or regret? If yes, explain.</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>COMMENTS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No known side effects has been reported</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Question</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>---</td>
<td>----------</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Might any aspect of your study reasonably be expected to place the participant at risk of criminal or civil liability? If yes, explain.</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td><strong>COMMENTS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Might any aspect of your study reasonably be expected to place the participant at risk of damage to their financial standing or social standing or employability? If yes, explain.</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td><strong>COMMENTS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Does the protocol require any physically invasive, or potentially harmful procedures [e.g. drug administration, needle insertion, rectal probe, pharyngeal foreign body, electrical or electromagnetic stimulation, etc?] If yes, please outline below the procedures and what safety precautions will be used.</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td><strong>COMMENTS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood will be collected by a qualified phlebotomist using a vacutainer system from the vena cephalica of the fasted elder. 5 ml EDTA blood, two 7ml clotted blood a 3 ml. glucose tube and 5 ml sodium citrate blood will be collected from each subject. Breakfast will be served after collection of the blood.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Will any treatment be used with potentially unpleasant or harmful side effects? If yes, explain the nature of the side-effects and how they will be minimised.</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td><strong>COMMENTS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Does the research involve any questions, stimuli, tasks, investigations or procedures which may be experienced by participants as stressful, anxiety producing, noxious, aversive or unpleasant during or after the research procedures? If yes, explain.</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td><strong>COMMENTS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Will any samples of body fluid or body tissues be required specifically for the research which would not be required in the case of ordinary treatment? If yes, explain and list such procedures and techniques.</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td><strong>COMMENTS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Question</td>
<td>Comments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>----------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Are any drugs/devices to be administered? If yes, list any drugs/devices to be used and their approved status.</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Will participants be fingerprinted or DNA &quot;fingerprinted&quot;? If yes, motivate why necessary and state how such is to be managed and controlled.</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Does the project involve genetic research e.g. somatic cell gene therapy, DNA techniques etc? If yes, list the procedures involved</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Is this research expected to benefit the subjects directly or indirectly? Explain any such benefits.</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Does the researcher expect to obtain any direct or indirect financial or other benefits from conducting the research? If yes, explain.</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>Will this research be undertaken on the behalf of or at the request of a pharmaceutical company, or other commercial entity or any other sponsor? If yes, identify the entity.</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>If yes to 29, will that entity undertake in writing to abide by Durban University of Technology's Research Committees Research Ethics Policy and Guidelines? If yes, do not explain further. If no, explain.</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>If yes to 30, will that entity undertake in writing to indemnify the institution and the researchers? If yes, do not explain further. If no, explain.</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COMMENTS</td>
<td>32</td>
<td>Does permission need to be obtained in terms of the location of the study? If yes indicate how permission is to be obtained.</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>COMMENTS</td>
<td>33</td>
<td>Does the researcher have indemnity cover relating to research activities? If yes, specify. If no, explain why not.</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>COMMENTS</td>
<td>34</td>
<td>Does the researcher have any affiliation with, or financial involvement in, any organisation or entity with direct or indirect interests in the subject matter or materials of this research? If yes, specify.</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>
The undersigned declare that the above questions have been answered truthfully and accurately

STUDENT NAME---------C.J. Grobler-----------------

SIGNATURE------------------------------------------- DATE--------13 September 2010----

PROMOTOR NAME---------Prof. W.H. Oldewage-Theron-----------------

SIGNATURE------------------------------------------ DATE--------13 September 2010--
Letter of Information and Consent

Title of the Research Study:
Impact of vitamins B12, B6 and folate supplementation on the cardiovascular risk markers in an elderly semi-urbanised black community.

Principal Investigator:
Mrs. Christa Grobler, student enrolled for the Doctorate Degree: Biomedical Technology at Durban University of Technology.

Brief Introduction and Purpose of the Study:
You are invited to be a volunteer for a research study. The information in this letter will help you understand what the research is about and how it will benefit you as an individual but also other elderly people in South Africa. If there are any questions, which are not clearly explained in this letter, do not hesitate to ask the staff or investigator.

Older people have a high risk for cardiovascular diseases (ex. heart attack and stroke). This study will help to understand some possible means to reduce this risk.

Outline of the Procedures:
This study will assess the effect vitamins B6, B12 and folate supplementation will reduce risk for heart attack and stroke in the elderly population of Sharpeville. Fasting blood will be collected twice (before the study start and after six months), by a qualified nursing
sister. Blood will be used only for nutritional markers. You are therefore requested not to eat or drink anything from the previous night 22:00. Blood pressure will be taken, participants will also been weighed and measurements taken by trained field workers.

You will receive two different vitamin supplementation tablets. These tablets contain vitamins B6, B12 and folate. They are commercially available tablets and are safe to be used without any side effects. Tablets need to be taken daily with breakfast for six months. You will receive tablets during our visits to the day care centre every month. After six months the measurements will be repeated in order to determine the effect the supplementation had on the risk for heart attack and stroke. Due to the vitamin content in the tablets the colour of your urine might change.

**Benefits:**
The new information gained from the study may help to address the high risk for cardiovascular disease in the elderly people of South Africa.

**Reason/s why the Subject May Be Withdrawn from the Study:**
Your participation in this trial is entirely voluntarily and you can withdraw at any time.

**Remuneration:**
There will be no remuneration for the participant.

**Costs of the Study:**
The patient will not be liable for any costs.

**Confidentiality:**
All information obtained in this trial will be strictly confidential. Data that may be reported in scientific journals or published will not include information that will identify you as a patient in this study.
Informed Consent Form

Persons to Contact in the Event of Any Problems or Queries:

Mrs. C.J. Grobler  Prof. W.H. Oldewage-Theron  Dr. Carin Napier  Prof. J.K. Adams
Principal  Promoter  Co/Promoter (1)  Co/Promoter (2)
Investigator

0169509210  0169509792  0313732326  0313735291

Statement of Agreement to Participate in the Research Study:

I,…………………………………………subject’s full name,
ID number…………………………………………….., have read this document in its entirety and understand its contents. Where I have had any questions or queries, these have been explained to me by ……………………………………………….to my satisfaction. Furthermore, I fully understand that I may withdraw from this study at any stage without any adverse consequences and my future health care will not be compromised. I, therefore, voluntarily agree to participate in this study.

Subject’s name  Subject’s signature
Date:........................

Researcher’s name Researcher’s signature
Date:........................

Witness name  Witness signature
Date:........................

Promoters name Promoters signature
Date:........................
Lengolo la tsebiso le boitlamo

Sehloho sa dipatlisiso:
Karolo eo phumantsho ya divitamin B12, B6 le folate di nang le yona ho ditshupetso tsa kotsi ya mahloko a tsamaelanang le pelo ho maqheku a batho ba batsho ba dulang makeisheneng.

Motho ya ka sehlohong sa dipatlisiso: Mofumahadi Chrisra Grobler eo yena a ingodiseditseng dithuto tse phahameng tsa lengolo la bongaka la Biomedical Technology (PhD), Durban University of Technology.

Tsebiso (ha kgutshwane) le sepheho sa dipatlisiso tsena:
O mengwa ho ba monka karolo ya sa lefuweng ho dipatlisiso tsena. Ditsebiso tse lengolong lena di tla o thusa hore o utlwisise hore dipatlisiso tsena di ka ha eng le hore o ka fola molemo o feng ka bowena le setjhaba sa maqheku sa Afrika Borwa ka kakaretso. Ha eba hona le dintho tse ebang ha di a hlahoswa hantle lengolong lena, o lokolohile ho botsa bathuso ba dipatlisiso ho o hlalosetsa.

Maqheku a kotsing ya ho tshwarwa ke mahloko a amanang le pelo jwale ka stroke le heart attack. Dipatlisiso tsena di tla thusa ka ho fumana mekgwa eka sebediswang ho fokotsa kotsi ena.

Mekgwa ya ho etsa dipatlisiso:
Dipatlisiso tsena di tla hlahlobo ha fokotseha ha kotsi ya mafu a pelo jwale ka heart attack le stroke ho batho ba fumantshwang divitamin B12, B6 le folate ho setjhaba sa maqheku a Sharpeville. Madi a motho ya sokang a eja letho tsatsing leo, a tla nkuwa makgetlo a mabedi. Lekgetlo la pele e tla ba pele ho qaleho ya dipatlisiso, ebe la bobedi ka mora kgwedi tse tsheletseng ka mora ho qalwa ha dipatlisiso. Madi a tla nkuwa ke mooki mme ona a tla sebediswa bakeng sa ho batlisisa ditshupetso tsa phepo fela. O koptjwa ke hona hore o seke wa ja kapa wa nwa bosiu bo ka pele ho nkiwa madi ho tloha ka hora ya leshome. Kgetello ya madi, boima ba moto le di tekanyetso tsa mmele di tla nkuwa ke bathuso ba rupelletsweng ba dipatlisiso.
Monka karolo o tla fumantshwa dipilisi tse pedi tse fapaneng tsa divitamin. Dipilisi tsena di na le vitamin B6, B12, le folate. Dipilisi tsena di a fumaneha le ho rekiswa hohle ka hona di bolokehile mme ha ho kotsi ho di nwa. Dipilisi tsena di tshwanetse ho nowa letsatsi le letsatsi ha mmoho le dijo tsa hosing bakeng la dikgwedi tse tsheletseng. O tla fumantshwa dipilisi tsena ka nako eo ba etsang dipatlisiso ba etelang lehae la tlhokomelo ya maqheku kgwedi le kgwedi. Mafelong a kgwedi tse tsheletseng ho tla nkuwa ditekanyetso hape hore ho tle ho fumanwe ha eba divitamin tsena di na le karolo eo di e tlisang ho phokotseho ya mahloko a heart attack le stroke. Mmala wa moroto o tla fetoha ka lebaka la keketseho ya divitamin e tla bang teng.

**Melemo ya dipatlisiso tsena ho monka karolo:**
Tsebo e tlang ho fumanwa ho tswana di dipatlisiso tsena e tla thusa phokotsong ya kotsi ena ya mahloko a pelo mqhekung a Afrika Borwa.

**Mabaka a ka etsang hore mo nka karolo a tloswe dipatliso tsena:**
Mo nka karolo o etsa hoo ka ho inehela mme a ka itokolla neng kapa neng ha a batla ho etsa jwalo.

**Mokgolo wa banka karolo:**
Ba nka karolo ha ba na ho lefuwa makgolo.

**Tefello ya dipatlisiso:**
Ba nka karolo ha ba lebellwa ho lefa letho.

**Ditaba tsa lekunutu:**
Sephetho sa dipatlisiso tsena se tla dula e le lekunutu. Sepetho seo se tlang ho sebediswa masedinyaneng a science ha se na ho kenywa letho le amangang le wena jwaleka mo nka karolo.
Foromo ya boitlamo.

Batho bao o ka ikamahanyang le bona bakeng sa ditlelebo le dipotso:

Mof. C.J Grobler    Prof W.H Oldewage-Theron    Dr C. Napier    Prof. J.K Adams
Ya ka sehlohung     mo-Promoter          Mothusa       Mothusa
                            promoter(1)          promoter(2)
0169509210           0169509792           0313732368  0313735291

Mantswe a tumellano ya boitlamo ba ho nka karolo dipatlisisong:
Nna, ..................................................mabitso a mo nka karolo ka botlalo, Nomoro ya boitsebiso.................................................., ke badile tsebiso ena ka botlalo le ho utlwisisa dikahare tsa yona. Moo e bang ke ne ke ena le dipotso kapa dingongoreho ke ile ka fumantshwa tlhalosetso e tletseng ke.............................. Hodimo ha moo, ke utlwisisa hore ke na le tokelo ya ho itokolla neng kapa neng ha ke ikutlwa ho etsa jwalo kantle le ho hlokofatswa mme seo ha se na ho ba le kotsi bophelong ba ka. Ka hoo, ke inehela le ho itlama ho nka karolo dipatlisisong tsena.

Lebitso la mo nka karolo...............................Tekeno.................................

Letsatsi.........................

Lebitso la mo etsa dipatlisiso...........................Tekeno.................................

Letsatsi.........................

Lebitso la paki.............................................................Tekeno.................................

Letsatsi.........................

Lebitso la mohlokomedi............................................Tekeno.................................

Letsatsi.........................
**SHARPEVILLE INTEGRATED NUTRITION PROJECT (SINP)**

**FIELDWORK CONTROL**

Subject ID number: 

<table>
<thead>
<tr>
<th>Stations</th>
<th>Activity</th>
<th>Baseline survey</th>
<th>Beginning of clinical trial</th>
<th>End of trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Station 1: Check/control</td>
<td>Handing out of file and check consent form and details</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Station 2: Socio-demographic and health data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Station 3: Clinical signs, blood</td>
<td>Oral temperature………</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Station 4: Café</td>
<td>Handing out of snacks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Station 5: Anthropometry</td>
<td>Weight………………….</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Station 6: Dietary intake</td>
<td>QFFQ</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Station 7: Nutrition education</td>
<td>Nutrition education information and training session</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Station 1: Check/control</td>
<td>Control that all fieldwork is complete</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# SHARPEVILLE INTERGRATED NUTRITION PROJECT
## ANTHROPOMETRIC, HEALTH, MEDICAL AND BEHAVIOURAL QUESTIONNAIRE

1. 
2. **Section A:**

3. **Sub ject ID** | **Age**
--- | ---
Height | m | Weight | kg
Gender | Male | Female

4. **Section B:**

5. **HEALTH QUESTIONNAIRE:**

6. **IS THERE A HISTORY OF THE FOLLOWING IN YOUR FAMILY?**

<table>
<thead>
<tr>
<th></th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Any skin disease?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Any affection of the skeleton and/or joints?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Any affection of the eyes, ears, nose or teeth?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Any affection of the heart or circulatory system?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Any affection of the chest or respiratory system?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Any affection of the digestive system?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Any affection of the urinary system and/or genital organs?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Any nervous affection or mental abnormality?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Any headaches</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Any other illness?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3. Have you experienced any of the following?

<table>
<thead>
<tr>
<th></th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>6. Weight loss during the past month?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. A recent change in appetite?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tiredness

Problems with the following:

- * chewing?
- * swallowing?
- * nausea?
- * diarrhoea?
- * vomiting?
- * constipation?

8. Follow a special diet?

If yes, specify…….

Allergic to any foods?

If yes, specify

4. Is anyone in the household physically disabled?

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
</table>

GIVE THE TYPE OF THE DISABILITY

-------------------------------------------------------------------

-------------------------------------------------------------------

5. Do you smoke at this moment?

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
</table>

5.1. Yes

5.2. No (Never smoked)

5.3. No (Stopped)

6. Does your spouse or partner smoke at this moment?

<table>
<thead>
<tr>
<th>Tick the correct block</th>
</tr>
</thead>
</table>

1. Yes

2. No

3. Not applicable
7. Do you make use of snuff at this moment? YES NO
   1. Yes
   2. No (Never used)
   3. No (Stopped)

8. Do you use alcohol on a regular basis? YES NO
   1. Yes
   2. No
   3. Not applicable

9. Do you use drugs other than medicine on a regular basis? YES NO
   1. Yes
   2. No
   3. Not applicable

10. Have you undergone any operations during the past five years? YES NO
    GIVE TYPE OF THE OPERATION
    ........................................................................................................
    ........................................................................................................
    ........................................................................................................

Section C:
MEDICATION AND HEALTH FACILITY QUESTIONNAIRE:

1. Do you use chronic medication? YES NO
   2. If no, go to the next block.
   3. If yes, what for/why?
       ........................................................................................................
       ........................................................................................................
       ........................................................................................................

2. Do you take any supplements? YES NO

3. If yes in previous question.
Specify the type | Vitamins, specify | Minerals, specify | Multivitamin | Other, specify
---|---|---|---|---

4. Which health facility is commonly used you?  
- 1. Private Doctor  
- 2. Clinic  
- 3. Hospital  
- 4. Traditional Healer  
- 5. Other (please state)

5. How do you travel to the health facility?  
- 1. On foot  
- 2. Taxi  
- 3. Bus  
- 4. Own transport  
- 5. Other (please state)

Thank you very much for your co-operation. We appreciate the time.

Mrs. C.J. Grobler  Prof. W.H. Oldewage-Theron  Dr. Carin Napier  Prof. J.K. Adams  
Principal  Promoter  Co/Promoter (1)  Co/Promoter (2)  
Investigator  0169509210  0169509792  0313732326  0313735291
ANNEXURE F
24 – HOUR RECALL

Subject ID number : ______________    Gender: Male/Female

Interviewer: ______________

Date: ______ / ______ / 2008

Tick what the day was yesterday:

<table>
<thead>
<tr>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
</tr>
</thead>
</table>

Would you describe the food that you ate yesterday as typical of your habitual food intake?

| Yes | No |

If not, why?

| I bought some food | My visitor brought me some food | Other reasons (pls. specify) |

I want to find out about everything you ate or drank yesterday, including food you bought. Please tell me everything you ate from the time you woke up to the time you went to sleep. I will also ask you where you ate the food and how much you ate.

<table>
<thead>
<tr>
<th>Time (approximately)</th>
<th>Place</th>
<th>Description of food</th>
<th>Amount</th>
<th>Amount in g (office use only)</th>
<th>Code (office use only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>From waking up to going to work, or starting day’s activities</td>
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</tr>
<tr>
<td>Time (approximately)</td>
<td>Place</td>
<td>Description of food</td>
<td>Amount</td>
<td>Amount in g (office use only)</td>
<td>Code (office use only)</td>
</tr>
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<tr>
<td>During the morning (after breakfast)</td>
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<tr>
<td>Middle of the day (Lunch time)</td>
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<tr>
<td>During the afternoon</td>
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<td>At night (dinner time)</td>
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</tr>
<tr>
<td>Time (approximately)</td>
<td>Place</td>
<td>Description of food</td>
<td>Amount</td>
<td>Amount in g (office use only)</td>
<td>Code (office use only)</td>
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</tbody>
</table>

**After dinner, before going to sleep**

|                      |       |                      |        |                               |                     |
|                      |       |                      |        |                               |                     |
|                      |       |                      |        |                               |                     |
|                      |       |                      |        |                               |                     |
|                      |       |                      |        |                               |                     |

* Do you take any vitamins (tablets or syrup)  | Yes  | No

Give the brand name and dose of the vitamin/tonic:
## Annexure G

**Sharpeville Integrated Nutrition Project**

**FFQ List of Foods and Food Groups Diversity**

Please indicate the food you ate during the past seven (7) days by a (x)

<table>
<thead>
<tr>
<th>Group 1: Flesh foods (meat, poultry, fish) diversity</th>
<th>Mother/GMother</th>
<th>Child</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pork</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tinned fish (pilchards, tuna)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish (fresh/whole)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minced meat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutton</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken runners and heads</td>
<td></td>
<td></td>
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<tr>
<td>Chicken livers</td>
<td></td>
<td></td>
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<tr>
<td>Goat (meat)</td>
<td></td>
<td></td>
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<tr>
<td>Tripe</td>
<td></td>
<td></td>
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<tr>
<td>Dried meat (biltong)</td>
<td></td>
<td></td>
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<tr>
<td>Viennas/polony/Russians</td>
<td></td>
<td></td>
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<tr>
<td>Sausage (wors)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steak</td>
<td></td>
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<tr>
<td>Other, specify</td>
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</tr>
</tbody>
</table>

**Group 2: Eggs diversity**

| Eggs                                                  |                |       |

**Group 3: Diary products diversity**

<p>| Milk, unpasteurized (cow/goat)                        |                |       |
| Evaporated milk (Ideal milk)                          |                |       |
| Maas/inkomasi                                        |                |       |
| Powdered milk                                        |                |       |
| Skim or low-fat milk (pasteurized)                    |                |       |
| Full cream milk (pasteurized)                         |                |       |
| Cheese                                               |                |       |
| Custard/Ultramel                                      |                |       |
| Ice cream                                            |                |       |
| Yoghurt/Yogisip                                      |                |       |
| Other, specify                                       |                |       |</p>
<table>
<thead>
<tr>
<th>Group 4: Cereals, roots and tubers diversity</th>
<th>Mother/GMother</th>
<th>Child</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pap (Maize)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macaroni/pasta/spaghetti</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize rice (mielierys)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samp (stampmielies)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bread (white or brown)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dumpling / “Vetkoek”</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biscuits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buns / bread rolls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mabela (soft porridge)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn flakes / Rice Krispies / Wheet Bix</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mageu</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potatoes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweet potatoes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Traditional beer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other, specify</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Group 5: Legumes and nuts                  |                |       |
| Dried beans                                |                |       |
| Dried peas                                 |                |       |
| Peanut butter                              |                |       |
| Peanut or any other nuts                   |                |       |
| Soya                                       |                |       |

| Group 6: Vitamin A rich fruits and vegetables diversity |                |       |
| Pumpkin                                               |                |       |
| Carrots                                                |                |       |
| Wild leafy vegetables (morogo)                        |                |       |
| Fresh and dried                                       |                |       |
| Spinach                                                |                |       |
| Butternut                                              |                |       |
| Apricots (Appelkoos)                                  |                |       |
| Peach (yellow cling)                                  |                |       |
| Mango                                                  |                |       |

<p>| Group 7: Other fruits (and juices) diversity         |                |       |
| Deciduous fruits                                     |                |       |
| Apple                                                 |                |       |
| Peaches                                               |                |       |
| Pear                                                  |                |       |
| Grapes (black/green)                                  |                |       |
| Plum                                                  |                |       |</p>
<table>
<thead>
<tr>
<th>Sub – tropical fruit</th>
<th>Mother/GMother</th>
<th>Child</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naartjie</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banana</td>
<td></td>
<td></td>
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<tr>
<td>Pineapple</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avocado</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blueberry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cherry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kiwi fruit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raspberry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watermelon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild watermelon(tsamma)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guava</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Juices**

Juice (100% pure juice e.g. Ceres/Liquifruit)

**Group 8: Other vegetables diversity**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Onions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cabbage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beetroot</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhubarb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turnips (raap)</td>
<td></td>
<td></td>
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<tr>
<td>Gem-squash (lemoenpampoen)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomatoes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green beans (fresh)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peas (fresh – green)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cauliflower</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chili (red/green)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lettuce</td>
<td></td>
<td></td>
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<tr>
<td>Mushroom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baby marrow</td>
<td></td>
<td></td>
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<tr>
<td>Green pepper</td>
<td></td>
<td></td>
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<tr>
<td>Sweet-corn (baby)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn-on-the-cob(white)</td>
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<td></td>
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<tr>
<td>Garlic</td>
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</tbody>
</table>

**Group 9: Oil and fat diversity**

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<tbody>
<tr>
<td>Butter</td>
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<tr>
<td>Sunflower oil</td>
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<td></td>
</tr>
<tr>
<td>Margarine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lard</td>
<td></td>
<td></td>
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<tr>
<td>Salad oil</td>
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</tbody>
</table>

Thank you very much for your co-operation. We appreciate the time.

**Abdulkadir Egal (Dr)**

**Wilna Oldewage-Theron (Prof)**
Principal Investigator: QwaQwa Integrated Nutrition Project
e-mail: abdul@vut.ac.za

Director: ISL
e-mail: wilna@vut.ac.za
ANNEXURE H

Socio-Demographic Questionnaire

This questionnaire covers certain aspects of your life, including work and personal details, health and illness, lifestyle and social life that is relevant to health. The answers to these questions will be kept strictly confidential and the information will not be identifiable from any reports or publications.

1. GENERAL INFORMATION

Date : ......................................................................................
Name : ......................................................................................
ID Number : ..............................................................................
Address : ......................................................................................
............................................................................................
............................................................................................
............................................................................................

Please answer all questions by marking the correct answer with X, except where otherwise indicated.

Example: In what town do you live?

<table>
<thead>
<tr>
<th>Johannesburg</th>
<th>Bloemfontein</th>
<th>Cape Town</th>
<th>Vanderbijlpark</th>
<th>Durban</th>
</tr>
</thead>
</table>

2. PERSONAL INFORMATION

2.1 Your role in the family

<table>
<thead>
<tr>
<th>Mother</th>
<th>Grandmother</th>
<th>Father</th>
<th>Grandfather</th>
<th>Other, specify..............................</th>
</tr>
</thead>
</table>

2.2 When were you born? Year: Month: Day:

2.3 How old are you? ________________ years

2.4 Gender:

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
</table>

2.5 Are you?
3. ACCOMMODATION AND FAMILY COMPOSITION

3.1 Where do you live?

<table>
<thead>
<tr>
<th>Town/City</th>
<th>Farm</th>
<th>Informal settlement</th>
<th>Rural village</th>
<th>Hostel</th>
<th>Other, specify</th>
</tr>
</thead>
</table>

3.2 Do other people live in your house?

☐ Yes
☐ No

3.3 How many people are living in your house?

1 2 3 4 5 6 7 8 9 10 10+

3.4 Please complete the table below on all members of the household

<table>
<thead>
<tr>
<th>Name of household member</th>
<th>Age (yrs)</th>
<th>Gender M/F</th>
<th>Family relationship</th>
<th>Does this person eat and sleep in this house at least 4 days a week?</th>
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</tbody>
</table>

3.5 Are all members permanent residents in this house?

☐ Yes  ☐ No

3.6 If yes, how long have you been staying permanent in this house?

☐ < 1 year  ☐ 1-5 years  ☐ >5 years

3.7 Do you have another home outside the Vaal Triangle?
3.8 In what type of house are you staying and indicate the number of rooms?

<table>
<thead>
<tr>
<th>Brick</th>
<th>Clay</th>
<th>Grass</th>
<th>Zinc/shack</th>
<th>&lt; 2 rooms</th>
<th>3-4 rooms</th>
<th>&gt; 4 rooms</th>
</tr>
</thead>
</table>

3.9. Are there other houses/shacks within the same yard of the main house?

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

3.10 How would you describe the place where you are currently living?

<table>
<thead>
<tr>
<th>Homeless</th>
<th>Living with parents</th>
<th>Living with relatives</th>
<th>Living with friends</th>
<th>Hostel accommodation</th>
<th>Squatter home</th>
<th>Rented house</th>
<th>Rented flat</th>
<th>Own house</th>
<th>Own flat</th>
<th>Other, specify…………………</th>
</tr>
</thead>
</table>

3.11 Do you have the following facilities at home?

3.11.1 Water

<table>
<thead>
<tr>
<th>Tap in the house</th>
<th>Tap outside the house (in yard)</th>
<th>Borehole</th>
<th>Spring / river / dam water</th>
<th>Fetch water from elsewhere</th>
</tr>
</thead>
</table>

3.11.2 Toilet facilities

<table>
<thead>
<tr>
<th>None</th>
<th>Pit latrine</th>
<th>Flush / sewage</th>
<th>Bucket system</th>
<th>Other, specify…………………</th>
</tr>
</thead>
</table>

3.11.3 Waste removal | Yes | No |
|---------------------|-----|----|
3.11.4 Tarred road in front of house  Yes  No
Gravel road in front of house  Yes  No

3.12 To what extent do you have problems with your housing (e.g. too small, repairs, damp, etc.)?

..............................................................................................................................
..............................................................................................................................

3.13. Do you have problems with the following?

<table>
<thead>
<tr>
<th>Mice / Rats</th>
<th>Cockroaches</th>
<th>Ants</th>
<th>Other pests, specify</th>
</tr>
</thead>
</table>

4. INCOME

4.1 How long have you been on pension?

<table>
<thead>
<tr>
<th>&lt; 6 months</th>
<th>6-12 months</th>
<th>1-3 years</th>
<th>&gt; 3 years</th>
</tr>
</thead>
</table>

4.2 Is your spouse (partner) in paid employment at present?

<table>
<thead>
<tr>
<th>Yes, full time, permanent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes, part-time, permanent (&lt; 25 hours p w)</td>
</tr>
<tr>
<td>Yes, temporary</td>
</tr>
<tr>
<td>No, unemployed</td>
</tr>
<tr>
<td>No, retired</td>
</tr>
<tr>
<td>No, other, specify</td>
</tr>
</tbody>
</table>

4.3 If YES, what is your spouse (partner)’s occupation or job?

..............................................................................................................................

4.4 What is the total income in the household per month?

<table>
<thead>
<tr>
<th>&lt; 500</th>
<th>R501-R1000</th>
<th>R1001-R1500</th>
<th>R1501-</th>
<th>R2001-R2500</th>
<th>&gt; R2500</th>
</tr>
</thead>
</table>

4.5 How often does it happen that you do not have enough money to buy food or clothing for you or your family?

<table>
<thead>
<tr>
<th>Always</th>
<th>Often</th>
<th>Sometimes</th>
<th>Seldom</th>
<th>Never</th>
</tr>
</thead>
</table>

4.6 How many people e.g. partner, relatives & others (including yourself) contributed to your household income from any source, (including wages/salary from paid employment, money from second or odd jobs income from savings investments, pension, rent or property, benefits and or maintenance etc.) in the last 12 months?
4.7 How often do you buy food?

<table>
<thead>
<tr>
<th>Every day</th>
<th>Once a week</th>
<th>Once a month</th>
<th>Other, specify…</th>
</tr>
</thead>
</table>

4.8 Where do you buy food?

<table>
<thead>
<tr>
<th>Spaza shop</th>
<th>Street vendor</th>
<th>Supermarket</th>
<th>Other, specify…</th>
</tr>
</thead>
</table>

4.9. How much money is spent on food PER WEEK? (Tick only one box)

<table>
<thead>
<tr>
<th>R 0 – R 50</th>
<th>R 51 – R 100</th>
<th>R 101 – R 150</th>
<th>R 151 – R 200</th>
<th>R 201 – R 250</th>
<th>R 251 – R 300</th>
<th>&gt; R 300</th>
<th>I do not know</th>
</tr>
</thead>
</table>

4.10 Do you buy the following regularly (per month)?

<table>
<thead>
<tr>
<th>Food item</th>
<th>Yes</th>
<th>No</th>
<th>Food item</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td></td>
<td></td>
<td>Cheese</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td></td>
<td></td>
<td>Eggs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutton</td>
<td></td>
<td></td>
<td>Fresh milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tripe</td>
<td></td>
<td></td>
<td>Powdered milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish</td>
<td></td>
<td></td>
<td>Condensed / Ideal milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canned fish eg pilchards</td>
<td></td>
<td></td>
<td>Cremora</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polony and other processed meat</td>
<td></td>
<td></td>
<td>Frozen vegetables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canned meat eg bully beef</td>
<td></td>
<td></td>
<td>Fresh vegetables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold drink</td>
<td></td>
<td></td>
<td>Canned vegetables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar</td>
<td></td>
<td></td>
<td>Fresh fruit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize meal</td>
<td></td>
<td></td>
<td>Canned fruit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oil</td>
<td></td>
<td></td>
<td>Fruit juice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter / margarine</td>
<td></td>
<td></td>
<td>Coffee, instant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut butter</td>
<td></td>
<td></td>
<td>Tea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish paste</td>
<td></td>
<td></td>
<td>Cheese curls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jam</td>
<td></td>
<td></td>
<td>Sweets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bread, brown</td>
<td></td>
<td></td>
<td>Chips</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bread, white</td>
<td></td>
<td></td>
<td>Maltabella</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5 EDUCATION AND LANGUAGE

5.1. What is the highest education you have?

<table>
<thead>
<tr>
<th>None</th>
<th>Primary School</th>
<th>Secondary school</th>
<th>College</th>
<th>Other post school</th>
</tr>
</thead>
</table>

5.2 What language is spoken mostly in the house?

<table>
<thead>
<tr>
<th>Sotho</th>
<th>Xhosa</th>
<th>Zulu</th>
<th>Pedi</th>
<th>Other, specify</th>
</tr>
</thead>
</table>

6 ASSETS

Tick one block for every question:

<table>
<thead>
<tr>
<th></th>
<th>Father</th>
<th>Mother</th>
<th>Child</th>
<th>Grandma</th>
<th>Grandpa</th>
<th>Other</th>
</tr>
</thead>
</table>

6.1 Who is mainly responsible for food preparation in the house?

6.2 Who decides on what types of food are bought for the household?

6.3 Who is mainly responsible for feeding/serving the child?

6.4 Who is the head of this household?

6.5 Who decides how much is spent on food?

6.6 How many meals do you eat at per day?

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>&gt; 3</th>
</tr>
</thead>
</table>

6.7 Where do you eat most of your meals?

<table>
<thead>
<tr>
<th></th>
<th>Home</th>
<th>Friends</th>
<th>Work</th>
<th>Buy</th>
<th>Other, specify</th>
</tr>
</thead>
</table>

6
6.8 Does your home have the following and how many?

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrical stove</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gas stove</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primus or paraffin stove</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microwave</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hot plate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Television</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refrigerator</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freezer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bed with mattress</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mattress only</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lounge suite</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dining room suite</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrical iron</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kettle, electrical</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Thank you very much for your co-operation.

Wilna Oldewage-Theron (Prof)
Activity leader: Sharpeville Integrated Nutrition Project

Tel: 016 950 9279
Fax: 016 950 9788
## ANNEXURE I

**SHARPEVILLE INTEGRATED NUTRITION PROJECT (SINP)**  
**SIGNS OF MALNUTRITION**

Subject ID number: .......................... Completed by..........................................................

<table>
<thead>
<tr>
<th>Signs/symptoms associated with malnutrition</th>
<th>Tick if yes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hair</strong></td>
<td></td>
</tr>
<tr>
<td>· Lack of natural shine, dull and dry</td>
<td></td>
</tr>
<tr>
<td>· Dyspigmented</td>
<td></td>
</tr>
<tr>
<td>· FLAG sign</td>
<td></td>
</tr>
<tr>
<td>· Easily plucked (no pain)</td>
<td></td>
</tr>
<tr>
<td><strong>Face</strong></td>
<td></td>
</tr>
<tr>
<td>· Scaling of skin around nostrils</td>
<td></td>
</tr>
<tr>
<td>· Swollen face</td>
<td></td>
</tr>
<tr>
<td>· Paleness</td>
<td></td>
</tr>
<tr>
<td><strong>Eyes</strong></td>
<td></td>
</tr>
<tr>
<td>· Pale conjunctiva</td>
<td></td>
</tr>
<tr>
<td>· Bitot’s spots</td>
<td></td>
</tr>
<tr>
<td>· Dryness of the eye</td>
<td></td>
</tr>
<tr>
<td>· Corneal xerosis (dullness)</td>
<td></td>
</tr>
<tr>
<td>· Corneal softening</td>
<td></td>
</tr>
<tr>
<td>· Redness and fissuring of eyelid corners</td>
<td></td>
</tr>
<tr>
<td>· White ring around the eye</td>
<td></td>
</tr>
<tr>
<td>· Small, yellowish lumps around eyes</td>
<td></td>
</tr>
<tr>
<td><strong>Lips</strong></td>
<td></td>
</tr>
<tr>
<td>· White or pink lesions at corners of mouth</td>
<td></td>
</tr>
<tr>
<td>· Magenta tongue</td>
<td></td>
</tr>
<tr>
<td>· Filiform papillae</td>
<td></td>
</tr>
<tr>
<td>· Atrophy or hypertrophy</td>
<td></td>
</tr>
<tr>
<td>· Red tongue</td>
<td></td>
</tr>
<tr>
<td><strong>Teeth</strong></td>
<td></td>
</tr>
<tr>
<td>· Mottled enamel</td>
<td></td>
</tr>
<tr>
<td>· Caries/cavities</td>
<td></td>
</tr>
<tr>
<td>· Missing teeth</td>
<td></td>
</tr>
<tr>
<td><strong>Gums</strong></td>
<td></td>
</tr>
<tr>
<td>· Spongy, bleeding</td>
<td></td>
</tr>
<tr>
<td>· Receding gums</td>
<td></td>
</tr>
<tr>
<td><strong>Glands</strong></td>
<td></td>
</tr>
<tr>
<td>· Front of neck swollen</td>
<td></td>
</tr>
<tr>
<td>· Swollen cheeks</td>
<td></td>
</tr>
<tr>
<td><strong>Nervous system</strong></td>
<td></td>
</tr>
<tr>
<td>· Psychomotor changes</td>
<td></td>
</tr>
<tr>
<td>· Mental confusion</td>
<td></td>
</tr>
<tr>
<td>· Sensory loss</td>
<td></td>
</tr>
<tr>
<td>· Motor weakness</td>
<td></td>
</tr>
<tr>
<td>· Loss of positional sense</td>
<td></td>
</tr>
<tr>
<td>· Loss of vibration</td>
<td></td>
</tr>
<tr>
<td>· Loss of ankle and knee jerks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Burning and tingling of hand and feet</td>
<td>□</td>
</tr>
<tr>
<td>Dementia</td>
<td>□</td>
</tr>
</tbody>
</table>
VITA FORCE VITAMIN B6-50mg

SCHEDULING STATUS: Not Scheduled.

PROPRIETARY NAME (and dosage form):
VITA FORCE Vitamin B6-50mg tablets

COMPOSITION:
EACH tablet contains: Pyridoxine HCl (Vitamin B6 50mg

PHARMACOLOGICAL CLASSIFICATION:
A22 1.4 Vitamins. Other.

PHARMACOLOGICAL ACTION:
Nutritional supplement.

INDICATIONS:
VITA FORCE VITAMIN B6-50mg is a high potency supplement to help reduce symptoms associated with premenstrual syndrome (PMS) and pregnancy induced nausea.

VITA FORCE VITAMIN B6-50mg plays a key role in nervous system, cardiovascular and skin health. An adequate intake of Vitamin B6 is important for the production of serotonin, known as the "feel good" hormone, which is responsible for mood elevation.

CONTRA-INDICATIONS:
Hypersensitivity to any of the ingredients contained in the product.

Vitamin B6 reduces the effects of Levodopa, therefore this product should not be administered to patients receiving Levodopa medication.

DOSE AND DIRECTIONS FOR USE:
One tablet to be taken once to three times a day with meals, or as directed by a healthcare professional.

DRUG INTERACTIONS:
Pyridoxine reduces the effect of Levodopa, but this does not occur if a dopa decarboxylase inhibitor is also given. Pyridoxine reduces the activity of amitriptyline. It has also been reported to decrease serum concentrations of phenobarbital and phenytoin. Many medicines may increase the requirements for pyridoxine, such medicines include: hydralazine, isoniazid, penicillamine and oral contraceptives.

SIDE-EFFECTS AND SPECIAL PRECAUTIONS:
Long term use of large doses of pyridoxine is associated with the development of severe peripheral neuropathies.

KNOWN SYMPTOMS OF OVERDOSE AND PARTICULARS OF ITS TREATMENT:
The treatment of overdose is symptomatic and supportive.

IDENTIFICATION:
Round, white, biconvex tablets.

PRESENTATION:
Packs of 100 tablets and 1000 tablets.

STORAGE INSTRUCTIONS:
Store below 25°C. Keep out of reach of children.

REGISTRATION NUMBER:
T1222(Act 101/1995)

NAME AND BUSINESS ADDRESS OF THE HOLDER OF THE CERTIFICATE OF REGISTRATION:
PharmaNatura
The Natural Medicine Company
1 Carey Street, Wynberg, Sandton
P.O. Box 494 Bergvlei 2012
Tel (011) 445 6000
Made in South Africa

DATE OF PUBLICATION OF THIS PACKAGE INSERT:
20 May 1991