NUTRITIONAL FACTORS INVOLVED IN DEVELOPMENT OF NEURAL TUBE DEFECTS IN OFFSPRING OF WOMEN RESIDING IN A HIGH RISK AREA

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Thesis presented for the degree of

DOCTOR OF PHILOSOPHY

in the Department of Chemical Pathology

SCHOOL OF PATHOLOGY, FACULTY OF HEALTH SCIENCE

UNIVERSITY OF LIMPOPO (*Turfloop Campus*)

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2009

DECLARATION

This thesis is presented in fulfillment of the requirements for the degree of Doctor of Philosophy (PhD) in the Department of Chemical Pathology, School of Pathology, Faculty of Health Science, University of Limpopo (Turfloop Campus). It has not, in whole or in part, been submitted before for any degree at this aor any other university. The university is empowered to reproduce all or part of the contents for the purpose of research.

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THIS THESIS IS DEDICATED TO:

ALMIGHTY GOD,

KING OF GLORY,

THANK YOU for giving me the aptitude, potency and insight

to do this work,

THANK YOU for being a faithful, prayer-answering GOD.

Against all odds, You kept me in the palm of Your hand with your

immeasurable love, abundant grace, everlasting mercies and favours.

And, through it all, I have learned to trust in You with all my heart.

ALL THE GLORY BE UNTO YOU

ETERNAL ROCK OF AGES, IN THE PRECIOUS NAME OF JESUS

Psalm 40: 1-5

ACKNOWLEDGEMENTS

This thesis would not have been complete, if it was not for several people who have contributed knowledge, assistance, support and encouragement. Therefore, I would like to extend my heartfelt gratitude and give credit to the following people:

- **Prof. Marianne Alberts**, **my main supervisor**, for her genuine interest in this research work and her patience in developing an upcoming researcher like me. Her brilliant knowledge and skills in research has contributed to this project. Thank you, Prof, for your efforts, support and patience. You are a career role model for young resolute researchers.

- **Dr. Nelia P Steyn, my co-supervisor**, for her momentous contributions and helpful feedbacks on the thesis. I will always be grateful to you.

- My beloved **grandmother,** Phildah Phyllis Nobesuthu, and loving **mother** Faith, for their unconditional love and support. Your parental care and guidance have brought out a better adult in me. Especially to you, Phildah, you are an exceptional role model and thank you for teaching me that God and education make a virtuous individual.

- Special thanks to my **family members;** aunts, uncles, brothers and sisters for their support**,** especially to aunt Pinky Mawasha for her thoughtfulness of me, being far from home, as well as adorable Nomvula (Puli).

- **My loving Pastors and mentors,** Uncle Felix Mbajiorgu, Dr. Funke Mbajiorgu, for their continuous prayers and Godly counsel, as well as elders and members of Redeemed Christian Church of God (RCCG, Trinity parish). I am also indebted to, Pastors, Dr Sindiswa Khoabane & Victor Adeoye and their families for encouragement, prayers and mentoring. I also appreciate the support I got from my **sisters, brothers and friends in the Lord**. Thank you for your encouragement, constructive criticisms, and your help and presence during sleepless nights spent writing this thesis.

- I would also like to thank the **Dikgale Demographic Surveillance Site population** and the **Indunas (leaders)** of the villages. Special thanks to all women of childbearing age who availed themselves to participate in the study and to **field workers**; Mary Mogashoa, Suzan Makgato-Mashangoane, Anna Makwela and others for their outstanding help. Also, my gratitude goes to several **nurses** at Seobi clinic (Moduane village) and Mankweng hospital as well as to **University of Limpopo nurses**; Ms Melba Mamabolo, Dr. Nancy (Mmapula) Malema, Dr. Sonto Maputle and Ms Eudoxia Raditlhalo.

- Many thanks to **Mrs Rita Olwagen,** from Department of Research and Development, Statistics, UL. "*Data collection without a good statistical analysis is a missed opportunity".* Your help was outstanding. Mag die HERE jou seën.

- To my **colleagues** in the Department of Medical Science, especially Mr Solly Choma, Dr. Lesly Mamabolo, Dr L Mpolokeng, Mr SA Dikotope and staff members of Department of Nutrition for advise. To Prof. Les Minter for counsel and editing of my thesis and article. To Department of Nutrition, North West University (Potchefstroom campus) for helping me with MRC food finder.

- Special thanks to **student laboratory assistants** of Departments of Medical Science and Optometry, for helping me to carry out my daily duties while I was writing this thesis and to those who acknowledged me on a daily basis as a mentor.

- To everyone who has shown interest in my work, that I forgot to mention, be rest assured that I acknowledge you.

- I am sincerely appreciative to **Lancet Laboratories** for analysis of plasma homocysteine, and **MRC Promec Unit, Tygerberg, South Africa** for analysis of morogo samples.

- Finally, I am also deeply thankful to **University of Limpopo**, **Research Department** and **National Research Foundation (NRF)** for funding.

ABSTRACT

AIM: This study aimed to assess the nutritional status of non-pregnant women of childbearing age residing in a rural area of Limpopo Province, South Africa, and the effect of fortification of staple foods on their folate and iron status.

The following objectives were carried out:

- To assess the socio-demographic status and maternity history of non-pregnant rural women of childbearing age.
- To determine the following anthropometric measurements; body weight, height, body mass index, waist and hip circumference, and waist hip ratio of women of childbearing age.
- To assess dietary intake of non-pregnant rural women of childbearing age using 24-hour recall and quantitative food frequency questionnaires prior to fortification of foods.
- To determine folate status of non-pregnant rural women of childbearing age by levels of serum and red blood cell folate, and assessing vitamin B_{12} and homocysteine levels before and after fortification of foods.
- To determine iron status of non-pregnant rural women of childbearing age by full blood count, serum ferritin, iron, total iron binding capacity, transferrin saturation and C–reactive protein before and after fortification of foods.
- To determine albumin and liver enzymes (ALP, ALT, AST and GGT) of women of childbearing age before and after fortification.
- To assess mycotoxins (i.e. fumonisins) in morogo samples collected randomly among participants from the study area.

STUDY DESIGN: This is a prospective cohort study, conducted in two different time-periods within the same area and population. The first phase of the study took place between November 2002 and April 2003 **(pre-fortification period)**. The second phase was a follow-up of the subjects that participated in the first phase, and was conducted from June 2004 to July 2004, approximately nine months subsequent to fortification of staple foods (**post-fortification period).** Fortification of foods in South Africa was officially mandated during October 2003.

STUDY SETTING: The study was conducted in Dikgale Demographic Surveillance site (Dikgale DSS), a rural area in the Capricorn district of Limpopo Province, South Africa. This rural site is situated ± 50 km northeast of Polokwane, the capital city of Limpopo Province and covers approximately 71 square km. Dikgale DSS consists of 8 villages situated close to one another and has a poor infrastructure in terms of sanitation, postal service, transport and roads. Unemployment is estimated at 50-60%.

STUDY POPULATION: The total population of Dikgale DSS was 8000 with 1 649 women of childbearing age (18 – 44 years) in 2000/2001. During October 2002, a random sample of 150 women (18-44 years of age) was recruited to participate in the study (pre-fortification period) in order to obtain a representative sample of 100. The women were recruited during house visits. Women who were lactating, on chronic disease medication, using oral contraceptives, tobacco and alcohol were excluded from the study. Pregnancy screening was carried out, using commercial pregnancy urine test-strips and women who were found to be pregnant were excluded from the study.

Eventually 120 women participated in pre-fortification period while only 80 of the same women who participated in the study prior to fortification were available in post-fortification period, since some of the women were pregnant or absent from Dikgale DSS.

OUTCOME MEASURES: Data collected included socio-demographic status, maternity history, anthropometric measurements, dietary intake using 24 hr recall and quantitative food frequency, food samples (i.e. morogo) and fasting blood samples. Socio-demographic status information factors assessed were age, marital status, occupation, level of education and household information. For maternity history, parity and number of stillbirths, miscarriage, perinatal mortality and cases and knowledge of neural tube defects (NTDs) were assessed. Anthropometric indices measured were body weight (W), height (H), waist and hip circumferences (WC and HC). From these anthropometric indices, body mass index (BMI) and waist-hip-ratio were calculated as W (kg)/ $[H\ (m)]^2$ and WC(cm)/HC(cm), respectively. Dietary intake was assessed using two of 24-hr recall questionnaires and one QFFQ questionnaire per individual.

From the fasting blood collected, biochemical parameters measured included serum folate (Sfol) and red cell folate (RCF), plasma homocysteine (*t*Hcy), vitamin B12 (VB12) serum ferritin (SF), iron (SIron), total iron binding capacity (TIBC), percentage transferrin saturation (%TS), C-reactive protein (CRP), full blood count (FBC), red blood cell (RBC), haemoglobin (HGB), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC), as well as

albumin (Alb) and liver enzymes (ALP, ALT, AST and GT). Access Immunoassay, 5-diff Coulter (Beckman Coulter) and ILAB 300 plus (Instrumentation Laboratory) as well as High Liquid Performance Chromatography (HPLC) at Lancet Laboratories and Dimension ES sample auto analyser, were used to analyse the above parameters. Morogo samples were also collected randomly, to assess the presence of mycotoxins (fumonisins) and the analysis was carried out at MRC Promec Unit, Tygerberg in Cape Town, South Africa.

RESULTS: High rates of unemployment (67%) and single-hood (64.3%) were observed. Although none of the women had tertiary education, a high percentage of the women (87.8%) completed secondary education. Electricity was used mostly for lighting (68.7%), and refrigerator use (64.3%). Wood (85.2%) and paraffin (58.3%) were used for cooking while water was mainly supplied by communal tap (67.8%). Large household sizes of 6-10 members (70.4%) and 11 members (17.4%) were observed. Parity of 1-4 was common (61%) compared to parity of ≥5 (11.3%), while 27% had no children. Unfavourable pregnancy outcomes observed among the women were stillbirths (7%), perinatal mortality (8%) and miscarriage (2.6%) and were more common in elder women compared to younger women. Most of the women had no knowledge of NTDs, and only 1 case of NTDs (0.9%) was reported.

Overweight (26.7%) and obesity (27.7%) were prevalent among women of childbearing age. Abdominal obesity (WC ≥88cm) was observed in 20.8% women while 14.9% had WHR ≥0.85. Energy, fiber and micronutrient intakes such as vitamin A, riboflavin,

niacin, vitamin B6, vitamin C, folate, iron, zinc and calcium, were inadequate among women of childbearing age whereas carbohydrate and protein (plant) intakes were adequate.

The prevalence of low Sfol (< 3ng/ml) in women of childbearing age, before fortification, was 27.6%, while after fortification none of the women had low Sfol. Low RCF (< 164 ng/ml) was observed in 26.4% of subjects before fortification and in 1.9% of subjects after fortification. The prevalence of elevated *t*Hcy decreased significantly from 8.6% to 0% after fortification. However, the percentage of women with VB12 deficiency (< 145 pg/ml) increased from 6.3% during phase 1 of the study to 11.3% during phase 2. The percentage of women with low SF levels was similar before and after fortification (25%) as well as the percentage of women (100%) with low TIBC (< 44.7µg/dl). The prevalence of low levels of SIron increased from 19% to 21.6% and %TS increased from 11.9% to 16.7%, though not significantly. Percentage of subjects with low RBC and HGB were not significant different after fortification of foods.

Levels of fumonisins exceeded the tolerance level of 100-200ng/g in some of the morogo samples. Fumonisin B1 **(**FB1) (38860 ng/g), FB2 (6511 ng/g) and FB3 (1565 ng/g) were present in one sample of *Cleome Gynandra* (CG) whereas in the other sample of CG, FB1 was present at level 143 ng/g but neither FB2 nor FB3 was detected in this sample. In *Vigna Unguiculata* (VU), FB1 was detected at level 1601 ng/g and FB2 at level 518 ng/g. Some of the morogo samples collected were cooked in a mixture of 2 species combined. A level of 661 ng/g for FB1, was observed in a combined sample of

Amaranthus Viridis (AV) and CG. FB1 was present at level 143 ng/g for AV mixed with tomatoes.

CONCLUSION: Socio-demographic data showed high prevalences of singlehood, unemployment, larger household and limited use of electrical appliances. The maternity history of women of childbearing age showed cases of stillbirth, miscarriage and prenatal mortality.

Inadequate intake of energy, fat, dietary fiber and micronutrients were observed. Plant proteins, carbohydrate-rich foods and low intake of dairy products were common while consumption of vegetables and fruits was poor. A higher prevalence of underreporting using 24 hour recall questionnaire was shown compared to the quantitative food frequency questionnaire. On the other hand, the prevalence of overweight and obesity was high whereas underweight was relatively low.

Folate status of women of childbearing age improved significantly after consumption of fortified foods and high levels of homocysteine decreased significantly. Poor iron status as measured by serum ferritin was similar before and after fortification. Low levels of vitamin B12 observed before fortification increased significantly after consumption of fortified foods. Possible contamination of morogo, one of the mostly consumed green leafy vegetable and one of the major sources of folic acid, with mycotoxins (fumonisins), was observed.

ORIGINAL PAPERS (PUBLICATIONS)

1. **Modjadji SEP**, Alberts M, Mamabolo RL Folate and iron status of South African non-pregnant rural women of childbearing age, before and after fortification of foods. *South African Journal of Clinical Nutrition* 2007; **20 (2)**: 62-67.

2. A.M. van der Walt, E van der Linde, M Alberts, **P Modjajdi**, SD Jivan and CC Bezuidenhout. Fuminisins-producing *fusarium* strains and fumonisins in traditional African vegetables (Morogo), *South African Journal of Science* 2006; **102**: 151 – 155.

PRESENTATIONS AT CONFERENCES

1. **Research day,** Department of Health and Welfare, Polokwane; **11/2008 – AWARD "2nd position)** iron fortification of foods in South Africa: Is it effective in women of childbearing age?

2. **Academic day, University of Limpopo** (Medunsa Campus); **08/2008**- AWARD "Best postgraduate student paper" [1st place], Nutritional status of non-pregnant women of childbearing age in a rural South African area: An imperative assessment.

3. **Academic day, University of Limpopo** (Medunsa Campus); **08/2007-** AWARD "Best postgraduate student paper" [2nd place], Folic acid fortification of foods in South Africa: Is it effective in women of childbearing age?

4. **Research day,** Department of Health - (Limpopo Province) – **10/2005,** Folate and iron status of women of childbearing age, before and after fortification of foods in South Africa.

5. **IDIFA (Initiative for the Development of Indigenous Food of Africa)** Workshop, Potchefstroom, South Africa – **08/2005**, Exposure to mycotoxins (fumonisins) present in indigenous green leafy vegetables: possible effect on folate status.

6. **FSASP (Federation of South African Societies of Pathology)** Conference (Pretoria, South Africa) – **07/2005**, Assessment of folate status and related parameters before and after fortification of staple foods in non-pregnant women of childbearing age in a rural area.

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ABBREVIATIONS

%TS	- Percentage transferrin saturation
µg	- Microgram
µmol/µM	- Micromole
24 hr recall	- 24 hour recall
5MTHF	- 5-methyl tetrahydrofolate
AIDS	- Acquired immunodeficiency distress syndrome
ALB	- Albumin
ALP	- Alanine aminotransferase
ALT	- Aspartate aminotransferase
ANOVA	- Analysis of variance
AST	- Aspartate transaminase
AV	- *Amaranthus viridis*
BMI	- Body mass index
BMR	- Basal metabolic rate
Ca	- Calcium
CDC	- Center for Disease Control and Prevention
CG	- *Cleome gynandra*
CHO	- Carbohydrates
CHOL	- Cholesterol
CI	- Confidence Interval
CRP	- C-reactive protein

cSHMT	-Serine hydoxymethyltransferase
Dcytb	- Diheme plasma membrane protein
DMT1	- Divalent metal transpoter
DNA	- Deoxyribose nucleic acid
DRI	- Dietary reference intake
DSS	- Demographic Surveillance Site
E	- Energy
EAR	- Estimated average requirement
EDTA	- Ethylene diamine tetra acetate
EI	- Energy intake
FACS	- Food advisory consumer service
FAD	- Flavin adenine dinucleotide
FAO	- Food and Agriculture Organization
FB	- Fumonisins
FB1	- Fumonisin B1
FB2	- Fumonisin B2
FB3	- Fumonisin B3
FB4	- Fumonisin B4
FBC	- Full blood count
FDH	- 10-formyl tetrahydrofolate dehydrogenase
FL	- Femto litre
FiGlu	- Formiminoglutamic acid
FMN	- Flavin mono nucleotide

g	- Grams
GGT	- γ-glutamyl transferase
Glu	- Glutamic acid
GLV	- Green leaf vegetables
GRAS	- Generally recognized as safe
H	- Body height
HC	- Hip Circumference
HCT	- Haematocrit
HGB	- Haemoglobin
H-iron	- Haem iron
HIV	- Human immunodeficiency virus
HoloTC	- Holotranscobalamin
IDA	- Iron deficiency anemia
IF	- Intrinsic factors
IQ	- Inter quartile ranges
IU	- International Unit
IUGR	- Intra uterine growth retardation
K	- Potassium
kg	- Kilogram
kJ	- Kilo joule
km	- Kilometer
L	- Liter
LBW	- Low birth weight

m	- Meter
MCH	- Mean cell haemoglobin
MCHC	- Mean cell haemoglobin concentration
MCV	- Mean cell/corpuscular volume
mg	- Milligram
Mg	- Magnesium
ml	- Millilitre
MMA	- Methyl melonic acids
Mol	- Molar
MRC	- Medical Research Council
MS	- Methionine synthase
MTHF	- Methyl tetrahydrofolate
MTHFR	- Methenyl tetrahydrofolate reductase
MTHFS	- Methenyl tetrahyrofolate synthetase
MUFA	- Mono unsaturated fatty acids
n	- Number of subjects
Na	- Sodium
NaFeEDTA	- Sodium iron ethylene diamine tetra acetate
NFCS	- National food consumption survey
ng	- Nano-gram per milliliter
NH-iron	- Non Haem iron
NMDA	- N-methyl-D-aspartate
NRC	- National Research Council

NTDs	- Neural tube defects
p-value	- Level of probability
P	- Phosphorus
PLP	- Pyridoxial phosphate
PUFA	- Poly unsaturated fatty acids
QFFQ	- Quantitative food frequency questionnaire
RBC	- Red blood cell/count
RCF	- Red cell folate
RDA	- Recommended daily allowances
RR	- Reference range
SA	- South Africa
SAM	- S-adenosylmethionine
SCF	-Scientific committee on food
SF	- Serum ferritin
SFA	- Saturated fatty acids
Sfol	- Serum folate
SIron	- Serum iron
SPPS	- Statistical package for social science
Stats	- Statistics
TC II	- Transcobalamin II
*t*Hcy	- Total homocysteine
THF	- Tetrahydrofolate
TIBC	- Total iron binding capacity

TP	- Total protein
U	- Units
UK	- United Kingdom
UN	- United Nations
US	- United States
USA	- United States of America
VB12	- Vitamin B12
Vit	- Vitamin
VU	- *Vigna Unguiculata*
W	- Body weight
WC	-Waist circumference
WHO	- World Health Organization
WHR	- Waist hip ratio
Zn	- Zinc
β	- beta
$\chi 2$	- Chi-square test

CHAPTER 1

1.1 INTRODUCTION

The nutritional status of women of childbearing age during the preconception period is important and remains a prognostic indicator of pregnancy outcome (Sargent & Schulken, 1994; Opare-Obisaw, 2003; Yekta, 2005). During pregnancy, women are at a high risk of developing nutritional problems (e.g. undernourishment, obesity, anaemia and nutrient deficiencies) (Mattson, 1998; Costello & Osrin; Opare-Obisaw, 2003, Foo et al., 2004). Increased needs are due to reproductive functions such as a high demand of nutrients like folate and iron (Bailey, 1990; Opare-Obisaw, 2003, Sheridan et al., 2003). Therefore, the nutritional status of a non-pregnant woman is likely to provide a better picture of early gestational environment than are measurements taken during pregnancy because it is unaffected by the pregnancy-induced expansion of volume, which dilutes most biochemical parameters (Scholl & Johnson, 2000).

Maternal obesity adversely affects pregnancy outcomes primarily through increased rates of chronic hypertension, pre-eclampsia, diabetes (pregestation and gestation), caesarean section and infections. Also, obesity may be an independent risk factor for NTDs, fetal mortality and preterm delivery, and the risk of stillbirths and neonatal death (AMCHP, 2006). Undernourished women who become pregnant are susceptible to unfavourable pregnancy outcomes such as neural tube defects [NTDs], perinatal mortality, low birth weight (LBW), pre-eclampsia and pre-term delivery leading to increased neonatal mortality (Mattson, 1998; Manning et al., 2000; Opare-Obisaw, 2003; Costello & Osrin,

2003; WHO, 2006). There is a high incidence of LBW infants in developing countries and high early neonatal mortality rate among these infants (Costello & Osrin, 2003). Neonatal mortality is higher in south-eastern Asia (46 cases per 1000 live births) compared to Europe (6 cases per 1000 live births) and North America (4 cases per 1000 live births) (Costello & Osrin, 2003). In southern Africa neonatal mortality is estimated at 18 cases per 1000 live births compared to West Africa (54 cases per 1000 live births) and East Africa (41 cases per 1000 live births) (Costello & Osrin, 2003). In South Africa (Limpopo Province), the incidence of LBW has been estimated at between 8.8 to 10.9% (Mostert 2000; Mamabolo et al., 2004).

Globally, birth defects (mainly NTDs) constitute a significant health problem and in many developed countries these defects account for one in every four infants' deaths (Botto et al., 2005). A high incidence of NTDs has been reported in China (11.4 cases/1000 live births) and India (6 cases/1000 live births) (Moore et al., 1997; Kulkarni & Jose, 1997).

In South Africa, a high incidence of NTDs has been reported in a rural area of Transkei district in Eastern Cape Province (61.3/10000 live births) (Ncayiyana, 1986) and in rural areas of Limpopo Province (35.5/10000) (Venter et al., 1995). In contrast, low incidences of NTDs have been reported in black urban populations in Pretoria (9.9/10 000) (Delport et al., 1995), Johannesburg (11.8/10 000) (Kromberg & Jenkins, 1982) and Cape Town (5.5 to 10.6/10 000) (Cornell et al., 1983; Buccimazza et al., 1994). Prevalence of NTDs

has been found to be higher in rural black compared to uban black, which most likely reflects nutritional differences (Viljoen et al., 1995).

Suboptimal maternal folate status has been implicated as the cause of NTDs due to the role of folate in cell division (Bailey & Gregory, 1999; Wartanowicz et al., 2001). Numerous studies have associated the high incidence of NTDs with folate deficiency, prevalent among women of childbearing age (Rush 1992; Mills et al., 1995; Jacques et al., 1999; Krishnaswamy & Nair 2001; Hetrampft & Cortés, 2004; Bailey, 2004; Chen & Rivera, 2004). Researchers in South Africa implicated folate deficiency as the cause of high rates of NTDs in Limpopo Province where the prevalence of folate deficiency ranges from 21%-48% (Baynes et al., 1986; Bourne et al., 1993; Venter et al., 1995; Ubbink et al., 1999; Mamabolo et al., 2004). With the confirmed association between folic acid and NTDs, it is important that dietary intake of folic acid before conception and during early pregnancy be sufficient to prevent occurrence of NTDs.

Folate deficiency within the body can arise not only from a dietary shortage, but also from the cell's inability to absorb or use folate and is normally caused by several factors, including fumonisins (Stevens & Tang, 1997). Fumonisins (FB1, FB2, FB3 and FB4) are toxic fungal metabolites produced by members of the genus *Fusarium.* Researchers have reported that high consumption of these mycotoxins (FB1), interfere with fatty substances (i.e. sphingolipids) in the cell's outer surface, which are responsible for folate absorption, leading to folate deficiency (Merrill et al., 1993; Stevens & Tang, 1997). A high

concentration of fumonisins is found in the maize consumed by many populations (Marasas, 1996; Rheeder et al., 2002; Bakker et al., 2003).

A major concern is the growing of traditional green leafy vegetables (morogo), such as *Amaranthus* in maize fields. Morogo is usually consumed with staple foods in South Africa, and is likely to be contaminated by fumonisins (van der Walt et al., 2006). It has been suggested that some birth defects unexplained by other known risk factors, may be caused by exposure to FB1, the most significant fumonisin in terms of toxicity and occurrence (Stevens & Tang, 1997; Bakker et al., 2003). Fumonisins B has also been linked to various human diseases, including hepatotoxicity and forms of cancer (Ferguson, 2002; Carratuuu et al., 2003).

In addition to folate deficiency, iron deficiency is the most common nutritional deficiency worldwide and high prevalences have been reported in developing countries, including the poorer populations of Africa, Asia and Latin America (Kuvibidila et al., 1994; Hurrell et al., 2002; Makola et al., 2003). The World Health Organization (WHO) refers to iron deficiency as the most common cause of anaemia: one-half of women of reproductive age suffer from iron deficiency (CDC, 2002; Hurrell et al., 2002). Higher prevalences of anaemia in non-pregnant women have been reported in Africa (43%) and Asia (44%) compared with other countries such as North America and Europe where prevalence of anaemia is less than 20% (WHO, 2000). Iron deficiency anaemia has also been implicated as a cause of perinatal complications such as LBW, premature delivery and perinatal mortality as well as maternal mortality (Hurrell et al, 2002; Carley, 2003).

Globally, micronutrients deficiencies affect about two billion people and evidence of inadequate intake of micronutrients exists in rural South Africa (Baynes et al., 1986; Patel et al., 1992; Dannhauser et al., 1999; Steyn et al., 2001; Bopape, 2003; FAO, 2004; Mostert et al., 2005). Micronutrients deficiencies continue to contribute to the burden of mortality and morbidity in South Africa (Steyn et al., 2007). In 1999, the first National Food Consumption Survey (NFCS) was undertaken in South Africa in 1 to 9 year old children (Labadarios et al., 2000). This survey showed that many micronutrients including iron, zinc, calcium, vitamin B6, folate, niacin, riboflavin, vitamin A, C and E were deficient in the diet, particularly in children. In addition, studies in adults have shown similar micronutrient deficiencies and support the findings in children (Steyn et al., 2001; Grobbelaar et al., 2004).

The initiation of national food mandatory fortification by the Department of health follows the outcomes of NFCS. To improve the nutrient intake of populations, fortification of foods has been used in some industrialized countries for many decades but has only recently been applied in developing countries such as South Africa, Nigeria and Zambia (DOH, 2003; FACS, 2004; Haas & Miller, 2006). In South Africa, fortification of maize and wheat was officially mandated in October 2003 and nutrients added, include vitamin A, thiamin, riboflavin, niacin, folic acid, pyridoxine, zinc and iron (FACS, 2004).

The effectiveness of fortifying food with folic acid has been well described in the USA (Jacques et al., 1999; Castilla et al., 2003; Bailey, 2004; Hetrampft & Cortés, 2004). Evidence that improved folate status due to the bioavailability of synthetic folic acid

results in significant reduction of NTDs, has been reported (MRC, 1991; Czeizel & Dudas, 1992; Berry et al., 1999; Jacques et al., 1999; Honein et al., 2001; Persad et al., 2002; Bailey et al., 2003; Castilla et al., 2003; Bailey, 2004; Hetrampft & Cortés, 2004). However, evidence of the efficacy of iron fortification, reported from many developed and developing countries is inconclusive, mainly due to the different forms and levels of iron used as well as to other factors such as the presence of iron absorption inhibitors (Hallberg et al., 1986; Walter et al., 1993; Fairweather-Tait, 1997; Milman et al., 1994 & 1999; Osler et al., 1999; Foo et al., 2004; Small 2004; Andang'o et al., 2007).

The magnitude of problems relating to the nutritional status (dietary and anthropometric) of pregnant women has been described extensively in many populations and the effect of food fortification (Opare-Obisaw, 2003; Bailey, 2004; Hetrampft & Cortés, 2004). In South Africa research has focused on the nutritional status of pregnant black women (Baynes et al., 1986; Bopape, 2003; Mamabolo et al., 2004; Mostert et al., 2005). However, relatively few studies were conducted on the adult black population, included non-pregnant women of childbearing age (Bourne and Steyn, 2000; Steyn et al., 2001; Ladzani 2005). Since fortification of foods was introduced during the present study period, it was decided that an investigation be done on the effects of food fortification on folate and iron status. To our knowledge, there has not been a study in South Africa that assessed the effect of fortification of staple food on the biochemical folate and iron status of women of childbearing age in a rural population.

AIM OF THE STUDY

- Assessment of the nutritional status of non-pregnant women of childbearing age residing in a rural area of Limpopo Province; South Africa, and the effect of fortification of staple foods on their folate and iron status.

HYPOTHESIS

- Folate status will be low in non-pregnant rural women of childbearing age.
- Introduction of fortified staple foods improves the folate and iron status of women of childbearing age.

OBJECTIVES OF THE STUDY

- To assess the socio-demographic status and maternity history and their effect on the folate and iron status of non-pregnant rural women of childbearing age

- To determine the following anthropometric measurements; body weight, height, body mass index, waist, hip and waist-hip ratio and their association with selected nutrient intakes of non-pregnant women of childbearing age.

- To assess dietary intake of non-pregnant rural women of childbearing age using 24-hour recall and food frequency questionnaires, prior to fortification of foods and its association with selected biochemistry

- To determine the folate status of non-pregnant rural women of childbearing age by levels of serum and red blood cell folate, vitamin B_{12} and homocysteine, before and after fortification of foods.

- To determine iron status of non-pregnant rural women of childbearing age by full blood count, serum ferritin, iron, total iron binding capacity, percentage transferrin saturation and C–reactive protein (as an inflammatory marker), before and after fortification of foods.

- To determine albumin of women of childbearing age, before and after fortification as well as liver enzymes (ALP, ALT, AST and GGT) in order to assess the effect of mycotoxins.

- To assess the presence of mycotoxins (i.e. fumonisins) in randomly collected morogo samples from participants in Dikgale Demographic Surveillance Site.

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1.2 LITERATURE REVIEW

1.2.1 OVERVIEW

Adequate nutrient status is essential for several functions such as normal reproduction, growth and development (Mahan & Escott-Stump, 2000). Deprivation of essential nutrients over extended periods causes the poor nutritional status currently prevalent in developing countries, especially in South Asia and sub-Saharan Africa, including South Africa (Vorster et al., 1997; Navaneetham & Jose, 2005). Nutrient requirements and eating behaviors are determined by a large number of factors, including physiological, pathological, psychological, cultural and socio-economic (Vorster et al., 1997). Contributing to poor nutrient intakes are inadequate income levels to meet basic needs, poor food security, poor access to health care, poor environment and sanitation, and large family size (Navaneetham & Jose, 2005). Most critical, lack or low levels of education and nutritional knowledge among women in the households are risk factors for poor dietary intake (Molteno & Kibel, 1989; Weimer, 1999).

Poor nutritional status among women of reproductive age predisposes them and their newborns to poor health (Opare-Obisaw, 2003; Nojilana et al., 2006; Ravishakar, 2006). This is particularly true for women who have experienced protein-energy malnutrition and inadequate micronutrient intake before conception (Galloway, 1994). For these reasons, adequate nutritional status is an important element of reproductive health (MI, 2000).

1.3 ASSESSMENT OF NUTRITIONAL STATUS

Comprehensive information on nutritional status is obtained through a combination of methods including (1) anthropometric measurements (2) biochemical analyses, (3) dietary intake and (4) clinical symptoms (Vorster et al., 1997).

1.3.1 Anthropometric measurements

Anthropometric measurements are used to assess body size, proportions and composition, and reflect food intake, overall health and welfare, among other aspects. The basic anthropometric measurements are body weight, height, circumferences such as waist and hip, and skin folds (Vorster et al., 1997). From the body weight and height, body mass index (BMI) is calculated using the formula: BMI (kg/m^2) = weight (kg)/height $(m)^2$ (WHO, 1998). The waist/hip ratio (WHR) is calculated from waist and hip circumference (Seidel et al., 1988).

1.3.1.1 Overview of anthropometry of populations

In South Africa, 5.6% of women are underweight while 26.1% are overweight [BMI = 25 – 29.9 kg/m^2) and 30.1% are obese (Steyn & Nel, 2006) compared to other countries such as Ghana. In Ghana, women of childbearing age are commonly underweight (BMI < 19 kg/m^2) (GSS & MI, 1999). On the whole, 40.5% of black South African women, have a waist circumference greater than 88 cm while 32% have a WHR greater than 0.85 (Steyn & Nel, 2006). However, obesity (BMI > 30 kg/m^2) is more prevalent in developed countries and is on the increase in developing countries (Puoane et al., 2002; Ronnenberg et al., 2003).

The prevalence of overweight and underweight among women aged 20 – 49 years from 36 developing countries (Mendez et al., 2005) are as follows:

Table 1.1 Prevalence of overweight and underweight in some developing countries

COUNTRY	YEAR	%OVERWEIGHT	%UNDERWEIGHT
AFRICA			
Benin	1996	10.5	15.5
Burkina Faso	1992	5.4	15.5
Central African Republic	1994	5.6	16.7
Cameroon	1998	19.5	5.9
Cote d'Ivoire	1994	8.4	12.0
Ghana	1998	12.2	12.6
Kenya	1998	15.3	12.1
Madagascar	1997	3.6	21.5
Malawi	1992	10.5	9.2
Mali	1996	6.1	14.6
Namibia	1992	15.2	16.5
Niger	1998	4.5	19.6
Nigeria	1992	23.4	13.3
Senegal	1998	11.0	14.4
South Africa	1998	55.8	5.7
Tanzania	1996	11.4	9.6
Uganda	1995	9.4	9.8
Zambia	1996	11.5	9.9
Zimbabwe	1994	23.7	4.9
NORTH AFRICA & MIDDLE EAST			
Egypt	1995	46.6	1.8
Jordan	1997	63.0	1.8
Morocco	1992	27.9	4.5
Turkey	1998	65.6	1.5
CENTRAL ASIA			
Kazakhstan	1999	36.3	6.0
Kyrgyz Republic	1997	34.5	4.4
Uzbekistan	1996	26.1	7.4
EAST & SOUTH ASIA			
China	1997	15.2	6.1
India	1999	5.6	48.2
LATIN AMERICA & THE CARIBBEAN			
Bolivia	1998	47.1	0.6
Brazil	1996	33.0	9.3
Colombia	2000	51.4	2.1
Dominican Republic	1996	40.2	6.2
Guatemala	1998	42.6	1.6
Haiti	1994	8.0	20.8
Mexico	1999	58.6	2.2
Guatemala	2000	43.3	0.7

1.3.2 Biochemical analyses

Concentrations of biochemical parameters in blood, plasma/serum or urine give useful information on the dynamics of a particular physiological or pathological condition and also on nutritional status. However, there is no single biochemical variable that will reflect total nutritional status. A number of variables are generally used in conjunction with other measures of nutritional status such as anthropometry, nutrient intakes and clinical signs of deficiencies (Vorster et al., 1997).

1.3.2.1 Overview of the biochemical status of populations

Low levels of micronutrients have been reported in both developed and developing countries. Folate and iron deficiencies are more common in women of reproductive age and pre-school children (Baynes et al, 1986; UN, 1992; Kruger et al., 1994; Voster et al., 1997; Ubbink et al., 1999; Bopape, 2003; Mamabolo et al., 2004).

1.3.3 Dietary intake

Measurements and analyses of diets to obtain nutrient intake gives valuable information on nutritional status (Vorster et al., 1997). The nutrient requirements for humans varies by age, gender and ethnicity, and mean reference values have been established to ensure adequate nutrient intake in populations (NICUS, 2003). A requirement is defined as the lowest continual intake level of a nutrient that, for a specified indicator of adequacy, will maintain a defined level of nutrient intake in an individual (IOM, 2000). Recommended Dietary Allowance (RDA) is the average daily dietary nutrient intake level sufficient to

meet the nutrient requirement of nearly all (97 – 98 %) healthy individuals in a particular life stage and gender group (IOM, 2002).

1.3.3.1 Assessment of dietary intake

Four methods used to assess dietary intake are 24 hour recall, the quantitative food frequency questionnaire (QFFQ), diet history and diet records (Margetts & Nelson, 1997; Vorster et al., 1997). Nutrient intake data depends to a large extent on the methods used to obtain information on dietary habits and intakes (Vorster et al, 1997). A survey of South African literature on dietary intakes showed that the 24hr recall method was used in larger surveys while QFFQ or diet history methods were used in smaller studies with a limited number of subjects (Vorster et al, 1997).

(a) 24-hour recall – is the most commonly used method of obtaining information about an individual's intake and is useful as a screening tool. An individual is asked to describe the types and amounts of food eaten in the previous 24 hour period. Nutrient intake is often under estimated and one recall may not represent a typical day's intake of an individual. This method is commonly used in populations with low literacy levels.

(b) Food frequency – In this method, an individual is asked to identify the foods eaten in a specified amount of time. This method often overestimates an individual's intake. It is useful as a screening tool for assessing the dietary intakes of groups of people and uses the least amount of interview time.

(c) Diet history - this entails a detailed interview with an individual and provides information about eating history and usual pattern of food intake. A diet history can be used in combination with 24hr recall, food record, or a QFFQ questionnaire, to obtain a fair picture of an individual's nutritional intake.

(d) Diet records – this method requires a subject/observer to record all foods offered to be eaten. To provide accurate intake information, foods should be weighed or measured and methods of food preparation described. It is considered the most accurate method and can be used to validate other methods. However, this method is expensive and requires literacy (Margetts & Nelson, 1997; Nutrpeds, 2001).

See Table 2.1 in Chapter 2 (Methodology) for details on advantages and disadvantages of dietary methods.

1.3.3.2 Overview of dietary intakes of populations

In developing countries, there are high prevalences of mineral deficiencies such as iron, zinc and iodine, particularly in young women (Mudalige & Nestel, 1996). In South Africa, several studies have reported evidence of poor nutrient quality with regard to energy, animal proteins and micronutrients, particularly folate, vitamin B6, vitamin C, vitamin D, iron and calcium (Badenhorst et al., 1993; Mostert et al., 2005; Steyn et al., 2001; Steyn & Nel, 2006). Mean intakes of niacin are generally low in South Africa whereas thiamin is on the borderline (Bopape, 2003; Mostert et al., 2005; Steyn & Nel,

2006). In addition, intakes of riboflavin are low in South Africa as well as in other parts of Africa, Guatemala, China and India.

1.4 MICRONUTRIENTS

(FOLATE, VITAMIN B12 AND IRON)

Micronutrient (vitamins and minerals) malnutrition is primarily related to inadequate dietary intake. Dietary surveys in developing countries have consistently shown that multiple micronutrient deficiencies are common and that low dietary intake and poor bioavailability of micronutrients account for the high prevalence of these multiple deficiencies (MI, 2000).

1.4.1 FOLATE

Folate exists in a number of different chemical forms and is also called folic acid. The chemical structure of folate is shown in Fig. 1 and comprises of pteridine, p-aminobenzoic and glutamic acid (Steegers-Theunissen, 1995; Bailey, 2004; Ziegler, 1996; Stover, 2004). The natural occurring folates consist of a large group of derivatives caused by removal or addition to the basic structure. Folates are available as small molecules with one to three side chains, i.e. mono and oligoglutamates, which are free folates, and as larger molecules, i.e. polyglutamates (Steegers-Theunissen, 1995; Stover, 2004).

The prime function of folate is to provide one-carbon moieties for the synthesis of three of the four DNA bases, i.e. guanine, adenine and thymine, as well as for synthesis of

other compounds. The one carbon moiety is derived from the conversion of serine into glycine, formiminoglutamic acid (FiGlu) into glutamic acid (Glu), or the cleavage of glycine (Steeger-Theunissen, 1995).

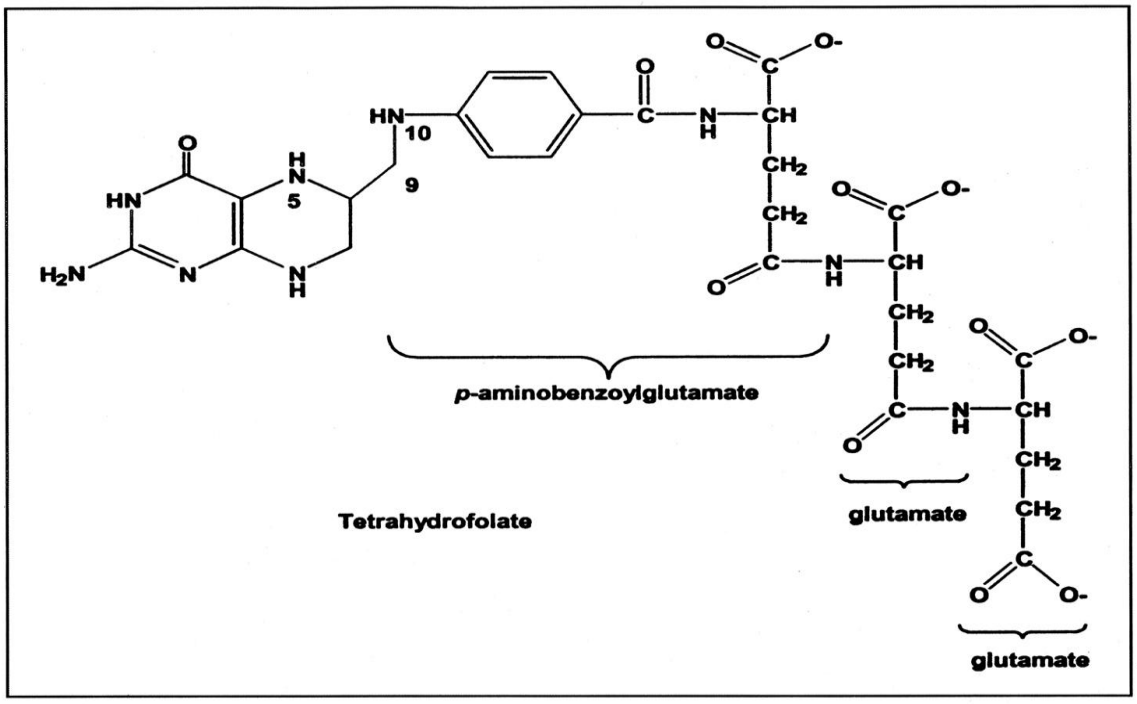

Fig. 1.1 Structure of tetrahydrofolate triglutamate (Stover, 2004)

1.4.1.1 Absorption of folate

The absorption process of dietary folate is divided into two steps. In the first step, polyglutamates are converted to monoglutamates by a conjugase present the in small intestine (jejunum). Only monoglutamates, or at most oligoglutamates, can be taken up by the intestinal cells. The second step includes absorption by active and passive transport of monoglutamates into the intestinal cells. In the intestinal cells, the absorbed monoglutamates are fully reduced to tetrahydrofolate (THF) by dihydrofolate reductase. Depending upon the folate supply, the monoglutamate is transported directly into the portal circulation, converted to 5-methylTHF (5MTHF), or converted directly into THF-

polyglutamate stores by the activity of the enzyme folylpolyglutamate synthase (Steegers-Theunissen, 1995; Stover, 2004).

The stored THF-polyglutamates can be degraded to THF-monoglutamates by intracellular conjugase and released into the circulation. 5MTHF is the predominant form of folate in serum and in many tissues. In the liver, polyglutamate derivatives of 5MTHF form the major storage molecules. About two-thirds of 5MTHF in serum is loosely bound to a non-specific, low affinity folate binder, such as albumin. A second folate binder has also been described. Although this binder has a high affinity for folate, its capacity to bind folate is low (Steergers-Theunissen, 1995; Stover, 2004).

1.4.1.2 Assessment of folate status

Folate is present in all body tissues and stored to the greatest extent in the polyglutamate form in liver, pancreas, kidneys and brain. Free folate and polyglutamates together represent total folate (Steegers-Theunissen, 1995). Folate status may be evaluated from serum/plasma, red blood cells (RBC) levels and dietary folate intake (Bailey 1990). The folate content of circulating RBC is a better index of folate status and reflects general tissue supply and represents long-term tissue stores, whereas plasma folate reflects recent intake (Krishnaswamy & Nair, 2001; Stanger, 2002). It is generally accepted that values of serum folate and RBC folate above 6.2ng/ml and 158ng/ml, respectively, are satisfactory (Krishnaswamy & Nair, 2001).

1.4.1.3 Metabolism of folate

Folate metabolism is required for the synthesis of purines, thymidylate and methionine (Fig. 2) (Stover, 2004). The principal function of folate coenzymes is to accept or donate one-carbon units in key metabolic pathways, and the polyglutamyl form of tetrahydrofolate (THF) serves as the central folate acceptor molecule in the one-carbon cycle. THF is converted to 5, 10-methylene-THF employing the 3-carbon of serine as a major carbon source, a crucial first step in the cycle. This one-carbon unit is transferred from serine to THF via pyridoxal phosphate (PLP)-dependent serine hydroxymethyltransferase (SHMT) to form 5, 10-methylene-THF and glycine (Bailey & Gregory, 1999). A portion of the 5, 10-methylene-THF produced undergoes irreversible enzymatic reduction to the methyl oxidation state, 5-methyl-THF, and the enzyme responsible for the conversion is methylene-THF reductase (MTHFR) (Bailey & Gregory, 1999). The N-5 methyl group of 5-methyl-THF can only be used metabolically in its transfer to homocysteine, which results in the (re) generation of methionine.

MTHFR plays a key role in one-carbon metabolism by converting methylene-THF to 5-methyl-THF, thus irreversibly directing this one-carbon moiety to the methylation of homocysteine to methionine synthesis by methionine synthase. Approximately 50% to 80% of the homocysteine generated is remethylated, depending on the dietary content of methionine and choline. In the methionine synthase reaction, a methyl group is removed from 5-methyl-THF, which functions as a substrate, and is sequentially transferred to the vitamin B-12 coenzyme before homocysteine, thus forming methionine. In addition to protein synthesis, methionine serves as a methyl group donor through conversion to 5-

adenosylmethionine (SAM), a key biological methylating agent involved in more than 100 methyltransferase reactions with a wide variety of acceptor molecules. The methionine synthase reaction also regenerates THF required for the formation of 5, 10-methylene-THF and 10-formyl-THF used directly in thymidylate and purine synthesis, respectively. In the thymidylate synthase reaction, 5, 10-methylene-THF donates its CH_2 unit (becoming the thymidine methyl group) (Bailey & Gregory, 1999).

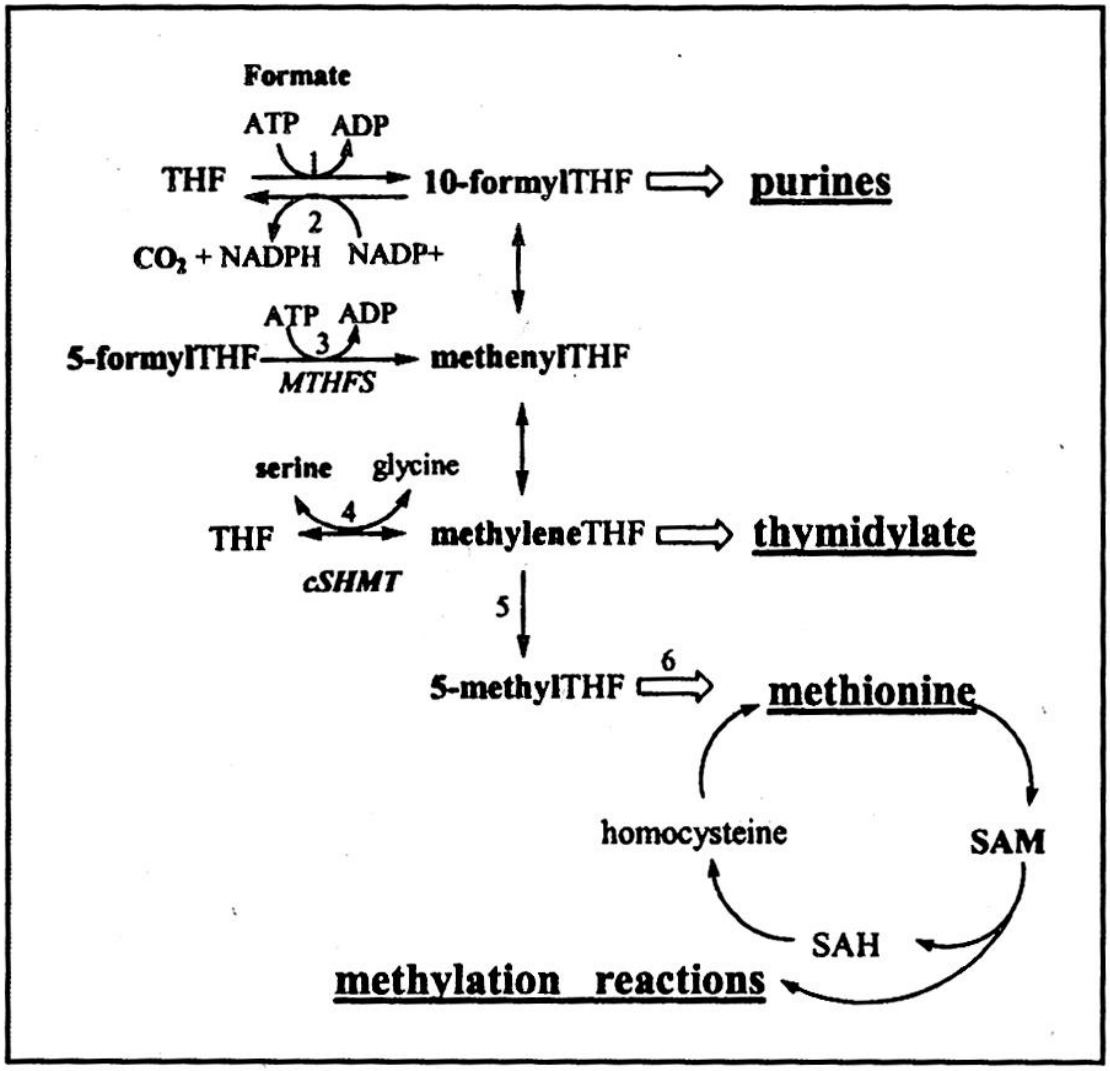

Fig. 1.2 Folate metabolism. Folate metabolism is required for purine, thymidylate and methionine biosyntheses. Methionine is converted to SAM, which donates methyl groups for other one-carbon transfer reactions. (1) 10-formylTHF synthetase, (2) 10-formylTHF dehydrogenase (FDH), (3) methenylTHF synthetase (MTHFS), (4) serine hydroxymethyltransferase (cSHMT), (5) methyleneTHF reductase (MTHFR), (6) methionine synthase (MS) (Stover, 2004)

1.4.1.4 Folate requirements

Sufficient intake of folic acid is essential to reduce the risk of chronic illness and birth defects (Bailey & Gregory, 1999). Folic acid is an important nutrient, especially for women of childbearing age (Burndage, 2002). In 1992, CDC recommended that all women of reproductive age consume 400μg of synthetic folic acid to prevent birth defects, which was reaffirmed and clarified by the Food and Nutrition Board of the Institute of Medicine (MMWR, 2004; Tanne, 2005). While a daily intake of 400μg has been suggested for non-pregnant women, pregnant women are expected to consume 600μg due to the rapid rate of maternal and fetal cell growth and development (Caudill et al., 1998; Berry et al., 1999; Bailey, 2000).

1.4.1.5 Dietary sources of folate

Food sources of folic acid include enriched grain products such as breakfast cereals, bread, pasta and rice, and natural sources such as dark green leafy vegetables and legumes, liver and kidney (Bailey & Gregory, 1999; Bailey et al., 2001; Burndage, 2002). Before fortification of foods, dark green leafy vegetables were considered the most accessible food source of folate frequently consumed with staple foods especially in the rural areas (Nesamvuni e al., 2001; Steyn et al., 2001; van der Walt et al., 2006), as well as meat, cereals, fruits and vegetables in the urban areas of South Africa (Charlton et al., 1997). To date, in South Africa, food sources of folic acid include all maize and wheat foodstuffs fortified with folic acid (1.50 mg/kg) (FACS, 2004).

Most naturally occurring folates are present as polyglutamates and are lost in storage and cooking, whereas the synthetic folic acid found in supplements and fortified foods exist as monoglutamates and exhibit greater bioavailability than naturally occurring folate (Steegers-Theunissen, 1995; Stover, 2004).

1.4.1.6 Folate deficiency

Folate deficiency can arise in a variety of settings such as low dietary intake, increased demands during growth periods, pregnancy and lactation, malabsorption, conditions of hemolysis such as hemolytic anaemia and malignancy such as leukemia (Krishnaswamy & Nair, 2001). Serum folate levels between 3ng/ml – 6.2ng/ml and RBC folate between 141ng/ml – 158ng/ml are considered to be low and suggestive of risk, hence values lower than these levels indicate clear deficiency of folate (Krishnaswamy & Nair, 2001).

Folate deficiency occurs in four sequential stages. In the first stage of early negative balance, a serum folate level of 3ng/ml indicates negative folate balance but does not provide information regarding body stores when evaluated alone; the second stage of negative folate balance is tissue depletion, which may be assessed by measuring red cell folate levels, and levels below 140ng/ml indicate reduced tissue stores; the third stage is observed through an abnormal deoxyuridine suppression test showing biochemical evidence of folate depletion. The test first becomes abnormal in the bone marrow cells, followed by the peripheral blood lymphocytes; the final stage shows impaired synthesis of DNA resulting in neutrophil hypersegmentation and the red or white cells become oval. In this stage mean cell volume (MCV) is increased and haemoglobin (HGB) concentration falls below normal (Herbert, 1987a).

In the presence of folate deficiency all reactions in one-carbon metabolism are compromised to varying degrees depending on the affinities of the enzyme for the folate molecules involved (Finkelstein, 1998). When reactions of one-carbon metabolism are affected by folate deficiency, various substrates and metabolic intermediates will accumulate and may have negative consequences (Boushey et al., 1995).

- Prevalence and consequences of folate deficiency

Deficiency of folate in women of childbearing age ranged from 19% to 31.4% in Costa Rica and 22% in South California, prior to fortification of foods (Jacques et al., 1999; Chen & Rivera, 2004). In India, folate deficiency during different gestational periods ranged from 40.5% to 53.3% (Raman et al., 1989; Neela & Raman, 1997). In South Africa (Limpopo Province), the prevalence of folate deficiency ranged from 10.3% to 48% in pregnant women (Baynes et al., 1986; Mamabolo et al., 2004). Ubbink *et al.* (1999) affirmed a folate deficiency of 21% in non-pregnant women.

After fortification of foods, significant improved folate status was observed among women of childbearing age in several countries such as Chile, Southern California and Costa Rica (CDC, 1999; Jacques et al., 1999; Caudill et al 2001; Ray et al., 2003; Hertrampft et al., 2003; Chen & Rivera, 2004; Hertrampft & Cortés, 2004;). The above authors attributed the improved folate status to the highly bioavailable synthetic folic acid found in fortified foods.

Clinical manifestations of advanced folate deficiency are macrocytic or megaloblastic anaemia and elevated levels of homocysteine (Krishnaswamy & Nair, 2001). In addition,

five known inherited disorders of folate metabolism causing deficiency include; 5,10-methylene-THF reductase deficiency; functional 5MTHF- homocysteine methyltransferase synthase (MS); glutamate formiminotransferase deficiency; hereditary malabsorption; dihydrofolate reductase deficiency. These inherited disorders, lead to megaloblastic anaemia, peripheral neuropathy, apnoea, speech abnormalities and developmental delays (Steegers-Theunissen, 1995).

The presence of folate deficiency during pregnancy increases the risks of neural tube defects (NTDs), Down's syndrome, spontaneous abortion, abruptio placenta and pre-eclampsia (Scholl et al., 1996; Bailey & Gregory, 1999; Stanger 2002). However, the role of folate deficiency in other birth outcomes such as reduced birth weight, pre-term delivery and perinatal mortality is unclear (Scholl & Johnson, 2000).

- *Folate deficiency: a risk factor for birth defects*

The role of folic acid in reducing the rate of NTDs has been well established (Wald et al., 2001). Folic acid deficiency is teratogenic and the mechanisms that are suggested to cause births defects (i.e. NTDs) include defective biosynthesis or methylation of DNA or elevated levels of homocysteine (Christensen & Rosenblatt, 1995).

1.4.1.7 Neural tube defects (NTDs)

NTDs are common congenital malformations that occur when the embryonic neural tube, which ultimately forms the brain and spinal cord, fails to properly close during the first few weeks of development. NTDs are among the most common of all human birth

defects, yet their etiological basis and embryology remain poorly understood. Numerous clinical studies indicate that NTDs are of a multifactorial origin, having both genetic and environmental components (Hendricks, 1999; MRC Vitamin Study Research Group, 1991; Finnell et al., 2000; Genelineau-van Waes & Finnell, 2001). The most common NTDs are anencephaly and meningomyelocele (Lemire, 1988).

- Prevalence of NTDs before fortification

There is a significant geographic variation in the prevalence of NTDs worldwide (Krishnaswamy & Nair, 2001) and incidence of NTDs varies among different ethnic and socio-economic groups (Reider, 1994).

In the general population of the USA, incidence of NTDs is less than 3 cases per 10 000 live births (Cifuentes, 2002). Some regions have strikingly high frequencies, such as 106 cases per 10 000 live births in Guatemala (Quetzaltenango), where the most frequent defect was myelomeningocele (Cifuentes, 2002). In the Texas-Mexico border, elevated NTDs rates have been noted, ranging from 27 cases/10 000 live births in 1990 -1991 to 15 cases/10000 live births few later. This rate is 3 to 5 times higher than those observed elsewhere in the USA (Hendricks et al., 1999). High incidence of NTDs the above mentioned regions is attributable to high consumption of maize/corn with possibility of contamination with fumonisins (Hendricks et al, 1999).

Countries of the British Isles are known to have high incidence rates of NTDs, as well as China and India (Lemire, 1988; Leech & Payne, 1991; Moore et al., 1997; Kulkarni &

Jose, 1997). Anencephaly is common in Northern Ireland and Wales (63.8 to 109/10000 births) (Lemire, 1988). The incidence rate of NTDs in China is estimated at 114 cases/10000 live births with higher rates in the rural areas of Northern China (57 - 73 cases/10 000) of NTDs (Lian et al., 1987; Moore et al., 1997). In India, cases of NTDs are estimated at 60 cases/10000 live births (Leech & Payne, 1991; Kulkarni & Jose, 1997).

In South Africa, a high incidence of NTDs has been reported in a rural area of Transkei district in Eastern Cape Province (61.3/10000 live births) (Ncayiyana, 1986) and in rural areas of Limpopo Province (35.5/10000) (Venter et al., 1995). In contrast, low incidences of NTDs have been reported in black urban populations in Pretoria (9.9/10 000) (Delport et al., 1995), Johannesburg (11.8/10 000) (Kromberg & Jenkins, 1982) and Cape Town (10.6/10 000) (Buccimazza et al., 1994). Very low incidence of NTDs has been reported in the mixed ancestry population of Cape Province; 7.1/10000 live births (Singer et al., 1979), Cape Town; 8.0/10000 live births (Cornell et al., 1983) and 9.8/10000 live births (Buccimazza et al., 1994). NTDs rates in the white population of South Africa have been reported to be 31.8/10000 (Singer et al., 1979), 36.0/10000 live birth (Cornell et al., 1983) and 25.4/10000 live births (Buccimazza et al., 1994).

Primary prevention of certain serious birth defects is now feasible globally. The groundbreaking studies of Smithells and colleagues, (1976; 1980; 1983), confirmed by other studies and randomized trials (MRC Vitamin Study Research Group, 1991; Czeizel & Dudas, 1992; Berry et al., 1999; Botto et al., 1999), showed that supplements

containing folic acid can reduce spina bifida and other NTDs by 80% or more when consumed before conception. Data from clinical trials support the hypothesis that reduction in risk may be specifically attributable to folic acid (Werler et al., 1993; Green, 2002). In a few countries; US, Canada, Chile and South Africa, recommendations to consume folic acid supplements are integrated with a policy of wide-spread fortification of flour and the effectiveness of this population-wide approach has been proven (Honein et al., 2001; Ray et al., 2002; Castilla et al., 2003).

In addition to the possible causes of folate deficiency, it is believed that the conversion of polyglutamate to a simpler form, monoglutamate, is impaired in genetically susceptible women (March of Dimes, 2000), which might explain why the reduction in the incidence is not 100%. An ecological study in South Africa conducted in twelve public hospitals in four provinces reporting rates of NTDs and other birth defects before and after fortification showed a significant decline in the prevalence of NTDs following folic acid fortification in South Africa (Sayed et al., 2008).

1.4.2 OTHER CAUSES OF NTDs

1.4.2.1 Dietary fumonisins

Given that folic acid deficiency is the best know risk factor for NTDs known at present, some birth defects unexplained by other known risk may be caused by the exposure to fumonisins (Stevens & Tang, 1997). Fumonisins are a family of mycotoxins that were first isolated in South Africa in 1988 from cultures of *Fusarium verticillioides* (formerly

Fusarium moniliforme) strain MRC 826, followed soon thereafter by elucidation of the structure of the isoforms fumonisin B1 (FB1) and B2 (FB2) (Gelderblom et al., 1988; Bezuidenhout et al., 1988). The fumonisins; FB1 and FB2, together with FB3, occur most abundantly in contaminated maize (Shepard et al., 1996). The joint FAO/WHO Expert Committed on Food Additives (2002) indicated that the maximum tolerable daily intake of fumonisins was 2 µg/kg body weight.

Therefore, if high intake of fumonisins due to contaminated food, is a risk factor for NTDs in humans, this association might be most evident among populations that consume the highest amounts of maize, such as those of Central and South America and parts of southern Africa and Asia (Ncayiyana, 1986; Dombrink-Kurtzman & Dvorak, 1999). Recently, a major concern is the contamination with fuminisins in traditional leafy vegetables (i.e. morogo) of rural communities of South Africa (van Der Walt et al., 2006).

1.4.2.2 Implications of fumonisins as a cause of NTDs

Recent evidence suggests that fungal toxins including fumonisins can be teratogenic, at least in part, through interference with the utilization of folic acid (Kellerman et al., 1990). A potential link between fumonisins, folate deficiency and an increased risk for NTDs seems plausible based on findings that demonstrated that receptor-mediated folate uptake was reduced by up to 50% in Caco-2 cells pretreated with fumonisins (Stevens & Tang, 1997; Chatterjee et al., 2001).

The placental, high affinity folate transporter is a glycosylphosphatidylinositolinositol (GPI)-anchored protein associated with membrane microdomains enriched in cholesterol and sphingolipids, which is critical for embryonic development (Luhrs & Slomiany, 1989; Brown & London, 1998; Piedrahita et al., 1999). Fumonisin affects the transporter by altering both its endocytic trafficking (Chatterjee et al., 2001) and the amount of the receptor that is available for transport. These findings provided a conceptual framework whereby exposure to fumonisin B1 (FB_1) might be a risk factor for NTDs by disrupting folate utilization via depletion of cellular sphingolipids needed for normal receptor function (Marasas et al., 2004).

1.4.2.3 Contamination of foods with fumonisins

Contamination of traditional maize-based human foodstuffs with fumonisins has been reported in South Africa, Canada, China, Egypt, Peru and Switzerland (Zhen et al., 1984; Sydenham et al., 1991; Stack et al., 1992). The greatest contamination of maize with fumonisins may be expected in countries with a dry, warm climate (e.g. Zimbabwe, Egypt) while the lowest would be expected in countries with cool, damp climates (e.g. Canada, New Zealand) (Nair, 1998). Fungal and mycotoxin contamination is affected by temperature and humidity, and occurs during crop growth, harvesting or storage (Turner et al., 1999).

In rural settings, green leafy vegetables (morogo) usually grow in proximity to homegrown maize, which is considered a likely source of fusarial contamination. Morogo is frequently consumed with grain-based staple diets, especially in the rural communities

of South Africa (van der Walt et al., 2006). The common occurrence of fumonisin-producing *Fusarium* strains (i.e. *F. verticillioides, F. proliferatum*, *F. oxysporum* and *F subglutinans)* have been well documented worldwide, including African countries such as Ghana, South Africa and Zimbabwe (Kpodo et al. 2000; Gamanya & Sibanada, 2001; Rheeder et al.,, 2002; Fandohan et al., 2003).

1.4.2.4 Absorption, distribution, metabolism and excretion of fumonisins

In all animal species studied, fumonisins are poorly absorbed from the digestive tract and are rapidly distributed and eliminated. The liver and the kidney retain most of the absorbed fumonisins (FB_1 mainly), and FB_1 persists longer in these organs than in plasma. Very low concentrations of FB1 have been recovered from the uterus and placenta of animals (rats and rabbits) but cannot be found in the fetus, indicating that there is no placental transfer (Bolger et al., 2001).

Although fumonisins are not metabolized by cytochrome P450 enzymes, FB_1 can alter the activity of these enzymes through mechanisms that alter sphingolipid biosynthesis. Fumonisins are structurally related to the sphingoid base. Removal of the tricarboxylic acid side chains, presumably by the microbial flora of the gut, converts FB_1 into a substrate for ceramide synthase. The product of the enzyme reaction is an inhibitor of the enzyme in vitro, like FB1 (Bolger et al., 2001).

1.4.2.5 Assessment and control of fumonisins

Toxicological studies have shown that liver and kidney are the target organs to assess the effect of FB1 since they retain most of the absorbed FB1 (Bogler et al., 2001). However,

human dietary intake of chemicals is usually estimated by combining data on concentrations of chemical in different food products and the consumption rate of these products (Kistemaker et al., 1998). Because fumonisins do not persist for long in the plasma due to poor absorption in the digestive tract, assessment of fumonisin contamination in food samples associated with contamination are usually used to analyse the fumonisin species (Meredith et al., 1999; Dombrink-Kurtzman, 1999).

1.4.2.6 Consequences of fumonisins contamination on human health

Epidemiological, animal and cell culture studies have linked dietary fumonisins B produced by grain-associated *Fusarium* strains to various human diseases including cancer, liver and kidkney disease (Guarro et al., 2000; Vismer et al., 2002; Dignani & Anaissie, 2004). However, only three genera of fungi producing mycotoxins are implicated in human health; Aspergillus, Fusarium (producing fumonisins) and Penicillium (Turner et al, 1999). Early signs of toxicity in the kidney are increased in free sphingo bases, renal tubule-cell apoptosis and cell regeneration (Bogler et al., 2001, SCF, 2000).

In China (Henan Province), with a high frequency of *Fusarium moniliforme* contamination the incidence of ooesophageal cancer is high. Fumonisins have been reported in Mexican tortillas (Dombrink-Kurtzman & Dvorak, 1999) and high rates of NTDs are major health concerns (Hendricks, 1999). In South Africa, contamination of FB1 (in maize) was reported in the Transkei (Ncayiyana, 1986; Rheeder et al., 1992). In this area, maize is frequently visibly mouldy and mouldy maize is used for homebrewed

beer because of the unique flavour the mould imparts (Norred, 1993). In the entire South Africa, Transkei has the highest prevalence of NTDs and ooesophageal cancer (Ncayiyana, 1986; Rheeder et al., 1992).

Experimental animal studies on the effect of FB1 reported several health consequences that might also affect human health. Those consequences include reduction in maternal body weight gain, maternal hepatotoxicity and lethality, increased embryonic resorptions, reduced offspring body weight and foetal malformations such as cleft plate, hydrocepahulus, malformed ribs, and incomplete digital and sutural ossification. FB1 also induces leukoencepholomalacia and pulmonary oedema (Marasas et al., 1988; Harrison et al., 1990). Association of FB1 with NTDs has been suggested in areas with corn/maize based diet such as Texas-Mexico (Hendricks et al., 1999).

1.4.3 HOMOCYSTEINE

Homocysteine is a sulfur-containing, nonproteogenic amino acid and was originally identified as biologically important in 1932 (Medina et al., 2001; Tighe et al., 2005). Homocysteine is biosynthesized from methionine, an essential amino acid involved in multiple fundamental biological processes including synthesis of proteins. Interest in homocysteine increased after the recognition of an inborn error in methionine metabolism, which resulted in homocystinuria, a condition characterized by excessive levels of homocysteine in the blood and urine. Later, the most common cause of

homocystinuria was identified as a deficiency of the enzyme cystathionine β-synthase (Tighe et al., 2005).

1.4.3.1 Homocysteine in plasma

Human plasma contains both reduced and oxidized species of homocysteine. The thiol group of homocysteine allows it to form a disulfur bond with other homocysteine molecules (leading to formation of homocystine), or with free cysteine or with albumin. The oxidized form of homocysteine is the major fraction and the reduced homocysteine represents not more that 1% of total plasma homocysteine. The sum of all the forms of homocysteine existing in plasma are usually called total homocysteine (Jacobsen, 1998; Medina et al., 2001).

Protein bound homocysteine represents 75% of total homocysteine and the remaining homocysteine exist as a free oxidized fraction (~30%) (Ueland & Refsum, 1989; Ueland et al., 1997). A rapid equilibrium exists between free and protein-bound homocysteine fractions *in vivo*. Thus transitorily increased free homocysteine, induced by an increase in homocysteine export or by methionine loading, becomes progressively bound to plasma protein in a redistribution which takes place in less than 24 hours (Ueland & Refsum, 1989).

Normal concentrations of total homocysteine in plasma are in the range of 5 – 16 µmol/L, although 10µmol/L should be considered the desirable upper limit (Medina et al., 2001). A level above 16 µmol/L represents hyperhomocysteinemia which is defined in three

ranges: moderate (16- 30µmol/L), medium (30 - 100µmol/L) and severe (>100µmol/L) hyperhomocysteinemia, reaching values as high as 500 µmol/L in patients with homocystinuria (Medina et al., 2001).

1.4.3.2 Homocysteine metabolism

The most important vitamins involved in homocysteine metabolism are folate, vitamin B12 (cobalamin) [Fig 1.3], vitamin B6 (pyridoxial-5'-phosphate) and riboflavin [Fig. 1.4] (Hustad et al., 2000; Medina et al., 2001; Brauer & Tierney, 2004).

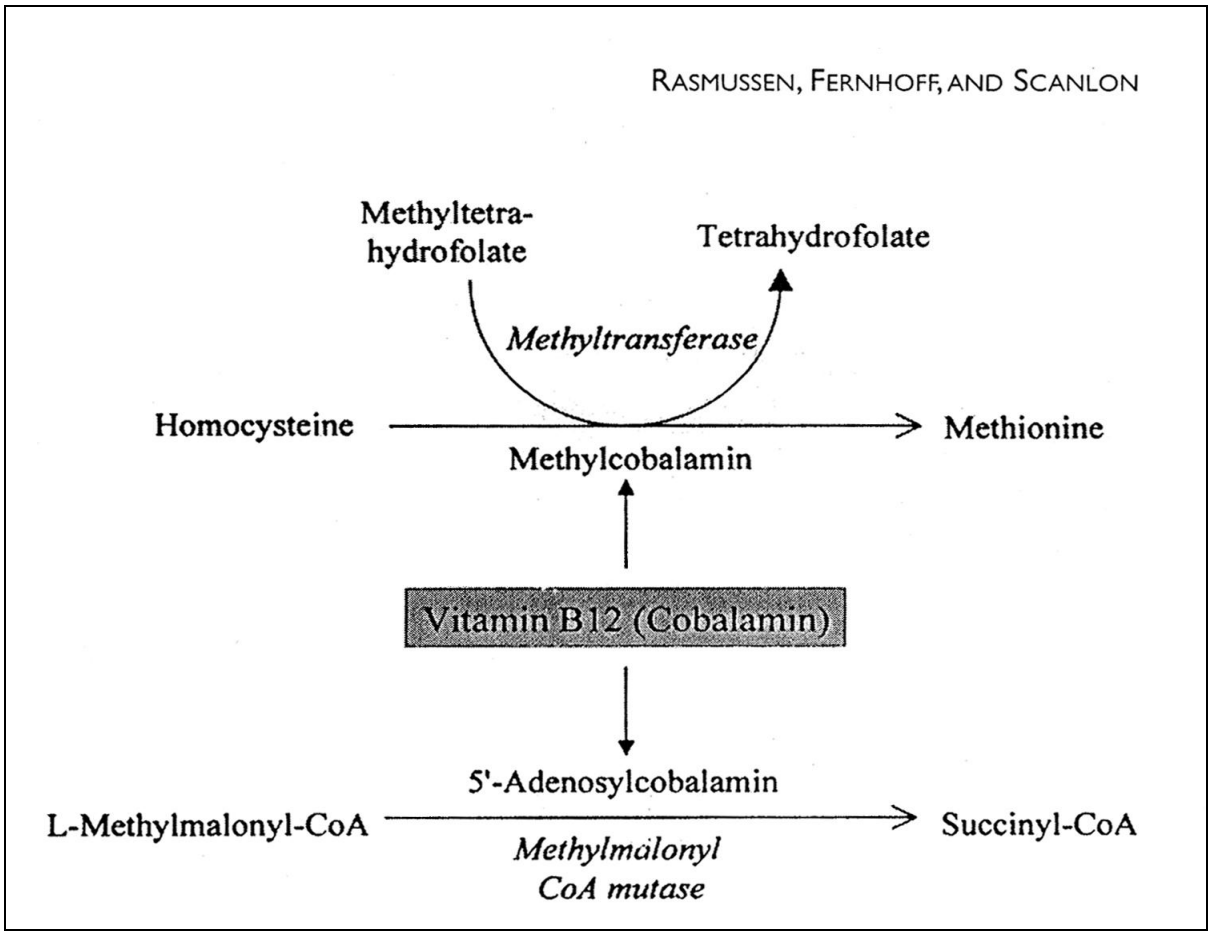

Fig 1.3 Vitamin B12 as a cofactor in homocysteine metabolism

Homocysteine is an important intermediate in one-carbon metabolism. Intracellular homocysteine is either converted to cysteine by cystathionine β–synthase, which requires pyridoxal 5'-phosphate (PLP; vitamin B6) as a cofactor and through the trans-sulfuration pathway or remethylated to methionine through the remethylation pathway (Fig 1.4). In most of the tissues, the remethylation pathway is catalysed by the enzyme methionine synthase, which requires cobalamin as a cofactor and 5-MTHF as a methyl donor. This explains why folate and cobalamin are major determinants of plasma homocysteine. The formation of 5-MTHF is catalysed by the enzyme 5,10-MTHFR (Allen et al., 1994; Hustad et al., 2000; Tighe et al., 2005).

Riboflavin is the precurssor of flavin mononucleotide (FMN) and FAD, which serve as cofactor for enzymes involved in the metabolism of vitamin B6, folate and cobalamin. FMN serves as a cofactor for pyridoxine-5'phosphate oxidase, which is important for the formation of the active form of vitamin B6, pyridoxial-5'-phosphate, whereas FAD is a cofactor for MTHFR (Hustad et al., 2000; McNulty et al., 2002). Both flavin coenzymes are involved in cobalamin metabolism and serve as cofactors for methionine synthase reductase. The role of flavoenzyme in the metabolism of several B vitamins points to the possibility that riboflavin status may influence homocyteine metabolism and thereby plasma *t*Hcy concentration (Hustad et al., 2000).

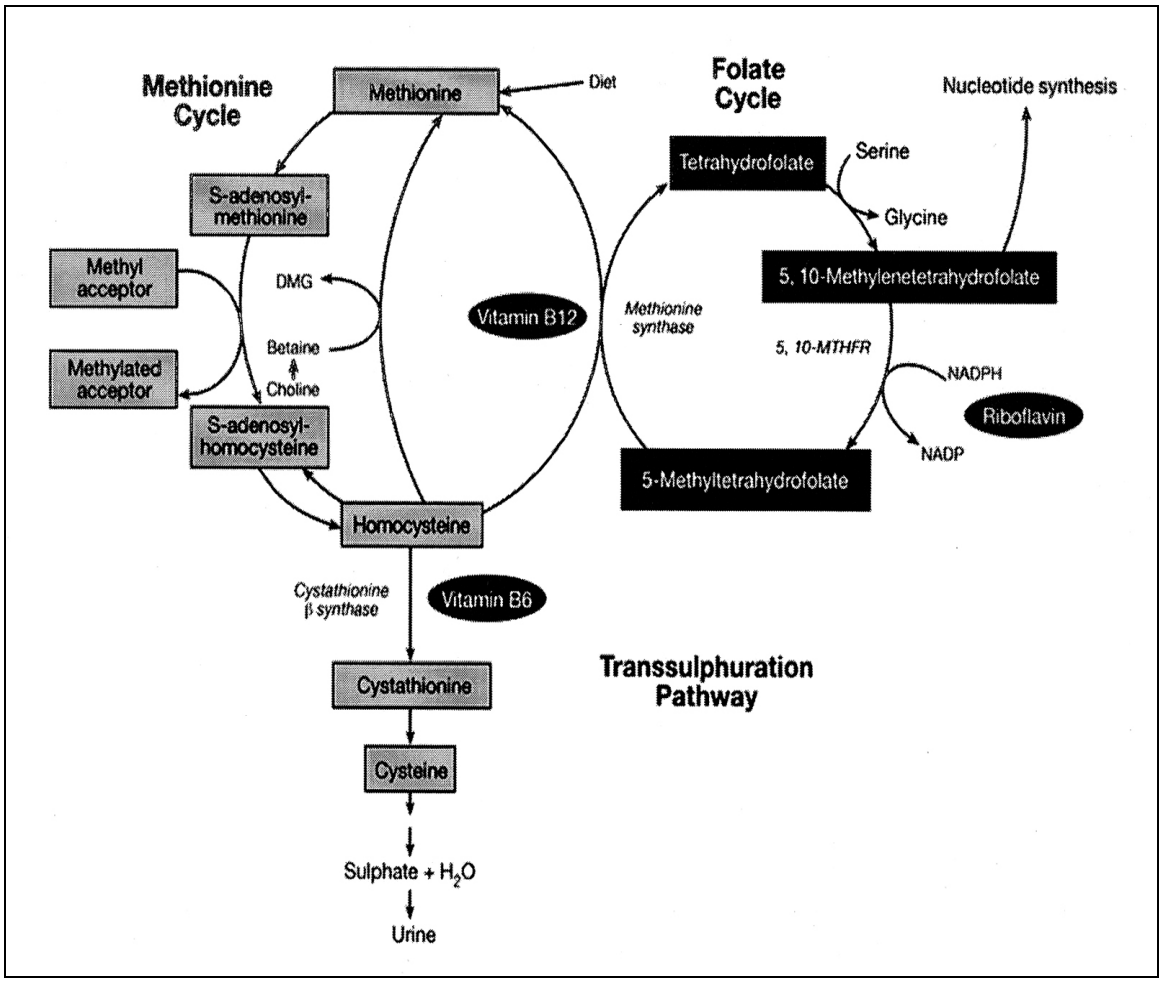

Fig. 1.4 Pathways of homocysteine metabolism (Tighe et al., 2005)

1.4.3.3 Impairment of homocysteine metabolism

Homocysteine metabolism is tightly regulated by the different affinities of methionine synthase and β–synthase for homocysteine (Finkelstein, 1990). Methionine synthase has a low K_m value for homocysteine while β–synthase has a high K_m value for homocysteine. Thus, at low homocysteine concentrations, methionine conservation is favored while at high homocysteine concentrations immediate and long-term drainage of homocysteine via the transsulfuration pathway is ensured (Finkelstein, 1990). As such, abnormal elevations of homocysteine in plasma and urine are the result of increased

levels of homocysteine export, and this reflects an imbalance between homocysteine production and metabolism (Medina et al., 2001).

1.4.3.4 Factors affecting homocysteine levels

Factors influencing plasma homocysteine levels include demographic, genetic and physiological factors, as well as acquired determinants, nutrition, disease, transplants and medication (Medina & Amores-Sánchez, 1999). Of interest are congenital and nutritional disorders, as well as renal failure, causing hyperhomocysteinemia (Medina et al., 2001).

There are many congenital metabolic disorders involving the homocysteine-methionine cycle that can contribute to elevated levels of homocysteine and reduced levels of methionine. The most common causes of elevated levels of homocysteine involve enzymes MTHFR and MS. Two polymorphisms have been found in the MTHFR enzyme gene. A nucleotide point mutation C677T, is the most common genetic defect for mild hyperhomocysteinemia. In this change, a single nucleotide results in valine substituting for alanine in the enzyme thereby generate a thermolabile variant with decreased enzyme activity. In the second MTHFR nucleotide point mutation, A1298C, changes amino acid glutamic acid to alanine (Frosst et al., 1995).

There are many distinct gene mutations described for cystathionine β-synthase that are inherited as recessive traits which can ultimately increase both homocysteine and methionine serum levels. Congenital hyperhomocysteinuria due to cystathionine β–synthase deficiency in homozygotes has an incidence rate of 1/200 000 and leads to the

most severe form of elevated homocysteine with serum levels exceeding 100μM/L (Mudd et al., 1985; Brauer & Tierney, 2004).

Nutritional disorders leading to the impairment of homocysteine regulation include vitamin B12, vitamin B6, folate and riboflavin deficiencies, as these are required as cofactors for enzymes in the homocysteine cycle. When there is a nutritional deficiency of folic acid, levels of the methyl donor 5'-methyl THF are insufficient to provide the carbon units needed to methylate homocysteine to methionine. With low levels of vitamin B12, there is also reduced activity of the cobalamin-dependant enzyme methionine synthase. Cystathionine β–synthase is also dependant on vitamin B6 as a cofactor and nutritional deficiency of vitamin B6 increases both homocysteine and methionine serum levels. Medication that interferes with folate metabolism (e.g. anticonvulsant) and vitamin B6 (oestrogen-containing oral contraceptives) can also cause levels of homocysteine to rise (Young & Blass, 1982; Masse et al., 1996; Hankey & Eikelboom, 1999; Hustad et al., 2000; Van Ede et al., 2002; Desouza et al., 2002).

1.4.3.5 Studies on homocysteine and the effect of fortification

A multi-ethnic study of healthy subjects in Los Angeles reported that black African and Asian Americans have slightly lower mean homocysteine concentrations compared to whites (Carmel et al., 1999). Low homocysteine levels have also been reported in Latin American women compared to men (Carmel et al., 1999). Bangladeshis have higher levels of homocysteine and genetic and dietary factors are implicated in this population (Obeid et al., 1998).

In South Africa, data on the effects of folic acid on the homocysteine status of women of childbearing age is scanty, however several studies has been conducted among men prior to fortification periods. Vermaak et al (1991) reported significantly lower concentrations of homocysteine in black South African men exposed to a Western diet for two years compared to whites (46% difference). In contrast, a study of Ubbink et al (1995) reported no significant difference of fasting plasma homocysteine between black and white South Africans. However, the mean maximum increase measured after methionine loading was significantly lower in blacks than in white subjects.

Several studies reported a significant reduction of homocysteine levels in participants after fortification of foods with folic acid, indicating an adequate folate status (Ward et al.,1997; Schorah et al., 1998; Caudill et al., 2001; Wald et al., 2001; Rydlewicz et al., 2002).

1.4.3.6 Consequences of elevated levels of homocysteine

In the last decade of the 20th century, the link between NTDs and disordered homocysteine metabolism was established (Steegers-Theunissen et al., 1991). Subsequently, it was shown that the risk of spina bifida is higher among infants of mothers with upper quartile homocysteine values (Mills et al., 1995).

Epidemiological and experimental studies have shown that certain *N*-methyl-D-asparatate (NMDA) receptor antagonists are associated with congenital defects of neural crest and neural tube derivatives, and that homocysteine may act as an antagonist of the NMDA

type of glutamate receptor (Kim & Pae, 1996; Rosenquit et al., 1996). Based on these data, it has been hypothesized that the mechanism of homocysteine teratogenicity may involve its ability to inhibit the activity of the NMDA receptor (Andaloro et al., 1998).

There is increasing evidence in the last few years linking hyperhomocysteinemia to obstetrical complications other than NTDs. These complications include the placental-mediated diseases such as recurrent early pregnancy loss, pre-eclampsia, abruption placenta and spontaneous abortion (Ray & Laskin, 1999; Vollset et al., 2000; Hague, 2003). There are inconsistencies in the mechanism of homocysteine and other pregnancy disorders. However, March of Dimes (2000) reported that it appears that high levels of homocysteine damages blood vessels in the placenta, whose function is to nourish and protect the fetus. Placental vessel impairment is known to cause preeclampsia and fetal growth restriction, and that may cause premature labor and delivery, which are a leading cause of pre-term birth. Several malformations and obstetric complications associated with homocysteine have been investigated in relation to the TT genotype of MTHFR 677C>T polymorphism, and weak associations with placenta-mediated disease, IUGR and LBW have been reported (Ray & Laskin, 1999; Nurk et al., 2004).

During the past few years, elevated blood levels of homocysteine have been linked to increased risk of premature coronary artery disease, stroke and thrombo-embolism even among people who have normal cholesterol levels (Barret, 2003). Elevated levels of homocysteine appear to contribute to atherosclerosis in at least three ways; a direct toxic effect that damages the cells lining the inside of arteries, interference with clotting

factors, and oxidation of low density lipoproteins (Barret, 2003; AHA; 2009). In the brain, elevated homocysteine levels are neurotoxic leading to hyperactivation of N-methyl-D aspartate receptors and apoptosis whereas in the vascular system increased levels increase proliferation of smooth muscles cells, increase platelet aggregation and the number of strokes and white matter lesions (Garcia & Zanibbi, 2004). According to the trecent statement by American Heart Association (AHA) high homocysteine level in the blood is a major risk factor for cardiovascular disease (AHA; 2009).

1.4.4 VITAMIN B12 (COBALAMIN)

Vitamin B12 is the generic name for a water-soluble group of cobalt-containing corrinoids with biological activity in humans. The body contains active cobalamins (mainly hydroxy-methyl-cobalamin and 5'-deoxyadenosyl-cobalamin), but also variable numbers of vitamin B12 analogues which are biologically inactive non-cobalamin corrinoids. Cyanocobalamin is normally not present in humans, except in very small amounts, especially in smokers (van Den Berg, 1993; Fairfield & Fletcher, 2002).

1.4.4.1 Dietary sources and recommended intake of vitamin B12

Humans cannot synthesize vitamin B12, hence a dietary supply is required. Vitamin B12 occurs naturally in animal tissues and dietary sources are meat, poultry, fish, milk, cheese and eggs (Scott, 1997; Fairfield & Fletcher, 2002).

In rural populations in South Africa, the major sources of vitamin B12 are not in the top 10 commonly eaten foods, however, eggs and chicken feature in the top 20 of food items (Vorster et al., 1997; Nesamvumi et al., 2001; Steyn et al., 2001), whereas in the urban populations, meat and milk are the most frequently consumed sources (Charlton et al., 1997). The RDA of vitamin B12 intake for women of childbearing age is 2.4µg/day (NICUS, 2003). The bioavailability of dietary vitamin B12 is estimated to be approximately 50% (IOM, 2001). On the basis of hematological evidence and serum B12, the EAR intakes for vitamin B12 are estimated at 2µg/day for men and women aged 19 – 50 years. The RDA for adult men and women age 19 years and above is 2.4µg/day. It is recommended that pregnant women consume 2.6µg/day (Bailey, 2004).

1.4.4.2 Absorption of vitamin B12

The absorption of vitamin B12 from foods is a complex process (Markle, 1996; Herrmann & Giesel, 2002). Vitamin B12 in food is released by the action of gastric acid and proteolytic enzymes in the stomach, where it is preferentially captured by haptocorrin, an R-binder protein present in the saliva and stomach. The haptocorrin-cobalamin complex is then degraded in the upper small intestine by a pancreatic enzyme and the alkaline pH. The released vitamin B12 is recaptured by another protein, the intrinsic factor (IF). In the terminal ileum, this complex is recognized and internalized by specific membrane receptors of the enterocytes and the absorption by this process is limited to less than 3µg/meal (Carmel, 1985).

In addition to this process of absorption, evidence supports the existence of an alternate system that is independent of IF or even an intact terminal ileum. Approximately 1% of a large oral dose of vitamin B12 is absorbed by this second mechanism. This pathway is important in relation to oral replacement (Elia, 1998).

Once in the enterocytes, the complex is degraded and vitamin B12 is transferred to a third binding protein, transcobalamin II (TC II) and the newly formed complex enters the portal circulation and is recognized by specific receptors present on all cell types. Part of vitamin B12 bound to TC II is called holotranscobalamin (holoTC) (Carmel, 1985). About 6-20% of total plasma vitamin B12 is present in the active form bound to TC II. The remaining major part of vitamin B12 is bound to transcobalamin I and II and it is supposed that this fraction is involved in transporting redundant vitamin B12 from cells back to the liver. Most of vitamin B12 is stored in the liver (Herrmann et al., 2003). Therefore, interruption of one or any combination of these steps of absorption, places a person at risk of developing vitamin B12 deficiency (Oh & Brown, 2003).

1.4.4.3 Importance of vitamin B12

Vitamin B12 functions primarily as a coenzyme in intermediary metabolism, especially in transmethylation reactions (C1-transfer), which are the thymidylate synthase reaction and methionine synthase reaction (Finkelstein, 1990; van Den Berg, 1993; Herbert, 1994). It also acts as a coenzyme in fat and carbohydrate metabolism (Fairfield & Fletcher, 2002). Together with folate and iron, vitamin B12 is involved in erythropoiesis;

a process by which haemopoetic tissue of bone marrow produces red blood cells (Koury&Ponka,2004).

1.4.4.4 Causes and stages of vitamin B12 deficiency

Causes of vitamin B12 deficiency are divided into three classes: nutritional deficiency, malabsorption syndromes and other gastrointestinal causes (Snow, 1999). The multifactorial causes of vitamin B12 deficiency include achlorhydria and decreased secretion of IF, chronic disease and inflammation, chronic polypharmacy, gastro-intestinal bleeding as well as poverty, physical inability to prepare food, alcoholism and inadequate dietary intake (Charlton et al., 1997). Major infectious organisms causing vitamin B12 deficiency includes bacterial *Helicobacter pylori* (a very common infection in populations living in poor sanitary conditions), the parasite Giardia and an overgrowth of bacteria in the upper intestine (Allen, 2001). Congenital transport-protein deficiencies, including TC II deficiency are rare cause of vitamin B12 deficiency (Oh & Brown, 2003).

Vitamin B12 deficiency can be divided into four stages. In stages 1 and 2 of vitamin B12 deficiency, the plasma and cell stores become depleted. A low plasma level of holoTC indicates these stages, and additionally in stage 2, a low level of holohaptocorrin. Stage 3 is characterized by functional imbalances and in this stage in addition to lower holoTC, increased plasma levels of homocysteine and methylmalonic acids (MMA) are observed. In stage 4 of vitamin B12 deficiency clinical signs become recognizable such as macro-ovalocytosis, elevated mean corpuscular volume (MCV) of erythrocytes or lowered haemoglobin (Herbert et al., 1990; Herbert et al., 1994; Dharmarajan et al., 2001).

1.4.4.5 Clinical assessment and diagnosis of vitamin B12 status

Assessment of vitamin B12 status has traditionally been based on low serum vitamin B12 levels along with clinical evidence of disease. Furthermore, measurements of metabolites such as methylmalonic acid and homocysteine are also early markers for tissue vitamin B12 deficiency, even before hematologic manifestations occur (Lindenbaum et al., 1990; Savage et al., 1994). However, elevated serum methylmalonic acid concentration is considered the most sensitive and specific indicator of vitamin B12 status in contrast to either low serum vitamin B12 or elevated homocysteine concentration (Lindebaum et al., 1994; Allen et al., 1994).

The diagnosis of vitamin B12 deficiency is based on the presence of typical neuropsychiatric symptoms associated with megaloblastic anaemia or macrocytosis and low serum vitamin B12. However, in the absence of anaemia and macrocytosis, difficulties are encountered if the serum vitamin B 12 concentration is borderline or in the presence of a purely psychiatric syndrome (Healton et al., 1991; Reynolds, 2006). If serum vitamin B12 concentration is equivocal, a raised plasma homocysteine or methylmalonic concentration confirms the presence of significant deficiency (Koury & Ponka, 2004). Vitamin B12 deficiency often presents as folate deficiency and therefore is commonly misdiagnosed. Elevated intakes of folate interfere with the clinical diagnosis of vitamin B12 deficiency by ameliorating vitamin B12 deficiency-induced megaloblastic anaemia, but do not impact on the irreversible progression of the neurological dysfunction and cognitive decline that result from vitamin B12 deficiency (Stover, 2004).

1.4.4.6 Intake of Vitamin B12

Inadequate intake of vitamin B12 is common in the vegans, elderly and alcoholics. Because this vitamin is found only in animal products, many poor populations, and populations that avoid animal products for religious or other reasons, consume little of vitamin B12 (Antony, 2001, 2003).

Adequate intake of vitamin B12 has been reported in the United States, Canada and Europe (Russel, 1992; Howard et al., 1998; Wolters et al., 2003). The average dietary vitamin B12 intake in the US is estimated to be approximately 5µg/day. Serum vitamin B12 concentrations are reported to be significantly lower in elderly population groups compared to younger groups (Allen et al., 1994; Lindenbaum et al., 1994; van Asselt et al., 1996; Wright et al., 1998). Studies in South Africa (Limpopo Province) have reported adequate intakes of vitamin B12 among children (Mamabolo et al., 2006), adolescent (Bopape, 2003), adult population (Steyn et al., 2001) including elders (Kruger et al., 1993; Charlton et al., 1995; 2001) and women of reproductive age (Mostert, 2000). However, inadequate intake of vitamin B12 still exists among some children, adolescent and elders (Steyn et al., 1990; Ladzani, 2005).

1.4.4.7 Vitamin B12 status, worldwide

Vitamin B12 deficiency exists in both developed and developing countries (Patel & Pettifor, 1992; Kruger et al., 1994; House et al., 2000). Studies among low-income people in Guatemala, Mexico, Nepal and Venezuela reported that 25 – 50% of individuals are deficient (Allen, 2001; Stabler & Allen, 2004). High prevalences of

vitamin B12 deficiency have been reported in Guatemala elders (38%) and in Indonesia (third of elders) (King et al., 1997; Juguan et al., 1999). Vitamin B12 status is a significant health problem in India due to both lacto vegetarianism and the expense and scarcity of meat (Antony, 2001, 2003). Vitamin B12 deficiency is also a major problem in northern Pakistan where a 56% prevalence of megaloblastic anaemia due to low intake of this vitamin has been reported (Modood-ul-Mannan et al., 1995).

Until recently, vitamin B12 deficiency was thought to be rare in Africa, despite the awareness that many diets were deficient in animal products (Stabler & Allen, 2004). The following prevalences of vitamin B12 deficiency have been reported among pregnant women; Malawi (49%) (van Den Broek & Letsky, 2000), Tanzania (Hinderaker et al., 2002) and Zimbabwe (13%) (Allain et al., 1997). In South Africa (Limpopo Province), vitamin B12 deficiency is estimated at 16.4% among women of childbearing age (Mamabolo et al., 2004) and ranges from 3% - 16% among South African elders (Charlton et al., 1997; Ladzani, 2005).

1.4.4.8 Consequences of vitamin B12 deficiency

Vitamin B12 deficiency has been implicated as one of the causative factors of NTDs in newborns. This was validated by the findings that showed increased levels of MMA in women carrying NTD-affected fetuses (Steen et al., 1998). Although the vitamin B12 deficiency-NTDs mechanism is not clear, it has been suggested that the deranged vitamin B12 metabolism and/or transport, may be the pathophysiologic mechanism involved in development of NTDs (Steen et al., 1998).

The classical disorder of malabsorption of vitamin B12 is pernicious anaemia, an autoimmune disease that affects the gastric parietal cells and destruction of these cells limits the production of IF and subsequently limits vitamin B12 absorption (Oh & Brown, 2003).

1.4.4.9 Vitamin B12 status and folic acid fortification

Presently, vitamin B12 does not form part of fortification of foods worldwide, except in the UK where it is included in voluntary fortification of several breakfast cereals to benefit vegans (BNF, 2004).

There is a major concern that folic acid fortification may mask symptoms of vitamin B12 deficiency, primarily in the elderly population (Allen et al., 2004). Several studies have associated low vitamin B12 with cognitive impairment and anaemia which usually become worse after the administration of high doses of folate (Savage et al., 1994; Savage & Lindebaum 1995). In the presence of vitamin B12 deficiency, high consumption of folic acid is harmful to the nervous system, but can improve anaemia (Reynolds, 2006). Consequently, a diagnosis of vitamin B12 deficiency becomes delayed allowing the severity of neurological impairment to progress with rising serum folate concentrations (Savage et al., 1994; Reynolds, 2006). It has been reported that the inverse correlation between the degree of anaemia and of neurological disability in vitamin B12 deficiency might be linked to the effect of folic acid on the blood and nervous system in the presence of vitamin B12 deficiency. In the presence of vitamin B12 deficiency, folic acid is harmful to the nervous system but can improve anaemia (Reynolds, 2006).

Consistent with these studies, recent reports have acknowledged the possibility that some sectors of the populations might suffer adverse affects from folic acid fortification (Ramos et al., 2005; Morris et al., 2005; 2006). Although, the findings are clear about the adverse effects of high folic acid intake in persons with low vitamin B12, challenging questions are being asked concerning the mechanism of interaction, causes of anaemia and cognitive impairment (Smith, 2007), which are not comprehensible at the moment.

Morris et al (2005) reported that an increased risk of cognitive decline in elderly people who consumed folic acid in doses > 400µg/d. However, an interaction between folate intake and vitamin B12 intake was observed such that the cognitive decline was less marked in those who also took high dose vitamin B 12-containing supplements. Other recent reports showed that subjects with a normal itamin B12 status, high serum folate (>59 nmol/l) was associated with protection from cognitive impairment. Similar results have been reported for Latinos living in California where higher red cell folate concentrations after fortification were associated with protection from cognitive impairment and dementia (Ramos et al., 2005; Morris et al., 2007). However, is subjects who had a low vitamin B12 status were much at risk of cognitive impairment and anaemia (Morris et al., 2007).

1.4.5 IRON

Iron is a trace element mostly present in the red blood cells as haemoglobin, in muscles as myoglobin and as iron containing enzymes. Functions of iron include involvement in energy metabolism, gene regulation, cell growth and differentiation, oxygen binding and

transport, muscle oxygen use and storage, enzymatic reactions, neurotransmitter synthesis and protein synthesis (CDC, 1998; Provan, 1999; Beard, 2001).

1.4.5.1 Dietary sources of iron

Dietary iron exists in two chemical forms called haem and non-haem. The non-haem form is present in plants (cereals, pulses, legumes, vegetables and fruits), muscle tissue and other animal products like eggs, milk and milk products, and it is stored in liver whereas haem iron is found in food sources such as meat, poultry and fish (Hallberg et al., 1997; FAO/WHO, 1998). Haem iron is more effectively absorbed than non-haem iron, as it is unaffected by enhancers or inhibitors which affects non-haem absorption (Beard & Tobin, 2000). The amount of ingested iron that enters the body beyond the intestinal cell defines iron bioavailability (FAO/WHO, 1988). Diets poor in haem iron and enhancers such as vitamin C provide little biovailable iron, which is the case in most populations in developing countries (Fernández-Ballart, 2000), including South Africa.

1.4.5.2 Absorption of iron

Iron enters the body by way of specialized transport mechanism in epithelial cells on the tips of the villi in the duodenum, and two pathways are involved (Skikne & Baynes, 1994).

Haem iron is taken up intact and then released from the porphyrin ring by haem oxygenase. All other forms of iron are transported into the cell by the divalent metal transporter (DMT1, previously named Nramp2 and DCT1), which is a proton symporter

that transports ferrous iron and other divalent metals. The iron released from haem and the iron imported by DMT1 enters a common pathway (Lynch, 2000). Most of the iron stored in ferritin is lost when the mucosal cell exfoliates. The mechanisms responsible for transport through the mucosal cell have not been identified, but a putative basolateral iron transporter, ferroportin 1 (also called Ireg 1 or MTP 1) has been discovered. Coexpression of hephaestin, a cytosolic or transmembrane copper protein and ceruloplasmin, is required for the efficient transfer of iron out of the enterocyte and for binding to transferrin (Lynch, 2000).

Regulation of iron absorption occurs primarily during entry of iron into the cell and at the time of exit into the body where it becomes bound to transferrin for delivery to various organ systems. For non-haem iron, uptake by the mucosal cells is the primary site of regulation (Nathanson & McLaren, 1987). When iron requirements are high, increased quantities of DMT1 are found on duodenunal enterocyte surfaces. Dietary iron absorption is increased approximately fourfolds once the iron stores are totally depleted (Lynch, 2002). Hepatocytes serve as a storage reservoir for iron, taking up dietary iron from portal blood and at times of increase demanded, releasing iron into circulation by way of ferroportin (Fleming & Bacon, 2005).

Iron absorption is the critical factor affecting iron homeostasis and the rate at which iron is absorbed is affected by the body's iron stores, the level of erythropoetic activity in bone marrow, the haemoglobin concentration, the blood oxygen content, and the presence or absence of inflammatory cytokines. More than one of these factors may act

simultaneously and some are interrelated (Fleming & Bacon, 2005). Excess iron absorption relative to body iron stores is the hallmark of hereditary hemochromatosis (Fleming & Bacon, 2005).

1.4.5.3 Assessment of iron status

Serum ferritin, plasma iron, serum transferrin receptors, haemoglobin and mean cell volume are the most commonly used laboratory measurements of iron status (Cook et al., 1992). Serum ferritin measures iron reserves and is therefore a better index of iron sufficiency than of iron deficiency. The serum ferritin levels are especially useful for monitoring long-term changes in the iron replete segment of populations. Once iron stores are fully depleted, further decline in body iron is accompanied by a reduction in the concentration of plasma iron, one of the earliest measurements of iron status. Plasma iron is usually measured in combination with transferrin, its specific plasma transport protein. Transferrin is often determined in the laboratory as total iron binding capacity (TIBC), which is the amount of added iron that can be specifically bound by transferrin (Cook et al., 1992; WHO/CDC, 2004).

There are marked effects of age and sex on serum ferritin levels, which reflect physiological variations in iron status. Serum ferritin is relatively high in newborns but falls rapidly to the iron deficient range during the first few months of life as iron stores are mobilized for the expanding red cell mass. The concentration increases slowly throughout childhood until late adolescence, but males' values increase roughly 3-times higher than in women. Serum ferritin levels remain lower in women during their

childbearing years, then rises through the fifth and sixth decades following menopause, a period of more favorable iron balance (Cook et al., 1992).

Ferritin is also a positive acute phase reactant and increases in the presence of various acute or chronic disease conditions (Torti & Torti, 2002; Chen et al., 2006). Elevated serum ferritin levels have been found in many chronic inflammation-related diseases (Kalantar-Zadeh et al., 2004; Chen et al., 2006). Inflammatory cytokines have been shown to induce ferritin synthesis in experimental models (Torti & Torti, 2002). Thus, serum ferritin may be an unreliable indicator of iron stores when there is infection or inflammation (Kalantar-Zadeh et al., 2004; Mandato et al., 2005).

Since the largest proportion of body iron is contained in blood, laboratory measurements to detect evidence of reduced haemoglobin formation in circulating red cells are important in the detection of overt iron deficiency. Hence, changes in circulating red cells provide useful information about iron status in nutritional surveys even in the absence of anaemia (Cook et al., 1992). A reduction in the size of circulating red cells, as shown by MCV, is a reliable index of reduced haemoglobin synthesis. Decreased ferritin levels illustrate iron deficiency and in pregnancy this is often accompanied by anaemia resulting in iron deficiency anaemia (Cook et al., 1992). Haemoglobin concentration is the most reliable indicator of anaemia at the population level as opposed to clinical measures which are subjective and therefore have more room for error (WHO, 2008). According WHO (2008), the haemoglobin threshold used to define anaemia for non-pregnant women is 120 g/L (12 g/dL).

1.4.5.4 Deficiency of iron and its consequences

Iron is stored in the body as the soluble protein complex ferritin and/or the insoluble protein complex hemosiderin, in the liver, bone marrow, spleen and skeletal muscle (CDC, 1998; Burke et al., 2001). The initial stage, iron depletion, occurs when stored iron in the bone marrow diminishes due to insufficient supply of iron. Generally this stage is asymptomatic, creates no overt effect on erythropoiesis, and escapes detection by haemoglobin or haematocrit screening (Pediatric Nurs, 2003). There is a net negative iron balance (Burke et al. 2001). Continued iron store depletion leads to the second stage, iron deficiency, during which storage levels become substantially reduced and haemoglobin synthesis begins to be affected. There is a difference between the body's need and the amount of iron actually stored. The final stage, iron deficiency anaemia, develops when iron stores are insufficient to maintain haemoglobin production. This advanced stage will be reflected in low haemoglobin and haematocrit values. Iron deficiency anaemia is present when haemoglobin concentrations of females over the age of 18 years are below 10.5 g/dl (FAO/WHO, 1988; CDCP, 1998; Burke et al., 2001; Pediatric Nurs, 2003).

The etiology of iron deficiency is attributable to poor diets that are low in iron bioavailability, malabsorption, which can be due to factors such as presence of iron absorption inhibitors, bleeding – e.g. menstruation, intravascular hemolysis, parasites, chronic renal failure and pregnancy (Small, 2004; Foo et al., 2004). Iron deficiency is associated with alterations in many metabolic processes, including mitochondrial electron transport, neurotransmitter synthesis, protein synthesis, organogenesis and thus, may cause birth defects (Beard et al., 1990; Allen, 2000; Lozoff et al., 2000).

1.4.5.5 Iron deficiency anaemia

Iron deficiency anaemia (IDA) is a worldwide problem that is prevalent in both developing and developed regions of the world, however, the prevalence is three to four times higher in developing than that in developed countries (Foo et al., 2004). Iron deficiency is not restricted to infants and women of childbearing age, but also affects school-age children, adolescents, elders and even adult males in developing countries (Mora & Mora, 1997). The highest incidence of anaemia is reported in South Asia (57% in females) and sub-Saharan Africa (48% in females), where a large portion of women of reproductive age and preschool children are affected (UN, 1992). In South African studies, iron deficiency anaemia is prevalent in pregnant women; 25% to 62.8% (Ross et al., 1981; Kruger et al., 1994; Dannhauser et al., 1999; Mamabolo et al., 2004) and elderly people (Charlton et al., 1997; Ladzani, 2005).

1.4.5.6 Consequences of iron deficiency anaemia

Women with iron deficiency anaemia have a threefold increase in LBW infants and greater than twofold increase in the risk of pre-term delivery (Beard , 1994). The major concern about the adverse effects of anaemia in pregnant women is that their babies are at greater risk of perinatal mortality and morbidity (CDC, 1998; Allen, 2000). High maternal mortality due to IDA has been reported in developing countries with ranges from 27 (India) to 194 (Pakistan) deaths per 10 000 live births (AbouZahr & Roysten, 1991). In Indonesia, maternal mortality among women with iron deficiency anaemia been reported to be 197/10 000 deliveries compared to 70/10 000 deliveries for non-

anemic women (Chi et al., 1981). In South Africa, about 13% of perinatal deaths and 9% of maternal deaths my have been due to IDA during the year 2000 (Nojilana et al., 2006).

Four basic complementary approaches to increase pre-pregnancy iron reserves and prevent gestational iron deficiency have been suggested: preventive supplementation and correction of iron deficiency and iron-deficiency anaemia with iron doses when detected and balanced multi-nutrients; the food fortification approach, where biovailable iron and other essential nutrients are added to staple foods or to special foods; the dietary approach, promoting meal preparation and ingestion of food products rich in haem iron, vitamin C and A or carotenes, while reducing consumption of tea, coffee and other beverages rich in polyphenols, and the correction of excessive iron losses or of conditions that impair iron absorption and utilization. Infections and any source of chronic inflammation and blood loss, as well as the presence of other nutritional deficiencies, must be corrected (Fernández-Ballart, 2000).

1.4.5.7 Effect of iron fortification on iron status

In addition to the use of iron supplements, consumption of fortified foods has also been suggested as a preventive measure against iron deficiency. However, inconclusive evidence has been presented concerning the effect of iron fortification on iron status in populations (Milman et al., 1999). It is generally assumed that food iron fortification is responsible in part for the marked reduction in the prevalence of iron deficiency anaemia that has occurred during the last 50 years. However, many other factors including improved living standards, increased meat intake, the widespread consumption of iron

supplements and the use of oral contraceptives may have played equally or more important roles (Lynch, 2002). In South Africa, a study reported that iron fortification is not effective in improving iron or HGB status (van Stuijvenberg et al., 2008).

Contradictory results reported after iron fortification arises due to the different types of fortificants used. The most widely used iron is elementary iron powders which have low bioavailability compared to other inorganic iron compounds such as ferrous sulphate and fumurate (Hurrell et al., 2002; Lynch, 2002). For iron to bind DMT1, it must be soluble and in the ferrous state. Non-haem food iron as well as fortification iron added in a form of elemental powders is dissolved in gastric juice primarily by the action of hydrochloric acid. All of the dissolved iron enters a common pool. Reduction of ferric iron is mediated by meal components such as ascorbic acid or by a putative di-haem plasma membrane protein (Dcytb) that is present on the duodenal mucosal cell surface (McKie et al. 2001). The ferrous iron is taken up from this common pool and transported into the enterocytes by DMT1. However, the DMT1 receptors must compete for the available iron with the iron inhibitors of iron absorption in food (Lynch, 2002).

The most important currently recognized inhibitors include: phytates, polyphenols, vegetable proteins and some animal proteins such as those found in milk products and eggs, and calcium (Skikne & Baynes 1994). The negative effects of inhibitors may be reduced markedly by enhancing factors in the diet that render the iron soluble and available to DMT1. The most thoroughly studied enhancer is ascorbic acid whereas other

organic acids including citric, lactic and malic acids probably have similar effects but are less well documented (Lynch, 2002).

1.5 FORTIFICATION OF FOODS

Strategies to combat micronutrient deficiencies worldwide include fortification of staple foods, supplementation, modification of traditional diets, and, recently, fortified beverages (Abrams et al., 2003).

For nearly 80 years, food fortification has been practiced worldwide and has proved to be one of the most cost-effective ways of improving the nutrient intake of populations (FACS, 2004). Food fortification is defined as the addition of specific micronutrients (vitamins and minerals) to specific foods and the food to be fortified depends on the eating habits of the population (FACS, 2004). Food fortification is practiced in several developed and developing countries; however, there are differences in the food vehicle, type and amount of micronutrients added, due to varying eating habits and type of deficiency disease. The nutritional status and nutritional needs of the population are used to determine the type and amount of micronutrients needed to fortify the food vehicle (FACS, 2004).

1.5.1. Use of fortification

One of the earliest successful fortification programmes was the addition of iodine to salt in 1924 to prevent goiter, cretinism and other symptoms of severe iodine deficiency. In the early 1930s, vitamin D was first added to cow's milk to aid in the absorption of calcium and phosphorus preventing the development of rickets. In 1938, voluntary enrichment of flours and breads was initiated to prevent development of deficiency diseases in the general population. Enrichment included thiamin for beriberi, niacin for pellagra, riboflavin essential for functioning of niacin and B6. The mandatory requirements were effective in 1943 (FDA, 1996).

Iron fortification started in 1941 and the USA was the first country to enrich low-extraction wheat flour with iron. By 1965, virtually all white wheat flour and wheat bread and most corn meal (86 – 94%) and macaroni products were fortified with iron, as well as cereal products (Bauernfiend & DeRitter, 1992). By 1979 most of the ready-to-eat breakfast cereals (92%) were also fortified with iron (together with other vitamins) to provide some 25% of the RDA per serving (Hayden, 1980). As commercial cereal-based weaning foods became popular, they were fortified with iron because infants are highly susceptible to iron deficiency (Lynch & Hurrell, 1990). Countries where iron fortification is mandatory include USA and Latin America (Chile, Costa Rica, El Savador and Panama) while it is voluntary in Mexico and Brazil (Hurrell et al., 2002; MI, 2002).

Folic acid fortification is currently mandatory in USA, Canada, Chile and Costa Rica (Botto et al., 2004). In 1996, the Food and Drug Administration (FDA) in the USA

implemented mandatory folic acid fortification supported by the Centers for Disease Control (CDC). All wheat flour products are fortified with folic acid at the level of 140 µg/100kg which is estimated to supply an extra 100 µg daily to the average diet (Sweeney et al., 2007). This mandatory fortification program was introduced as a strategy to improve folic acid intake, especially in women of childbearing age (Sweeney et al., 2007).

In the UK, mandatory and voluntary fortification of foods has long been practiced (Hurrell et al. 2002). Margarines are fortified with vitamin A and D in the UK, wheat flour is fortified with iron, thiamin, niacin and all types of flour with calcium. Voluntary fortification of many breakfast cereals with iron, and foods produced for vegans such as soya products are fortified with vitamin B12 as well as calcium (BNF, 2004).
Mandatory fortification of staple foods (usually flour) with folic acid has been seriously considered in a number of European countries including Denmark, Germany, Ireland, Norway, Poland, Switzerland and the UK, but has not as yet been introduced. The pros and cons of fortification are still under debate in several European countries. However, it is believed that the UK and Republic of Ireland will likely introduce mandatory fortification in the next year or so (Sweeney et al., 2007).

Since 400 µg of folic acid per day is needed to prevent NTDs, one to three months into pregnancy, the Australian and New Zealand governments published guidelines for women recommending folic acid supplements prior to pregnancy in 1992. In 1995, both governments amended their food regulations to permit voluntary fortification of flour as a

public health measure initiated to prevent NTDs. However, fortification is voluntary and too few products have been fortified to be effective in providing sufficient folate for all women of reproductive age (Lindeberg, 2004). In New Zealand, there was no fortification of foods with iron by 1992, while in Australia some foods such as breakfast cereals and breads have long been fortified with iron (Ball & Bartlet, 1999; Lindeberg, 2004).

Successful programmes of fortification of foods have been reported in Asian countries. About 35% of flour in Philippines is fortified with vitamin A since 1998 whereas all flour in Indonesia is fortified with iron, folic acid and zinc. Iron fortification programmes are being introduced in central Asia and in countries such as India, Nepal, Bangladesh, Vietnam and Sri Lanka (Hurrell et al., 2002; Johnson, 2005).
Mandatory fortification of foods has been introduced in several African countries such as South Africa, Zambia, Malawi, and Nigeria (FACS, 2004; Johnson, 2005).

One of the outcomes of the National Food Consumption Survey (NFCS) in South Africa (Labadarios et al., 2000) was the initiation of a national mandatory food fortification programme by the Department of Health. Since maize meal and bread were the most commonly consumed staple foods, it was decided to use them as food vehicles for fortification (DOH, 2003). In South Africa, mandatory fortification of maize and wheat foodstuffs with folic acid, thiamin, vitamin A, niacin, riboflavin, pyridoxine, iron and zinc was introduced in October 2003 (Table 1.1) (FACS, 2004).

Table 1.2 Nutrients and quantities used in food fortification in South Africa

Fortification	Unit	Wheat foodstuff	Maize foodstuff	Unsifted maize meal
Vitamin A	IU/kg	5400.00	6400.00	6400.00
Thiamin	mg/kg	3.60	3.58	3.58
Riboflavin	mg/kg	2.00	1.85	1.85
Niacin	mg/kg	31.00	28.50	28.50
Folic acid	mg/kg	1.50	1.50	1.50
Pyridoxine	mg/kg	3.20	3.20	3.20
Iron	mg/kg	43.00	37.00	14.00
Zinc	mg/kg	20.00	18.50	18.50

(Department of Health, South Africa, 2002), **Abbreviations**: IU/kg – International units per kilogram and mg/kg – milligrams per kilogram

Fortification programme was considered to be a sustainable and relatively inexpensive way to eradicate vitamin and mineral deficiencies without changes in traditional food consumption (Kotiah, 2000). However, the effectiveness of this mandatory fortification process in the dosage supplied to the average South African adult has not been evaluated (Steyn et al.,2007).

1.5.2 Efficacy of fortificants

1.5.2.1 Folic acid fortification

The synthetic form of folic acid used as a fortificant in fortified foods is more bioavailable compared to the naturally occurring folates and has resulted in improved folate status worldwide (Caudill et al., 2001; Chen & Rivera, 2004; Hertrampft & Cortés, 2004). In several countries such as Canada and South America, folic acid fortification has caused a rise in blood folate levels as well as reduction in the incidence of NTDs (Honein et al., 2001, Hirsch et al., 2002; Meyer & Siega-Riz, 2002; Ray et al. 2002; Castilla et al., 2003). After fortification was implemented, A 28% reduction of NTDs was reported in the US (MMWR, 2004). In Canada 46% reduction for all NTDs was reported (De Wals et al., 2007). In South Africa, a decline of 30.5% of NTDs has been reported with

significant declines in NTDs perinatal deaths (65.9%) and in NTD infant mortality (38.8%) (Sayed et al., 2008).

1.5.2.2 Iron fortification

Different types of iron fortificants have been used in iron fortification. There are two categories of iron compounds used for fortification: inorganic iron compounds and protected iron compounds (Dary et al., 2002).

- Inorganic iron compounds

Inorganic compounds that can be used in iron fortification include ferrous sulphate, ferrous fumarate, ferric pyrophosphate, ferric orthophosphate and elemental iron. Three types of elemental iron are used in fortification; (i) H-reduced, CO-reduced and atomized-reduced, (ii) electrolytic iron and (iii) carbonyl iron. These compounds are widely used by the food industry because they are generally inert and have very little effect on the sensory properties of foods. They have advantage of causing few, if any, color and flavor problems in stored food vehicles. They are inexpensive and suitable for fortification of staple foods – wheat and maize flour (Hurrell et al., 2002). However, the contribution to iron nutriture is questionable because of their very low solubility and absorption (Dary et al., 2002; Fairweather-Tait & Teucher, 2002). The inefficiency of iron fortification has been well described in European and African countries (Hallberg et al., 1986; Walter et al., 1993; Millman et al., 1994 & 1999; Andang'o et al., 2007).

- Protected iron compounds

The protected iron compounds include chelates and encapsulated compounds. Iron chelate, sodium iron ethylenediaminetetraacetate (NaFeEDTA) is a good fortificant and its bioavailability is 2 – 3 folds higher than ferrous sulphate. However, NaFeEDTA may cause unacceptable color change in some food vehicles (Hurrell, 1997) and is expensive (Dary et al., 2002). Other chelate compounds are ferrous bisglycinate and ferric trisglycinate which have tendency of causing unwanted reactions and are expensive (Bovell-Benjamin et al., 2000).

At present, NaFeEDTA is not widely used as a fortificant in many countries, mainly because of its relatively high cost and because it has yet to gain GRAS (generally recognized as safe) approval (FairweatherTait & Teucher, 2002). Efficacy of NaFeEDTA/Na_2EDTA as a food additive has been proven in countries in Asia (Vietnam and Sri Lanka) and Africa (South Africa and Kenya) (MacPhail et al., 1994; Hettiarachchi et al., 2004; Van Thuy et al., 2005; Andang'o et al., 2007). But there are concerns that prolonged use of NaEDTA may lead to trace element depletion, particularly of zinc (MacPhail et al., 1994). Data on long term use of Na2EDTA is scanty, but fortification trials using NaFeEDTA in humans extending over 2 years have failed to produce changes in serum zinc concentration in the participants (Viteri et al, 1983; Ballot et al., 1989).

On the other hand, encapsulated compounds such as ferrous sulphate and ferrous fumarate are commercially available for food fortification. Encapsulated ferrous sulphate

has been shown to be highly efficacious in improving the iron status of children consuming fortified salt, but is expensive (Zimmermann et al., 2004).

1.6. SUMMARY

Since the preconception period is vital for favourable pregnancy outcome, the adequate nutrient intake is important. The introduction of food fortification promises improvement of nutrient intake including folate. Concern has been raised on the bioavailability of iron compounds used in food fortification as several studies did not find an improvement after fortification. Iron deficiency being the most common nutritional deficiency worldwide, efforts have to be made to ensure the effectiveness of iron fortification. There is a need for vitamin B12 fortification populations with high vitamin B12 deficiency.

Due to folic acid fortification, incidence of NTDs has decreased in several countries worldwide resulting in a better pregnancy outcome. Implicated in the aetiology of NTDs are mycotoxins (fumonisins), known to interfere with folate metabolism and affect organs including liver. Therefore, it is important to determine serum proteins and liver enzymes.

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CHAPTER 2

2.1 METHODOLOGY

Originally, the present study was:

- To determine the presence of mycotoxins in mealie meal used in urban and rural areas
- To compare the folate status of women of childbearing age residing in urban and rural areas

It was later observed that the population in rural areas no longer grind maize for their own consumption, but purchase commercial mealie meal, the same as women in urban areas. Thus, no comparison could be made. Since morogo is common in the diet of rural populations, samples were collected to investigate contamination with mycotoxins and the possible effects on the liver.

Fortification of foods was introduced on October 2003 and it was decided that the folate and iron status of women of childbearing age from a rural area be compared for two periods: before and after fortification.

2.1.1 STUDY AREA

The present study was conducted in the Dikgale Demographic Surveillance Site (Dikgale DSS), a rural area situated in the Capricorn district of Limpopo Province, South Africa. Limpopo Province, formerly known as Northern Province, is one of the nine provinces of South Africa and is situated in the far northern part of the country (Fig. 2.1). The total population of the province is estimated at 5.4 million (Stats SA, 2006). The province is considered one of the most impoverished regions in South Africa and has a high rate of unemployment approximately 31.3% (Stats SA, 2005) and a high level of illiteracy estimated at 33.4% (Aitchison & Harley, 2004).

Dikgale DSS (Fig. 2.2) is situated ± 50 km northeast of Polokwane (formerly known as Pietersburg), the capital city of Limpopo Province and covers an area of approximately 71 square km. Dikgale DSS consists of 8 typical rural villages of varying size; Mantheding, Maselaphaleng, Madiga, Ga-Ntsime, Ga-Maphoto, Moduane, Ga-Tjale and Sefateng. The dwelling units consist of a mixture of shacks, traditional mud huts and conventional brick houses. The infrastructure of Dikgale DSS is poor in terms of sanitation, postal service, transport and roads. Unemployment is estimated at 50-60%. All inhabitants of this area are Northern Sotho-speaking people (Alberts, 1999).

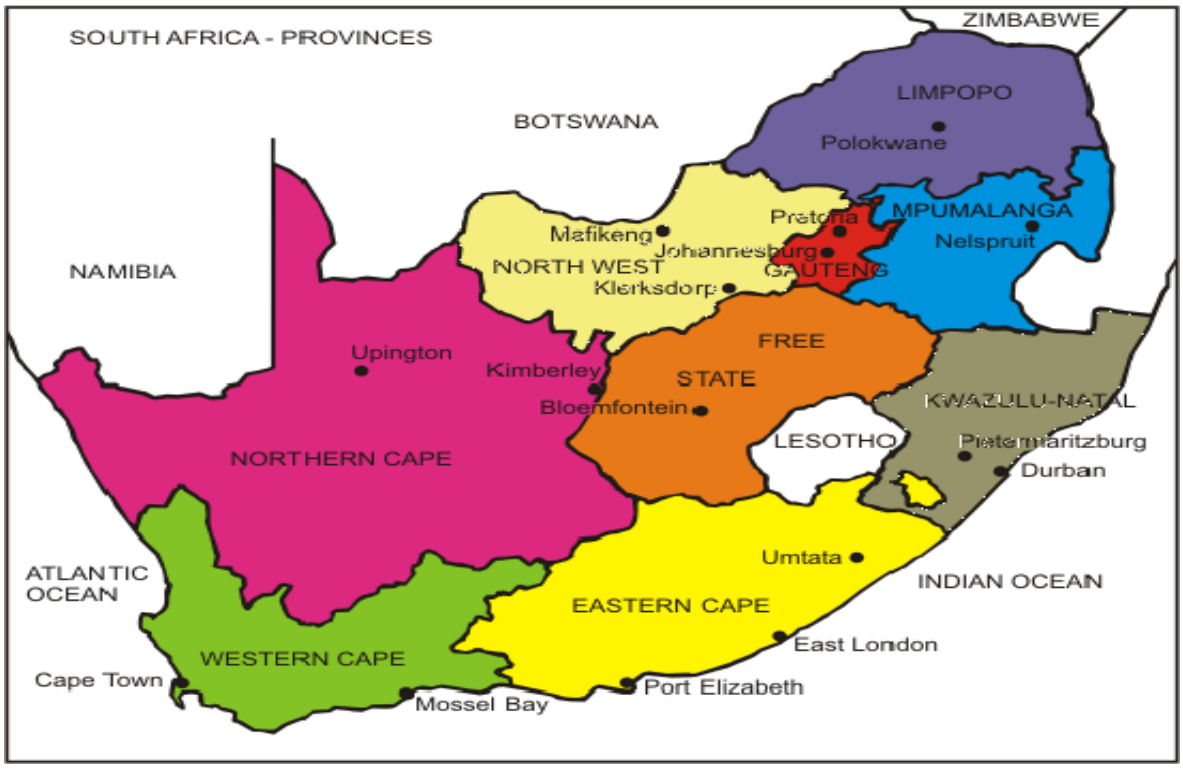


Figure 2.1 Geographical map of South Africa showing the provinces (www.places.co.za)

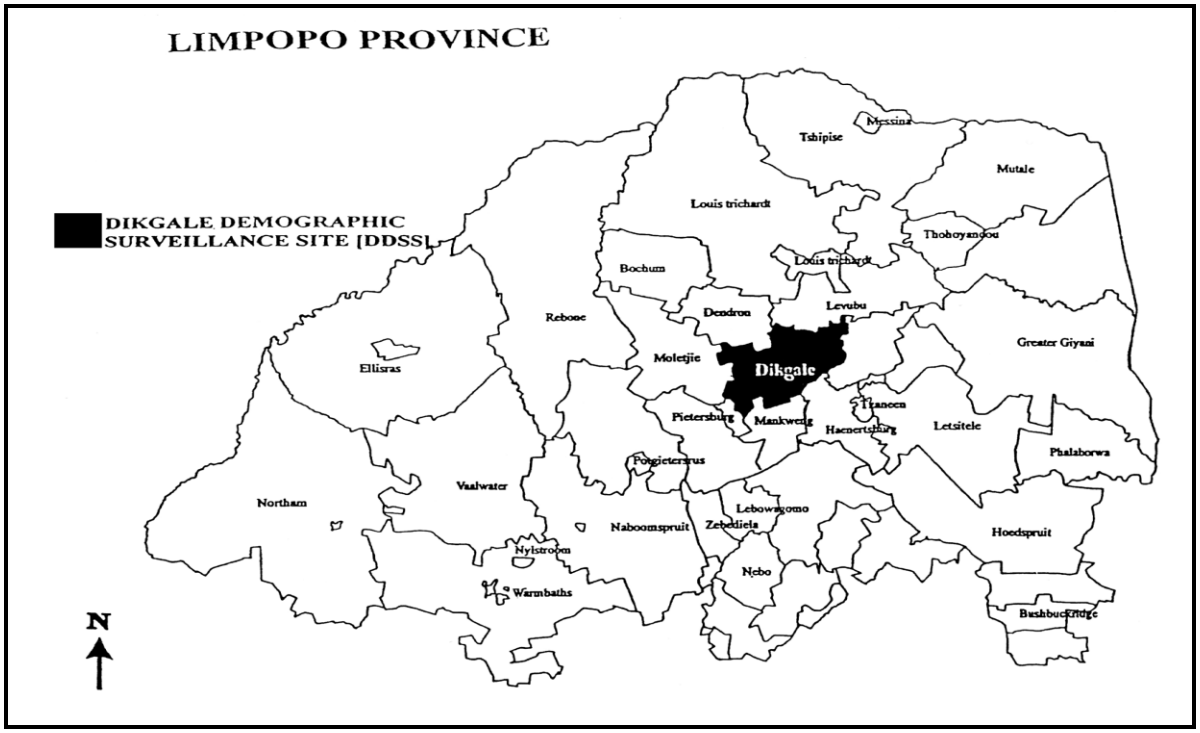


Figure 2.2 Limpopo province map showing Dikgale DSS and neighboring places (Steyn et al., 2001)

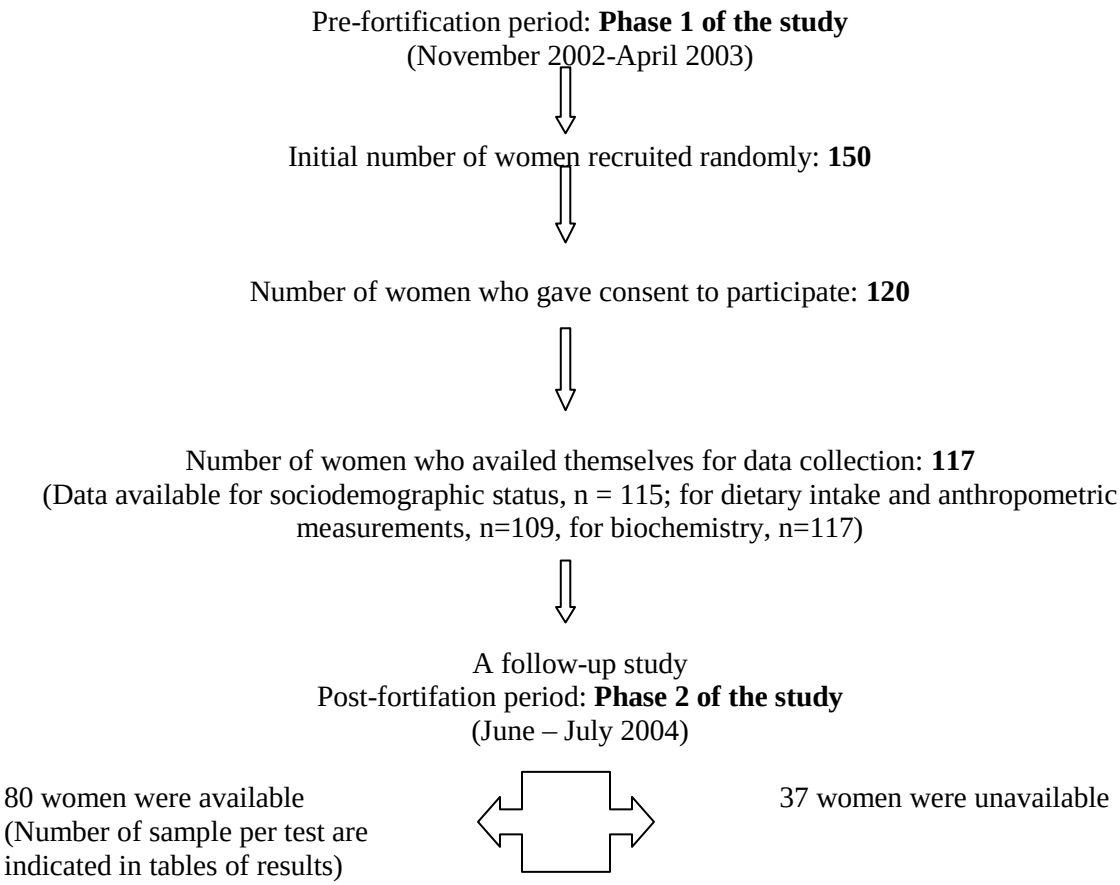
2.1.2 STUDY POPULATION

The total population of Dikgale DSS was 8000 with 1 649 women of childbearing age (18 – 44 years) in 2000/2001. During October 2002, a random sample of 150 women (18-44 years of age) were recruited to participate in the study (pre-fortification period) in order to obtain a representative final sample of 100. Sample size was calculated using the formula: $n \geq [1.96\ (\sigma/\delta)]^2$, where 1.96 is a constant when using 95% confidence interval, n stands for sample size, σ stands for estimated standard deviation of the population and δ stands for mean estimated using 95% confidence interval (Daly & bourke, 2000). In a random selection method, each individual from the population of women of childbearing age secured an equal chance of being in the sample. The sampling frame consisted of a list of all women of childbearing age and the sample was drawn using a table of random numbers. Recruitment was carried out through home visits. During the visits, the project leader thoroughly explained the aim and objectives of the study to every subject (Appendix I) using their language (northern sotho/sepedi).

Approximately nine months after fortification of foods **(post-fortification period)** was introduced, a follow-up on the 120 women participating in the first phase of the study **(pre-fortification period)** was done. Women were re-visited in their homes and asked to participate in the follow-up study. Not all women who initially participated in the study were available for the follow up study. During re-visits, the exclusion criteria applied in the first phase of the study were still applicable, and some women were found to be pregnant, lactating or on medication while others had left the study area and couldn't be traced.

2.1.3 STUDY DESIGN

A prospective cohort study design was used. The study was conducted in two different time-periods within the same area and population. The first phase of the study took place between November 2002 and April 2003. This period was prior to mandatory fortification of foods with folic acid and other essential nutrients; hence, it is referred to as a **pre-fortification period**. The second phase was a follow-up of the subjects that participated in the first phase, and was conducted from June 2004 to July 2004. This phase was carried out approximately nine months subsequent to fortification of staple foods and hence is called the **post-fortification period.** Fortification of foods in South Africa was officially mandated during October 2003. Order of events was as follows:



- ***Description of cohort designs***

Cohort methods are used to determine the incidence and natural history of a condition. Cohort studies can be either prospective or retrospective. In a prospective study, a variety of variables that might be relevant to the development of a certain condition are investigated. Over a period of time the participants in the sample are observed to see whether they develop the outcome of interest. Conversely in a retrospective study, data already collected can be used for other research purposes. The methodology is the same as that of the prospective study, but, a cohort is "followed up" retrospectively (Mann, 2003).

- ***Advantages and disadvantage of using cohort designs***

The advantage of using cohort design is that it measures potential causes before the outcome has occurred. The study can demonstrate that these causes preceded the outcome, thereby avoiding debate as to which is cause and which is effect. A further advantage is that a single study can examine various outcome variables (Mann, 2003).

One of the disadvantages with a prospective study is the loss of some subjects prior to the follow up stage of the study. This can significantly affect the outcome and the rarer the condition the more significant the effect is. The retrospective study has a disadvantage in the subjects with the outcome of interest are more likely to remember selected past history, or exaggerate or minimize what they now consider to be risk factors (recall bias) (Mann, 2003).

2.1.4 EXCLUSION CRITERIA

To avoid interference with the results, women who were pregnant and lactating, using anticonvulsant drugs, oral contraceptives, intrauterine devices, or tobacco and alcohol were excluded from the study:

- ***Use of medication for chronic illness***

Anticonvulsant drugs are reported to be the most common drugs interfering with both folate and homocysteine metabolism (Ueland & Refsum, 1989; Mayer et al., 1996; Refsum, 2002; Monograph 2005). Proposed mechanisms are inhibition of the deconjugation of polyglutamates, inhibition of absorption of the monoglutamate by the intestinal epithelium, reduction of serum and tissue concentration of folate, and interference with the drug-metabolizing system in the liver (Lambie et al., 1985; Monograph, 2005). Anticonvulsant drugs are also associated with development of macrocytosis and megaloblastic anaemia (Collins et al., 1988), hence, they disturb vitamin B12 metabolism (Dharmarajan et al., 2001).

- ***Use of oral contraceptives and intrauterine devices-***

Oestrogen-containing oral contraceptives affect levels of homocysteine and folate (Van Ede et al., 2002; Desouza et al., 2002). It is believed that use of oral contraceptives causes low folate concentration in serum and erythrocytes, and an increased formiminoglutamate (Lambie et al., 1985). Oral contraceptives may interfere with absorption of ptereoylpolyglutamates, and cause malabsorption of dietary folate by

inhibition of jejunal folate conjugase and alter the metabolism of homocysteine (Lambie et al., 1985; Wong & Kang, 1988; Monograph, 2005).

- ***Tobacco and alcohol use***

Tobacco and alcohol are recognized as factors that may have a negative effect on folate status and homocysteine levels. Alcohol is reported to interfere with several aspects of normal folate transport to tissues, and the normal pattern of folate storage and release by the liver. Alcohol blocks the release of folate from the hepatocyte, thereby disrupting the folate supply to tissues and rapidly producing a defect in cell proliferation (Hillman & Steinberg, 1982; Bailey, 1990; Refsum, 2002)

- ***Women lactating***

During lactation there is a high demand for folate such as an increased need to replace the quantity of folate secreted daily in breast milk plus the amount necessary to maintain normal folate status (Bailey, 1990)

- ***Use of oral supplements-***

Use of folic acid supplements improves folate status (Bailey, 1999).

2.1.5 SELECTION AND TRAINING OF FIELDWORKERS

The need for fieldworkers to assist in this project was advertised. Fieldworkers were selected among a group of people who were interested in assisting with the fieldwork. Fieldworkers were required to have grade 12 as the lowest education qualification, be

able to speak Northern-Sotho (Pedi) as their first language, have a general knowledge of the lifestyle of the study population, and have practical experience of fieldwork and of administrating dietary questionnaires. The fieldworker-training manual adopted from the National Food Consumption Survey (NFCS) done by Labadarios et al (2000) was used to train field workers (Appendix VIII).

A manual with standard procedures of measuring was used to train field workers on how to complete the questionnaires to collect the data needed on socio demographic status, maternity history, dietary intake and anthropometric measurements. The manual included the pictures on nutritional status assessment and procedures on how to complete each questionnaire. The field workers fiets worked together under supervision until they had mastered the required skills. Two field workers assisted each other by interviewing one subject. Nutritional status measurements included anthropometric measures (weight, height, waist and hip circumferences. Field workers were assessed for competence as per a written and practical examination before they started collecting data. In order to ensure quality control, interviewers repeated interviews in the presence of the projecte leader (researcher) and findings were compared. Inconsistencies were simplified in this manner.

2.1.6 ETHICAL AND LEGAL CONSIDERATION

The study proposal was submitted to the University of Limpopo Ethics Committee and Department of Health and Welfare Research Committee, Limpopo Province for approval. The aim and objectives of the study as well as the procedures to be employed were

explained to each of the participants using their native language; northern Sotho (Appendix I). A written consent form was obtained from each participant (Appendix II).

2.1.7 DATA COLLECTION

2.1.7.1 Socio-demographic status and maternity history

To assess the background of women of childbearing age, socio-demographic data was collected using a questionnaire (Appendix III). The questionnaire used to assess socio-demographic status in this study was adapted from a questionnaire used in the Risk factors for coronary heart disease in the black population of the Cape Peninsula (BRISK study) (Steyn et al., 1991). This questionnaire included questions on socio-demographic aspects and on risk factors for CVD. This questionnaire is used routinely in the Dikgale Demographic Surveillance site for assessing changes in the socio-demographic status of the population. Socio-demographic information such as age, marital status, occupation, level of education and household information was recorded.

In addition to the socio-demographic information, the maternity history profile [parity, age of children, cases of stillborns, miscarriages/spontaneous abortion, death of newborns immediately after birth (perinatal mortality), and cases and knowledge of neural tube defects (NTDs)] of women of childbearing age was assessed using a Maternity History Questionnaire, adopted from questionnaire used in Dikgale DSS (Appendix IV).

2.1.7.2 Dietary intake assessment

For nutritional status assessment, dietary intake of the study population was assessed to approximate the quantity of food consumed in terms of recommended dietary allowances (RDAs) and/or dietary reference intakes (DRIs). Dietary intake was assessed only at baseline. The visits for to collected dietary information were done on a daily basis for different participants. During interviews, dietary kits were used (Appendix VI). Globally, there are several methods available to assess dietary intake. According to Myers et al (2004), the commonly used dietary assessment methods are diet history, 24 hour recall, food frequency (quantified) and food records/diaries (weighted). Table 2.1 shows the various methods available and their advantages and disadvantages. The main disadvantage of using weighed diet records and/or the diet history methods is that they are not suitable for use in illiterate populations (Margetts & Nelson, 1997). As such for this study the quantitative frequency and 24-hour recall (repeated) methods were selected. The procedure of repeating a 24-hour recall and administering QFFQ ensures that seasonal foods are being captured.This was based on the fact that these had been specifically developed from use in South African children and they include most of the foods consumed nationally (Labadarios et al., 2000; 2005).

To obtain dietary intake information, each participant was required to complete two 24 hour recall questionnaires (1 questionnaire in relation to dietary intake during the week days, and the other in relation to dietary intake on Sunday) and one QFFQ (concurrent with the first 24 hr recall).

Table 2.1: Comparison of the dietary methods available to determine food intake

Dietary Method	Advantages	Disadvantages
Diet records (Weighed)	☑ Most accurate method ☑ Can be used to validate other methods ☑ Suitable for studies on individuals	☒ Expensive ☒ Non-random sampling and sample size is limited ☒ Literacy essential ☒ Distortion of eating habits due to recording process ☒ Meals away from home create problems
Diet history	☑ Usual eating pattern determined ☑ Large sample sizes possible ☑ Lower respondent burden than with weighed records ☑ Random sampling possible ☑ Once-only interview that can be conducted anywhere ☑ No change in normal eating habits	☒ Interview bias ☒ Long interview ☒ Heavy demands on the interviewer ☒ Experienced interviewer required ☒ High respondent burden
Food frequency (quantified)	☑ Low burden on respondent ☑ Usual pattern determined ☑ Large sample sizes possible	☒ Experienced interviewer required ☒ Heavy demands on interviewer ☒ Overestimation of intakes
24-Hour recall	☑ Low respondent burden ☑ Takes relatively little time ☑ Low refusal rate ☑ Cost-effective ☑ Suitable for large sample sizes ☑ Representative of intakes of groups ☑ Reliable for groups with a relatively monotonous diet ☑ Random sampling possible	☒ Relies on memory ☒ One recall not representative of the individual's intake. ☒ underestimation of intakes

Source: (Margetts and Nelson, 1997)

(A) Quantitative food frequency questionnaire (QFFQ)

The usual dietary habits of women of childbearing age were assessed using QFFQ (Appendix VII). Trained fieldworkers (trained using manual in Apppendix IX) administered the questionnaire to participants. During interviews, interviewees were asked to indicate the kinds of foods consumed and how often the foods were consumed, as the interviewer mentioned the listed food and beverage items of the questionnaire. Food models and standard household measuring equipment (such as spoons and ladles) were used to estimate size and volume of food consumed by participants. The amount

and type of the foods eaten were listed on the questionnaire according to the times they were consumed either per day, per week or per month. Foods hardly eaten were recorded under "seldom/never" (Labadarios et al., 2000). One-hundred and twenty two food items and 13 food groups were included in the NFCS questionnaire.

- ***Advantages and disadvantages of QFFQ***

QFFQ is frequently used to grade individuals according to the distribution of their food intake and is considered useful in terms of describing associations of food consumption, disease patterns and food preferences, and it sheds light on the frequency of food consumption (Labadarios and Haffeejee, 1990; Margetts and Nelson, 1997). QFFQ is advantageous because a large sample size can be used and usual patterns of intake can be determined. However, the disadvantages of using QFFQ include the need for an experienced interviewer, overestimation of food. QFFQ has a very high respondent burden and takes about 45-60 minutes to complete compared to 24HR which takes 20-30 minutes. In addition, the limitations of QFFQ are that it relies on the memory and cooperation of the subjects. QFFQ may over or underestimate dietary intakes. Furthermore, QFFQ is very time consuming and may not be suitable for all population groups (Margetts & Nelson, 1997). The 24-hr recall questionnaire has been used previously in Dikgake DSS as by the same field workers in the present study.

- ***Development and validation of QFFQ***

The QFFQ used in this present study was adapted from the National Food Consumption Survey (NFCS) in 1999 (Labadarios et al, 2000). The original QFFQ was designed and

validated by MacIntyre (1998), MacIntyre (2001) and MacIntyre (2001) for surveys on African urban and non-urban adults. Therefore, since the validity of QFFQ was tested and shown to provide valid and reliable data during dietary intake assessment in the NFCS (MacIntyre, 1998; Labadarios et al, 2000), it was regarded as suitable for the provision of valid and reliable data for women of childbearing age in the present study without repeating the process of validation.

Since the validity of QFFQ was tested when the NFCS was undertaken, it was deemed costly to repeat the process for this survey as it was shown to provide valid and reliable data for caregivers regarding the children (Labadarios, 2000; MacIntyre, 1998). The difference between the original questionnaire and adapted one used by the NFCS group was that NFCS version had more food items added to it but these were mainly infant food (Labadarios, 2000).

The relative validity of the QFFQ was tested during the NFCS by means of a comparison with the three-day weighed records on a sample of mothers/caregivers/children in the Limpopo and Western Cape Provinces. For all nutrients the QffQ gave higher intakes than the 24 hour recalls. Bland-Altman (B-A) plots for the completed data, after axcluding outliers, showed wide limits of agreement (Labadarios, 2000).

(B) The 24-hour recall questionnaire (24hr-recall)

The 24-hour recall questionnaire (Appendix VIII) was used to determine the dietary intake of women of childbearing age in this study. Each participant was interviewed

twice on two different days: one-week day and one weekend day. During the interview, participants were asked to describe all the food and beverages consumed during the previous 24 hours and the interviewer recorded the answers on the questionnaire. The first interview was conducted on a weekday visit at their respective homes. The second interview was conduct on Monday of the following week. In order to ensure true reporting of data in this study, food portions were estimated using dietary aids. This questionnaire has been widely used as a method of determining dietary intake of groups (Willet, 1990; Nelson & Bingham, 1997).

- ***Advantages and disadvantages of 24-hour recall***

The 24-hour recall questionnaire defines and quantifies food intake for a specific day before the interview. This method is suitable for a population with a high rate of illiteracy such as the population in this study, as it recalls food consumed the previous day before the interview. The use of 24-hour recall questionnaire is well established in assessing and estimating the average intake of groups as well as of large groups, particularly when using more than 1 questionnaire (Block, 1982; Margetts and Nelson, 1997). The 24-hour recall is cost effective, relatively rapid and less time consuming, requiring about 10-20 minutes to complete and the method is not biased (Labadarios and Haffejee, 1990; Margetts and Nelson, 1997). However, this method depends largely on the memory, cooperation and communication of an individual and on the skill of the interviewer (Margetts and Nelson, 1997).

The limitations of 24-hour recall method are that it does not represent typical or long-term dietary intake and it is known that dietary intake varies from day to day (Willet, 1990). For this reason, 24-hour recall cannot be used to rank individuals on specific nutrients. Errors may also arise if the interviewer fails to probe for details on food/portions eaten. Therefore, the procedure of repeating a 24-hour recall lessens the effects of these limitations.

- ***Development and validation of 24-hour recall***

Originally the 24-hour recall pre-coded questionnaire was developed and validated in 12 year-old children and their mothers/caregivers/grandmothers in a study done in the Western Cape Province of South Africa among all ethnic groups (Steyn, 1986). The NFCS recently modified the questionnaire but the structural integrity remained the same (Labadarios et al., 2000). The South African Food Composition Table (1998) was used to code each food item on the questionnaire. The 24-hour recall questionnaire contained common food portion sizes to assist the interviewer when calculating portion size of items eaten. Breakfast was defined as the first meal of the day, lunch as the afternoon and supper as the evening meal. Snacks were regarded as any other food item consumed in-between breakfast, lunch and supper. The last page of the questionnaire has an empty column where additional food items could be added.

The relative validity of the 24 hour recall questionnaire was tested during the NFCS by means of a comparison with the three-day weighed records on a sample of mothers/caregivers/children in the Limpopo and Western Cape Provinces. For all nutrients the QffQ gave higher intakes than the 24 hour recalls. Bland-Altman (B-A)

plots for the completed data, after axcluding outliers, showed wide limits of agreement (Labadarios, 2000).

- ***Reproducibility of the dietary intake tools used***

Both Steyn (1986) and MacIntyre (1998) tested the 24-hour recall questionnaire and the QFFQ for reproducibility during their original development. The adapted versions were also tested in the NFCS, with good correlation coefficients. In this study, the training of fieldworkers optimized their ability to obtain accurate information. However this does not imply that the data collection was accurate. There might have been errors that occurred which were beyond anyone's control. However the questionnaires were repeated on a selected random sample of children.

2.1.7.3 Anthropometric Measurements

For nutritional status assessment, anthropometric measurements (body weight, height, waist and hip circumference) taken from women of childbearing age were determined according to standard procedures (WHO, 1998) at the same time of administration of the first dietary questionnaire. Anthropometric measurements were taken only at baseline. Measurements were recorded on the anthropometry sheet (Appendix V).

- ***Body Weight and Height***

Body weight was measured on a calibrated digital bathroom scale to the nearest 0.1 kg and height was measured to the nearest 0.5 cm with a stadiometer (Norton & Olds, 1996). From the body weight and height of each participant, body mass index (BMI) was

calculated as weight in kilograms divided by height in meters squared. BMI (kg/m^2) = weight (kg)/height $(m)^2$ (WHO, 1998). Normal BMI is within 19 to 24 kg/m^2 whereas underweight was defined as BMI<18.5 kg/m^2, overweight as BMI: 25 to 29.9 kg/m^2 and obesity as BMI ≥ 30 kg/m^2 (WHO, 1998).

- ***Body waist and hip circumferences***

Waist circumference was measured at the midpoint between the lower rib margin and the iliac crest, and hip circumference was measured at the maximal circumference of the buttock with a non-stretchable tape measure. Circumferences were measured with cross-hand technique, with the tape held at the right angles to the body segment that was being measured with no indentation of the skin (Norton & Olds, 1996). The cutoff point for central obesity was a waist circumference ≥ 88 cm. The waist-hip-ratio (WHR) was computed as the waist circumference divided by the hip circumference. The WHR cutoff point for women was 0.85 (WHO, 1998).

2.1.7.4 Blood collection

An overnight (10 – 12 hours) fasting blood sample was collected into ethylenediamine-tetra-acetic acid (EDTA) silicon tubes from 120 women prior to fortification of foods **(pre-fortification period: phase 1)** to measure biochemical variables. For total homocysteine (*t*Hcy) analysis, blood in the ETDA tube kept in a cooler box full of ice, was centrifuged within 2 hours of collection using a mini centrifuge and plasma was stored in a cooler box with crushed ice mixed with potassium nitrate salt to slow the melting process. This was done to avoid elevation of levels of homocysteine. Samples

were analysed by Lancet laboratories, using HPLC (High Performance Liquid Chromatography). To assess folate status, serum folate (Sfol) collected in a silicon tube and red blood cell folate (RCF) from where whole blood was collected in an EDTA tube, were analysed in addition to vitamin B12 (VB12) collected in a silicon tube, using Access Immunoassay (Beckman Coulter). Iron status was determined using Access Immunoassay (Beckman Coulter) and ILAB 300 plus (Instrumentation Laboratory) by measuring serum ferritin (SF), serum iron (Siron), percentage transferrin saturation (%TS) and total iron binding capacity (TIBC) while C-reactive protein (CRP) was analysed to assess the inflammation/infection status of individuals. For all analysis, serum controls were used.

Full blood count (FBC) parameters measured include red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) and were determined on 5-diff Coulter (Beckman Coulter). Other biochemical parameters include albumin (ALB) and liver enzymes [γ-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)] which were determined on Dimension ES.

On arrival at the laboratory, plasma stored in the cooler box was placed in a freezer at – 80 ^{0}C. For determination of RCF, 50µl of whole blood (EDTA) was mixed with 1000µl red cell lysing agent and stored at –80^{0}C until further analysis. Full blood count was determined from whole blood (EDTA) within 6 – 8 hours of arrival at the laboratory and

thereafter the blood was centrifuged and plasma stored at – 80^0C until used. From the silicon tubes, serum was obtained after centrifugation and stored at – 80^0C until used. Approximately nine months after introduction of fortified foods **(post-fortification period: phase2)**, blood was collected from the same women who participated in the initial phase of the study.

Normal levels of folate were equal to or greater than (≥) 3ng/ml while serum folate deficiency was said to be severe if lesser than (<) 2.5ng/ml or moderate if the levels <3ng/ml. Red cell folate deficiency was present if the level was <164ng/ml and considered normal when ≥164ng/ml. Women with serum ferritin levels between 12µg/ml and 20µg/ml were said to be moderately iron depleted, while those with levels <12µg/ml were considered to be severely iron depleted. Serum ferritin levels were considered normal when ≥ 20µg/ml. CRP ≥ 10 mg/l showed the possibility of inflammation. Vitamin B12 deficiency was said to be present in the women when the levels were <145pg/ml and normal when ≥145pg/ml (Assay manual, Beckman Coulter). Hyperhomocysteinemia was whem *t*Hcy > 12.4 µmol/L. Liver enzymes were used to assess the effect of mycotoxins whereas GGT below 55U/L confirmed that alcohol was not regularly consumed.

Change in the prevalences either reduced or increased for the variable: Sfol, RCF, VB12, Siron, %TS, RBC, HGB, *t*Hcy between the phase 1(pre-fortification) and phase 2 (post-fortification) was calculated as follows:

$$\frac{\text{Phase 2} - \text{Phase 1}}{\text{Phase 1}} \times 100 = \% \text{ of reduction/increase}$$

The reference ranges (RR) for various variables were adopted from assay manuals (Table 2.2):

TABLE 2.2 Normal, below and above reference ranges (RR) for variables

VARIABLES	NORMAL RR	BELOW RR		ABOVE RR
Sfol (ng/ml)	≥ 3ng/ml	2.5– 2.99ng/ml	< 2.5ng/ml	-
RCF (ng/ml)	≥ 164ng/ml			-
SF (µg/ml)	≥ 20µg/ml	12– 19.9µg/ml	< 12µg/ml	-
SIron (µg/dl)	9.0 – 30.4 µg/dl	< 9 µg/dl		> 30.4 µg/dl
TIBC (µg/dl)	44.7 – 80.5 µg/dl	< 44.7 µg/dl		> 80.5 µg/dl
%TS	≥ 16 %	< 16 %		-
CRP	< 10 mg/l			≥ 10 mg/l
VB12 (pg/ml)	180 – 914 pg/ml	< 145 pg/ml		-
*t*Hcy (µmol/L)	4.5 – 12.4 µmol/L	< 4.5 µmol/L		> 12.4 µmol/L
RBC(10^6/µL)	4 – 6.2 X 10^6/µL	< 4 X 10^6/µL		-
HGB (g/dl)	11 – 18.8 g/dl	< 11 g/dl		-
HCT (%)	35 – 55 %	<35 %		> 55 %
MCV (fl)	80 – 100 fl	< 80 fl		> 100 fl
MCH (pg)	26 – 34 pg	< 26 pg		> 34 pg
MCHC (g/dl)	31 – 35 g/dl	< 31 g/dl		> 35 g/dl
ALB (g/L)	34 – 50 g/L	< 34 g/L		> 50 g/L
GGT (U/L)	5 – 55 U/L	< 5 U/L		> 55 U/L
AST (U/L)	15 – 37 U/L	< 15 U/L		> 37 U/L
ALT (U/L)	30 – 65 U/L	< 30 U/L		> 65 U/L
ALP(U/L)	50 – 160 U/L	< 50 U/L		> 160 U/L

Abbreviations: RBC - red blood cell count, HGB – haemoglobin, HCT – haematocrit, MCV – mean cell volume, MCH – mean cell haemoglobin and MCHC – mean cell haemoglobin concentration. The reference ranges for biochemical parameters were adopted from the assay kits from Beckman Coulter, Access Immunoassay, Beckman, Lancet Laboratories and Dimension ES

2.1.7.5 Calculations of coefficient variations (CVs) of intra and inter assays

The intra-assay CV was determined by measuring one sample four times within one run whereas the inter-assay CV was determined by measuring one sample four times on different runs. The following formula was used: CV = SD X 100/mean. Accepted CV should be below 10%.

Table 2.3 Coefficient variations [CVs) for variables: intra and inter assays

VARIABLES	INTRA-ASSAY CV (%)	INTER-ASSAY CV (%)
SFol (ng/ml)	2.7	6.1
SF (µg/ml)	1.5	3.8
CRP (mg/l)	8.6	9.3
VB12 (pg/ml)	2.6	4.5
tHcy (µmol/L)	2.2	5.2
RBC (10^6/ µl)	1.3	4.0
HGB (g/dl)	0.9	2.9
HCT (%)	1.1	4.6
MCV (fl)	0.5	2.0
MCH (pg)	0.8	4.2
MCHC (g/dl)	1.2	4.3
Alb (g/L)	1.3	3.9
GGT (U/L)	3.8	5.5
AST (U/L)	1.2	4.3
ALT (U/L)	6.1	9.7
ALP (U/L)	1.2	8.8

2.1.7.6 Food collection

Ten samples of morogo (*Amaranthus viridis, Cleome gynandra and Vigna ungiuculata)* were collected from participants by the project leader and field workers to determine the presence of mycotoxins. About 300 - 500g of each sample cooked and dried in the sun was collected. Prepared morogo samples are routinely stored in containers inside the house until used. The samples were collected randomly from the houses of the participants chosen randomly from the study population and if the sample was absent, an adjacent household was chosen. During collection of morogo, questions about the kind of sample, preparation and preservation were asked (Appendix X). Samples were kept in -80^{0}C refrigerator not more than 2 months until couriered to Promec Unit at Cape Town for analysis. No visible signs of contamination on the samples were observed. There is no tolerance level of fumonisins in morogo but a tolerance level of fumonisins defined in maize foods is 100-200 µg/kg. The collection of samples started during the harvest period of maize (November 2002-April 2003), hence maize was not available during the initial state, since it is never kept in the homes, but sent to milling factories, unlike morogo which is available throughout the year by preservation.

2.1.8 DATA ANALYSIS

- *Cleaning of data*

To ensure that all the questionnaires were properly entered, the project leader re-checked them to correct errors where necessary. The socio-demographic and maternity history questionnaires were coded to facilitate easy analysis before being entered into the computer.

2.1.8.1 Statistical tests

- *Dietary intake analysis*

The MRC food finder III, version 1.0.9 programme was used to enter the quantity of foods recorded on dietary questionnaires (QFFQ and 24-hour recalls) and convert them into energy intake and nutrient values. Information entered in the food finder programme included: personal information of individuals (date of birth and gender), observational information (anthropometrics: body weight and height), and food items and quantities consumed. For the two 24-hour recall assessments and average nutrient values were calculated.

The energy intake and nutrient values were captured using Statistical Package for Social Science (SPSS) 12. Descriptive statistics for nutrient intakes were analysed. Means of nutrients using 24hr recall and QFFQ were compared using paired sample-t-test whereas means of nutrient intakes by age were compared using analysis of variance (ANOVA). Percentages of subjects with nutrient intakes below/above the cut-off points were computed and compared using chi-square (χ^2) test. Results are presented as means [95% confidence interval (CI)]. Statistical significance was set at a probability level (p) of 0.05.

RDAs/DRIs used were taken from the food finder programme (MRC), Dietary Reference Intakes (NICUS, 2003) and Recommended Dietary Allowance (NRC, 1989).

The percentage of under- and over-reporters was calculated using the formula for energy intake divided by basal metabolic rate (EI/BMR) (Goldberg et al., 1991). BMR was determined using the Schofield (1985) formula for ages 19 – 30 and 31 - 60 years:

Females: 0.062 x weight + 2.036 (19 – 30 years)

: 0.034 x weight + 2.538 (31 – 60 years)

These categories were compared for the two questionnaires (QFFQ and 24 hr recall) and statistical significance was set at a probability level (p) of 0.05. As suggested by Goldberg et al (1991), under-reporting was established when the ratio of EI: BMR was less than 1.2 while over-reporting was established at EI/BMR $\geq$ 2.4 (Johansson et al., 1998).

- *Socio-demographic status and maternity history analysis*

Statistical analysis was done using SPSS 12 for Windows. Cross-tabs and the Chi-square ($\chi 2$) test were used to analyse the socio-demographic status and maternity history information. Results are presented as percentages (number of subjects), [%(n)]. Participants were categorised into age groups, ANOVA was computed, and the differences between the various age groups tested by the Bonferroni method. Statistical significance was set at a probability level (p) of 0.05.

- *Anthropometrics measurement analysis*

Body weight, height, waist and hip circumferences were captured in SPSS 12, data was computed to calculate BMI and WHR, and the results are presented as means [95% confidence interval (CI)]. Cross-tabs was used to determine the percentages of

participants with WHR and BMI below or above the given reference ranges and compared using the Chi-square ($\chi 2$) test and results are presented as percentages (number of subjects), [%(n)]. Participants were categorised into age groups, ANOVA was computed, and the differences between the various age groups tested by the Bonferroni method. Pearson correlation was used to correlate anthropometry with selected nutrients. Statistical significance was set at probability level (p) of 0.05.

- *Biochemical parameter analysis*

Statistical Package for Social Sciences [SPSS] for windows, version 12, was used to analyze the biochemical parameter data. Tests of normality [Kolmogorov-Smirnov and Shapiro-Wilk] and frequencies of variable distributions were analyzed. Variables that were not normally distributed were logarithmically transformed to approximate normality. Statistical analyses performed included descriptive statistics, Pearson correlations, paired-sample *t* test and chi square (χ^2).

Means of variables from the pre and post-fortification periods were compared using Paired-sample *t*-test and the changes in mean averages in the two periods were calculated. Results are presented as median difference (IQ range). Percentages of subjects with variables below/above the cut-off points before and after fortification were determined and compared using Chi-square (χ^2), and results are presented as percentages (number of subjects), [%(n)]. Statistical significance was set at probability level (p) of 0.05.

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CHAPTER 3

RESULTS

Ultimately out of 150 women, 120 non-pregnant women participated in the study prior to fortification of foods and gave written consent. The other 30 women couldn't participate in the study for the following reasons:

- **Exclusion criteria** – even though some showed an interest in participation, the criteria used in the study excluded them (see 2.4).
- **Religious reasons** - some reported that the churches they attend do not allow them to be pricked/punctured with needles for blood donation.
- **Cultural reasons** - some reported that their ancestors are not happy when they give unknown people their blood.
- **Fear –** because of the HIV/AIDS epidemic in South Africa and its social connotations there is a great reluctance among adults to donate blood.

During a follow-up study, approximately nine months after fortification was introduced, 20 women were unavailable to re-participate in the study, owing to pregnancy or absence from Dikgale DSS.

However, due to the destruction of blood samples not all biochemical tests could be carried out on all blood samples. Number of test done as follows:

TWO PHASES OF THE STUDY

Phase1 – Pre fortification period	**Phase 2 – Post fortification period**
Tests in all = 117, except for:	Tests done in all = 80, except for:
*t*Hcy = 35	*t*Hcy = 35
RCF = 73	RCF = 53
SIron =56	SIron = 42
TIBC =56	TIBC = 42
CRP =96	CRP = 49
Alb = 107	Alb = 67
GGT = 115	GGT = 76
AST = 106	AST = 78
ALT = 11	ALT = 74
ALP = 111	ALP = 73

Homocysteine tests could not be done in all subjects because of the prolonged power failure which affected most of the samples. Samples were also affected during analysis of albumin and liver enzymes (GGT, AST, ALT and ALP) due to the malfunctioning of the Dimension ES machine used.

Results for women of childbearing age are presented as follows:

- **SECTION A – PRE-FORTIFICATION PERIOD (Phase 1)**

  A1. Socio-demographic status and maternity history

  A2. Anthropometric measurements

  A3. Dietary intake using 24hr and QFFQ questionnaires

  A4. Association between anthropometry and selected nutrients

  A5. Biochemistry results

  A6. Socio-demographic status indicators on folate and iron status

  A7. Association between dietary intake and biochemistry

- **SECTION B - POST-FORTIFICATION PERIOD (Phase 2)**

  B1 - Biochemistry results

- **SECTION C – PHASE 1 AND PHASE 2**

  C1 - Comparison of biochemistry results before and after fortification

- **SECTION D – FOOD SAMPLE ANALYSIS**

  D1 - Analysis of mycotoxins in morogo [green leafy vegetables (GLV)]

SECTION A

PRE – FORTIFICATION PERIOD (PHASE 1)

A1. SOCIO-DEMOGRAPHIC STATUS AND MATERNITY HISTORY

3.1 Age distribution of women of childbearing age

Complete information on socio-demographic status was available for 115 women since other women were absent to be interviewed. The non-pregnant women of childbearing age participating in this study were aged between 18 and 44 years. Results are presented as medians (95% CI). The women were divided into three age categories; 18 – 24 [20.6 (20.0; 21.1), n=47], 25 – 34 [30.0 (29.0; 31.1), n=37] and 35 – 44 [39.2 (38.3; 40.1), n=31] years. The highest percentage of participants was in the youngest age group (18 – 24 years) and the lowest number of participants in the older group (35 – 44 years) (Fig. 3.1).

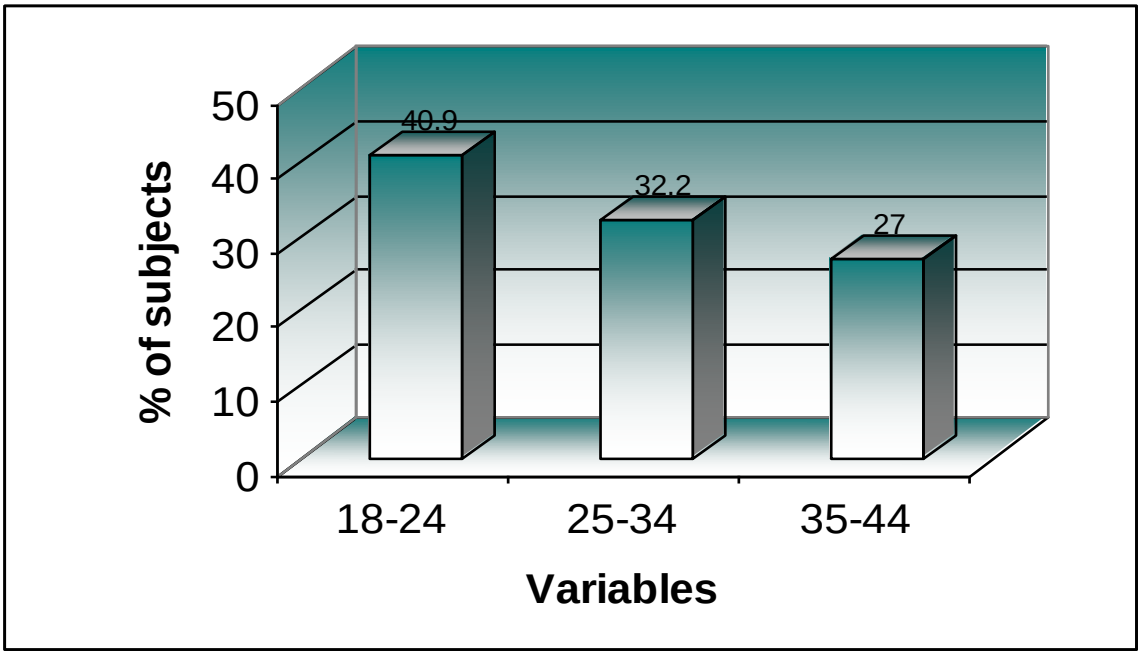

Figure 3.1 Percentage distribution of women of childbearing age by age categories (n=115)

3.2 Socio-demographic status and maternity history

Table 3.1 shows socio-demographic status and maternity history profiles of women of childbearing age. The highest percentage of women lived in households with 6-10 members (70.4%) compared to the percentage of women living in households with 1–5 members (12.2%) and in households with ≥ 11 members (17.4%). The majority of women (64.3%) who participated in the study were not married, 33% were married and 2.6% were widows. Most of the women were not working (67% whereas 12% were employed and 21% were school pupils. Most women had a secondary education (87.8%), 12.2% had primary education and none had tertiary education.

Electricity supply was available to 68.7% of women whereas 31.3% had no access. Thirty-five percent of women were using a refrigerator. Three methods of water supply were available in the study area. Communal taps were the most commonly used (67.8%) followed by tap in the yard (24.3%) and a shared tap (2 – 5 families) was the least used (7.8%).

Most of the participants had 1 to 4 children (61%) while 11% of women had ≥ 5 children and 27% had no children. Still births were reported by 8 women (7%) and miscarriage by 3 women (2.6%). Cases of perinatal mortality (death of their newborns immediately after birth) were reported by 9 women (7.8 %).

Table 3.1 **Percentage distribution of women of childbearing age by selected demographic and maternity history characteristics (n-115)**

Age category		Parity	
18 – 24	40.9 % (47)	0	27 % (31)
25 – 34	32.2 % (37)	1 – 4	61 % (71)
35 – 44	27 % (31)	5+	11.3 % (13)
Marital status		Number of still birth	
Single	64.3 % (74)	0	93 % (107)
Married	33 % (38)	1	3.5 % (4)
Widow	2.6 % (3)	>1	3.5 % (4)
Occupation/employment		Number of miscarriage	
Not working	67 % (77)	0	97.4 % (112)
Pupils	21 % (24)	1	2.6 % (3)
Employed	12 % (14)	>1	0
Level of education		Number of perinatal mortality	
Primary	12.2 % (14)	0	92.2 % (106)
Secondary	87.8 % (101)	1	5.2 % (6)
		>1	2.6 % (3)
Highest qualification		Knowledge of neural tube defects	
Post-graduate	0	Yes	30.4 % (35)
Graduate	0	No	69.6 % (80)
Formal training	4.3 % (5)		
Informal training	13 % (15)		
No training	82.6 % (95)		
Electricity use		Cases of neural tube defects	
Yes	68.7 % (79)	0	99.1 % (114)
No	31.3 % (36)	1	0.9 % (1)
Water supply			
Tap outside	24.3 % (28)		
Shared tap	7.8 % (9)		
Communal tap	67.8 % (78)		
Use of refrigerator			
Yes	35.7 % (41)		
No	64.3 % (74)		
No. of household members			
1 – 5	12.2 % (14)		
6 – 10	70.4 % (81)		
≥11	17.4 % (20)		

In Table 3.2, socio-demographic status of participants was analyzed by age categories and compared. Significant differences were observed for marital status ($p<0.0001$) and occupation ($p<0.0001$).

A high percentage of single women (97.9%) was observed in the youngest age group (18–24 years) compared to the middle aged [25–34 years (64.9%)] and older age groups [35–44 years (12.9%)]. Only 1 participant in the younger group was married compared to 35.1% and 77.4% of middle aged and older women, respectively. Widows (9.7%) were observed in the older group only.

Many of the participants of the youngest group were pupils (48.9%) compared to only 1 woman of middle aged group and none from the older age group. High percentages of women not working were observed in age groups; 25–34 (78.4%), and 35–44 (83.9%). Very few of young participants (4.3%) were employed compared to middle age (18.9%) and older age (16.1%) women. No significant differences in level of education, highest qualification and household information were observed. However, 6.4% in the youngest group had formal training, compared to middle aged (2.7%) and older age (3.3%) women.

Table 3.2 **Comparison of socio-demographic status by age (n=115)**

Demographic variables	18 – 24 years (n=47), % (n)	25 – 34 years (n=37) % (n)	35 – 44 years (n - 31) % (n)	P-value
Marital status				
Single	97.9% (46)	64.9% (24)	12.9% (4)	
Married	2.1 (1)	35.1% (13)	77.4% (24)	
Widow	0	0	9.7% (3)	< **0.0001**
Occupation				
Not working	46.8% (22)	78.4% (29)	83.9% (26)	
Pupils	48.9% (23)	2.7% (1)	0	
Employed	4.3% (2)	18.9% (7)	16.1% (5)	< **0.0001**
Level of education				
Primary	6.4% (3)	16.2% (6)	16.1% (5)	
Secondary	93.6% (44)	83.8% (31)	83.9% (26	0.29
Highest qualification				
Post- graduate	0	0	0	
Graduate	0	0	0	
Formal training	6.4% (3)	2.7% (1)	3.2% (1)	
Informal training	4.3% (2)	24.3% (9)	12.9% (4)	
No training	89.4% (42)	73.0% (27)	83.9% (26)	0.09
Electricity use				
Yes	66% (31)	59.5% (22)	83.9% (26)	
No	34.0% (16)	40.5% (15)	16.1 (5)	0.30
Water supply				
Tap outside	19.1% (9)	24.3% (9)	32.3% (10)	
Shared tap	8.5% (4)	8.1% (3)	6.5% (2)	
Communal tap	72.3% (34)	67.6% (25)	61.3% (19)	0.78
Use of refrigerator				
Yes	27.7% (13)	37.8% (14)	45.2% (14)	
No	72.3%(34)	62.2% (23)	54.8% (17)	0.27
No. of household members				
1 – 5	10.6% (5)	10.8% (4)	16.1% (5)	
6 – 10	70.2% (33)	64.9% (24)	77.4% (24)	
≥ 11	12.1% (9)	24.3% (9)	6.5% (2)	0.38

Comparison of maternity history by age is presented in Table 3.3. Significant differences were observed for parity ($p<0.0001$), stillbirths ($p=0.01$) and perinatal mortality ($p=0.007$).

Most of the youngest participants had no children (52.4%) compared to 8.1% of women aged 25 – 34 years and 3.2% of women aged 35 – 44 years. High percentages of women with parity of 1 to 4 and or above 5 were observed in the middle and older age group whereas 20% of the youngest women reported parity of 1 – 4 and none had 5 or more children.

Stillbirths and perinatal mortality were more common in the older age groups and were significantly different compared to young and middle aged women. Seven older women reported perinatal mortality compared to one case in the other age groups. Stillbirths were also observed in 7 older women but none in women aged 25 – 34 and only one case in the younger group. No significant difference in the number of miscarriages was observed.

Table 3.3 **Comparison of maternity history by age (n=115)**

Demographic variables	**18 – 24 years (n=47), % (n)**	**25 – 34 years (n=37) % (n)**	**35 – 44 years (n - 31) % (n)**	**P-value**
Parity				
0	57.4% (27)	8.1% (3)	3.2% (1)	
1 – 4	42.6% (20)	86.5% (32)	61.3% (19)	
≥ 5	0	5.4% (2)	35.5% (11)	< **0.0001**
Number of stillbirth				
0	97.9% (46)	100% (37)	77.4% (24)	
1	2.1% (1)	0	9.7% (3)	
>1	0	0	12.9% (4)	**0.01**
Number of miscarriage				
0	97.9% (46)	97.3% (36)	96.8% (30)	
1	2.1% (1)	2.7% (1)	3.2% (1)	
>1	0	0	0	0.96
Number of perinatal mortality				
0	97.9% (46)	97.3% (36)	77.4% (24)	
1	2.1% (1)	2.7% (1)	12.9% (4)	
>1	0	0	9.7% (3)	**0.007**
Number of neural tube defects				
0	97.9% (46)	100% (37)	100% (31)	0.48
≥1	2.1% (1)	0	0	

3.2.1 Materials used for cooking in the study area

Materials used when cooking in the study area included wood, paraffin, electricity, coal and gas (Fig 3.2). The majority of women (85.4%) used wood for cooking. Paraffin (58.3%) and electricity (40.9%) were also commonly used for cooking. The least used materials were coal (15.7%) and gas (2.6%).

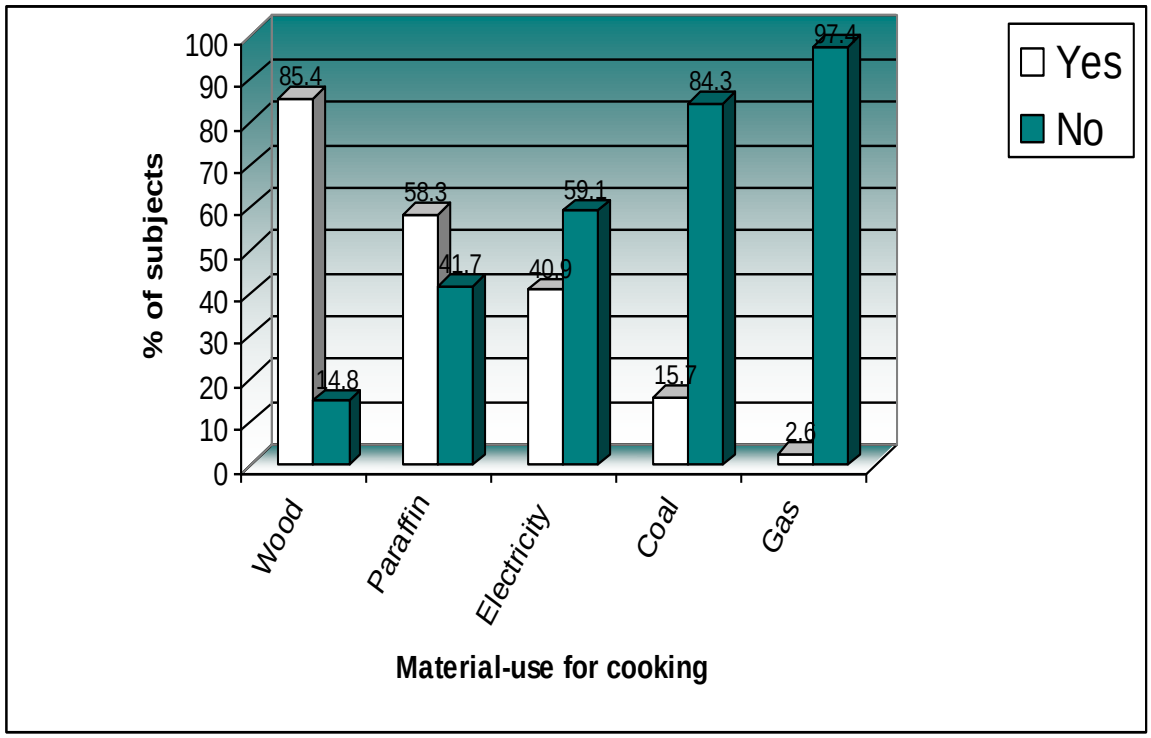

Figure 3.2 Materials used for cooking by participants (n = 115)

A2. ANTHROPOMETRY OF WOMEN OF CHILDBEARING AGE

3.3 Anthropometric measurements

Of the 120 women who participated in the study, 101 had complete information of dietary intake and anthropometry. Missing information was due to the absence of participants when remaining interviewes were to be conducted. Questionnaires were analyse two years after the study had started, as such it it was impossible to retrace women with missing information because of the developments that might have occurred in two years time. As such, women with missing and/or incomplete dietary intake and anthropometry were excluded.

BMI defines whether an individual has a normal body mass (18.5 ≤ to 24.9 kg/m^2), is underweight (<18.5 kg/m^2), overweight (25-29.9 kg/m^2) or obese (≥30 kg/m^2). Abdominal obesity in women is indicated by waist circumference ≥ 88 cm (WHO, 1998) or WHR ≥0.85. Descriptive statistics of anthropometric measurements of women are presented in Table 3.4. Body weight and height measurements were used to calculate the body mass index (BMI). Mean BMI of women was 26.9 kg/m^2(25.7; 28.0) indicating overweight in general, whereas the mean waist [79.6 cm (77.2; 81.9)] was normal as well as WHR [0.79 (0.78; 0.80)], although a maximum value of 1 was observed.

Table 3.4 **Descriptive statistics of anthropometric measurements of women of childbearing age (n=101)**

Variables (unit)	Mean	95%CI	Minimum	Maximum
Weight (kg)	66. 3	63.6; 69.1	42.0	117.0
Height (m)	1.57	1.56; 1.59	1.16	1.77
BMI (kg/m^2)	26.9	25.7; 28.0	18.8	52.4
Waist (cm)	79.6	77.2; 81.9	62.0	132.0
Hip (cm)	100.1	98.1; 102.2	78.0	139.0
WHR	0.79	0.78; 0.80	0.67	1.00

Abbreviations: BMI – body mass index, WHR – waist hip ratio, kg – kilogram, m – meter, cm – centimeters, and CI – confidence interval

Table 3.5 shows the percentage of participants with anthropometric variables that are normal, above, or below the cut off points. None of the women was underweight (BMI ≤18.5 kg/m^2) whereas 26.7% and 27.7% of women were overweight and obese, respectively. WHR was above the cut off point (≥ 0.85) in 14.9% of women while 20.8% of women had waist circumferences above the cut off point (≥ 88 cm).

Table 3.5 **Percentage of participants within, below or above the cut-off points of anthropometry (n=101)**

Variables (unit)	Cut-off points	% (n)
BMI (kg/m2)	< 18.5	0
	18.5 ≥ to 24.9	45.5% (46)
	25 – 29.9	26.7% (27)
	≥ 30	27.7% (28)
Waist (cm)	< 88	79.2% (80)
	≥ 88	20.8% (21)
WHR	< 0.85	85.1% (86)
	≥ 0.85	14.9% (15)

Abbreviations: BMI – body mass index, WHR – waist hip ratio, n = number of subjects

Table 3.6 shows comparison of means of anthropometric measurements of women by age. Significant differences of weight, BMI, waist, hip and WHR were observed when comparing the three age groups. The mean weight of young women was significantly different from the means of other two groups. The mean BMI of younger participants was significantly lower compared to the women in the middle and older groups. Older women showed high mean waist circumference (86.6cm) and WHR (0.82) compared to younger participants. No significant differences in the means of anthropometry measures were observed between the 25 – 34 and 35 – 44 year age groups. Means of height were similar for all the age groups.

Table 3.6 **Comparison of anthropometric measurements of participants by age group (n=101)**

Variables (unit)	**18 – 24 years (n = 41) Mean (95%CI)**	**25 – 34 years (n = 32) Mean (95%CI)**	**35 – 44 years (n = 28) Mean (95%CI)**	**P^A – value**	**P^B – value**
Weight (kg)	59.8 (56.8; 62.7)	68.1 (63.1; 73.0)	73.9 (68; 80)	**<0.0001**	P_1= **0.02** P_2 <**0.0001** P_3= 0.21
Height (m)	1.57 (1.54; 1.59)	1.58 (1.56; 1.59)	1.57(1.56; 1.60)	0.76	P_1= 1.00 P_2=1.00 P_3=1.00
BMI (kg/m2)	24.5 (23.1; 25.9)	27.4 (25.4; 29.3)	29.8 (27.2; 32.3)	**0.001**	P_1= 0.09 P_2 <**0.0001** P_3= 0.273
Waist (cm)	72.9 (70.9; 75.0)	81.7 (77.8; 85.5)	86.8 (81.4; 92.2)	**<0.0001**	P_1= **0.002** P_2 <**0.0001** P_3 = 0.18
Hip (cm)	95.3 (92.9; 97.6)	101.3 (97.3; 105.3)	105.9 (101.6; 110.2)	**<0.0001**	P_1= **0.03** P_2 < **0.0001** P_3= 0.218
WHR	0.76 (0.75; 0.78)	0.81 (0.78; 0.83)	0.82 (0.79; 0.84)	**<0.0001**	P_1= **0.007** P_2= **0.001** P_3= 1.00

Abbreviations: P^A-value – show p-value within three groups, P^B–value – show p-values between groups, P_1 – p-value between groups;18 – 24 years and 25 – 35 years, p_2-value – p-value between groups; 18 – 24 years and 35 – 44 years, and p_3 – value – p-value between groups; 25 – 34 years and 35 – 44 years.

In Table 3.7 percentage of women with anthropometry within, above or below the reference ranges were compared. High percentages of women with obesity were observed in middle and older age groups. Waist circumference was above normal in 21.9% of women (25 – 34 years) and 25.0% of older women. High percentages of women with abdominal obesity were observed in middle (31.3%) and older (35.7%) women.

Table 3.7 **Percentage of participants within, below and above the reference ranges of anthropometry by age**

Variables (unit)	**18 – 24 years (n=41) % (n)**	**25 – 34 years (n=32) % (n)**	**35 – 44 years (n=28) % (n)**	**P-value**
BMI (kg/m2)				
< 18.5	0	0	0	
19 – 24.9	68.3% (28)	37.5% (12)	21.4% (6)	
25 – 29.9	22.0% (9)	28.1% (9)	32.1% (9)	
≥ 30	9.8% (4)	44.4% (11)	46.4% (13)	**0.001**
WHR				
< 0.85	97.6% (40)	68.8% (18)	64.3% (18)	
≥ 0.85	2.4% (1)	31.3% (10)	35.7% (10)	**0.01**
Waist (cm)				
< 88	97.6% (40)	78.1% (25)	75.0% (21)	
≥ 88	2.4% (1)	21.9% (7)	25.0% (7)	**0.001**

Abbreviations: BMI – body mass index, WHR – waist hip ratio, n = number of subjects

A3. DIETARY INTAKE OF WOMEN OF CHILDBEARING AGE USING 24 HR RECALL AND QFFQ

To obtain dietary intake information, each participant was required to complete two 24 hour recall questionnaires (1 questionnaire in relation to dietary intake during the week days, and the other in relation to dietary intake on Sunday) and one QFFQ (concurrent with the first 24 hr recall).

3.4 Dietary intake using 24hr recall questionnaire

Table 3.8 shows descriptive statistics of mean nutrient intakes of women using two 24 hr recalls. Mean energy intake (6906kJ/day) was below the RDA of 9205 kJ/day and ranged from 3782 kJ/day to 9574 kJ/day. Mean intakes of total protein (50.7g/day) was at the RDA and carbohydrates (266.9 g/day) was above the RDA. However, the intake of plant proteins was much higher than the animal protein intake. Mean total fibre intake (20.4 g/day) was also below the RDA (25 g/day) and ranged from 0.9 g/day to of 39.6 g/day. Mean intakes of vitamins A (589.5µg/day), thiamin (1.0 mg/day), riboflavin (0.8 mg/day), niacin (9.6mg/day), vitamin B6 (0.6 mg/day), folate (176.9µg/day), vitamin C (31.0 mg/day) and vitamin D (2.2 µg/day) were below the RDA while mean intake of vitamin B12 (4.2µg/day) was above the RDA. Mean intakes of most minerals were below the RDA except for phosphorus (795.4 mg/day) and magnesium (314.2 mg/day) which were above RDA.

Table 3.8 **Descriptive statistics of selected nutrient intake of women of childbearing age (n=101) using 24 hour recall**

Variables (unit)	Mean	95%CI	Median	Minimum	Maximum	RDAs	%RDA
Energy(KJ)	6906	6664; 7148	6889	3782	9574	9205[A]	75
Tproteins(g)	50.7	48.1; 53.2	49.6	5.1	89.4	50[A]	101.4
Aproteins(g)	18.01	15.8; 20.3	17.5	0.0	54.2	*	
Pproteins(g)	32.7	31.2; 34.3	32.7	16.6	53.6	*	
CHO (g)	266.9	256.5; 277.3	270.0	142.8	360.2	130[B]	205
Tsugars (g)	20.7	16.8; 24.7	16.1	0.0	146.2	*	
Asugar (g)	24.3	20.5; 28.1	20.2	0.0	144.0	*	
Tfibre (g)	20.4	19.0; 21.8	19.5	0.9	39.6	25[B]	81.6
Starch (g)	9.6	5.8; 13.4	0.9	0.0	116.2	*	
Fat (g)	30.9	28.2; 33.6	28.7	10.9	73.7	*	
Chol (mg)	115.6	90.6; 140.6	57.0	0.0	495.0	< 300[C]	38.5
Vit A(RE)(µg)	589.5	326.2; 825.8	133.0	0.0	8583.0	800[A]	74.8
Vit E (mg)	6.5	5.4; 7.7	3.9	1.0	23.9	8[A]	81.5
Thiamin(mg)	1.0	0.9; 1.0	1.0	0.5	1.6	1.1[A]	90.9
Riboflavin(mg)	0.8	0.5; 1.1	0.5	0.2	14.8	1.3[A]	61.5
Niacin(mg)	9.6	8.8; 10.4	9.9	0.6	22.2	15[A]	64
Vit B_6 (mg)	0.6	0.6; 0.7	0.6	0.2	1.3	1.6[A]	37.5
Folate (µg)	176.9	155.1; 198.8	152.0	51.0	726.0	400[B]	44.2
Vit B_{12}(µg)	4.2	1.8; 6.5	0.8	0.0	98.4	2[A]	210
Vit C (mg)	31.0	24.3; 37.8	22.0	0.0	257.0	60[A]	51.6
VitD (µg)	2.2	1.7; 2.7	1.0	0	9.9	5[A, C]	44
Iron (mg)	7.9	7.2; 8.5	7.4	3.0	27.8	15[A]	52.7
H-iron (mg)	0.3	0.2; 0.4	0.0	0.0	2.6	*	
NH-iron (mg)	2.1	1.8; 2.4	1.9	0.2	6.4	*	
Ca (mg)	221.3	196.1; 246.4	193.0	8.10	752.0	1000[A]	22.1
Zn (mg)	6.1	5.7; 6.6	5.7	1.0	15.3	8[A]	76.3
Na (mg)	916.1	811.3; 1021.0	896.0	18.6	2660.0	< 2400[C]	38
K (mg)	1512.4	1443.9; 1581.0	1474.0	669.0	2551.0	1600-2000[C]	94.5
Mg (mg)	314.2	291.6; 336.7	295.0	135.0	1129.0	310[A]	101
P (mg)	795.43	753.1; 837.7	779.0	381.0	1912.0	700[A]	113

Abbreviations: Tproteins - total proteins, Aproteins - animal proteins, Pproteins - plant proteins, CHO - carbohydrates, Tsugars – total sugar, Asugar - added sugar, Tfibre - total fibre, Chol - total cholesterol, Vit – vitamin, H-iron – haem iron, NH-iron - non haem iron, Ca – calcium, Zn – zinc, Na – sodium, K – potassium, Mg – magnesium and P – phosphorus. A indicate the RDAs taken from MRC food finder programme version 1.0.9, B indicate the RDAs taken from nutrition information centre of the University of Stellenbosch, 2003 and C indicate the RDAs adopted from Recommended Dietary Allowances, National Research Council, 10th edition. ***** show that RDAs are not available.

3.5 Dietary intake using QFFQ

Table 3.9 shows descriptive statistics of nutrient intakes of women using QFFQ. Mean energy intake (8823kJ/day) was below the RDA of 9205 kJ/day ranging from 5727 kJ/day to 11934kJ/day. Mean intakes of total protein (61.1g/day) and carbohydrate (350.4 g/day) were above the RDA. However, the intake of plant proteins was much higher than the intake of animal proteins. Mean total fibre intake was normal (26.3 g/day) and ranged from 1.9 g/day to 39.7 g/day.

Mean intakes of vitamin A (534.2µg/day), vitamin E (6.2 mg/day), riboflavin (0.73 mg/day), niacin (12.1 mg/day), vitamin B6 (0.8 mg/day), folate (228.8µg/day), vitamin C (27.8 mg/day) and vitamin D (2.8 µg/day) were below the RDA. Mean intakes of thiamin (1.30 mg/day) and vitamin B12 (5.5µg/day) were above the RDA. However, thiamin and vitamin B12 showed values below the RDAs. Vitamins such as vitamin A, thiamin, riboflavin, niacin, folate, vitamin C and vitamin D showed intakes above the RDA whereas vitamin B6 showed an intake below RDA.

Mean intakes of iron (10.2 mg/day), calcium (248.6 mg/day), zinc (7.5 mg/day) and Na intake (1524.8 mg/day) minimum whereas phosphorus (1010.8 mg/day) and magnesium (391.9 mg/day) were above the RDA, and potassium (1802.7 mg/day) was normal.

Table 3.9 **Descriptive statistics of selected nutrients intake of women of childbearing age using QFFQ, (n=101)**

Variables (unit)	Mean	95%CI	Median	Minimum	Maximum	RDAs	%RDA
Energy(kJ)	8823	8579; 9068	9046	5727	11934	9205[A]	96
Tproteins(g)	61.1	59.4; 62.8	60.8	33.4	88.3	50[A]	122
Aproteins(g)	15.9	15.9; 18.4	15.8	5.6	40.3	*	
Pproteins(g)	43.2	41.6; 44.8	45.2	16.9	66.1	*	
CHO (g)	350.4	336.2; 364.6	376.5	39.8	479.0	130[B]	279.5
Tsugars (g)	38.7	34.52; 43.0	42	5.5	186.0	*	
Asugar (g)	46.3	36.2; 56.4	40.1	11.4	521.0	*	
Tfibre (g)	26.3	25.2; 27.4	27.2	1.9	39.7	25[B]	105.2
Starch (g)	8.5	4.6; 12.4	4.3	1.0	119.4	*	
Fat (g)	35.0	33.0; 36.9	33.9	16.9	66.1	*	
Chol (mg)	163.1	143.3; 182.9	139.0	20.0	581.0	< 300[C]	54
Vit A(RE)(µg)	534.2	394.7; 673.7	151.0	3.6	2820.0	800 [A]	66.8
Vit E (mg)	6.2	5.6; 6.9	5.6	1.7	26.3	8 [A]	77.5
Thiamin(mg)	1.3	1.2; 1.3	1.4	0.7	1.9	1.1 [A]	118
Riboflavin(mg)	0.7	0.7; 0.7	0.6	0.3	2.6	1.3 [A]	53.9
Niacin(mg)	12.1	11.6; 12.5	11.8	3.6	19.8	15[A]	80.7
Vit B_6 (mg)	0.8	0.8; 0.9	0.8	0.34	1.6	1.6 [A]	50
Folate (µg)	228.8	210.5; 247.0	194.0	72.0	591.0	400 [B]	57.2
Vit B12(µg)	5.5	2.8; 8.2	2.2	0.4	133.0	2 [A]	275
Vit C (mg)	27.8	24.6; 31.0	24.0	7.0	80.0	60 [A]	46.3
Vit D (µg)	2.8	2.6; 3.1	2.7	0.3	6.9	5 [A,C]	56
Iron (mg)	10.2	8.5; 11.8	9.1	5.3	89.0	15 [A]	68
H-iron (mg)	0.3	0.2; 0.4	0.1	0.0	2.2	*	
NH-iron (mg)	1.8	1.6; 2.0	1.5	0.7	5.6	*	
Ca (mg)	248.6	234.8; 262.5	242.0	97.0	700.0	1000 [A]	24.9
Zn (mg)	7.5	7.3; 7.5	7.5	3.3	12.3	8 [A]	93.8
Na (mg)	1524.8	1448.1; 1601.4	1536.0	14.9	3094.0	< 2400 [C]	63.5
K (mg)	1802.7	1752.9; 1852.5	1816.0	1235.0	2660.0	1600-2000 [C]	
Mg (mg)	391.9	378.6; 405.2	405.0	218.0	562.0	310 [A]	112.7
P (mg)	1010.8	976.7; 1044.8	1039.0	9.7	1327.0	700 [A]	144.4

Abbreviations: Tproteins - total proteins, Aproteins - animal proteins, Pproteins - plant proteins, CHO - carbohydrates, Tsugars – total sugars, Asugar - added sugar, Tfibre - total fibre, Chol - total cholesterol, Vit – vitamin, H-iron – haem iron, NH-iron - non haem iron, Ca – calcium, Zn – zinc, Na – sodium, K – potassium, Mg – magnesium and P – phosphorus. A indicate the RDAs taken from MRC food finder programme version 1.0.9, B indicate the RDAs taken from nutrition information centre of the University of Stellenbosch, 2003 and C indicate the RDAs adopted from Recommended Dietary Allowances, National Research Council, 10th edition. ***** show that RDAs are not available.

3.6 Comparison of dietary intake using 24hr recall and QFFQ

Table 3.10 compares the mean nutrient intakes of women using 24 hr recall and QFFQ. Mean energy intake was significantly higher with QFFQ (8823 kJ/day) than with 24 hr recall (6906 kJ/day). However in both methods, energy intakes were below the RDA. Mean total protein intake was significantly higher with QFFQ compared to 24 hr recall whereas mean animal protein was significantly lower with 24 hr recall than with QFFQ. Both questionnaires indicated that mean intake of total protein was above the RDA. No significant differences in mean plant proteins were observed. Mean intakes of carbohydrates, total sugars, added sugar and total fibre were significantly higher with QFFQ than with 24 hr recall. However, the mean intake of total fibre with QFFQ was above the RDA than was the case with 24 hr recall and the mean intake of carbohydrate was above the RDA with both questionnaires. Mean intakes of fat and cholesterol were significantly different and higher with QFFQ compared to 24 hr recall.

Mean intakes of micronutrients; thiamin, niacin, vitamin B6, folate and vitamin D were significantly different except for vitamin A, vitamin E, riboflavin, vitamin B12 and vitamin C. Higher intakes of thiamin, niacin, vitamin B6, folate and vitamin D were observed when using QFFQ. Mean intakes of vitamin B6 and folate were below RDA with both questionnaires. Mean intakes of minerals; iron, calcium, sodium, potassium, magnesium and phosphorus were significantly higher when using QFFQ compared to 24 hr recall except for intakes of haem-iron, non-haem iron and zinc.

Table 3.10 **Comparison of mean intakes of selected nutrients of women of childbearing age (n=101) using 24 hour recall and food frequency**

Variables (unit)	**24 hour recall Mean (95%CI)**	**Food frequency Mean (95%CI)**	**P-value**
Energy(kJ)	6906 (6664; 7148)	8823 (8579; 9068.0)	**<0.0001**
Tproteins(g)	50.7 (48.1; 53.2)	61.1 (59.4; 62.78)	**<0.0001**
Aprotein (g)	18.1 (15.8; 20.3)	15.9 (15.9; 18.4)	**<0.0001**
Pprotein (g)	32.7 (31.2; 34.3)	43.2 (41.6; 44.8)	0.751
CHO (g)	266.9 (256.5; 277.3)	350.4 (336.2; 364.6)	**<0.0001**
Tsugars (g)	20.7 (16.8; 24.7)	38.8 (34.5; 43.0)	**<0.0001**
Asugar (g)	24.3 (20.5; 28.2)	46.3 (36.2; 56.4)	**<0.0001**
Tfibre (g)	20.4 (19.0; 21.8)	26.3 (25.2; 27.4)	**<0.0001**
Starch (g)	9.6 (5.8; 13.4)	8.5 (4.6; 12.4)	**0.001**
Fat (g)	30.9 (28.2; 33.6)	35.0 (33.0; 36.9)	**0.002**
Chol (mg)	115.6 (90.6; 140.6)	163.1 (143.3; 182.9)	**<0.0001**
Vit A(RE)(µg)	589.5 (326.2; 825.8)	534.2 (394.7; 637.7)	0.70
Vit E (mg)	6.5 (5.4; 7.7)	6.2 (5.6; 6.9)	0.66
Thiamin(mg)	1.0 (1.0; 1.1)	1.3 (1.3; 1.3)	**<0.0001**
Riboflavin(mg)	0.8 (0.5; 1.1)	0.7 (0.7; 0.7)	0.62
Niacin(mg)	9.6 (8.8; 10.4)	12.1 (11.6; 12.5)	**<0.0001**
Vit B_6 (mg)	0.6 (0.6; 0.7)	0.8 (0.8; 0.9)	**<0.0001**
Folate (µg)	176.9 (155.1; 198.8)	228.8 (210.5; 247.0)	**<0.0001**
Vit B12(µg)	4.2 (1.8; 6.5)	5.5 (2.8; 8.2)	0.38
Vit C (mg)	31.0 (24.3; 37.8)	27.8 (16.1; 24.6)	0.41
VitD (µg)	2.2 (1.7; 2.7)	2.8 (2.6; 3.1)	**0.03**
Iron (mg)	7.9 (7.2; 8.5)	10.2 (8.5; 11.8)	**0.01**
H-iron (mg)	0.3 (0.2; 0.4)	0.3 (0.2; 0.4)	0.80
NH-iron (mg)	2.1 (1.8; 2.4)	1.8 (1.6; 2.0)	0.16
Ca (mg)	221.3 (196.1; 246.4)	248.6 (234.3; 262.)	**0.05**
Zn (mg)	6.1 (5.7; 6.6)	7.5 (7.3; 7.5)	0.07
Na (mg)	916.1 (811.3; 1021.0)	1524.79 (1448.13; 1601.43)	**<0.0001**
K (mg)	1512.4 (1443.9; 1581.0)	1802.7 (1752.9; 1852.5)	**<0.0001**
Mg (mg)	314.2 (291.6; 336.7)	391.9 (378.6; 405.2)	**<0.0001**
P (mg)	795.4 (753.1; 837.7)	1010.8(976.7; 1044;8)	**<0.0001**

Abbreviations: A indicate the RDAs taken from MRC food finder programme version 1.0.9, B indicate the RDAs taken from nutrition information centre of the University of Stellenbosch, 2003 and C indicate the RDAs adopted from Recommended Dietary Allowances, National Research Council, 10th edition, Tproteins - total proteins, Aproteins - animal proteins, Pproteins - plant proteins, CHO - carbohydrates,Tsugars – total sugars, Asugar - added sugar, Tfibre - total fibre, Chol - total cholesterol, Vit – vitamin, H-iron – haem iron, NH-iron - non haem iron, Ca – calcium, Zn – zinc, Na – sodium, K – potassium, Mg – magnesium and P – phosphorus. A indicate the RDAs taken from MRC food finder programme version 1.0.9, B indicate the RDAs taken from nutrition information centre of the University of Stellenbosch, 2003 and C indicate the RDAs adopted from Recommended Dietary Allowances, National Research Council, 10th edition. ***** show that RDAs are not available.

In Table 3.11, percentages of women with nutrient intakes within, below or above the RDA% are compared. RDA% of 80-100% indicates optimal intake of nutrients whereas %RDA of 67-79.9% indicate inadequate intake, though at a lower risk, and RDA% < 67% shows absolute inadequate intake (Kruger et al., 1994; Lee & Nieman, 1996). Significant differences in the percentage of women with values within, above or below RDA% were observed between 24 hr recall and QFFQ for energy, Tprotein, Tfiber, vitamin A, thiamin, riboflavin, niacin, vitamin B12, vitamin E and minerals; iron, zinc, sodium, potassium and magnesium. Most of the women (88%) consumed optimal energy with QFFQ compared to 24 hr recall (36%). Carbohydrate intake was optimal in 100% (24 hr recall) and 98% (QFFQ) of women. Cholesterol consumed with both 24 hr recall (75.2%) and QFFQ (73.3%) was below 300 mg/day as required.

The percentage of women with inadequate intakes were observed for vitamins A (82.2%), riboflavin (79.2%), niacin (51.5%), vitamin B6 (96%), folate (90.1%), vitamin B12 (59.4%), vitamin C (73.3%), vitamin D (72.3%), vitamin E (58.4%), iron (85.1%), zinc (42.6%), calcium (98%) and sodium (90.1%) when using 24 hr recall. When using QFFQ, high prevalences of women with inadequate intakes were observed for vitamin A (71.3%), Riboflavin (73.3%), vitamin B6 (91.1%), folate (79.2%), vitamin C (776.2), vitamin D (75.2%), vitamin E (45.5%) and for minerals, iron (68.3%), calcium (99%) and sodium (62.4%).

No significant differences were observed with nutrient intakes within, below or above %RDA between 24 hr recall and QFFQ, when comparing age groups.

Table 3.11 **Comparison of percentage of women (n=101) with nutrient intake normal, below or above the RDA percentage**

Variables (unit)	24 hr recall RDA%			QFFQ RDA%			P-value
	80-100% [% (n)]	67-79.9% [% (n)]	<67% [% (n)]	80-100% [% (n)]	67-79.9% [% (n)]	<67% [% (n)]	
Energy(kJ)	35.6 (36)	35.6 (36)	28.7 (29)	71 (88)	20 (9)	12.1 (4)	**<0.0001**
Tproteins(g)	82.1 (83)	9.9 (10)	7.9 (8)	98 (99)	1.0 (1)	1.0 (1)	**0.001**
CHO (g)	100 (101)	0	0	98 (99)	0	2.0 (2)	0.25
Tfibre (g)	44.6 (45)	24.8 (25)	30.7 (31)	90.1 (99)	5.0 (5)	5.0 (5)	**< 0.0001**
Chol (mg)	17.8 (18)	6.9 (7)	75.2 (76)	17.8 (18)	8.9 (9)	73.3 (74)	0.87
Vit A(RE)(µg)	14.9 (15)	3.0 (3)	82.2 (83)	28.7 (29)	0	71.3 (72)	**0.02**
Thiamin(mg)	74.3 (75)	13.9 (14)	11.9 (12)	97.0 (98)	2.0 (2)	1.0 (1)	**<0.0001**
Riboflavin(mg)	18.8 (19)	2.0 (2)	79.2 (80)	14.9 (15)	11.9 (12)	73.3 (74)	**0.02**
Niacin(mg)	20.8 (21)	27.7 (28)	51.5 (52)	47.5 (48)	39.6 (40)	12.9 (13)	**0.0001**
Vit B_6 (mg)	1.0 (1)	3.0 (3)	96 (97)	2.0 (2)	6.9 (7)	91.1 (92)	0.36
Folate (µg)	6.9 (7)	3.0 (3)	90.1 (91)	12.9 (13)	7.9 (8)	79.2 (80)	0.09
Vit B12(µg)	38.6 (39)	2.0 (2)	59.4 (60)	77.2 (78)	3.0 (3)	19.8 (20)	**< 0.0001**
Vit C (mg)	21.8 (22)	5.0 (5)	73.3 (74)	14.9 (15)	8.9 (9)	76.2 (77)	0.28
VitD (µg)	19.8 (20)	7.9 (8)	72.3 (73)	13.9 (14)	10.9 (11)	75.2 (76)	0.45
Vit E (mg)	36.6 (37)	5.0 (5)	58.4 (59)	32.7 (33)	21.8 (22)	45.5 (46)	**0.002**
Iron (mg)	8.9 (9)	5.9 (6)	85.1 (86)	6.9 (7)	24.8 (25)	68.3 (69)	**0.001**
Ca (mg)	0	2.0 (2)	98.0 (99)	0	1.0 (1)	99.0 (100)	0.50
Zn (mg)	36.6 (37)	20.8 (21)	42.6 (43)	86.1 (87)	11.9 (12)	2.0 (2)	**< 0.0001**
Na (mg)	4.0 (4)	5.9 (6)	90.1 (91)	5.9 (6)	31.7 (32)	62.4 (63)	**< 0.0001**
K (mg)	76.2 (77)	15.8 (16)	7.9 (8)	98.0 (99)	2.0 (2)	0	**< 0.0001**
Mg (mg)	80.2 (81)	6.9 (7)	12.9 (13)	96.0 (97)	4.0 (4)	0	**< 0.0001**
P (mg)	89.1 (90)	5.9 (6)	5.0	97.0 (98)	2.0 (2)	1.0 (1)	0.08

Abbreviations: Tfibre - total fibre, Chol - total cholesterol, Vit – vitamin, H-iron – haem iron, NH-iron - non haem iron, Ca – calcium, Zn – zinc, Na – sodium, K – potassium, Mg – magnesium and P – phosphorus. A indicate the RDAs taken from MRC food finder programme version 1.0.9, B indicate the RDAs taken from nutrition information centre of the University of Stellenbosch, 2003 and C indicate the RDAs adopted from Recommended Dietary Allowances, National Research Council, 10^{th} edition. ***** show that RDAs are not available.

Contribution of carbohydrates, proteins and fat intake to total energy intake for both 24 hr recall and QFFQ are presented in Figures 3.3 and 3.4, Tables 3.12 and 3.13.

Carbohydrates contributed 62% (4282 kJ) and 64% (5647 kJ) of total energy intake using 24 hr recall and QFFQ respectively. Percentage energy contributions for proteins were 13% (897 kJ) and 12% (1059 kJ) and for fats were 17% (1174 kJ) and 15% (1323 kJ), when using 24hr recall and QFFQ, respectively. According to Food and Agricultural Organization and the World Health Organization (WHO, 2003), carbohydrates should contribute 55 – 75% of energy intake, protein 10 - 15% and fat 15 – 30%. Results are shown in Fig 3.3, 3.4 and Table 3.12,

In Table 3.13, significant differences in the contribution of saturated and unsaturated fatty acids to total energy intakes were observed between 24 hr recall and QFFQ. The prudent guidelines (PG) from the MRC food finder III, 1.0.9, for fatty acid contribution to total energy intake are as follows, SFA; <10%E, MUFA; +10%E and PUFA; ~10%E. In the present study, saturated fatty acid (SFA) contributed 7% (24 hr recall) and 8.5% (QFFQ), ($p=0.002$) to total energy intake. Mono-unsaturated fatty acids (MUFA) contributed 12.8% and 10% when using 24 hr recall and QFFQ, respectively, however, no significant difference was observed. Poly-unsaturated fatty acids contributed 10.2% (24 hr recall) and 11.2 (QFFQ) to total energy intake, ($p=0.001$).

Table 3.12 **Percentage contribution of carbohydrate, protein and fat to total energy intake (2hr recall and QFFQ)**

Variables	**%**	**24 hr-recall**	**QFFQ**
EI (kJ)		**6906**	**8823**
CHO:	**55% – 75%**	=266.9g 266.9g x 16 kJ/g x 100 6906 4270 kJ x 100 6906 kJ = 0.618 x 100 = **62%**	=350.5g 350.5g x 16 kJ/g x 100 8823 5608 kJ x 100 8823 kJ = 0.635 x 100 = **64%**
Proteins:	**10% - 15%**	= 50.7g 50.7g x 17 kJ/g x 100 6906 861.9 kJ x 100 6906 kJ = 0.125 x 100 = **13%**	= 61.1g 61.1g x 17 kJ/g x 100 8823 1038.7 kJ x 100 8823 kJ = 0.117 x 100 = **12%**
Fat:	**15% - 30%**	= 30.9g 30.9g x 37 kJ/g x 100 6906 1132.2 x 100 6906 = 0.164 x 100 = **17%**	= 35.0g 35.0g x 37 kJ/g x 100 8823 1295 x 100 8823 = 0.148 x 100 = **15%**
Total:		62% + 13% + 17% **= 92%**	64% + 12% + 15% = **94%**

Abbreviations: EI – enegy intake, QFFQ – quantitative frequency questionnaire, CHO – carbohydrates, RDA – recommended dietary allowance

Table 3.13 **Percentage contribution of saturated and unsaturated fatty acids to total energy intake, n = 101**

Variables	**PG**	**24 hr recall % (95%CI)**	**QFFQ % (95%CI)**	**P-value**
%E-SFA	< 10%E	7.0 (6.3; 7.7)	8.5 (8.0; 9.0)	**0.002**
%E-MUFA	+ 10%E	12.8 (7.2; 18.6)	10.0 (9.3; 10.7)	0.06
%E-PUFA	~ 10%E	10.2 (9.0; 11.4)	11.2 (10.4; 12.0)	**0.001**

Abbreviations: PG – prudent guideline, %E – percentage of total energy, SFA – saturated fatty acids, MUFA – mono-unsaturated fatty acids and PUFA – poly-unsaturated fatty acids

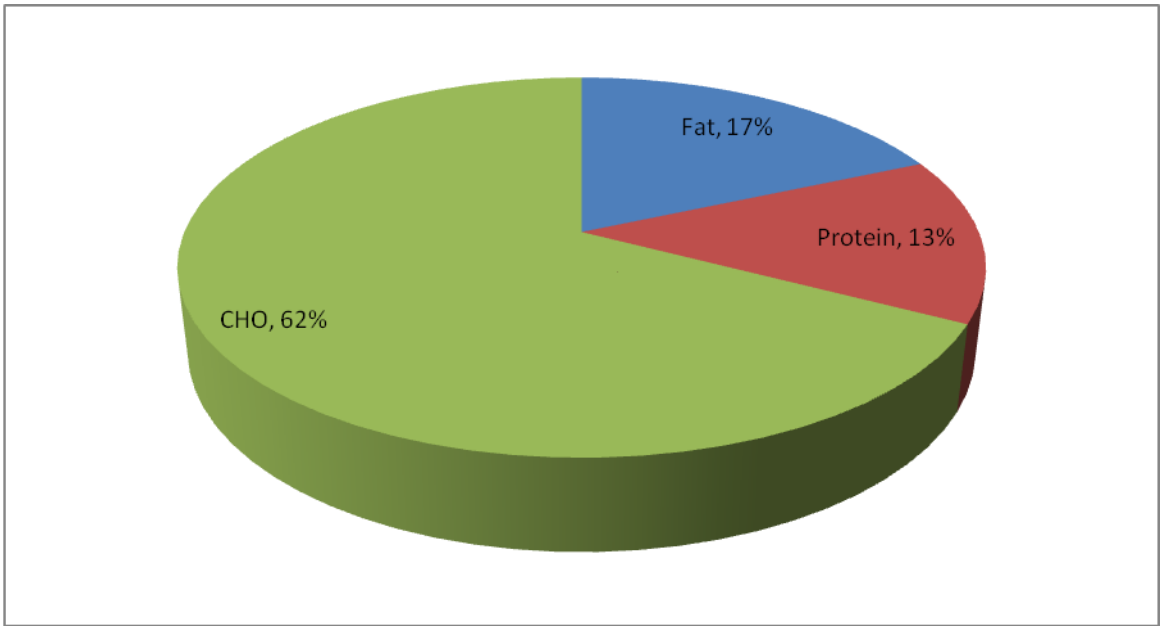

Figure 3.3 Contribution of total energy intake using 24 hr recall

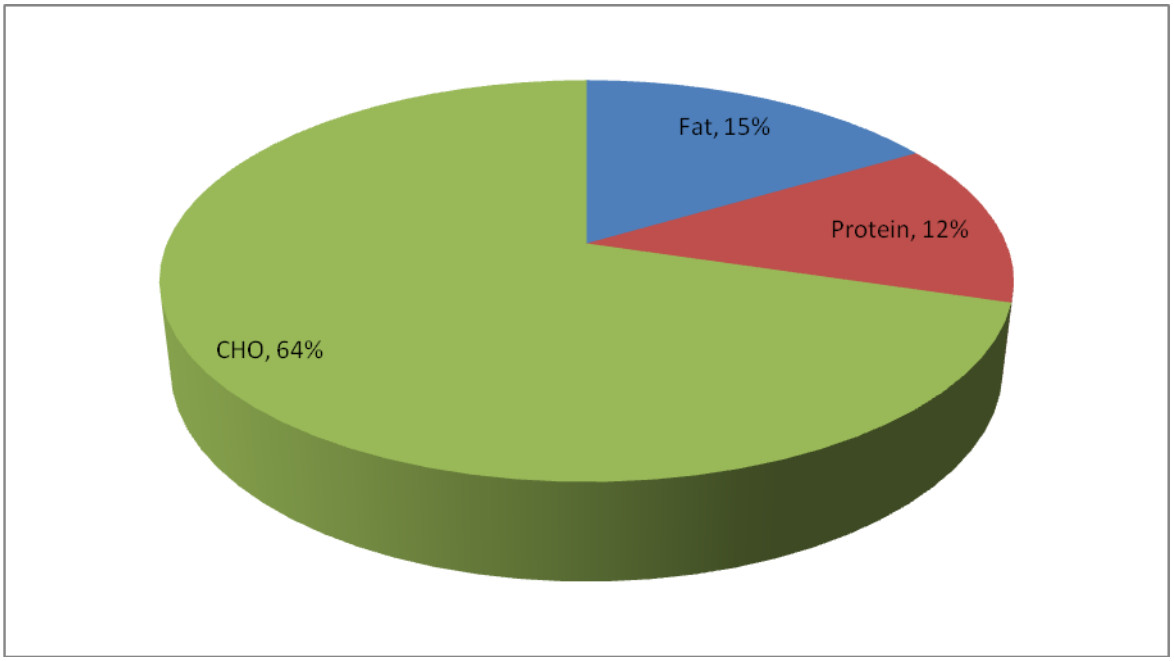

Figure 3.4 Distribution of total energy intake using QFFQ

3.7 Under-reporting of energy intake

Underreporting was ascertained by computing a ratio of energy intake (EI) to basal metabolic rate (BMR). Underreporting was established when the ratio of EI:BMR was less than 1.2 (Goldberg et al, 1991) while a ratio ≥ 1.2 indicate a normal reporting.

Formula to determine underreporting (Brieftel et al., 1997) = $\frac{\text{EI (total kJ)}}{\text{BMR}_{est}}$

Table 3.14 **Formula to determine BMR* (Schofield, 1985)**

Age group	Females	Males
19 – 30 years	0.062 x wt** + 2.036	0.063 x wt** + 2.896
31 – 60 years	0.034 x wt** + 2.538	0.048 x wt** + 3.653
Over 60 years	0.038 x wt** + 2.755	0.049 x wt** + 2.459

* BMR: Basal metabolic rate expressed in MJ/24 hours, ** wt: weight expressed in kg

Table 3.15 shows that 54.5% of women were classified as under-reporters when using 24hr recall whereas 8.9% of women underreported with QFFQ. Underreporting was significantly higher using the 24 hour recall compared to QFFQ, $p<0.0001$.

Table 3.15 **Reporting of energy intake of women of childbearing age**

EI/BMR	**24 hr recall % (n)**	**QFFQ % (n)**	**P-value**
< 1.2	54.5 (55)	8.9 (9)	< 0.0001
≥ 1.2	45.5 (46)	91.1 (92)	

Abbreviation: EI – energy intake, BMR – basal metabolic rate, n = number of subjects, QFFQ – quantitative food frequency questionnaire

3.8 Commonly consumed food using QFFQ

Food items commonly consumed by women of childbearing age residing in Dikgale DSS using QFFQ included maize meal, as a staple food, tea, as the most consumed beverage, brown bread, chicken feet, pilchard (tin fish in tomato sauce) and eggs among others. Green leafy vegetables (morogo and spinach), cabbage, potatoes, tomatoes and onions were the most commonly consumed vegetables whereas banana and apple were the most commonly consumed fruits. Chicken heads, chicken and boerewors were also among the most common foods eaten.

A4. ASSOCIATION BETWEEN ANTHROPOMETRY AND DIETARY INTAKE

Women were divided into three groups of BMI; 18.5-24.99 kg/m^2, 25-29.99 kg/m^2 and ≥30 kg/m^2 and variables related to body weight were selected for association.

Table 3.16 shows the mean (95%CI) energy, carbohydrate and fat intakes of women categorized according to the BMI using 24 hr recall. No significant differences in energy, carbohydrate and fat intake were observed among and within the groups.

Table 3.17 shows the mean (95% CI) energy, carbohydrates and fat of women by BMI using QFFQ. No significant differences of energy, carbohydrates and fat intakes by BMI were observed for the three groups.

Table 3.16 **Comparison of energy, carbohydrate and fat intakes (24 hr recall) by BMI**

Variables (unit)	**19 - 24kg/m^2 n=46 Mean (95%CI)**	**25 – 29.9kg/m^2 n=27 Mean (95%CI)**	**≥ 30 kg/m^2 n=28 Mean (95%CI)**	**P^A-value**	**P^B-Value**
Age (years)	26 (23.5; 28.0)	29 (26.3; 32.61)	33 (30.2; 35.8)	**0.001**	P_1=0.14 P_2<**0.0001** P_3=0.26
Energy (kJ/day)	7063 (6708.9; 7418.9)	7077.8 (6607.5; 7548;1)	6481.2 (5998.7; 6963.6	0.10	P_1=1.00 P_2=0.14 P_3=0.21
CHO (g/day)	272.0 (256.0; 287.9)	270.1 (252.1; 288.0)	255.5 (233.2; 277.8)	0.40	P_1=1.00 P_2=0.59 P_3=0.93
Fat (g/day)	33.1 (28.7; 37.5)	32.8 (27.6; 38.0)	25.5 (21.2; 29.8)	**0.05**	P_1=1.00 P_2=0.06 P_3=0.15

Abbreviations: P^A-value – show p-value within three groups, P^B–value – show p-values between groups, P_1 – p-value between groups;19-24.99 kg/m^2 and 25-29.99 kg/m^2, p_2-value – p-value between groups; 19-24.99 kg/m^2 and ≥30 kg/m^2, and p_3 – value – p-value between groups; 24.99-29.99 kg/m^2 and ≥30 kg/m^2, CHO – carbohydrates n= no. of subjects

Table 3.18 **Comparison of energy, carbohydrate and fat intakes (QFFQ) by BMI**

Variables (unit)	**19 - 24kg/m^2 N=46 Mean (95%CI)**	**25 – 29.9kg/m^2 N=27 Mean (95%CI)**	**≥ 30 kg/m^2 N=28 Mean (95%CI)**	**P^A-value**	**P^B-Value**
Age (years)	26 (23.5; 28.0)	29 (26.3; 32.61)	33 (30.2; 35.8)	**0.001**	P_1=0.14 P_2<**0.0001** P_3=0.26
Energy (kJ/day)	8658.7 (8267.2; 9050.3)	8884.0 (8371;7 9396.4)	9035.6 (8633.7; 9437.6)	0.43	P_1=1.00 P_2=0.62 P_3=1.00
CHO (g/day)	340.2 (318.2; 362.1)	348.6 (314.9; 382.3)	368.8 (349.4; 388.1)	0.25	P_1=1.00 P_2=0.30 P_3=0.90
Fat (g/day)	34.2 (31.5; 37.1)	36.0 (31.9; 40.1)	35.1 (31.4; 38.8)	0.76	P_1=1.00 P_2=1.00 P_3=1.00

Abbreviations: P^A-value – show p-value within three groups, P^B–value – show p-values between groups, P_1 – p-value between groups;19-24.99 kg/m^2 and 25-29.99 kg/m^2, p_2-value – p-value between groups; 19-24.99 kg/m^2 and ≥30 kg/m^2, and p_3 – value – p-value between groups; 24.99-29.99 kg/m^2 and ≥30 kg/m^2, CHO – carbohydrates, n= no. of subjects

The Pearson correlation coefficients of age and anthropometry with 24 –hr recall energy, carbohydrates and fat intake are presented in Table 3.18. Age correlated significantly positive with weight, BMI, waist and WHR (P<0.0001) whereas weight correlated significantly positive with BMI, waist and WHR (P<0.0001) and BMI with waist and WHR (P<0.0001). A significant negative correlation was observed between waist and fat intake (P=0.02) as well as between WHR and fat intake (P=0.007). Energy correlated significantly with carbohydrates and fat (P<0.0001).

Table 3.18 **Correlation coefficients of age and anthropometry with energy, carbohydrate and fat intakes (24 hr recall)**

Variables (unit)	Age	Weight	BMI	Waist	WHR	Energy	CHO
Weight (kg)	0.44”						
BMI (kg/m^2)	0.39”	0.90”					
Waist (cm)	0.52”	0.87”	0.83”				
WHR	0.41”	0.41”	0.43”	0.65”			
Energy (kJ/day)	0.005	-0.14	-0.11	-0.14	-0.11		
CHO (g/day)	0.08	-0.09	-0.11	-0.05	0.01	0.86”	
Fat (g/day)	-0.17	-0.21’	-0.12	-0.24’	-0.27”	0.50”	0.04

Abbreviations:’ – correlation is significant at the 005 level (2-tailed) and ” – correlation significant at the 0.01 level (2-tailed), BMI- bodya mass index, WHR – waist hip ratio, CHO- carbohydrates

The Pearson correlation coefficients of age and anthropometry with energy, carbohydrates and fat intake are presented in Table 3.19. Age correlated significantly positive with weight, BMI, waist and WHR (P<0.0001) whereas weight correlated significantly positive with BMI, waist and WHR (P<0.0001) and BMI with waist and WHR (P<0.0001). Energy correlated significantly positive with carbohydrates and fat (P<0.0001). No significant correlations were observed between anthropometry and nutrients.

Table 3.19 **Correlation coefficients of age and anthropometry with energy, carbohydrates and fat intake (QFFQ)**

Variables (unit)	Age	Weight	BMI	Waist	WHR	Energy	CHO
Weight (kg)	0.44”						
BMI (kg/m^2)	0.39”	0.90”					
Waist (cm)	0.52”	0.87”	0.83”				
WHR	0.41”	0.41”	0.43”	0.65”			
Energy (kJ/day)	-0.02	0.07	0.15	-0.008	-0.06		
CHO (g/day)	-0.06	0.14	0.23	0.10	0.03	0.85”	
Fat (g/day)	0.08	0.02	0.02	-0.08	0.04	0.43”	0.15

Abbreviations:’ – correlation is significant at the 005 level (2-tailed) and ” – correlation significant at the 0.01 level (2-tailed,) BMI- bodya mass index, WHR – waist hip ratio, CHO- carbohydrates

A.5 BIOCHEMISTRY RESULTS

3.9 Biochemical variables of women before fortification of foods

During phase 1 of the study, before food fortification was introduced in South Africa, fasting blood samples were collected from 120 women of childbearing age to assess folate and iron status as well as other biochemical variables. Folate status was assessed by serum and red cell folate and vitamin B12 and plasma homocysteine levels while iron status was assessed by measuring serum ferritin, iron, and total iron binding capacity, percentage transferrin saturation, C - reactive protein and full blood count.

Complete biochemical results were available for 117 women except for the indicated variables in relevant tables. Table 3.20 shows descriptive statistics of biochemical variables before fortification was introduced. Means of serum folate [3.7ng/ml (3.4; 3.9)] and red cell folate [262ng/ml (233; 291)] were within the normal ranges. Means of vitamin B12 and plasma homocysteine levels were also normal. Means of serum ferritin [40.9µg/l (33.2; 48.7)] and CRP [4.9mg/l (2.8; 6.8)] were normal. Means of serum iron [14.1µg/dl (12.4; 15.9)] and transferrin saturation [%TS, 39% (32.1; 43.5)] were within the reference ranges whereas mean total iron binding capacity [TIBC, 41.0µg/l (38.2; 43.8)] was below the reference range. Full blood count indices were within the reference range.

Table 3.20 **Descriptive statistics of biochemical parameters in women of childbearing age prior to fortification of foods**

Variables (unit) (n)	Mean (95%CI)	Median	Minimum	Maximum	RR
Age (years) (117)	28.7 (27.2; 30.2)	27	18	44	18 – 44
Sfol (ng/ml)† (117)	3.7 (3.4; 3.9)	3.6	1.0	10.0	≥3
RCF (ng/ml) † (73)	262 (233; 291)	230	61	793	≥ 164
SF (µg/l) † (117)	40.9 (33.2; 48.7)	33.9	2.4	273.6	≥ 20
CRP (mg/l) † (96)	4.8 (2.8; 6.8)	2.3	0	76.5	< 10
VB12 (pg/ml) † (117)	317.6 (291.0; 344.2)	298.0	114.0	829.0	180–914
*t*Hcy(µmol/L)† (35)	8.5 (7.3; 8.9)	8.0	4.3	16.7	4.5 – 12.4
SIron (µg/dl)† (56)	14.1 (12.4; 15.9)	13.6	2.4	34.9	9.0 – 30.4
TIBC (µg/dl)† (56)	41.0 (38.2; 43.8)	38.3	24.3	66.5	44.7 – 80.5
%TS (%)† (56)	39 (32.1; 43.5)	36	4	100	≥ 16
RBC (10^6/µL) (117)	4.5 (4.4; 4.6)	4.5	2.6	6.1	4.0 – 6.20
HGB (g/dl) (117)	13.2 (12.9; 13.5)	13.5	7.6	18.3	11.0 – 18.8
HCT (%) (117)	38.9 (39.8; 39.1)	39.7	24.1	55.3	35.0 – 55.0
MCV (fl) † (117)	86 (84.8; 87.6)	88	63	99	80.0 – 100.0
MCH (pg) † (117)	29 (26.6; 29.8)	30	19	34	26 – 34
MCHC (g/dl) † (117)	33 (33.6; 33,9)	34	31	35	31 – 35

Abbreviations: † - variable with non-Gaussian distribution. n – number of subjects, CI- confidence interval, RR – reference range, Sfol – serum folate, RCF – red blood cell folate, VB12 – vitamin B12, *t*Hcy – plasma homocysteine, SF– ferritin, CRP – C-reactive protein, SIron – serum iron, TIBC – total iron binding capacity, %TS – percentage transferrin saturation, RBC – red blood cells count, HGB – haemoglobin, HCT – haemotocrit and MCV – mean corpuscular volume, MCH – mean cell haemoglobin and MCHC – mean cell haemoglobin concentration.

Table 3.21 shows the percentages of women with values normal, below or above the reference ranges. Low serum and red cell folate were observed in 28.3% and 21.9% of women, respectively. Low vitamin B12 levels were observed in 14.5% and high levels of homocysteine were observed in 8.6% of women. Severe iron depletion was observed in 28.2% (serum ferritin levels below 12µg/l) whereas CRP values above the reference range were seen in 6.3% (6) of women. Of the 6 women with elevated CRP levels above 10 mg/l, four had serum ferritin levels above 20µg/l. Low TIBC was present in 71.4% of women whereas low serum iron levels were observed in 26.8% and low %TS in 17.9% of women. Haemoglobin was below the reference range in 9.4% of women and none had MCV values above 100fl.

Table 3.21 **Percentages of subjects with variables below, within and above the cut-off point, prior to fortification of foods**

Variables (unit) (n)	**Cut-offs**	**Below [%(n)]**	**Normal [% (n)]**	**Above [%(n)]**
Sfol (ng/ml) (117)	< 2.5 2.5 – 2.99 ≥ 3	18.8% (22) 9.4% (11)	71.8% (84)	
RCF (ng/ml) (73)	< 164 ≥ 164	21.9% (16)	78.1% (57)	
VB12 (pg/ml) (117)	< 145 180 - 914 > 914	14.5% (17)	85.5% (100)	0
*t*Hcy (µmol/L) (35)	< 4.5 4.5 – 12.4 > 12.4	5.7% (2)	85.7% (30)	8.6% (3)
SF(µg/l) (117)	< 20.0 < 12.0 12.0 – 19.9 ≥ 20	39.3% (46) 28.2% (33) 11.1% (13)	60.7% (71)	
CRP (mg/l) (96)	≥ 10 < 10		93.8% (90)	6.3% (6)
SIron (µg/dl) (56)	< 9.0 9.0 – 30.4 > 30.4	26.8% (15)	71.4% (40)	1.8% (1)
TIBC (µg/dl) (56)	< 44.7 44.7 – 80.5 > 80.5	71.4% (40)	28.6% (16)	0
%TS (%) (56)	< 16 ≥ 16	17.9% (10)	82.1% (46)	
RBC (10^6/µl) (117)	< 4.0 4.0 – 6.20	9.4% (11)	90.6% (106)	
HGB (g/dl) (117)	<11.0 11.0 – 18.8	9.4% (11)	90.6% (106)	
HCT (%) (117)	< 35.0 35 - 55 > 55.0	14.5% (17)	84.6% (99)	0.9% (1)
MCV (fl) (117)	< 80 80 – 100 > 100	15.4% (18)	84.6% (99)	0
MCH (pg) (117)	< 26.0 26 - 34 >34	12.8% (15)	84.6% (99)	2.6% (3)
MCHC (g/dl) (117)	< 31.0 31 - 35 > 35.0	0.9% (1)	98.3% (115)	0.9% (1)

Abbreviations: n – number of subjects, CI- confidence interval, RR – reference range, Sfol – serum folate, RCF – red blood cell folate, VB12 – vitamin B12, *t*Hcy – plasma homocysteine, SF– ferritin, CRP – C-reactive protein, SIron – serum iron, TIBC – total iron binding capacity, %TS – percentage transferrin saturation, RBC – red blood cells count, HGB – haemoglobin, HCT – haemotocrit and MCV – mean corpuscular volume, MCH – mean cell haemoglobin and MCHC – mean cell haemoglobin concentration.

Pearson correlations were done to assess the relationships between variables prior to fortification of foods. Correlation coefficients are presented in Table 3.22.

Age correlated significantly with BMI, waist and WHR (p=0.001), as well as with serum ferritin and C-reactive protein (p=0.001). BMI correlated significantly positively with waist and WHR. Positive correlations were observed between WHR with serum folate and VB12.

Serum folate correlated positively with TIBC (p=0.05) and negatively with serum ferritin and MCH (p=0.03). RCF correlated positively with vitamin B12 (p=0.002) and negatively with serum ferritin (p=0.008) and MCV (p=0.03). No significant correlations were observed between plasma homocysteine and other biochemical variables. C-reactive protein correlated significantly negatively with %TS.

Table 3.22 **Correlation coefficients of selected variables in women of childbearing age prior to fortification of foods**

Vs	Age	BMI	Waist	WHR	Sfol	RCF	VB12	*t*Hcy	SF	CRP	SIron	TIBC	%TS	RBC	HGB	HCT	MCV	MCH
BMI	0.38”																	
Waist	0.49”	0.86”																
WHR	0.39”	0.47”	0.70”															
Sfol	-0.18	0.09	0.00	0.21’														
RCF	-0.15	0.09	0.00	-0.04	0.38”													
VB12	0.04	0.08	0.04	0.27”	0.09	0.35”												
*t*Hcy	0.25	0.15	0.19	0.20	-0.25	-0.29	-0.25											
SF	0.21’	0.17	0.16	0.05	-0.23”	-0.31”	0.07	0.17										
CRP	0.23’	0.18	0.22	0.17	-0.02	-0.12	0.15	0.18	0.11									
SIron	0.07	-0.02	-0.01	-0.07	-0.19	0.16	-0.04	0.07	0.53”	-0.23								
TIBC	-0.13	0.04	.03	0.07	0.27’	0.30	0.05	0.09	-0.57”	0.16	-0.32’							
%TS	0.10	-0.02	-0.02	-0.08	-0.25	0.03	-0.05	0.09	0.65”	-0.27’	0.93”	-0.64”						
RBC	-0.01	-0.02	-0.04	-0.18	0.10	-0.08	-0.01	0.21	0.06	-0.08	0.03	0.22	0.02					
HGB	0.02	0.01	0.00	-0.17	-0.08	-0.19	-0.09	0.18	0.49”	-0.09	0.54”	-0.39”	0.59”	0.60”				
HCT	0.01	0.02	0.00	-0.16	-0.06	-0.19	-0.07	0.21	0.44”	-0.08	0.48”	-0.32’	0.51”	0.69”	0.99”			
MCV	0.14	0.06	0.07	-0.01	-0.18	-0.26’	-0.09	-0.09	0.53”	0.03	0.58”	-0.41”	-0.63”	-0.21”	0.61”	0.53”		
MCH	0.12	0.03	0.03	-0.06	-0.19’	-0.20	-0.14	0.13	0.56”	0.01	0.65”	-0.44”	0.70”	-0.20’	0.62”	0.52”	0.94”	
MCHC	0.05	-0.05	-0.09	-0.12	0.17	-0.15	-0.11	-0.01	0.51”	-0.10	0.57”	-0.53”	0.66”	-0.19’	0.53”	0.39”	0.74”	0.82”

Abbreviations: Vs – variables, ’ – correlation is significant at the 005 level (2-tailed) and ” – correlation significant at the 0.01 level (2-tailed), BMI – body mass index, WHR – waist hip ratio, Sfol – serum folate, RCF – red blood cell folate, VB12 – vitamin B12, *t*Hcy – plasma homocysteine, SF– ferritin, CRP – C-reactive protein, SIron – serum iron, TIBC – total iron binding capacity, %TS – percentage transferrin saturation, RBC – red blood cells count, HGB – haemoglobin, HCT – haemotocrit and MCV – mean corpuscular volume, MCH – mean cell haemoglobin and MCHC – mean cell haemoglobin concentration.

Table 3.23 shows descriptive statistics of serum protein and liver enzymes prior to fortification of foods. Protein measured was albumin (Alb) as well as liver enzymes, γ-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP).

Mean albumin [42.4 (41.5; 43.3)] was within the reference range. Means of GGT [23.1 U/L (21.3; 24.9)], AST [31.2 U/L (29.4; 33.0)], and ALP [64.4 U/L (60.3; 68.4)] were within the normal ranges while mean ALT [27.0 UL (24.9; 29.2)] was below the reference range.

Table 3.23 **Descriptive statistics of albumin and liver enzymes of women of childbearing age prior to fortification of foods**

Variables (unit) (n)	**Mean (95%CI)**	**Median**	**Minimum**	**Maximum**	**RR**
Alb (g/L) † (107)	42.4 (41.5; 43.3)	42.3	31.2	56.6	34 – 50
GGT (U/L) † (115)	23.1 (21.3; 24.9)	23.0	7.0	76.0	5 – 55
AST ((U/L) † (106)	31.2 (29.4; 33.0)	33.0	5.0	54.0	15 – 37
ALT (U/L) † (111)	27.0 (24.9;29.2)	26.0	4.0	67.0	30 – 65
ALP (U/L) † (111)	64.4 (60.3; 68.4)	61.0	24.0	142.0	50 – 160

Abbreviations: † indicate variables with non-Gaussian distribution. n – number of subjects per test. Alb – albumin, GGT – γ-glutamyl transferase, AST – aspartate aminotransferase, ALT – alanine aminotransferase and ALP – alkaline phosphatase

Table 3.24 presents the percentages of women with variables below or above the cut-off points. Relatively low levels of albumin (< 34g/L) were observed in 4.7% (5) of women. Only one woman had an elevated level of GGT and ALT (0.9%) whereas AST was elevated in 21.7% of women.

Table 3.24 **Percentages of subjects with albumin and liver enzymes levels below and above the cut-off point prior to fortification of foods**

Variables (unit) (n)	**Cut-off points**	**Below %(n)**	**Above %(n)**	**RR**
Alb (g/L) (107)	< 34.0 > 50	4.7% (5)	-	34 – 50
GGT (U/L) (115)	< 5.0 > 55.0	-	0.9% (1)	5 – 55
AST ((U/L) (106)	< 15.0 > 37.0	-	21.7% (23)	15 – 37
ALT (U/L) (111)	< 30.0 > 65.0	-	0.9% (1)	30 – 65
ALP (U/L) (111)	< 50.0 > 160.0	-	0	50 – 160

Abbreviations: † indicate variables with non-Gaussian distribution. n – number of subjects per test. Alb – albumin, GGT – γ-glutamyl transferase, AST – aspartate aminotransferase, ALT – alanine aminotransferase and ALP – alkaline phosphatase

A6. ASSOCIATION BETWEEN SOCIO-DEMOGRAPHIC STATUS ON FOLATE AND IRON

The association between socio-demographic status indicators and, folate and iron were assessed using serum folate and ferritin, respectively. Subjects susceptible to folate and iron deficiency were used as references showed by 1 in the odds ratios. Results are presented in Tables 3.25 and 3.26. There was no association between selected socio-demographic indicators and folate levels. With regard to marital status, widows (3) were excluded due to the small number. CRP was not used in this case because of the small samples size.

Table 3.25 **Folate status in relation to selected socio-demographic indicators**

Exposure Variable	Level	n	Odds ratios	95% CI	P-value
Age (yrs)	18-24	45	1		
	25-34	36	0.663	0.155-2.829	
	35-44	31	0.229	0.036-1.457	0.26
Marital status	Married	37	1		
	Single	72	0.340	0.0819-9.516	0.14
Occupation	Not working	74	1		
	Pupils	24	2.263	0.477-10.743	
	Employed	14	1.503	0.347-6.507	0.53
Education	Primary	13	1		
	Secondary	99	0.475	0.696-9.516	0.16
Parity	0	29	1		
	1-4	70	0.760	0.189-3.063	
	5	13	1.127	0.134-9.487	0.83
Electricity Cooking	No	46	1		
	Yes	66	0.581	0.212-1.590	0.29
Refrigerator	No	73	1		
	Yes	39	2.390	0.792-7.210	0.12

Table 3.26 shows the association between selected socio-demographic status and iron status. There was no association between selected socio-demographic indicators and folate status, except for age. Middle aged and older women had a significantly lower risk of iron deficiency than the younger women.

Table 3.26 **Iron status in relation to selected socio-demographic indicators**

Exposure Variable	Level	n	Odds ratios	95% CI	P-value
Age (yrs)	18-24	45	1		
	25-34	36	9.250	1.809-47.310	
	35-44	31	3.452	0.502-23.714	**0.02**
Marital status	Married	37	1		
	Single	72	2.922	0.588-14.513	0.19
Occupation	Not working	74	1		
	Pupils	24	2.289	0.663-7.900	
	Employed	14	1.166	0.245-5.549	0.42
Education	Primary	13	1		
	Secondary	99	0.475	0.083-2.705	0.40
Parity	0	29	1		
	1-4	70	1.291	0.381-4.376	
	5	13	4.482	0.486-41.373	0.38
Electricity Cooking	No	46	1		
	Yes	66	0.522	0.191-1.423	0.20
Refrigerator	No	73	1		
	Yes	39	0.961	0.349-2.648	0.94

A7. ASSOCIATION BETWEEN DIETARY INTAKES AND BIOCHEMICAL VARIABLES

Correlations between nutrients associated with some biochemical variables of women of childbearing age were done and non parametric test (Spearman correlation) was used. No important correlations between biochemical variables and selected nutrients in 24 hour recall were observed. Results are presented in Table 3.27

Table 3.27 **Correlation coefficients of selected nutrient intake (24-hr recall) and biochemistry of women of childbearing age**

Vs	Sfol	RCF	*t*Hcy	VB12	SF	SIron	HGB	Alb	DVA	DVB2	DVB6	Dfol	DVB12	DIron	Haem	DTP
RCF	0.31”															
*t*Hcy	-0.40’	-0.32														
VB12	0.09	0.41”	-0.18													
SF	-0.15	-0.30”	0.05	0.06												
SIron	-0.17	0.23	-0.28	-0.08	0.43”											
HGB	-0.10	-0.15	0.16	-0.04	0.51”	0.53”										
Alb	-0.13	-0.09	0.27	-0.10	0.28”	0.18	0.43”									
DVA	-0.12	0.12	0.16	0.13	-0.26”	-0.18	-0.26”	0.04								
DVB2	-0.06	0.08	0.16	0.04	-0.11	-0.06	-0.05	0.07	0.39”							
DVB6	0.11	0.19	-0.02	-0.10	-0.16	-0.01	0.00	-0.12	0.23”	0.45”						
Dfol	-0.05	0.15	-0.02	-0.10	-0.13	0.11	-0.12	-0.02	0.29”	0.34”	0.51”					
DVB12	-0.04	0.13	-0.08	0.06	-0.03	-0.08	0.01	0.07	0.23’	0.67”	0.26”	0.20’				
DIron	-0.05	0.17	-0.03	-0.11	-0.09	0.04	-0.04	0.06	0.46”	0.59’	0.48”	0.48”	0.32’			
H-Iron	0.11	0.23’	0.04	0.11	-0.16	-0.96	-0.05	-0.04	0.22’	0.25”	0.29”	0.23”	0.41”	0.16		
DTP	0.09	0.09	-0.04	0.03	-0.01	0.10	-0.04	-0.07	-0.03	0.36”	0.45”	0.19’	0.41”	0.31”	0.28”	
PP	0.10	0.02	-0.15	-0.13	0.02	0.19	0.10	0.08	0.01	0.16	0.43”	0.42”	-0.12	0.52”	-0.15	0.43”

Abbreviations: Vs – variables**, ”** indicate significant difference at level 0.01(1-tailed) and **’** – indicate significant difference at level 05 (1-tailed), RCF – red cell folate, tHcy – plasma homocysteine, VB12 – vitamin B12, SF – serum ferritin, SIron – serum iron, HGB – haemoglobin, Alb – albumin, DVA – dietary vitamin A, DVB – dietary vitamin B2, DVB6 – dietary vitamin B6, Dfol – dietary folate, DVB12 – dietary vitamin B12, DIron – dietary iron, H-Iron – haem iron DTP – dietary total protein, PP – plant protein

Table 3.28 presents the correlation coefficients between selected dietary intakes and biochemical variables of women of childbearing age. A positive significant correlation between Sfol and Dfol (QFFQ), (p=0.008) was observed. Results are presented in Table 3.29

Table 3.28 **Correlation coefficients of selected nutrient intake (QFFQ) and biochemistry of women of childbearing age**

Vs	Sfol	RCF	*t*Hcy	VB12	SF	SIron	HGB	Alb	DVA	DVB2	DVB6	Dfol	DVB12	DIron	Haem	DTP
RCF	0.31”															
*t*Hcy	-0.40’	-0.32														
VB12	0.09	0.41”	-0.18													
SF	-0.15	-0.30”	0.05	0.06												
SIron	-0.17	0.23	-0.28	-0.08	0.43”											
HGB	-0.10	-0.15	0.16	-0.04	0.51”	0.53”										
Alb	-0.13	-0.09	0.27	-0.10	0.28”	0.18	0.43”									
DVA	0.10	0.21’	-0.10	0.05	-0.19’	-0.23	-0.22’	-0.09								
DVB2	0.11	-0.09	-0.10	0.02	-0.18’	-0.10	-0.13	-0.11	-0.01							
DVB6	0.11	-0.05	0.06	-0.09	-0.15	-0.11	-0.04	0.00	0.08	0.54”						
Dfol	0.23”	0.14	-0.31’	0.00	-0.26’	0.02	-0.17’	-0.21’	-0.01	0.71”	0.60”					
DVB12	0.09	0.04	0.03	0.06	-0.27”	-0.21	-0.21’	-0.07	0.12	0.74”	0.43”	0.60”				
DIron	0.03	-0.06	-0.07	-0.15	-0.14	-0.01	-0.06	-0.05	-0.15	0.68”	0.53”	0.62”	0.42”			
Haem	0.15	0.21’	-0.22	0.06	-0.28”	-0.25’	-0.24”	-0.20’	-0.03	0.067”	0.36”	0.65”	0.72”	0.32”		
DTP	0.03	-0.17	0.14	-0.17’	-0.19’	-0.18	-0.08	-0.08	-0.05	0.44”	0.44”	0.44”	0.28”	0.67”	0.14	
PP	-0.05	-0.19	0.23	-0.23’	-0.03	-0.03	0.11	-0.06	-0.14	-0.07	0.14	0.02	-0.31”	0.34”	-0.37”	0.60”

Abbreviations: Vs – variables**, ”** indicate significant difference at level 0.01(1-tailed) and **’** – indicate significant difference at level 05 (1-tailed), RCF – red cell folate, tHcy – plasma homocysteine, VB12 – vitamin B12, SF – serum ferritin, SIron – serum iron, HGB – haemoglobin, Alb – albumin, DVA – dietary vitamin A, DVB – dietary vitamin B2, DVB6 – dietary vitamin B6, Dfol – dietary folate, DVB12 – dietary vitamin B12, DIron – dietary iron, H-Iron – haem iron DTP – dietary total protein, PP – plant protein

SECTION B

B1 – BIOCHEMISTRY RESULTS

3.10 Biochemical variables of women after fortification of foods

Approximately nine months after fortification of food was introduced, a follow-up study was carried out on women who participated prior to fortification of foods (Phase 1)**.** As explained in the methodology section, during re-visits, the exclusion criteria applied in phase 1 of the study were still applicable. Nutritional status assessment was not done on the follow-up study as explained in methodology. Therefore, some women were found to be pregnant, lactating or on medication while others had left the study area and couldn't be traced. As a result, phase 2 of the study had 80 participants. The numbers of tests for biochemical variables are indicated in the tables.

Table 3.29 shows descriptive statistics of biochemical variables after fortification was introduced. Means of serum [10.8ng/ml (10.1; 11.5)] and red cell folate [467ng/ml (431; 503)] were normal, while a minimum value of 159ng/ml (RCF) was observed, and none of the women had low levels of folate. Means of vitamin B12 and plasma homocysteine were also normal and no maximum value for homocysteine above the reference range was observed.

Mean serum ferritin (44.9µg/l) was normal with a maximum value of 301.4µg/l and mean CRP (3.0mg/l) was normal with a maximum value of 12mg/l. Mean serum iron (13.8µg/dl) was within the reference range, whereas mean TIBC (41.2µg/dl) was below the reference range and mean percentage transferrin saturation (%TS, 36%) was normal with a minimum value of 3.8%. Means of HGB [13.7 g/dl (13.3; 14.0) and MCV [86.6 (85.3; 88.2)] were within the reference range.

Table 3.29 **Descriptive Statistics of biochemical parameters of women of childbearing age after fortification of foods**

Variables (unit) (n)	**Mean (95%CI)**	**Median**	**Minimum**	**Maximum**	**RR**
Age (years) (80)	32.1 (30.2; 33.9)	34.0	20	46	20 – 46
Sfol (ng/ml) (80)	10.8 (10.1; 11.5)	10.5	3.7	17	≥3
RCF (ng/ml)† (53)	467 (431; 503)	435	159	1026	≥ 164
SF (µg/l) † (80)	44.9 (34.6; 55.2)	30.2	3.2	301.4	≥ 20
CRP (mg/l) † (49)	3 (2.4; 4.1)	3	0	12	<10
VB12 (pg/ml) † (80)	278.6 (252.4; 304.5)	258.5	105.0	572.0	180 –914
*t*Hcy(µmol/L) † (35)	6.1 (5.7; 6.6)	6.1	3.2	8.9	4.5 – 12.4
SIron (µg/dl) † (42)	13.8 (12.2 ;15.4)	14.2	4.6	23.3	9.0 – 30.4
TIBC (µg/dl) † (42)	41.2 (38.3; 44.2)	38.9	25.2	66.2	44.7 – 80.5
% TS (%)† (42)	36(31; 42)	35	7	87	≤ 16
RBC (10^6/µL) † (80)	4.7 (4.6; 4.9)	4.7	2.8	6.0	4.0 – 6.20
HGB (g/dl) † (80)	13.7 (13.3; 14.0)	14	8	16	11.0– 18.8
HCT (%)† (80)	41.1 (40.1 42.1)	42	24	49	35.0– 55.0
MCV (fl) † (80)	86.8 (85.3; 88.3)	88	62	97	80.0– 100
MCH (pg) †(80)	29.2 (28.4; 29.6)	30	18	33	26 –34
MCHC (g/dl) † (80)	33.3 (33.1; 33.4)	33	29	36	31 – 35

Abbreviations: † indicate variables with non-Gaussian distribution. n – number of subjects, CI- confidence interval, RR – reference range, Sfol – serum folate, RCF – red blood cell folate, SF – ferritin, CRP – C-reactive protein, SIron – serum iron, TIBC – total iron binding capacity, %TS – percentage transferrin saturation, VB12 – vitamin B12, *t*Hcy – plasma homocysteine and RBC – red blood cell count, HGB – haemoglobin, HCT – haemotocrit, MCV – mean cell volume, MCH – mean cell haemoglobin and MCHC - mean cell haemoglobin concentration.

Table 3.30 shows the percentages of women with values below or above the reference ranges. None of the women had serum folate levels below 3ng/ml whereas low levels of RCF were observed in 1 woman (1.3%). Low vitamin B12 levels were observed in 9 women (11.3%) while none of the women had elevated levels of homocysteine. Low ferritin levels were seen in 25% of women whereas CRP above the reference range was seen in 1 woman (2.3%). Low TIBC was observed in 19% of women while low %TS was observed in 16.7% of women. Low levels of haemoglobin were seen in 5% of women and none of the women had MCV above 100fl.

Table 3.30 **Percentages of subjects with biochemical variables below and above the cut-off point after fortification of foods**

Variables (unit) (n)	**Cut-offs**	**Below (n)**	**Above (n)**
Sfol (ng/ml) (80)	< 2.5 2.5 – 2.99 ≥ 3	0 0	
RCF (ng/ml) (53)	< 164 ≥ 164	1.3% (1)	
VB12 (pg/ml) (80)	< 145 180 - 914 > 914	11.3% (9)	 0
*t*Hcy(µmol/L) (35)	< 4.5 > 12.4	5.7% (2)	
SF (µg/ml) (80)	< 20.0 < 12.0 12.0 – 19.9 ≥ 20	37.5% (30) 25.0% (20) 12.5% (10)	
CRP (mg/l) (49)	≥ 10 < 10		2.3% (1)
SIron (µg/ml) (42)	< 9.0 9.0 – 30.4 > 30.4	19.0% (8)	 0
TIBC(µg/ml) (42)	< 44.7 44.7 – 80.5 > 80.5	73.4% (31)	 0
% TS (%) (42)	< 16 ≥ 16	16.7% (7)	
RBC (10^6/µl) (80)	< 4.0 4 – 6.20	2.5% (2)	
HGB (g/dl) (80)	<11.0 11 – 18.8	5.0% (4)	
HCT (%) (80)	< 35.0 > 55.0	8.8% (7)	
MCV (fl) (80)	< 80 80 – 100 > 100	13.8% (13.8)	 0
MCH (pg) (80)	< 26.0 >34	13.8% (11)	
MCHC (g/dl) (80)	< 31.0 31 -35 > 35.0	2.5% (2)	 1.3% (1)

Abbreviations: † indicate variables with non-Gaussian distribution. n – number of subjects, CI- confidence interval, RR – reference range, Sfol – serum folate, RCF – red blood cell folate, SF – ferritin, CRP – C-reactive protein, SIron – serum iron, TIBC – total iron binding capacity, %TS – percentage transferrin saturation, VB12 – vitamin B12, *t*Hcy – plasma homocysteine and RBC – red blood cell count, Hb – haemoglobin, HCT – haemotocrit, MCV – mean cell volume, MCH – mean cell haemoglobin and MCHC - mean cell haemoglobin concentration.

Pearson correlations were carried out to assess the relationships between variables after fortification of foods. Correlation coefficients are presented in table 3.31.

Serum folate correlated positively with vitamin B12 (p=0.04), and negatively with MCV (p=0.03), MCH (p=0.02) and MCHC (p=0.02). RCF correlated significantly with vitamin B12 (p=0.05). No significant correlations were observed between plasma homocysteine and other biochemical variables.

Significant correlations were observed among iron status parameters; serum ferritin, serum iron, %TS, Hb, HCT, MCV, MCH, MCHC, TIBC (p=0.01).

Table 3.31 **Correlation coefficients of selected variables in women of childbearing age after fortification of foods**

Vs	Age	Sfol	RCF	VB12	*t*Hcy	SF	CRP	SIron	TIBC	%TS	RBC	HGB	HCT	MCV	MCH
Sfol	0.09														
RCF	-0.09	0.41”													
VB12	0.08	0.23’	0.28’												
*t*Hcy	-0.12	-0.24	-0.18	-0.14											
SF	0.06	-0.17	-0.17	0.23’	-0.08										
CRP	0.36’	0.19	-0.03	-0.04	0.03	0.02									
SIron	-0.12	-0.09	-0.35	-0.02	-0.14	0.71”	-0.05								
TIBC	0.07	0.07	0.10	0.09	-0.13	-0.64”	-0.15	-0.43”							
%TS	-0.12	-0.10	-0.32	-0.05	-0.06	0.79”	-0.09	0.93”	-0.72”						
RBC	0.02	0.23	0.11	-0.01	-0.03	0.04	-0.08	0.20	-0.23	0.24					
HGB	-0.07	0.01	0.00	-0.09	-0.01	0.43”	-0.15	0.58”	-0.58”	0.70”	0.61”				
HCT	-0.07	0.07	0.01	-0.05	-0.02	0.38”	-0.08	0.53”	-0.53”	0.62”	0.70”	0.98”			
MCV	-0.05	-0.23’	-0.03	-0.15	-0.02	0.48”	-0.06	0.52”	-0.47”	0.58”	-0.24’	0.59”	0.47”		
MCH	-0.05	-0.27’	-0.04	-0.17	0.01	0.49”	-0.09	0.51”	-0.48”	0.58”	-0.26’	0.58”	0.45”	0.98”	
MCHC	-0.01	-0.25’	-0.04	-0.20	0.01	0.33”	-0.28	0.44”	-0.45”	0.51”	-0.25’	0.34”	0.13	0.64”	0.71”

Abbreviations: Vs – variables**, ”** indicate significant difference at level 0.01(2-tailed) and **’** – indicate significant difference at level 0.05 (2-tailed). Sfol – serum folate, RCF – red blood cell folate, SF – ferritin, CRP – C-reactive protein, SIron – serum iron, TIBC – total iron binding capacity, %TS – percentage transferrin saturation, VB12 – vitamin B12, *t*Hcy – plasma homocysteine and RBC – red blood cell count, Hb – haemoglobin, HCT – haemotocrit, MCV – mean cell volume, MCH – mean cell haemoglobin and MCHC - mean cell haemoglobin concentration.

Table 3.32 shows descriptive statistics of albumin and liver enzymes after fortification of foods. All mean values were within the normal reference ranges.

Table 3.32 **Descriptive statistics of albumin and liver enzymes of women of childbearing age after fortification of foods**

Variables (unit) (n)	**Mean (95%CI)**	**Median**	**Minimum**	**Maximum**	**RR**
Alb (g/L) (67)	36.7 (36.0, 37.5)	36.2	30.1	53.1	34 – 50
GGT (U/L) (76)	20.1 (17.8; 22.4)	18.5	3.0	53.	5 – 55
AST ((U/L) (78)	32.1 (29.6, 34.5)	32.5	9.0	67.0	15 – 37
ALT (U/L) (74)	30.2 (28.0, 32.4)	28.0	12.0	61.0	30 – 65
ALP (U/L) (73)	55.6 (50.9; 60.3)	54.0	17.0	135.0	50 – 160

Abbreviation: n= number of subjects**,** Alb – albumin, GGT – γ-glutamyl transferase, AST – aspartate aminotransferase, ALT – alanine aminotransferase and ALP – alkaline phosphatase, RR- reference range

Table 3.33 presents the percentages of women with variables below or above the reference ranges. None of the women had low albumin levels. None of the women had elevated GGT, ALT and ALP, however, AST levels were higher in 25.6% of women.

Table 3.33 **Percentages of albumin and liver enzymes below, within and above the cut-off point in women of childbearing age after fortification of foods**

Variables (unit) (n)	Cut-offs	Below	Above	RR
Alb (g/L) (67)	< 34.0 34 - 50 > 50	0	 -	34 – 50
GGT (U/L) (76)	< 5.0 5 - 55 > 55.0	-	 0	5 – 55
AST ((U/L) (78)	< 15.0 15 - 37 > 37.0	-	 25.6% (20)	15 – 37
ALT (U/L) (74)	< 30.0 30 – 65 > 65	-	 0	30 – 65
ALP (U/L) (73)	< 50.0 50 – 160 > 160	-	 0	50 – 160

Abbreviation: n = number of subjects Alb – albumin, GGT – γ-glutamyl transferase, AST – aspartate aminotransferase, ALT – alanine aminotransferase and ALP – alkaline phosphatase, RR – reference range

SECTION C – PHASE 1 and PHASE 2

C1 – COMPARISON OF BIOCHEMISTRY RESULTS (PHASE 1 and PHASE 2)

3.11 Comparison of biochemistry results before and after fortification of foods

In this section, biochemical variables of women of childbearing age analyzed before (Phase 1) and after (Phase 2) fortification, were compared. Available results for women in phase 2 were matched with phase 1 results and the numbers of tests are indicated in the tables.

Variables with non-Gaussian distribution were logarithmically transformed for statistical analysis (sample paired-*t*-test) and results are presented as medians [inter-quartile ranges (IQ)] of variables in women of childbearing age, before and after fortification. Sfol and RCF were significantly increased after fortification whereas means of VB12 and *t*Hcy decreased significantly. Differences of 7.2ng/ml (5.1; 9.3) and 199ng/ml (130; 336) were observed for Sfol and RCF, respectively. *t*Hcy decreased with 2.0µmol/L (-3.8; -0.6) after fortification, whereas VB12 decreased with 24.5pg/ml (75.0; 27.3). Results are presented in Table 3.34

No significant changes in SF, SIron, TIBC, %TS and CRP were observed after fortification. Mean levels of RBC, HGB and HCT were significantly increased after fortification. There were no significant differences in means of MCV and MCH after fortification. Due to insufficient blood, not all biochemical tests could be carried out on all blood samples.

Table 3.34 **Comparison of biochemical and hematological variables of women of childbearing age before fortification and after fortification**

Variables(unit)(n)	**RR**	**Pre-fortification period**	**Post-fortification period**	**Median Difference**	**P–value**
Sfol (ng/ml) (80)	≥3	3.9 (2.9; 4.2)	10.5 (8.5; 13.5)	7.2 (5.1; 9.3)	**<0.0001**
RCF (ng/ml) (53)	≥ 164	227 (154; 301)	429 (367; 610)	199 (130; 336)	**<0.0001**
VB12 (pg/ml) (80)	180 –914	266.0 (196.3; 394.0)	258.5 (182.0; 363.5)	-24.5 (-75.0; 27.3)	**0.02**
*t*Hcy (µmol/L) (35)	4.5 – 12.4	8.0 (7.2; 9.9)	6.1 (5.4; 6.9)	-2.0 (-3.8; -0.6)	**<0.0001**
SF(µg/ml) (80)	≥ 20	35.3 (12.0; 59.0)	30.2 (11.8; 55.4)	0.7 (-8.4 12.0)	0.99
CRP (mg/l) (43)	< 10	1.9 (1.2; 3.9)	2.4 (1.1; 3.5)	0.0 (-1.1; 1.4)	0.89
SIron(µg/dl) (42)	9.0 – 30.4	13.4 (10.2; 18.2)	14.2 (9.1; 17.6)	0.1 (-4.1; 4.2)	0.67
TIBC (µg/dl) (42)	44.7 - 805	40.5 (35.7; 46.6)	38.9 (35.8; 44.6)	0.4 (-3.7; 5.0)	0.89
% TS (%) (42)	≥ 16	34 (27; 49)	35 (21; 48)	-1.2 (-13.3; 13.7)	0.89
RBC (10^8/µl) (80)	4.0 – 6.2	4.6 (4.3; 4.8)	4.7 (4.5; 5.1)	0.2 (-0.0; 0.5)	**0.03**
HGB (g/dl) (80)	11.0 – 18.8	13.4 (12.7;14.2)	14.0 (13.1; 14.6)	0.5 (-0.3; 1.1)	**<0.0001**
HCT (%) (80)	35.0 – 55.0	40.0 (37.7; 41.8)	41.4 (39.1; 44.3)	2 (-0.6; 3.7)	**0.04**
MCV (fl) (80)	80.0–99.0	89.0 (82.0; 92.0)	88.0 (84.0; 92.0)	0.0(-1.00; 2.00)	0.69
MCH (pg) (80)	26 –34	30.2 (27.8, 31.2)	29.8 (28.1; 31.1)	-0.5 (-1.0; -0.5)	0.48
MCHC(g/dl) (80)	31 – 35	33.9 (33.4; 34.2)	33.5 (32.9; 33.9)	-0.4 (-0.7;-0.1)	**0.002**

Abbreviations: Sfol – serum folate, RCF – red blood cell folate, SF – ferritin, CRP – C-reactive protein, SIron – serum iron, TIBC – total iron binding capacity, %TS – percentage transferrin saturation, VB12 – vitamin B12, *t*Hcy – plasma homocysteine and RBC – red blood cell count, HGB – haemoglobin, HCT – haemotocrit, MCV – mean cell volume, MCH – mean cell haemoglobin and MCHC - mean cell haemoglobin concentration, n=number of subjects

In Table 3.35, percentages of women with variables below and above the cut-off points, before and after fortification are compared.

Percentages of subjects with low levels of Sfol and RCF decreased significantly after fortification of foods from 27.6% to 0% and 26.4% to 1.9%, respectively. The prevalence of elevated *t*Hcy decreased significantly from 8.6% to 0% after fortification. However, the percentage of women with VB12 deficiency almost doubled during phase 2 of the study. No change in the prevalence of low SF and TIBC were observed after fortification, while the prevalence of low levels of serum iron increased from 19% to 21.6% and %TS increased from 11.9% to 16.7%, though not significantly. Elevated levels of SF were observed in 5% of women before fortification and 2.5% after fortification. Percentage of subjects with low RBC and HGB were not significantly different after fortification of foods, however, RBC decreased from a prevalence of 5% to 2.5% and HGB from 7.5% to 5%. Low levels of HCT, MCV, MCH and MCHC increased after fortification, though not significantly.

Table 3.35 **Comparison of percentage of women with variables below or above the cut-off points before and after fortification**

		Pre-fortification period		Post-fortification period		
Variables (unit) (n)	Cut-offs	Below % (n)	Above % (n)	Below % (n)	Above % (n)	P-values
Sfol (ng/ml) (80)	< 2.5 2.5 – 3.0	16.3% (13) 11.3% (9)	-	0	-	< 0.0001
RCF (ng/ml) (53)	< 164.0	26.4% (14)	-	1.9% (1)	-	< 0.0001
*t*Hcy(µmol/L) (35)	4.5 – 12.4		8.7% (3)		0	< 0.0001
VB12 (pg/ml) (80)	< 145.0	6.3 (5)	-	11.3% (9)	-	**0.16**
SF (µg/ml) (80)	< 12.0 12 –19.9 >150	25% (20) 8.8% (7)	- 5% (4)	25% (20) 12.5% (10)	- 2.5% (2)	**0.74**
CRP (mg/l) (43)	≥ 10	-	2.3% (1)		0	**1.000**
SIron(µg/dl) (42)	< 9.0	19% (8)		21.4% (9)		**1.000**
TIBC (µg/dl) (42)	< 44.7	100% (42)	-	100% (42)	-	**Å**
% TS (%) (42)	< 16	11.9% (5)	-	16.7% (7)	-	**0.76**
RBC (10^8/µl) (80)	< 4.0	5.0% (4)	-	2.5% (2)	-	**0.41**
HGB (g/dl) (80)	< 11.0	7.5% (6)	-	5.0% (4)	-	**0.51**
HCT (%) (80)	< 35.0	7.5% (6)	-	8.8% (7)	-	**0.58**
MCV (fl) (80)	< 80.0	11.3% (9)	-	13.8% (11)	-	**0.63**
MCH (pg) (80)	< 26.0	11.3% (9)	-	13.8% (11)	-	**0.55**
MCHC (g/dl) (80)	< 31.0	1.3% (1)	--	2.5% (2)	-	**0.84**

Abbreviations: Sfol – serum folate, RCF – red blood cell folate, SF – ferritin, CRP – C-reactive protein, SIron – serum iron, TIBC – total iron binding capacity, %TS – percentage transferrin saturation, VB12 – vitamin B12, *t*Hcy – plasma homocysteine and RBC – red blood cell count, Hb – haemoglobin, HCT – haemotocrit, MCV – mean cell volume, MCH – mean cell haemoglobin and MCHC - mean cell haemoglobin concentration, n= number of subjects.

From Figure 3.5, it can be deduced that low levels of Sfol and RCF were reduced by 100% and 92.8%, respectively, after consumption of fortified foods. Elevated levels of homocysteine were reduced by 100% while the percentage of women with VB12 deficiency increased.

Change in the prevalence for Sfol and RCF were calculated using the formula:

$$\frac{\text{Phase 2} - \text{Phase 1}}{\text{Phase 1}} \times 100 = \% \text{ of reduction/increase}$$

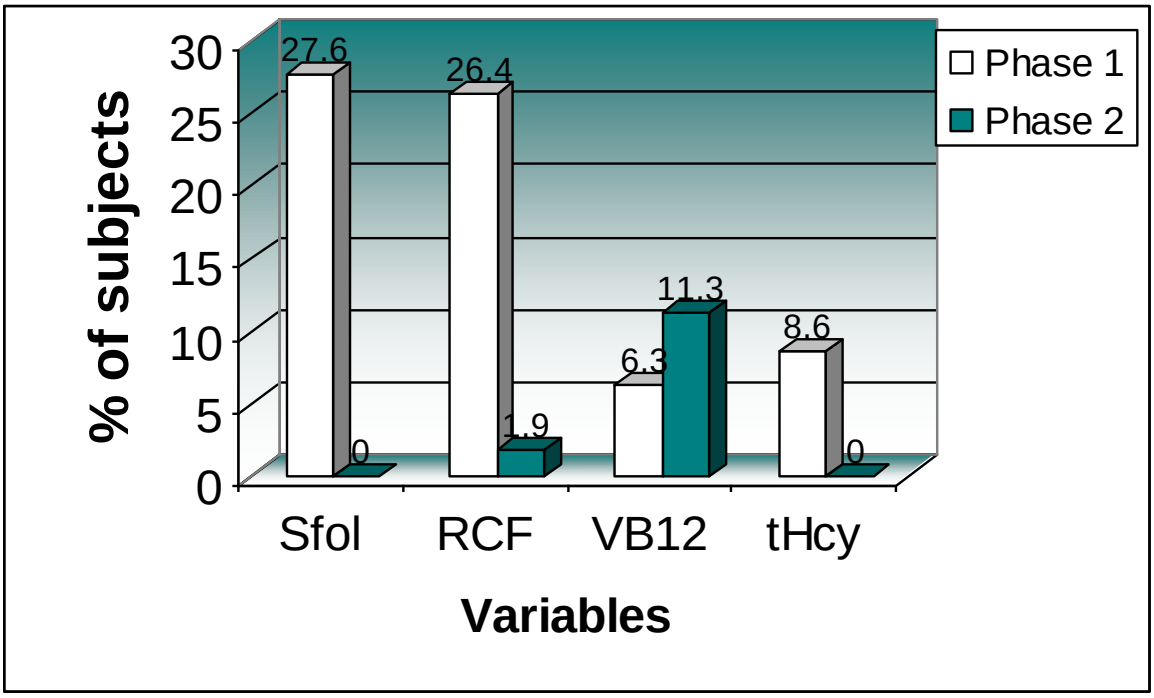

Fig 3.5 Percentages of women with folate and related variables below the cut-off points

Figure 3.6, indicates that no reduction in percentage of subjects with severe iron depletion (SF < 12g/ml) and low levels of TIBC were observed after consumption of fortified food. Percentages of subjects with low serum iron and %TS increased by 28% and 40%, respectively.

Change in the prevalence for Siron and %TS were calculated using the formula:

$$\frac{\text{Phase 2} - \text{Phase 1}}{\text{Phase 1}} \times 100 = \% \text{ of reduction/increase}$$

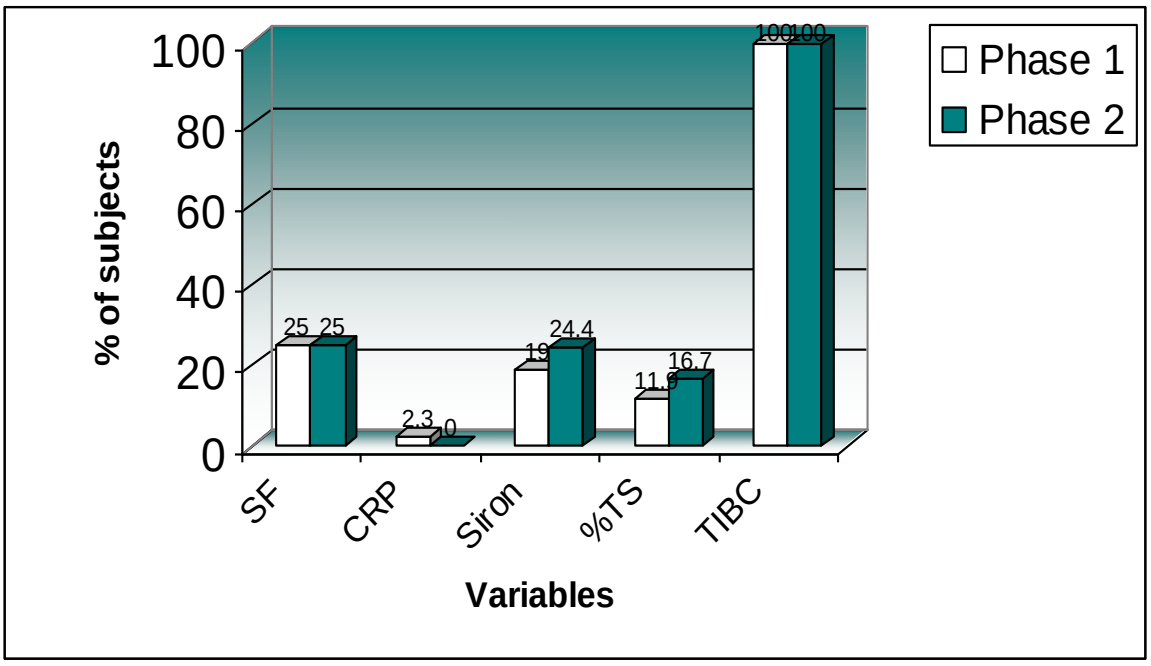

Fig 3.6 Percentages of women with iron variables below the cut-off points

Fig 3.7 shows that the percentages of women with low RBC and HGB were reduced by 50% and 33% respectively after fortification, while the percentage of women with low levels of MCV, MCH and MCHC increased slightly.

Change in the prevalence for Sfol and RCF were calculated using the formula:

$$\frac{\text{Phase 2} - \text{Phase 1}}{\text{Phase 1}} \times 100 = \% \text{ of reduction/increase}$$

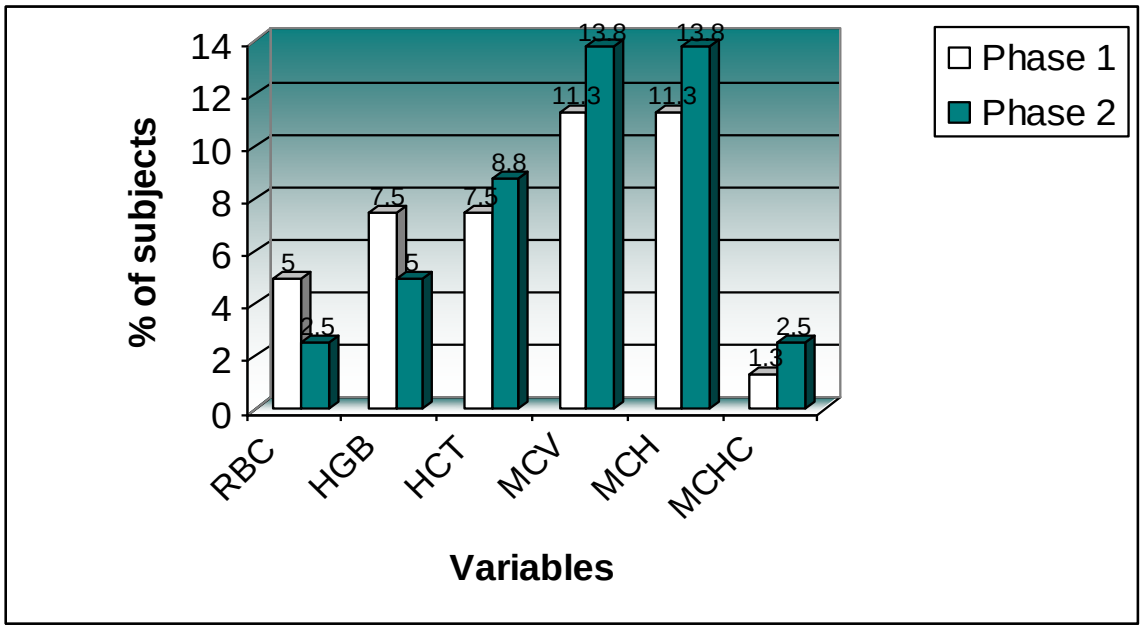

Fig 3.7 Percentage of women with full blood count indices below cut-off points

Table 3.36 presents a comparison of the means of serum albumin and liver enzymes of women of childbearing age before and after fortification. No significant difference of mean Alb was observed. However, during phase 2 of the study, mean level of Alb increased. GGT, AST and ALP levels were higher during phase 1 and decreased significantly during phase 2 of the study, whereas ALT increased significantly.

Table 3.36 **Comparison of mean albumin and liver enzymes of women of childbearing age before and after fortification of foods**

Variables (unit) (n)	**Pre-fortification Period Median (IQ)**	**Post-fortification Period Median (IQ)**	**P-value**	**RR**
Alb (g/L) (67)	36.8 (36.0; 37.5)	43.1(41.8; 44.3)	0.27	34 – 50
GGT (U/L) (76)	22.0 (17.0; 29.0)	18.5 (12.3; 25.8)	**<0.0001**	5 – 55
AST ((U/L) (78)	33.0 (27.0; 37.5)	32.0 (25.0; 37.5)	**0.03**	15 – 37
ALT (U/L) (74)	26.0 (19.5; 33.5)	28.0 (23.5; 36.0)	**0.003**	30 – 65
ALP (U/L) (73)	66.0 (49.5; 76.0)	54.0 (42.0; 65.5)	**<0.0001**	50 - 160

Abbreviation: n = number of subjects Alb – albumin, GGT – γ-glutamyl transferase, AST – aspartate aminotransferase, ALT – alanine aminotransferase and ALP – alkaline phosphatase, RR – reference range

Percentages of women with levels of variables below or above the cut-off points are presented in Table 3.37. None of the women had low albumin levels. No significant differences in the percentages of women with GGT, AST, ALT and ALP for 2 periods of the study were observed.

Table 3.37 **Comparison of percentages of subjects with low levels of albumin and elevated levels of liver enzymes**

	Pre-fortification period		Post-fortification period			
Variables (unit) (n)	Below % (n)	Above % (n)	Below % (n)	Above % (n)	P- value	Cut Offs
Alb (g/L) (67)	-	-	-	-	-	**<34**
GGT (U/L) (76)	-	1.3% (1)	-	2.6% (2)	0.22	**> 55**
AST ((U/L) (78)	-	24.7% (18)	-	24.7% (18)	0.90	**> 37**
ALT (U/L) (74)	-	-	-	-	-	**> 65**
ALP (U/L) (73)	-	-	-	-	-	**> 160**

Abbreviations: ** Shows a significant difference at the level 0.01 (2-tailed) and * Shows a significant difference at the level 0.05 (2tailed), n = number of subjects Alb – albumin, GGT – γ-glutamyl transferase, AST – aspartate aminotransferase, ALT – alanine aminotransferase and ALP – alkaline phosphatase

SECTION D – FOOD SAMPLES ANALYSIS

D1 - ANALYSIS OF MOROGO SAMPLES

3.12 Analysis of mycotoxins in morogo [(i.e. green leafy vegetables (GLV)]

During data collection, morogo samples were collected randomly from the 117 women of childbearing age who participated in the study. Difficulties to obtain morogo samples during collection were experienced because morogo is an important part of the diet, and regularly consumed with maize meal in this rural area. Only 10 samples were obtained and most of the consumed species of morogo in the area were available among the collected samples, which are *Amaranthus viridis* (AV) [Theepe, (sotho name)], *Cleome gynandra* (CG) [lerotho (sotho name] and *Vigna unguiculata* (VU) [monawa (sotho name].

The cooked morogo samples collected (n=10) were analyzed for mycotoxins at Promec Unit, MRC, Tygerberg. A tolerance level of mycotoxins (fumonisins) has not been defined in morogo, however, a tolerance level of 100-200 μg/kg (100-200ng/g) of fumonisins in maize intended for human consumption has been suggested (Merrill et al., 1993).

Table 3.38 presents the results of mycotoxins present in morogo. Levels of mycotoxins in most of the analyzed morogo samples were above the indicated reference range (100-200µg/kg) recommended for human consumption in maize foods. Variations in the levels of fumonisins were observed in the same type of morogo. Fumonisin B1 (FB1= 38860 ng/g), fumonisin B2 (FB2= 6511 ng/g) and fumonisin B3 (FB3 = 1565 ng/g) were present in one sample of *Cleome Gynandra* (GC) whereas in the other sample of CG, only FB1 was present at a level of 143ng/g. Neither FB2 nor FB3 was detected in this sample. In *Vigna Unguiculata* (VU), FB1 was detected at a level of 1601ng/g and FB2 at a level of 518ng/g. Some of the morogo samples collected were a mixture of 2 species combined. A level of 661ng/g for FB1, was observed in a combined sample of *Amaranthus Viridis* (AV) and CG. FB1 was present at a level of 143ng/g for AV mixed with tomatoes.

Table 3.38 **Levels of fumonisins (FB1, FB2 and FB3) in morogo samples**

Sample Code	**Species**	**FB1 (ng/g)**	**FB2 (ng/g)**	**FB3 (ng/g)**	**Total (ng/g)**
001A	AV + tomato	Nd	Nd	nd	Nd
001B	AV + tomato	143	Nd	nd	143
002A	CG	1932	568	nd	2500
002B	CG	38860	6511	1565	46937
003A	VU	Nd	Nd	nd	Nd
003B	VU	1601	518	nd	2119
004	AV + CG	Nd	Nd	nd	Nd
005	CG + VU	Nd	Nd	nd	Nd
006	VU + pumpkin	Nd	Nd	nd	Nd
007	AV + CG	661	Nd	nd	661

Abbreviation: nd - not detected, FB1 –fumonisin B1, FB2 - fumonisins B2 and FB3 - fumonisin B3, CG – *Cleome gynandra*, VU – *Vigna Unguiculata*, AV- *Amaranthus Viridis*

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CHAPTER 4

DISCUSSION

4.1 SOCIO DEMOGRAPHIC STATUS and MATERNITY HISTORY

4.1.1 Socio-demographic status

Most of the women in this study were unmarried particularly women in the 18-24 years age group and in 25–34 years age group. Unmarried women are common in South Africa as in other sub-Saharan countries such as Botswana and Namibia (Family planning methods and practice: AFRICA; Udjo, 2001; Steyn & Nel, 2006). South Africa has the highest mean age at marriage in the world while in other African countries such as Nigeria and Ghana, 75% of women marry before the age of 20 years (Family planning methods and practice: AFRICA; Udjo, 2001).

Although none of the women in this study obtained tertiary education, most of them completed secondary education. Research has indicated a low percentage of secondary and tertiary education among rural South African women (Steyn & Nel, 2006). In other African countries such as Ethiopia, Kenya and Mozambique, a large percentage of women with no formal schooling or primary education only, still exist and illiteracy is greater among rural females (Alonso et al., 2001; Berhane & Byass, 2001; Collinson et al., 2001; Statistics South Africa, 2001; Kruger et al., 2002; Steyn & Nel, 2006). Majority of women were not working in the present study population as has also been reported in other studies undertaken in South Africa and other African countries, particularly among

the low skilled and poorly educated (Teal, 2000). This could be due to the fact that job opportunities are scarce for people without skills.

The majority of women in this study lived in households with six or more people: large family sizes are common in rural families. Wood and paraffin were the fuels mostly used for cooking, while electricity was not commonly used. Electrical appliances such as refrigerators were not commonly used, indicating a possible risk of food spoilage. The supply of water was through communal taps, the most common access to water in South Africa (Steyn & Nel, 2006). Sanitation in general was poor in the study area as is the case in most African rural areas.

4.1.2 Maternity history

Of the participants, about 60% of women aged 18-24 years old had no children while 35.5% of women in the 35-44 years age group had a mean parity of 5. The total fertility rate in South Africa is one of the lowest in sub-Saharan countries and fewer than three births per woman are observed nationally and is declining (Kaufman, 2001). In our study, a mean parity of 2.25 was observed. Among the 253 births reported by women in the present study, seven cases of stillbirths and seven cases of perinatal deaths occurred. Perinatal mortality and stillbirth rates are high in Africa and Asia while lower rates are observed in northern and southern Africa compared to other parts of Africa and relatively low rates are recorded in Europe, North America and Oceania (WHO, 2006).

4.2 ANTHROPOMETRY, ENERGY AND MACRONUTRIENT INTAKES

4.2.1Anthropometric measurements

The importance of monitoring and assessing the nutritional status of populations is well established. In quantitative terms, body weight, height and body mass index (BMI) are indicators of the body size, and are indispensable in the analysis of body composition and nutritional status (Herrera et al., 2003). Anthropometric measurements have been associated with various health conditions and BMI is by far the most widely used measurement reflecting general obesity and underweight (Lemos-Santos, et al., 2004). For instance, a BMI above 30 kg/m2 is associated with development of diabetes mellitus and hypertension (Aneja et al., 2004; Lele RD, 2007).

A mean BMI of 26 kg/m^2 was observed in our study population indicating overweight and this is consistent with the findings of the South African Demographic Survey which reported a mean BMI of 26kg/m^2 among women (Puoane et al., 2002). A trend of BMI increasing with age was observed in the present study, confirmed by a significant positive correlation between age and BMI. Overweight was present in 26.7% of women while obesity was present in 27.7%, which is similar to other studies in South Africa (Kruger et al., 2002; Steyn & Nel., 2006). It is worth noting that the prevalence of obesity in South Africa is higher than that reported in other African countries, particularly in women. Socio-economic status has been implicated in overweight and obesity, indicating that higher rates are found among groups with lower educational and income levels, and in high poverty areas which predisposes them to the consumption of energy dense food and reduced level of physical activity (Drewnowski, 2007). However, in this study energy

intake was lower than recommended. The discrepancies could be due to inaccurate information of energy intake. Hence, further studies using more accurate measures should be undertaken.

Underweight was not prevalent in the present study which is similar to findings in other studies in South Africa (Kruger et al., 2002; Puoane et al., 2002; Steyn & Nel, 2006). Underweight among women of childbearing age is well documented in other African countries like Ghana (GSG & MI, 1999) and Ethiopia, Mali and Senegal (Goedecke et al., 2006/7) as well as in south and south-eastern Asia (40-42%) (Khoi et al., 1995; Habibullah, 2001). Underweight (low BMI) is an indicator of chronic energy deficiency (CED), an important measure of nutritional risk of women during reproductive age (Shetty & James, 1994). On the contrary, mean waist and WHR increased with age. This is consistent with what has been reported in South Africa (Steyn & Nel., 2006) and was confirmed by significant positive correlations of age with waist and WHR. Overall percent of 55% for overweight and obesity was observed but percentage of abdominal obesity was low. High BMI could still be due to heavy bone structure or mucles (Zlatarić et al, 2002;

Maternal obesity adversely affects pregnancy outcomes primarily through increased rates of chronic hypertension, pre-eclampsia, diabetes (pregestation and gestation), caesarean section and infections. Also, obesity may be an independent risk factor for NTDs, fetal mortality and preterm delivery, and the risk of stillbirths and neonatal death (AMCHP, 2006). Whilst a low pre-pregnant BMI is one of the strongest predictors of adverse pregnancy outcome such as preterm birth, IUGR and LBW, the mechanisms of the

association of low BMI with adverse pregnancy outcome are not clear (Neggers & Goldenberg, 2003; Ronnenberg et al., 2003). Poor pregnancy outcomes reported in South Africa include, LBW, stunting, underweight and overweight, and wasting (Labadarios & Van Middelkoop, 1995; Labadarios et al., 2000; Mamabolo et al., 2004; Mamabolo et al., 2006).

4.2.2 Energy intake of women of childbearing age and its association with their anthropometry

Energy intake according to the 24 hr recall and QFFQ was inadequate, and has been reported by other studies (Ladzani et al., 1998; Steyn et al., 2001; Kruger et al., 2002; Bopape, 2003; Mostert et al., 2005). Inadequate intake of energy among women of reproductive age is common in sub-Saharan Africa and south and south-eastern Asia (McGuire & Popkins, 1989; Khoi et al., 1993). Many African women are reported to consume less than recommended allowance for calories and this predisposes them to CED. CED is defined as a physical state in which energy intake is similar to the energy consumption, but the individual suffers from low weight and body energy reserves (Ferro-Luzzi, 1990; Herrera et al., 2003). CED is an indication of insufficient household food supply and is prevalent in women of childbearing age. Furthermore, CED is particularly pronounced in rural women and women who are illiterate and women living in low socioeconomic background (Ravishankar, 2006). However it is not common in the present study area.

The reported low energy intake in spite of the high prevalences of overweight and obesity, and the low prevalence of underweight observed in our study is a reflection of

the difficulty experienced in obtaining accurate information on food intake. It has been documented that several dietary assessment methods fail to measure the true intake for an individual (Sempos et al., 1999) and under-reporting of energy intake is common in many populations, affecting interpretations of dietary data (Johansson et al., 1998). The level of under-reporting is known to differ according to the dietary assessment method used (Black et al., 1991; González et al., 2000). Dietary methods such as 24 hour recall, tend to give lower energy intake data than other methods (Bingham, 1987), hence that might have contributed to the apparently high prevalence of inadequate energy intake in our study. A higher percentage (54.4%) of women in our study underreported energy intake with 24 hr recall compared to 8.9% of women who underreported with QFFQ.

4.2.3 Macronutrient intake of women of childbearing age and the association with their anthropometry

The contribution of macronutrients (carbohydrate, protein and fat) consumed by participants in this study were within the recommendations of Food and Agricultural Organization and the World Health Organization (WHO, 2003). Consistent with other studies, carbohydrate intakes (266.6-350.4 g/day) of women of childbearing age in our study was above the RDA. In Limpopo Province, high carbohydrate intakes have been reported in women of childbearing age (Bopape, 2003; Mostert et al., 2005) as well as in rural South African women (Steyn & Nel., 2006). Rural black subjects, compared to other populations, have relatively greater carbohydrate intakes in all age and gender groups (Vorster et al., 1997). This could be due to the fact that maize meal, a staple food, is consumed regularly in large quantities, especially in rural areas, as well as bread and sugar, which are consumed with tea, the common beverage in our study population.

Therefore, clearly, our findings conform to those obtained in other dietary studies of the adult population of Dikgale reflecting prudent diet among women due to high carbohydrate consumption (Steyn et al., 2001; Mostert et al., 2005).

Although, high consumption of carbohydrate is known to be associated with high BMI (Willet, 1998; González et al, 2000), no significant correlation between carbohydrate intake and BMI was observed in this study as was reported by other researchers (Romieu et al., 1988; Gonzáles et al., 2000). A significant positive correlation between energy and carbohydrate intake was observed.

Total protein intake ranged from 50.7g/day to 61.1 g/day; which exceeded the RDA. Although, the intake of total protein exceeded the RDA, protein intake may have been imbalanced since the diet contained relatively low animal food sources. The main food sources of protein were plant foods, as was found in previous studies conducted among blacks in rural South Africa (Patel et al., 1992; Bourne et al., 1994; Steyn et al., 1998; Mostert et al., 2005). The normal levels of serum albumin observed in the present study reflect a diet with adequate protein intake.

Fat intake was low among women of childbearing age in this study and was consistent with other findings (Steyn et al., 2001; Kruger et al., 2002; Mostert et al., 2005). The low fat diet consumed by black population is more prudent than the diets of Whites, Indians and Coloureds (Vorster et al., 1997; Steyn et al., 2001; Steyn & Nel., 2006), although a trend towards higher fat intake with urbanization has been observed (Kruger et al., 2002). Dietary fat is the most energy-dense macronutrient, and high fat intake, when associated

with over consumption of energy, has been considered an important factor in the development of obesity (González et al., 2000). However, no significant differences in fat intake were observed by BMI categories in this study and no significant correlation between BMI and fat intake was observed. The THUSA study, conducted in North-West Province of South Africa, reported significant positive correlation between BMI and fat intake among black women (Kruger et al., 2002). Fat intake has been significantly correlated with BMI in several populations (Romieu et al., 1988; Sichieri, 2000). The presence of low fat intake in spite of the high prevalence of overweight and obesity, confirms the possibility that obesity is known to occur even with relatively low fat intake and which has been documented in other studies (Willet, 1998; Kruger et al., 2001).
While on the other hand, high carbohydrate intake and low basal metabolic rate (BMR) conyribute to high prevalence of overweight and obesity.

Means of cholesterol intake observed in this study were ranging from 115.6mg/day to 163.1mg/day. These findings are similar to those of other studies in South Africa (Walker & Walker, 1991; 1992; Kruger et al., 1993; Charlton et al., 2001; Steyn & Nel, 2006). The contribution of saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs) contributing to the total energy intake in this study met the prudent guidelines.

4.3 MICRONUTRIENT INTAKE and BIOCHEMICAL STATUS

4.3.1 Vitamins: A, thiamin (B1), riboflavin (B2), niacin (B3), B6, C, D and E

Inadequate intakes of fat soluble (vitamin A, vitamin D and vitamin E) and water soluble (riboflavin, niacin, vitamin B6, and vitamin C) vitamins were observed in this study. The

low intakes of vitamin A among women of childbearing age were estimated at 82% and 71% with 24 hour recall and QFFQ, respectively, as was the case in other studies (Steyn et al., 2001; Mostert et al., 2005; Steyn & Nel., 2006).

The richest source of vitamin A is fish-liver oil as well as milk products, including whole milk, butter, cream and cheese, and certain green leafy vegetables and grasses (McLaren, 1981; Streyer, 1988; Olson, 1999; De Pee et al; 1999; Tang et al., 1999). Except for the frequent consumption of pilchards (tinned fish) and green leafy vegetables in our study population, dairy products such as milk, cheese and butter were not frequently consumed food. Also, the high percentage of households who lacked refrigerators in this population, indicate that dairy products were not stored by most of the families in our study population.

Low intake of vitamin E among women of childbearing age was observed in our study and ranged from the prevalence of 45.5% to 58%. Other dietary studies in Limpopo Province have reported high prevalences of low intakes of vitamin E among women of childbearing age (Mostert et al., 2005). Even though it is believed that the intake of vitamin E in most situations is adequate (Gey et al., 1993), in our study vitamin E intake was not sufficient. Inadequate intake of vitamin E could result in vitamin E deficiency, which can cause the accumulation of lipid peroxidation products, a risk factor of pre-eclampsia (Jain & Wise, 1995). In addition, low vitamin D intakes of women of childbearing age in our study ranged from 72% to 75%, which has also been reported in the general population of South Africa (Steyn & Nel., 2006). Although inadequate intake of vitamin D was considered a problem earlier (Allen, 2001), vitamin D deficiency has

declined considerably in recent years (Muller & Krawinkel, 2005). Vitamin D deficiency predisposes the fetus to future development of a broad variety of diseases including diseases of the immune system, such as atopic dermatitis or autoimmune disease (Adorini et al., 2003; Zella et al., 2003).

Mean intakes of riboflavin, niacin, vitamin B6, and vitamin C were inadequate in this study, while mean intake of thiamin was adequate. Similar findings were also observed in other South African studies (Bourne et al., 1993; Kruger et al., 1993; Charlton et al., 2001; Steyn et al., 2001; Bopape, 2003; Ladzani, 2005; Mostert et al., 2005; Steyn & Nel., 2006). Dietary sources of these micronutrients, including fruits and vegetables, were lacking in the present study population, hence the poor status observed. In addition to dietary sources, thiamin, riboflavin, niacin and pyridoxine (vitamin B6) are part of a fortification programme in South Africa since 2003, which may have improved the nutrient status. Of major concern is the low intake of vitamin C in South Africa (Vorster et al., 1997; Mostert, 2000; Steyn et al., 2001; Bopape, 2003) and vitamin C is not part of fortification in most countries, including South Africa. Vitamin C deficiency is associated with increased risk of infections, premature rupture of the foetal amniotic membranes, premature birth and eclampsia in pregnant women (IOM, 2000).

Poor vitamin B6 status has been reported among Indonesia and Vietnamese populations as well as in women of childbearing age in Egypt (Allen, 2001). In other parts of Africa, Guatemala, China and India, those mostly affected by low intakes of vitamin B6 are women of childbearing age (Bates et al., 1981; Bamji & Lakshmi 1998). Vitamin B6 intake reflects variety in the diet since this vitamin is found in several foods from both

animal and plant origin, however, it is clear that our study population lacks variety in its diet, which is the case in rural populations of South Africa (Vorster et al., 1997; Steyn et al., 2001; Ladzani, 2005). Vitamin B6 participates in methionine synthesis and influences metabolism of homocysteine and PUFA, among other functions (Tsuge et al., 2000).

Low riboflavin intakes are generally associated with low intake of dairy products, which was the case in the present study. Riboflavin deficiency in women of childbearing age is an independent risk factor of pre-eclampsia (Wacker et al., 2000). Riboflavin is the precursor of flavin mononucleotide (FMN) and FAD, which serves as cofactors for enzymes involved in metabolism of vitamin B6, folate and vitamin B12 (Hustad et al., 2000). FMN serves as a cofactor for pyridoxine-5' phospahate oxidase, which is important for the formation of the active form vitamin B6, whereas FAD is a cofactor of MTHFR (Hustad et al., 2000). The role of flavoenzymes in the metabolism of several B-vitamins suggests that riboflavin status may influence homocysteine metabolism (Hustad et al., 2000). Studies have shown that low dietary intake of riboflavin is associated with elevated plasma homocysteine (Hustad et al., 2000; Jacques et al., 2001; McNulty et al., 2002). However, no significant correlation was observed between riboflavin intake and biochemical parameters, including homocysteine in this study as was the case in another report (Jacques et al., 2001). Riboflavin correlated positively significant with protein intake, B6, folate, B12 and niacin intakes among other nutrients.

Niacin deficiency is mainly associated with the consumption of a diet that is high in refined carbohydrates, which is the case in the present study, in which participants followed a maize based diet. It is also common in individuals with impaired absorption of

the amino acid, tryptophan, and a precursor for niacin or nutrients lacking tryptophan. Insufficiencies of riboflavin and vitamin B6 are implicated in the clinical manifestations of niacin, since they are needed for conversion of L-tryptophan to niacin equivalents (McCormick, 1988; McCormick, 1997). A low periconceptional intake of niacin is associated with a 2-5 fold increased risk of spina bifida (Groenen et al., 2004).

4.3.2 Folate, B12 and homocysteine

Mean intakes of folate were inadequate in this study and 79.2% to 90.1% of women had low intakes of folate, whereas the mean intakes of B12 were adequate, yet high percentages of women (19.8% to 59.4%) with low intake were observed. These findings are consistent with other dietary studies conducted in South Africa, which reported a high prevalence of low folate intake among women of childbearing age and in the adult population (Bourne et al., 1993; Kruger et al., 1993; Charlton et al., 2001; Steyn et al., 2001; Bopape, 2003; Ladzani, 2005; Mostert et al., 2005; Steyn & Nel., 2006). In addition to dietary folate, the folate status was also assessed using biochemical parameters: serum folate and red cell folate in this study indicated a high prevalence of folate deficiency prior to fortification of foods. This is consistent with previous studies undertaken in South Africa as well as in other countries such as United States (Baynes et al, 1986; Ubbink et al., 1999; Ray et al., 2003; Hetrampft et al., 2003; Mamabolo et al., 2004).

Poor folate status is associated with low consumption of dietary folate sources such as liver, mushroom and fresh green leafy vegetables, especially spinach and broccoli (Mahan & Arlin, 1992). In the study population, a high consumption of green leafy

vegetables (spinach and morogo) was reported, as is the case in other rural parts of Limpopo Province, but folate status was still found to be poor (Nesamvuni et al., 2001; Steyn et al., 2001). In a wide variety of foods, folate occurs in a polyglutamate form, a reduced form which is heat labile and easily oxidized. Between 50-95% of folate is lost during home preparation and food processing, with considerable losses also occurring during the storage of vegetables at room temperature and by cooking at very high temperatures (Hercberg & Galan, 1992; Mahan & Arlin, 1996).

In addition to the possible causes of folate deficiency, it is believed that the conversion of polyglutamate to a simpler form, monoglutamate, is impaired in genetically susceptible women (March of Dimes, 2000). This has never been reported to be common in our study area. Then again, inadequate intake of iron and vitamin C leading to a deficient state in the host can impair folate absorption (Mahan & Escott-Stump, 2000). The present study showed positive significant correlations between serum folate and iron, vitamin B6 and vitamin B12 intakes, among other nutrients. Folate deficiency during pregnancy increases the risk of NTDs, Down's syndrome, spontaneous abortion, abruption placenta and pre-eclampsia while in other birth outcomes such as reduced birth weight, pre-term delivery and perinatal mortality, the role of folate is still unclear (Scholl et al., 1996; Bailey & Gregory, 1999; Scholl & Johnson, 2000). Deficiency of folic acid is teratogenic and although the exact mechanism is not known, defective biosynthesis or methylation of DNA or elevated levels of homocysteine have been implicated (Christensen & Rosenblatt, 1995).

After fortification of foods with folic acid and other vitamins was introduced in South Africa, folate status of women improved. Mean serum and red cell folate levels increased significantly and no woman in this study had low serum folate and only one woman had a low RCF. Percentages of women with low levels were reduced by 100% and 92.8%, for serum folate and RCF, respectively. Our findings are consistent with other studies conducted in various parts of the world. A significant reduction in folate deficiency was reported after fortification in several such as Chile, Southern California and Costa Rica (Jacques et al., 1999; Caudill et al., 2001; Chen & Riviera, 2004; Hetrampft & Cortés, 2004). The improved folate status in the above studies was attributed to the high consumption of fortified foods which have increased the bioavailability of synthetic folic acid compared to the naturally occurring folate, and this might also explain improvement of folate status in this present study. A significant decline in the prevalence of NTDs following fortification in South Africa has been reported (Sayed et al., 2008). However, there is a major concern about high folic acid consumption, especially, in the populations with vitamin B12 deficiency and among elders.

Mean intakes of vitamin B12 were above the RDA, yet there were women with inadequate intakes in this study. Although adequate mean intakes of vitamin B12 are reported in rural South Africa among different population groups (Steyn & Nel., 2006), it is possible that individuals or specific groups such as those living in the extreme poverty, could have low intakes because of insufficient intakes of animal foods. Prior to fortification of foods, biochemically, vitamin B12 deficiency was present in 6.3% of women of childbearing age in this study and after fortification of foods, the prevalence of vitamin B12 deficiency increased to 11.3%. High prevalences of vitamin B12 deficiency

have been reported in South Africa. (Joosten et al., 1993; Charlton et al., 1997; Ladzani, 2005; Mamabolo et al., 2004).

The adverse effects of folic acid fortification on vitamin B12 status have been well described, especially in populations with a high prevalence of vitamin B12 deficiency (Ramos et al., 2005; Reynolds, 2006). To explain the effect of folic acid fortification on vitamin B12 status, there is a possible suggestion that large quantities of folic acid could stimulate blood cell production, using vitamin B12 stores and causing vitamin B12 deficiency. This could aggravate neurologic damage in individuals and mask anaemia (Choumenkovitch et al., 2002). Although rare, vitamin B12 deficiency is implicated as one of the causative factor of NTDs, but the mechanism is unclear (Steen et al., 1998).

Vitamin B12 is present in animal proteins and some is synthesized by the bacteria in the colon (Mahan & Arlin, 1992). Although plant proteins were more commonly consumed than animal proteins, the consumption of eggs and pilchards might explain some of the reported mean vitamin B12 intake above the RDA. No significant correlation between B12 intake and serum B12 was observed. B12 deficiency is caused in most cases by factors other than dietary (Mahan & Escott-Stump, 2000). Parasitic infections and low secretion of intrinsic factor (IF) may lead to B12 deficiency. In our study area, parasitic infections are virtually absent due to the aridity of climate, although *Giardia* has been reported in children (Vermeulen et al., 1997). Previous studies in Africa, the United Kingdom and United States have shown that black people have higher serum B12 than whites (Fernandez-Costa & Metz, 1982; Kwee et al., 1985; Stabler et al., 1999). It has also been reported that pernicious anaemia is less common in blacks compared to whites

(Drum & Jankowiski, 1981; Kwee et al., 1985) and thus does not represent a major health problem in the black population.

Mean homocysteine levels, an amino acid related to riboflavin, vitamin B6, folate and vitamin B12 metabolism, were within the reference ranges in women of childbearing age, prior to fortification of foods. However, 8.7% of women had elevated levels of homocysteine. Data on the effect of folate consumption on plasma homocysteine levels of women of childbearing age in South Africa is rare (Ubbink et al., 1995) but black South Africans have low levels of homocysteine. Intakes of folate, B6, B12 and riboflavin also, affect the level of plasma homoscysteine (Medina et al., 2001; Hustad et al., 2000). An elevated concentration of plasma total homocysteine can be a sensitive marker for both poor folate and poor B12 status (Wolters et al., 2003). In the present study, no significant correlations between plasma homocysteine and dietary and biochemical nutrients were observed, except an unexpected significant negative correlation with serum folate.

Homocysteine methylation involves MTHFR, an enzyme responsible for converting methylene-THF to 5-methyl-THF. The one-carbon moiety is directed to cause the irreversible conversion of homocysteine to methionine. Folate is involved during this reaction and riboflavin serves as a cofactor of MTHFR while vitamin B12 is required (Bailey & Gregory, 1999; Wolters et al., 2003). Alternatively, homocysteine can be metabolized to cystathionine, requiring vitamin B6 (Narayanasamy et al., 2007). Plasma homocysteine levels are influenced by demographic, genetic and nutritional factors, among others (Medina et al., 2000, Brauer & Tierney, 2004). It has been suggested that

hyperhomocysteinemia damages the blood vessels in the placenta, whose role is to nourish and protect the fetus. Placental vessel impairment is known to cause pre-eclampsia and fetal growth restriction, which may cause premature labor and delivery, a leading cause of newborn death or lasting disability (March of Dimes, 2000).

After consumption of fortified foods, no women in our study population had high levels of homocysteine. In countries such as southern California and Chile, increased consumption of foods fortified with folic acid and food supplements have been shown to decrease plasma homocysteine levels. These findings have been reported among women of childbearing age from different backgrounds; i.e. socio-economically advantaged and/or disadvantaged (Caudill et al., 1999; Hertrampf et al., 2003).

4.3.3 Iron, calcium and zinc

In this study, 68.3 to 85.1% of women of childbearing age exhibited low iron intake. In Limpopo province (Capricorn district), Mostert et al (2005) reported low iron intake in 73.9% to 76.1% of women of childbearing age. Among pregnant adolescents, the prevalence of low iron intake was 98.4% (Bopape, 2003). In rural South African women, the prevalence of low iron intake was 79.4% (Steyn & Nel., 2006). Biochemical assessment of iron status in this study showed a high prevalence of iron deficiency (25%) as measured by serum ferritin among women of childbearing age, before fortification. In addition to a high prevalence of iron deficiency, a low percentage of transferrin saturation was observed before fortification (19%). In Limpopo Province, the prevalence of iron deficiency among pregnant women of childbearing age was estimated at 46% to 50.9% (Bopape, 2003; Mamabolo et al., 2004).

The causes of iron deficiency are multifactorial and often concurrent. Inadequate intake, poor bioavailability and the ingestion of soil have been implicated as major causes (O'dea, 2001). Low iron intake has been attributed by Steyn et al (2001) to the poor consumption of major haem iron sources such as meat. Meat is not frequently consumed in this study population, probably due to the fact that it is expensive to purchase. With regard to the foods either enhancing or inhibit iron absorption, the population showed low intakes of natural foods containing vitamin C and high consumption of tea and green leafy vegetables. Vitamin C is reported to be the most effective enhancer of iron absorption and on the other hand tea and green leafy vegetables are inhibitors of iron absorption since they contain high levels of polyphenols and phytate (Ballot et al., 1987; Steyn et al., 1993). These dietary substances could be among the factors responsible for the high prevalence of iron deficiency in this study population before and after fortification.

Poor iron intake is a cause of iron deficiency, a risk factor associated with anaemia, but our study did not show any significant correlation between serum ferritin, serum iron and iron intake. Iron deficiency and iron deficiency anaemia are worldwide problems in both developing and developed countries, affecting mostly women of childbearing age (Hurrell et al., 2002; Bovell-Benjamin et al., 2003; Foo et al., 2004). However, prevalences are higher in developing countries compared to developed countries (Foo et al., 2004).

Before implementation of iron-fortified foods by national governments, including South Africa, iron supplementation was used as a strategy to reduce iron deficiency (Bovell-

Benjamin et al., 2003). Studies conducted in the Capricorn district of Limpopo Province which evaluated the effectiveness of iron supplements, reported contradictory results (Malope, 2001; Mamaboo et al., 2004). Among pre-school children, the consumption of iron supplements improved HGB levels and all iron parameters (Malope, 2001), while on the other hand, the routine dispensing of iron supplements to pregnant women by health care centers was unable to reduce the high prevalence of iron deficiency (59%) (Mamabolo et al., 2004). The latter study attributed this to non-compliance of the women taking the iron supplements. In the present study, despite the consumption of fortified foods, no significant improvement of iron status was observed. The slight improvement observed for full blood count indices, especially, RBC and HGB, could be attributable to the increased consumption of vitamin A from fortified foods, as suggested by Malope (2001).

Very few data on the effect of iron fortification exist in South Africa, however, consistent with other studies, no improvement of iron status was reported by the use of electrolytic iron fortificant in South Africa and other countries (Hallberg et al., 1993; MacPhail et al., 1994; Milman et al., 1994; 1999; Van Thuy et al., 2005; Andango et al., 2007; van Stuijvenberg et al., 2008). Several types of iron fortificants exist; inorganic and organic iron compounds (Dary et al., 2002). Electrolytic iron used in fortification is an inexpensive inorganic iron compound which does not significantly interfere with the color and flavor of foods, but has low solubility and absorption, making it inefficient in populations with diets containing phytates and polyphenols (Dary et al., 2002; Fairweather-Tait & Teucher, 2002). Therefore, consumption of food fortified with

electrolytic iron used in mandatory fortification in South Africa, may explain our findings.

While iron requirements are important in women of childbearing age, they are greater for pregnant women (Sheridan et al., 2003). Pregnant women with iron deficiency anaemia have a threefold increase in the risk of LBW infants and a greater than twofold increase in the risk of pre-term delivery (Beard, 1994). Babies born of women in this condition are at greater risk of perinatal and neonatal mortality (Allen, 2000). High maternal mortality caused by iron deficiency anaemia is prevalent in developing countries such as South Africa, India, Indonesia and Pakistan (Chi et al., 1981; Abouzhar & Roysten, 1991; Nojilana et al., 2006).

In this study, in addition to iron deficiency, iron overload reflected by high serum ferritin > 150 µg/l (Choma et al., 2007) was observed in 5% of women before fortification and reduced to 2.5% after fortification. Before mandatory fortification, iron overload among women in Dikgale was estimated at 12.3% (Choma et al., 2007), which is higher compared to our findings. Iron overload is a public health problem in rural areas of Limpopo Province of South Africa (van der Westhuyzen et al., 1986; Friedman et al., 1990). Iron overload in women of childbearing age causes poor pregnancy outcomes (pre term delivery, infection, LBW and poor neonatal vitality and health (Casanueva & Viteri, 2003). Elevated ferritin due to inflammation may cause an increase in CRP. However, the percentage of women with elevated CRP was relatively low in this study. Elevated CRP is attributed to infectious diseases common in rural communities, including HIV (Phatlane, 2003).

Among other minerals, zinc and calcium intakes were inadequate in this study population. Low calcium and zinc intakes have been reported in rural areas of Limpopo Province (Vorster et al., 1997; Mostert et al., 2005; Mamabolo et al., 2006; Steyn & Nel, 2006). It is estimated that 82% of pregnant women in the world have inadequate regular intake of zinc and likely suffer the health consequences of zinc deficiency (Caulfield et al., 1998). The main cause of human zinc deficiency is a diet that is low in highly bioavailable zinc, but it also may be caused by illness that impairs food intake, provoking catabolism or malabsorption, or increase zinc excretion (Sandstead et al., 1998). Many staple foods in developing countries including cereals, maize and vegetables, are relatively good sources of zinc, but even when the net intake appears adequate by most recommendations, compromised zinc status is common (Gibson et al., 1998). Populations in south-east Asia and sub-Saharan Africa are at greater risk of zinc deficiency, and diets rich in plant-based foods is implicated in the cause of poor biovailability of zinc due to their high level of phytates which are known to form complexes with zinc that reduce its absorption (Ferguson et al., 1988; 1993; Manary et al., 2000).

Zinc-rich foods are expensive, hence, zinc fortification is an important consideration, especially because daily intakes appear to be more useful physiologically than intermittent doses (Scalgueiro et al., 2002). In South Africa, zinc is part of fortification in maize and wheat foodstuffs (FACS, 2002), however, the levels of plasma zinc were not analyzed in this study. Probably zinc in fortified foods may be the key to overcoming a natural nutritional problem. Zinc plays a role in protein synthesis, gene expression, cell division and enhances vitamin D effects on bone metabolism (Brandão-Neto et al., 1995; Polysangam et al., 1997; Rosado, 1998). It also participates in the metabolism of

carbohydrates, lipids and proteins, which in turn leads to good food utilization (Brandão-Neto et al., 1995). Zinc has a profound influence on rapidly growing tissue, therefore, its effect in reproduction is significant (Opare Obisaw, 2003). Consequences of maternal zinc deficiency in pregnant women are spontaneous abortion, congenital malformation, LBW and IUGR, pre and post term delivery, prolonged or inefficient first stage labor, protracted second stage labor, premature rupture of membranes, pregnancy related toxemia and pre-eclampsia (Maeda & Tanaka, 1996; Scalgueiro et al., 2002).

In South Africa, the intake of calcium is low in all populations and low consumption of milk and milk products are implicated (Vorster et al., 1997; Steyn et al., 2001). Inadequate intake of calcium is prevalent in developing countries particularly in Asia, Africa and Latin America compared to the high intakes reported in North America and Europe (FAO, 1998). Calcium, the most abundant mineral in the human body, plays an essential role in bone and tooth formation, blood clotting, muscle contraction, nerve transmission and regulation of energy metabolism (FNB 1997). In women of childbearing age, there is a need for calcium storage during pregnancy to support the demands of lactation later (Ziser & Whitney, 1997). A 40% increase in dietary calcium required during pregnancy is needed for development and growth of skeletal tissue and tooth formation especially in the third trimester of pregnancy (Williams, 1993; Wardlaw et al., 1994). Low calcium intake is associated with poor pregnancy outcomes such pre-eclampsia (Levine et al., 1997).

4.4 THE EFFECTS OF SOCIO-DEMOGRAPHIC STATUS INDICATORS ON SELECTED NUTRIENTS

No significant effect of the socio-demographic status indicators on serum folate and iron levels was observed (Table 3.26 & 3.27) in this study, except for age in relation to iron status. Socio-demographic status has been reported to influence nutrient intakes (Molteno & Kibel, 1989; Weimer, 1999; Barquera et al., 2003; Navaneetham & Jose, 2005; Ravishankar, 2006), however, no effect of sociodemographic status on nutrient intake was observed in the present study.

4.5 MYCOTOXINS AND LIVER ENZYMES

The chemical analysis of morogo revealed fumonisin contamination in four of the ten samples, irrespective of the plant species or the locations where samples were collected. Fumonisins in amounts totaling nearly 47 000ng/g (47 000 µg/kg) were found, especially in *Gleome gynandra.* This amount as well as several of the reported levels exceeded the tolerance level defined for fumonisins in maize foods (100–200 ng/g). There is no data at all on fumonisins in morogo in South Africa, except in maize foods (Marasas et al., 2004; Shepard et al., 2005). However, more serious work on mycotoxins contamination in morogo samples in rural areas in relation to folate status in needed.

The level of fumonisns observed in some of the morogo samples might have important consequences in poor rural communities where it is commonly consumed. It has been suggested that mycotoxins may interfere with folate metabolism, especially in pregnant women, thereby increasing the prevalence of NTDs (Marasas et al., 2003). On the other

hand, it is believed that when consumption of folate is high, actions of fumonisins might be suppressed. Therefore, possibly, with improved folate status among women of childbearing age after consumption of fortified foods, no severe effects may be present. However, this still requires critical evaluation in relation to human health.

Researchers have linked dietary fumonisins to various diseases. It has been suggested that fumonisin B1 (FB1) exposure induces oxidative stress which can damage DNA indirectly, and this acts as mechanism for FB1-associated liver-toxicity (Lerda et al., 2005). Injured liver results in cytolysis and necrosis, and causes liberation of various enzymes such as AST, ALT, and GGT; which are of clinically significance in assessing hepatocellular and hepatobiliary disease (Bishop et al., 2000). Means of liver enzymes levels in this study were within the reference range. GGT was normal, except in one woman, indicating that there was no excessive intake of alcohol. Isolated non-specific increases in aminotransferases are common and may reflect the use of herbal remedies (Marshall & Bangert, 2004).

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CHAPTER 5

CONCLUSION

The socio-demographic results in the present study indicate a high prevalence of singlehood, unemployment, large households and limited use of electrical appliances. No significant correlations between socio-demographic status indicators, and folate and iron status were observed in the present study.

The dietary intake as reported by the participants showed low intakes of energy, fat and dietary fiber and of the micronutrients reported in this study. Micronutrient inadequacies reflect poor consumption of fruits and vegetables, which was the case among women of childbearing age in this study. The diets comprised mainly of plant proteins, carbohydrates rich foods and low intake of dairy products and this clearly shows poor nutrient quality and imbalanced diet, which might be an indication of poor nutrition knowledge and poor economic conditions.

The apparent anomaly of inadequate intake of energy, fat, dietary fibre and the observation of overweight and obesity could be attributable to inaccurate information reported on dietary intake. However, reported adequate carbohydrates might be due to high consumption of mealie meal and bread whereas the adequate protein in the absence of meat and dairy product intake might be due to high plant protein consumption.

A higher prevalence of underreporting using 24 hour recall was shown in this study compared to the quantitative food frequency questionnaire. Although energy and fat intakes of women of childbearing age were low in this study, high prevalences of overweight, obesity and abdominal obesity were still observed, especially in the older women whilst the prevalence of underweight was low.

Inadequate dietary folate intake was confirmed by the poor folate status assessed by serum and red cell folate prior to fortification of foods but improved after consumption of fortified foods. Hence, this study showed a significant improvement of folate status in women of childbearing age, after fortification of maize and wheat foodstuff in South Africa. Poor iron status as measured by serum ferritin was observed prior to fortification of foods. However, no significant improvement of iron status after fortification was observed in this study. The slight observed improvement of red blood count and haemoglobin may be attributable to fortification with vitamin A. With regard to vitamin B12, the percentage of women with low levels of vitamin B12 before fortification increased after consumption of fortified foods.

It is well documented that nutritional status of women of childbearing age during preconception period is an important indicator of pregnancy outcomes. As such under-nourishment and pre-pregnancy overweight and obesity observed among women in this study might predispose them to unfavourable pregnancy outcome. Cases of stillbirth, miscarriage and perinatal mortality were reported in this study population.

The observed high levels of fumonisins in a small number of samples and in various species of Morogo in this study are alarming, since fumonisins are known experimentally to interfere with folate metabolism and can lead to neural tube defects (NTDs). Nonetheless, mandatory fortification causing high intake of folic acid might outweigh the actions of fumonisins. But with few samples used in this study, this calls for more serious work on mycotoxins contamination in Morogo.

CHAPTER 6

RECOMMENDATIONS

- Taking into consideration the high rate of unemployment among women, it is important to devise strategies to improve work opportunities in rural areas.

- Continued detailed research in the area of pre-pregnancy nutritional status among women of childbearing age (18-44 years) is needed to ensure favourable pregnancy outcomes for women.

- Women of childbearing age need to be educated about the importance of eating healthy foods and include nutrients related to favourable pregnancy outcomes, such as iron, calcium and vitamins.

- A follow-up study is needed, using more sensitive measures of iron status, to determine, the efficacy of iron fortification in this population. Most importantly, is the necessity to review the effectiveness of the type of iron fortificant used in the fortification program in South Africa.

- Our findings with regard to vitamin B12 support the recommendations made in several studies, i.e. that every food fortified with folic acid should be fortified with vitamin B12.

- Critical evaluation of the effect of fumonisins on folate metabolism and contamination in morogo is required.

APPENDICES

I – An explanation sheet for women of childbearing age

II – Consent form

III – Socio-demographic questionnaire

IV – Maternity history form

V- Athropometric measurements sheet

VI – Dietary kit

VII – Quantitative food frequency questionnaire

VIII – 24 hour recall questionnaire

IX – Field workers training manual

X – Green leafy vegetables (morogo) questionnaire

XI – Principles of biochemical parameters

APPENDIX I

AN EXPLANATION SHEET FOR WOMEN OF CHILDBEARING AGE

AN EXPLANATION SHEET FOR WOMEN OF CHILDBEARING AGE

The Department of Medical Sciences, University of the North is involved with a study to assess the nutritional status (anthropometry and dietary intake), folate and iron status of non pregnant women of childbearing age.

The procedures to be followed in this project will be:

- Administering dietary, socio-demographic status and maternity history questionnaires to non-pregnant women of childbearing age
- Measuring anthropometric indices (body weight, height, waist and hip circumferences)
- Fasting blood samples will be collected from women of childbearing age for further analysis
- Food sample of cooked green leafy vegetables (GLV), morogo from few selected women of childbearing age

You are invited to participate in this research project. Participation is voluntary and you are free to withdraw from the project at any time. If at any stage you have queries or questions about the research, or would like to obtain more information about this study, please feel free to contact me and I will gladly answer your questions.

Yours sincerely

Ms SEP MODJADJI – Tel: 015 268/3051/3398

Department of Medical Sciences, University of the North, P/Bag X1106 Sovenga 0727

APPENDIX II

CONSENT FORM

CONSENT FORM

Statement by Researcher

I have fully explained to the participant the aim, objectives and procedures of the study, including collection of a blood sample. I have answered to the best of my ability any questions that have arisen regarding the study and the procedures.

Signed by researcher: _____

Date: _____

Statement by the participant

I have been fully informed as to aim, objectives and procedures to be followed and understand them. In signing this form, I agree to this investigation and understand that I am free to refuse or withdraw this consent regarding my participation in the study, at any time.

Signed: _____

Date: _____

Witness: _____

APPENDIX III

Socio-demographic status questionnaire

Interviewer_____ Date _____

Name of a participant_____Age _____

Area _____ Duration of stay in the area _____

A. MARITAL STATUS

Married _____ 1

Single _____ 2

Widow _____ 3

Divorced _____ 4

B. HOUSEHOLD INFORMATION

i) Do you have electricity? _____ Yes [1] ____ No [2]

ii) What do you use for cooking?

Electricity _____ 1

Gas _____ 2

Paraffin _____ 3

Wood _____ 4

Coal _____ 5

iii) Which method of water supply do you use?

Tap in house _____ 1

Tap outside _____ 2

Shared Tap _____ 3

Communal Tap (or more)_____ 4

iii) Do you have a refrigerator/deep freeze? _____ Yes [1] ____ No [2]

iv) Are you employed? (Include self-employed

E.g. TV repair, dressmaking etc.) _____Yes [1] _____No [2]

v) If yes, in which sector? _____ Formal _____ Informal

vi) If you are not employed, are you a Domestic engineer _____ 1

Student _____2

Other? _____ 3

vii) How many are you in a family? (All members

including those who are in e.g. Johannesburg) _____

viii) How many are employed:

Migrants workers (those who are far from home) _____ M

Permanent workers (those who are sleeping home daily)_____**P**

C. **EDUCATIONAL INFORMATION**

i) What is the highest standard you passed at school? _____

ii) Do you have other training or education____ Yes [1] _____ No [2]

ii) If yes, indicate your highest qualification

Formal training after school _____ 1

(e.g. NTC diploma, Nursing)

Graduate training _____ 2

Postgraduate training/education _____3

Informal training _____ 4

Other (specify) _____ 5

APPENDIX IV

MATERNITY HISTORY FORM

Interviewer _____ Date _____

Name of a participant _____ Age _____

Area _____ Duration of stay in the area_____

i) Parity (i.e. babies you have) _____

ii) Age of children _____

iii) Number of live births _____

iv) Number of still births _____

v) Do you know the reason why you had stillbirths? ___ Yes [1] ___ No [2

vi) Number of spontaneous abortions/miscarriages _____

vii) Do you know why you had spontaneous abortion/miscarriage

___ Yes [1] ___ No [2]

viii) Number of deaths after births _____

ix) Do you know the causes of deaths after births _____

x) Do you know what neural tube defects (NTD) are? ___ Yes [1] ___ No [2]

xi) If yes, what are NTD? _____

xii) Number of neural tube defects (NTD) among your babies_____

xiii) Do you know why you had a baby affected with NTD? __ Yes [1] _ No [2]

APPENDIX V

Anthropometric measurements sheet

Interviewer_____ Date _____

Name of a participant_____Age _____

Area _____ Duration of stay in the area _____

Body weight (W):…….. (kg)

Body height (H): …….. (m)

Waist circumference: …… (cm)

Hip circumference:…… (cm)

APPENDIX VI

DIETARY KIT

The diet kit will consist of a box with the following:

Plastic measuring jug (500 ml)

Measuring cups (250 ml, 125 ml, 100 ml, 50 ml, 25 ml)

Measuring spoons (12.5 ml, 10 ml, 5 ml, 2,5 ml, 1 ml)

Tablespoon, serving spoon, teaspoon

Enamel mug

Glass (150 ml, 250 ml)

Enamel plate

Table knife

Dehydrated bread slices (10 mm, 60 mm)

Dehydrated bread (scone shape, 6 cm and 8 cm)

Tennis ball (for fruit portion)

Wax maize porridge models (½, 1 and 2 cups)

Plastic containers 500 ml each of (maize meal, samp, dehydrated vegetables)

Kitchen scale

Ruler, pencil, eraser

APPENDIX VII

QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE

INTRODUCTION

Greetings

Thank you for availing yourself to participate in this study. We would like to find out what types of food you eat and drink. This information will help us to know you are eating adequately and are well nourished and healthy.

Please think carefully about the food and drinks you consumed during the past four weeks. I will go through a list of foods and drinks with you and I would like you to tell me:

- If you eat the food;
- How much of the food do you eat at a time;
- How the food is prepared
- How many times a day do you eat the food, if not every day, how many times a week or a month do you eat the food.

THERE ARE NO RIGHT OR WRONG ANSWERS. EVERYTHING YOU TELL ME IS CONFIDENTIAL.

IS THERE ANYTHING YOU WANT TO ASK ME NOW?

ARE YOU WILLNG TO GO ON WITH THE QUESTIONS?

DATE ……. /……../…….

ID NUMBER: _____

SURNAME: _____

PARTICIPANT'S NAME: _____

CODES USED

Measures

1t=1 rounded teaspoon
1T = 1 rounded tablespoon (15ml)
1SP= 1 roundserving spoon (30ml)
c= measuring cup (250m)
s/s= small size
m/s= medium
L/s= large
E= enriched
P= plain

Milk

SM= skim milk
WM= whole milk
BL= blend
CON= condensed milk
ND= non-dairy

Bread

Wh= white
Br= brown
Ww= wholewheat

Meat

F= with fat
FT= fat trimmed

Oil/Fat

B=butter
HM= hard margarine
Med= medium fat/light
PM= polyunsaturated
SO= sunflower oil
WF= white fat
PB= peanut butter

BR= breakfast (up to 09h00)
IS= in between snack
L= lunch (midday) (12h00-14h00)
D= dinner (evening) (17h00-19h00)
AD= after dinner
Comm= commercial
Home= homemade
Pot= potato
Cab= cabbage
Carr= carrot
Fill= filling
Usually= at least 4x/week

***May be more than one place;** **COPYRIGHT RESERVED: NATIONAL FOOD CONSUMPTION SURVEY 1999.** **Tick one box only**

QUESTION	YES	NO	DON'T KNOW	REMARKS / OTHER		
7. Do you eat maize porridge?	1	2	3	If yes, what type do you have at home now?		
				Give brand name 1	Don't know 2	Grind self 3
8. If brand name is given, do you usually use this brand?	1	2	3			

9. Where do you get maize-meal from?	Shop: 1 Specify type:	Employer 2	Harvest /grind self 3	Other 4 Specify:	Don't know 5	NA 6

10. Where do you usually store your maize-meal?						NA
11. What amount of maize meal do you store (kg)?						NA
12. For how long do you usually store maize meal (days)?						NA
13. How long do you usually cook the porridge (minutes)?						NA
14. Is the maize porridge cooked on:	Open fire 1	Electric stove / plate 2	Gas Stove 3	Paraffin Stove 4	Other Specify: 5	NA 6

15. Do you eat breakfast	Regularly (4 or more times a week)	Sometimes (1-3 times a week)	Never
	1	2	3

INSTRUCTIONS TO FIELDWORKERS:

CIRCLE THE CHOSEN ANSWER AND FILL IN THE AMOUNT AND TIMES EATEN IN THE APPROPRIATE COLUMNS.

I will ask you about the type and the amount of food you have been eating during the last 6 months. Please tell me if you eat the food, how much and how often you eat.

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/ NEV
PORRIDGE	**Maize-meal Porridge**	Stiff (Pap) –Plain -Enriched	**3400** **4278**	1c stiff = 250 g 1T = 75g						
		Soft (Slappap) – Plain -Enriched	**3399** **4277**	1c soft = 250g 1T = 75g						
		Crumbly (Phutu) – Plain -Enriched	**3401** **4279**	1 c crumbly = 140 g 1T = 30g						
	Sour Porridge	Maize with Vinegar Maize Fermented Mabella with Vinegar Mabella Fermented	**P0001** **P0002** **P0003** **P0004**	½c = 125g 1c = 250g						
	Mabella Porridge/Cornrice	Stiff Soft	**3437** **3437**	½ c = 125g						
	Maltabella Porridge	Stiff Soft	**3241** **3241**	½ c = 125g						
	Oats Porridge	Brand Name:	**3239**	2c = 125g						
	Other Cooked Cereals	Specify Type:								
	Milk on Porridge	None								
	(Circle type usually used)	Whole/Fresh Sour 2% Fat Free / Skim Milk Blend	**2718** **2787** **2772** **2775** **2771**	little = 30g med = 60g much = 125g						

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/ NEV
		Soy Milk	**2737**							
		Condensed (Whole, Sweet)	**2714**	1t = 10g						
		Condensed (Skim, Sweet)	**2744**							
		Evaporated Whole	**2715**	1t = 3g						
		Evaporated Low Fat	**2827**							
		Non-Dairy Creamer	**2751**	1t = 4g						
	Is sugar added to porridge? (Circle type usually used)	None								
		White Brown	**3989 4005**	1t sugar = 6g						
		Syrup	**3988**	1t honey/syrup = 15g						
		Honey	**3984**							
		Sweetener: Type	**P0016**							
	FOOD	**DESCRIPTION**	**CODE**	**QUANTITY (g/ml)**	**AMOUNT USUALLY EATEN (HHM)**	**AMOUNT USUALLY EATEN (g)**	**P/D**	**D/W**	**P/M**	**SEL/ NEV**
		None								
	Is fat added to porridge?									
	(Circle type usually used)	Animal Fat (Butter)	**3479**	1t marg/oil = 5g						
		Hard Margarine	**3484**							
		Soft Margarine (PM)	**3496**							
		Soft Margarine (Med)	**3531**							
		Sunflower Oil	**3507**							
		Peanut Butter	**3485**	1t = 12g						
	Breakfast Cereals	Specify types usually eaten		**(See Manual)**						
	Baby/Infant Cereals (Circle Type)	Mixed Dry (Nestum 2) Mixed Dry (Purity) Wholewheat Dry (Purity)	**2834 2842 2861**	1t = 2g 1T = 8g ½ c = 20g						
		Rice and Maize Dry (Nestum)	**2835**							
		Rice Dry (Purity)	**2862**							
		Wheat Dry (Nestum 1)	**2832**							
		With Milk Dry (Cerelac)	**2836**							
		Junior Cereal Dry	**2833**							

BREAKFAS

		Other									

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/ NEV
STARCHES	Pastas	Macaroni Spaghetti Plain	**3262 3262**	1T = 35g; 1SP = 70g; ½ c = 90g						
		Spaghetti and Tomato Sauce	**3258**	1T =45g; 1SP =80g; ½ c=125g						
		Other: Specify								
	Do you add fat to any of these starchy foods?	Yes ____ No ____ If yes, specify types, amounts and to which food?		**(See Manual)**						
	How many times a week does the child eat the above starchy foods?									

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/ NEV
BREADS AND SPREADS	**Now we come to bread and bread spreads:**									
	Bread/Bread Rolls	White Brown	**3210 3211**	Wh+Br 10mm = 30g Wh + Br 20mm = 60g Wh + Br 30mm = 100g ½ loaf = 400g						
		Whole Wheat	**3212**	Ww 10mm = 35g						
	Other Breads (Specify Types)	Raisin Maize Meal	**3214 3278**	m/s = 30g; L/s = 50g						
		Sweetcorn	**3379**							
		Rye	**3213**							
		Pumpernickel	**3283**							
		Other								
	How many times per week do you eat bread? _____									
	Dumpling	(Depends on specific areas)		**(See Manual)**						
	Vetkoek	(Depends on specific areas)		8 cm diam = 60g						
	Provita		**3235**	6g						
	Crackers	Cream Crackers	**3230**	8g						
		Refined (eg. Tuc)	**3331**	4g						

	Wholewheat	**3391**	8g							
Pizza	(Specify Toppings)		**(See Manual)**							
Hot Dogs	(Specify Sausage)		**(See Manual)**							

	FOOD	**DESCRIPTION**	**CODE**	**QUANTITY (g/ml)**	**AMOUNT USUALLY EATEN (HHM)**	**AMOUNT USUALLY EATEN (g)**	**P/D**	**D/W**	**P/M**	**SEL/ NEV**
	Hamburgers	(Specify Meat)		**(See Manual)**						
	Are any of the following spreads on the your bread? **Fat Spreads: (Tick box)**	Butter Butro Animal Fat (Beef Tallow) Lard	**3479** **3523** **3494** **3495**	1t = 5g						

	FOOD	**DESCRIPTION**	**CODE**	**QUANTITY (g/ml)**	**AMOUNT USUALLY EATEN (HHM)**	**AMOUNT USUALLY EATEN (g)**	**P/D**	**D/W**	**P/M**	**SEL/ NEV**
BREADS AND SPREADS		Hard Margarine Soft Margarine (PM) Soft Margarine (Med)	**3484** **3496** **3531**							
	PeanutButter		**3485**	1t = 12g						
	Sweet Spreads	Jam Syrup Honey	**3985** **3988** **3984**	1t = 15g						
	Marmite/OXO	Marmite Oxo	**4030** **4029**	thin = 2g; med = 4g; thick=7g						
	Paste	Fish Paste Meat Paste	**3109** **2917**	thin = 5g; med = 7g; thick = 10g						
	Cheese (Specify Types)	Cheddar Gouda	**2722** **2723**	grated: med = 10g; thick = 15g cubes = 30g; slice = 8g; cheezi = 20g						
		Cottage Low-Fat Cheese Cream Cheese Other	**2760** **2725**	med = 20g; thick = 30g thin = 10g; med = 20g						
	Cheese Spreads (Specify Types)		**2730**	med = 12g; thick = 25g						
	Atchar		**3117**	1T = 14g; 1SP = 60g						

	Other Spreads (Specify Types)									

You are being very helpful. Can I ask you about protein foods? These are: meat, beans, chicken, fish and eggs.

CHICKEN		Boiled with skin Boiled without skin Fried in batter/crumbs Fried – not coated Roasted/grilled with skin Roasted/grilled without skin	**2926 2963 3018 2925 2925 2950**	Breast + skin = 125g Thigh =80g Drumstick = 42g Foot = 30g Wing = 30g						
	Chicken Bones Stew	(Specify ingredients)	**P0048**							
	Chicken Heads		**2999**							

	FOOD	**DESCRIPTION**	**CODE**	**QUANTITY (g/ml)**	**AMOUNT USUALLY EATEN (HHM)**	**AMOUNT USUALLY EATEN (g)**	**P/D**	**D/W**	**P/M**	**SELNEV**
	Chicken Stew	With Vegetables	**3005**	1SP = 90g;						
		With Tomato & Onion	**2985**	½ c = 125g						
	Chicken Feet		**2997**	Foot = 30g						
	Chicken Offal	Giblets	**2998**	stomach = 20g						
	Chicken Liver		**2970**	Liver = 30g						
	Chicken Pie	Commercial or homemade	**2954**	med = 150g						
RED MEAT	**Beef**	Roasted with Fat	**2944**	120 x 60 x 5 = 35g						
		Roasted, Fat Trimmed	**2960**	120 x 60 x 10 = 70g						
		Rump, Fried with Fat Rump, Fried, Fat Trimmed	**2908 2959**	S/s 130 x 70 x 15 = 125g L/s 165 x 70 x 30 = 270g						
		Stewed/Boiled With Fat (Cabbage)	**3006**	1SP = 105g; ½ c = 125g						
		Stewed/Boiled Without Fat (Vegetables)	**2909**							
		Mince With Tomato and Onion	**2987**	1T=40g;1SP=85g ; ½ c=100g						
		Other Preparation Methods:								
	Mutton	Fried/Grilled: With Fat Fried/Grilled: Without Fat	**2927 2934**	Loin chop = 60g; Rib chop = 40g						
		Stew: Plain	**2974**	1SP = 105g;						

	Stew: Irish (Vegetables) Stew: Curry Stew: Greenbean	**2916** **3039** **3040**	½ c = 125g							
	Other Preparation Methods:									
Pork	Fried/Grilled: With Fat Fried/Grilled: Without Fat Roast With Fat Roast Without Fat Other Preparation Methods:	**2930** **2977** **2958** **2978**	Chop: 115 x 80 x 20 = 100g Schnitzel: 115 x 80 x20 = 110g Roast: 110x 65 x 5 = 30g 1SP = 105g; ½ c = 125g							
Goat	Fried/Grilled: With Fat	**P0008**	120 x 60 x 5 = 35g							

FOOD	**DESCRIPTION**	**CODE**	**QUANTITY (g/ml)**	**AMOUNT USUALLY EATEN (HHM)**	**AMOUNT USUALLY EATEN (g)**	**P/D**	**D/W**	**P/M**	**SEL/NEV**
	Fried/Grilled: Fat Trimmed Stewed (Plain) Stewed (With Vegetables) Other Preparation Methods:	**P0009** **4281** **4282**	120 x 60 x 10 = 70g 1SP = 105g ½ c = 125g						

FOOD	**DESCRIPTION**	**CODE**	**QUANTITY (g/ml)**	**AMOUNT USUALLY EATEN (HHM)**	**AMOUNT USUALLY EATEN (g)**	**P/D**	**D/W**	**P/M**	**SEL/ NEV**
Offal	"Vetderm" Fried Liver: Beef (Fried) Liver: Sheep (Fried) Kidney (Beef) Kidney (Sheep) Tripe, Beef, Cooked in Milk Heart (Beef) Heart (Sheep)	**P0023** **2920** **2955** **2923** **2956** **2951** **2968** **2969**	1SP = 105g; ½ c = 125g 80g 55g 85g 30g 1SP = 105g; ½ c = 125g 60g 60g						

GENERAL MEAT:		Lung (Beef)	**3019**	60g							
	Wors/Sausage	Fried	**2931**	Thin x 200mm = 45g; Thick x 165mm = 90g							
	Bacon	Fat Lean	**2906** **2915**	1 rasher = 10g							
	Cold Meats	Polony Ham Viennas Other	**2919** **2967** **2936**	Slice 5mm thick = 8g Comm slice = 16g Med slice = 25g 100mm = 30g; 150mm =40g							
	Canned Meats	Bully Beef Other (Specify)	**2940**	138 x 85 x 3 = 20g; ½ c = 100g							
	Meat Pie	Bought (Steak & Kidney) Other (Specify)	**2957**	120g							

	Legumes (Specify dried beans/peas/legumes)	Stews (Bean, Potato & Onion)	**3178**	1T=60g; 1SP = 120g; ½c=125g						
		Soups: Commercial Split Pea Lentil Beef & Vegetables Bean	**3165** **3157** **3153** **3159** **3145**	½ c = 125g 1T=35g; 1SP = 80g; ½ c = 130g						
		Legume Salad	**3174**	1T=40g; 1SP=105g; ½ c=135g						
	Soya Products e.g. Toppers / Imana	(Specify)	**3196**	1SP = 85g; ½ c = 120g						

	FOOD	**DESCRIPTION**	**CODE**	**QUANTITY (g/ml)**	**AMOUNT USUALLY EATEN (HHM)**	**AMOUNT USUALLY EATEN (g)**	**P/D**	**D/W**	**P/M**	**SEL/N EV**
	Fried Fish (Fresh or Frozen, Fried in Sun Oil)	With Batter/Crumbs Without Batter/Crumbs	**3094** **3084**	Small 50 x 55 x 30 = 60g; Med 100 x 55 x 30 = 120g						
	Canned Fish	Pilchards in Brine Pilchards in Tomato Sauce Pilchards, Mashed Sardines in Oil	**3055** **3102** **3102** **3104**	1 Pilchard = 75g 1 SP = 85g; ½ c = 100g Ss = 7g; L/s = 25g						

FISH		Sardines in Tomato Sauce	**3087**							
		Tuna in Oil	**3093**	¼ c = 50g						
		Tuna in Brine	**3054**							
		Other (Specify)								
	Pickled Fish/Curried Fish		**3076**	1 SP = 95g; ½ c=140g						
	Do you remove fish bones before eating canned fish? Yes ___No __									
	Fish Cakes	Fried: Oil/Butter/Margarine	**3098**	65 x 15mm = 50g						
	Fish Fingers	Fried: Oil/Butter/Margarine	**3081**	85mm = 35g						

	FOOD	**DESCRIPTION**	**CODE**	**QUANTITY (g/ml)**	**AMOUNT USUALLY EATEN (HHM)**	**AMOUNT USUALLY EATEN (g)**	**P/D**	**D/W**	**P/M**	**SEL/NEV**
EGGS	**Eggs**	Boiled/Poached	**2867**	1 egg = 50g						
		Scrambled in Oil In Butter In Margarine	**2889 2886 2887**	1T = 35g; 1SP = 80g; ½c=115g (approx. 2 eggs)						
		Fried in Oil In Butter In Margarine In Bacon Fat	**2869 2868 2877 2870**	1 egg = 52g						
		Curried	**2902**	1 egg + sauce (1T) = 75g						

How many times a week do you eat meat, beans, chicken, fish or eggs? _____

Are there any other foods in this category that you eat? If yes, please list these foods/dishes YES NO

FOOD	**DESCRIPTION**	**CODE**	**AMOUNT USUALLY EATEN (HHM)**	**AMOUNT USUALLY EATEN (g)**	**TIMES EATEN**			
					Per day	**Days per week**	**Per month**	**Seldom/ Never**

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL /NEV
We now come to vegetables:										
VEGETABLES	**Cabbage**	Boiled, Nothing Added Boiled with Potato, Onion and Fat Fried, Nothing added Boiled, then fried with potato, onion Other	**3756** **3813** **3812** **3815**	1T=30g; 1SP=55g; ½ c=80g 1T=35g; 1SP=75g; ½ c=80g 1T=30g; 1SP=55g; ½ c=80g 1T=35g; 1SP=75g; ½ c=80g						
	Spinach/Marog/Imifino/ Amaranth Leaves Other Green Leafy Vegetables: **List Names**	Boiled, nothing added Boiled, fat added Boiled with Onion, Potato and Fat Boiled with Peanuts	**3980** **3898** **3901** **P0015**	1T=40g; 1SP=105g; ½ c=90g 1T=40g; 1SP=105g; ½ c=90g 1T=50g; 1SP=105g; ½ c=110g 1T=55g; 1SP=120g; ½ c=105g						
		Other:								

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/NEV
	Tomato and Onion "Gravy"/ Relish/Chow/Sheshebo	Home Made with Sugar Home Made, no Sugar Canned	**3910** **3925** **4192**	1T = 35g; 1SP = 75g; ½ c = 140g						
	Pumpkin (Specify Type)	Boiled, nothing added Cooked in Fat and Sugar	**4164** **3893**	1T = 45g; 1SP = 85g; ½ c = 105g						
		Other								
	Carrots	Boiled, Sugar and Fat With Potato/Onion (HM) Raw, Salad (Sugar added) Chakalaka	**3818** **3822** **3721** **P0046**	1T = 25g; 1SP=50g; ½ c = 85g 1T=35g; 1SP=70g; 1/2 c=105g 1T = 25g						

	Other									

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/NEV
VEGETABLES	**Mealies/Sweet Corn**	On Cob	**3725**	1T =30g; 1SP = 60g; ½ c =95g						
		Off Cob – Creamed, Sweet Corn	**3726**	1T = 55g; 1SP = 125g; ½ c = 135g						
		Off Cob – Whole Kernel Canned	**3942**							
		Other								
	Beetroot	Cooked (No Sugar)	**3698**	1T=40g; 1SP = 70g; ½ c = 80g						
		(With Sugar)	**3699**							
		Salad (Grated)	**3699**	1T = 25g; 1SP = 65g						
	Potatoes	Boiled/Baked with Skin Without Skin	**4155 3737**	S/s = 60g; m/s = 90g						
		Mashed (WM)	**3876**	1T=50g; 1SP = 115g; ½ c = 125g						
		Roasted	**3878**	1 med = 70g						
		French Fries/Potato Chips	**3740**	½ c = 50g; med = 80g						
		Salad	**3928**	1T = 45g; 1SP = 105g; ½ c = 120g						
		Other								
	Sweet Potatoes	Boiled/Baked with Skin	**3748**	1T = 50g; 1SP = 110g; ½ c = 145g						
		Without Skin	**3903**							
		Mashed (With Sugar)	**3749**							
		Other								

FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g) P/DD/WP/MSEL/NEV
Green Beans	Green, Frozen	**4123**	1T = 25g; 1SP=60g; 1/2 c=80g		
	Cooked, Potato & Onion (HM)	**3792**	1T = 40g; 1SP = 75g; ½ c =120g		

	Other									
	Green, Frozen, Boiled	**4146**	1T=30g; 1SP = 65g;							
		1001								

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/NEV
	Peas	Green, Frozen with Sugar, Boiled	**3720**	½ c = 85g						
		With Sugar and Butter	**3859**							
	Green Peppers	Raw	**3733**							
		Cooked	**3775**							
	Brinjal/Egg Plant	Cooked Fried in Oil	**3700 3802**	1 slice = 20g (70 mm) + batter = 30g						
		Stew (oil, onions, tomato)	**3798**	1T=50g; 1SP=100g;1/2c=130g						
	Mushrooms	Raw Sauteed in brick margarine	**3842 3839**	1T=30g; 1SP = 65g; 1/2c = 80g						
		Sauteed in oil	**3841**							
	Onions	Sauteed in Sun Oil	**3730**	1T = 50g						
	Salad Vegetables	Raw Tomato	**3750**	Med = 120g; slice = 15g						
		Lettuce	**3723**	1 med leaf = 30g						
		Cucumber	**3718**	Med slice = 10g; thick = 15g						
		Avocados	**3656**	¼ avo (80 x 50mm) = 40g						
	Other Vegetables: Specify									
VEGETABLE	**If you fry vegetables or add fat, specify type of fat usually used**	Butter Butro Animal Fat (Beef Tallow)	**3479 3523 3494**	1t = 5g						
		Lard	**3495**							
		Hard Margarine (Brick)	**3484**							
		Soft Margarine (Tub, PM)	**3496**							
		Soft Margarine (Med)	**3531**							

	Vegetable Purees with or without meat for babies or infants: (Specify)	First Food average Vegetable (Jar) Junior Food Veg (Jar) Junior Food Veg Plus Meat Infant Dinner, Beef and Veg Infant Dinner, Chicken and Veg Infant Dinner, Mixed Veg	**2851** **2849** **2848** **2841** **2840** **2839**	1t = 5g 1T = 15g ½ c = 47g						
		Other								

	FOOD	**DESCRIPTION**	**CODE**	**QUANTITY (g/ml)**	**AMOUNT USUALLY EATEN (HHM)**	**AMOUNT USUALLY EATEN (g)**	**P/D**	**D/W**	**P/M**	**SEL/NEV**
DRESSINGS	**Mayonnaise/Salad Dressing**	Mayonnaise – Bought - Home-made	**3488** **3506**	1t = 10g 1T = 40g						
		Cooked Salad Dressing Salad Dressing, low-oil Salad Dressing, French	**3503** **3505** **3487**	1t = 5g; 1T = 15g						
		Oil – Olive Oil - Sunflower Oil - Canola	**3509** **3507** **4280**	1t = 5g; 1T = 15g						
FRUIT	**How many times a week do you eat vegetables? _____ How many times will this be fresh? _____ Canned _____ Frozen _____** **I will now ask about fruit**									
	Apples	Fresh Canned, Pie, Unsweetened	**3532** **4216**	1T=60g; ½ c = 120g; 1 med = 150g (52 x 66)						
	Bananas		**3540**	1 med = 75g						
	Oranges/Naartjies		**3560**	Med (7cm) = 180g						
	Grapes		**3550**	Med bunch = 230g; ½ c = 90g						
	Peaches	Fresh Canned in Syrup	**3565** **3567**	1 med = 150g (60 x 65)						
	Apricots	Fresh Canned in Syrup	**3534** **3535**	1 med = 35g						

	Mangoes	Fresh Canned in Syrup	**3556 3633**	135mm = 350g						
	Pawpaw		**3563**	Wedge 165 x 26 x 27 = 90g						
	Pineapple	Raw Canned in Syrup	**3581 3648**	1 slice (85 x 10mm) = 40g						
	Guavas	Fresh Canned in Syrup	**3551 3553**	Med (6cm) = 95g						
	Pears	Fresh Canned in Syrup	**3582 3583**	1 med (80 x 65mm) = 165g						

	FOOD	**DESCRIPTION**	**CODE**	**QUANTITY (g/ml)**	**AMOUNT USUALLY EATEN (HHM)**	**AMOUNT USUALLY EATEN (g)**	**P/D**	**D/W**	**P/M**	**S E L/ N E V**
	Wild Fruit and Berries: (Specify Type)									

	FOOD	**DESCRIPTION**	**CODE**	**QUANTITY (g/ml)**	**AMOUNT USUALLY EATEN (HHM)**	**AMOUNT USUALLY EATEN (g)**	**P/D**	**D/W**	**P/M**	**S E L/ N E V**
	Dried Fruit (Also as Snacks)	Raisins	**4232**	1 handful = 27g						
		Prunes (Raw) Prunes (Cooked with Sugar)	**4230 3564**	1T = 50g; ½ c = 110g; 1 = 12g						
		Peaches (Raw) Peach (Cooked with Sugar)	**3568 3569**	1 med = 150g (60 x 65)						
		Apples (Raw)	**3600**	1T=60g; ½ c = 120g; 1 med = 150g (52 x 66)						
		Dried Fruit Sweets	**3995**	**(See Manual)**						
		Other								
	Fruit Purees for Babies or Infants (Specify Types)	First Food Average (Jar) Junior Fruit (Jar) Strained Fruit (Jar) Infant Dinner, Guava and Custard	**2852 2863 2854 2837**	Jar = 200g 1t = 11g ½ c = 125g						

		Other								
	Other Fruit									
	How many times a week do you eat fruit? _____ How many times will this be fresh _____ Canned _____ Frozen _____									
DRINKS	**Tea**	Ceylon Rooibos	**4038 4054**	Teacup = 180ml; mug = 250ml						

	FOOD	**DESCRIPTION**	**CODE**	**QUANTITY (g/ml)**	**AMOUNT USUALLY EATEN (HHM)**	**AMOUNT USUALLY EATEN (g)**	**P/D**	**D/W**	**P/M**	**SEL/NEV**
	Sugar Per Cup of Tea	Specify Type: White Brown	**3989 4005**	1t sugar = 6g						
	Milk per Cup of Tea	Fresh/Long Life Whole Fresh/Long Life 2% Goat Fresh/Long Life from (skimmed)	**2718** **2772** **2738 2775**	20ml – tea in cup 35ml – tea in mug 40ml – coffee in cup 75ml – coffee in mug						

	FOOD	**DESCRIPTION**	**CODE**	**QUANTITY (g/ml)**	**AMOUNT USUALLY EATEN (HHM)**	**AMOUNT USUALLY EATEN (g)**	**P/D**	**D/W**	**P/M**	**SEL/NEV**
		Whole Milk Powder Reconstituted (Specify Brand)	**2831**	1t = 4g						
		Skimmed Milk Powder, reconstituted (Specify Brand)	**2719**	1t = 4g						
		Milk Blend, reconstituted (Specify Brand)	**2771**	20ml – tea in cup 35ml – tea in mug 40ml – coffee in cup 75ml – coffee in mug						
		Whitener/non-dairy creamer	**2751**	1t = 4g						

		(Specify Brand)								
		Condensed Milk (Whole) Condensed Milk (Skim)	**2714** **2744**	1t = 10g						
		Evaporated Milk (Whole) Evaporated Milk (Low-Fat)	**2715** **2827**	1t = 3g						
		None								
	Coffee		**4037**	Teacup = 180ml; mug= 250ml						
	Sugar per Cup of Coffee	Specify Type: White Brown	**3989** **4005**	1t sugar = 6g						
	Milk per Cup of Coffee	Specify Type		**(See Manual)**						
DRIN	**Milk as such:**	Fresh/Long Life/ Whole	**2718**	To drink ½ c = 125ml						

	FOOD	**DESCRIPTION**	**CODE**	**QUANTITY (g/ml)**	**AMOUNT USUALLY EATEN (HHM)**	**AMOUNT USUALLY EATEN (g)**	**P/D**	**D/W**	**P/M**	**SEL/NEV**
	What type of milk does the child drink as such?	Fresh/Long Life/2% Fresh/Long Life/Fat Free (skimmed) Goat Sour/Maas	**2772** **2775** **2738** **2787**	Baby bottle = 250ml						
		Brand: Infant Formulas (Specify)								
	Milk drinks. Specify Brands, including milk supplements and type of milk used	Nestle Drinking Chocolate	**4287**	1t = 5g						
		Malted Milk Beverage, no Sugar (eg Milo)	**2735**	1t = 5g						
		Flavoured Milk:	**2774**	Carton = 250ml; S/s plastic = 350 ml						
		Other								

	FOOD	**DESCRIPTION**	**CODE**	**QUANTITY (g/ml)**	**AMOUNT USUALLY EATEN (HHM)**	**AMOUNT USUALLY EATEN (g)**	**P/D**	**D/W**	**P/M**	**SEL/**

										NEV
DRINKS	**Yoghurt**	Drinking Yoghurt Thick Yoghurt: Plain, Fat-Free WM Plain - Fruit, Low Fat	**2756** **2778** **2757** **2732**	S/s = 175ml Yogisip = 350ml ½ c = 125g						
		Other								
	Squash	Sweeto, Sixo	**3982**	Small glass = 150ml Medium glass = 250 ml Large glass = 500 ml S/s bottle = 350ml L/s bottle = 500ml S/s can = 350ml						
		Oros/Lecol with Sugar Artificial Sweetener	**3982** **3990**							
		Kool Aid	**3982**							
		Other								
	Fruit Juice	Fresh/Liquifruit/Ceres/Purity	**2866**	1 Liquifruit s/s = 250ml						

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/ NEV
		"Tropica"/mixture with milk	2791	1 Liquifruit L/s = 500 ml S/s bottle = 350ml L/s bottle = 500ml S/s can = 350ml						
	Fruit Syrups	Average	2865	1t = 5g						
		Guava Syrup	2864							
	Fizzy Drinks (e.g. Coke, Fanta)	Sweetened	3981	S/s bottle = 350ml L/s bottle = 500ml S/s can = 340ml						
		Diet	3990							
	Magou/Motogo		4056	1 carton = 500 ml						
	Alcoholic Beverages such as Sorghum Beer	Specify: Sorghum Beer	4039	(See Manual)						
	Other (Please Specify)									
SNACKS	**Please indicate what types and amounts of snacks, puddings and sweets the child eat:**									
	Potato Crisps		3417	(See Manual)						
	Peanuts	Roasted Unsalted	3452							
		Roasted, Salted	3458							
	Cheese Curls (Nik Naks, etc.)	Average	3267							
		Savoury	3418							
	Popcorn	Plain	3332							
		Sugar Coated	3359							
	Peanuts and Raisins (mixed)	Roasted, Salted	P0047							
	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/ NEV
	Chocolates	Specify types and names: Assorted	3992							
	Candies	Sugus, gums, hard sweets (Specify)	3986							
	Sweets	Toffee, fudge, caramels (Specify)	3991							
	How many times a week does the child eat snack food? _____									
CAKES, BISCUITS AND	**Biscuits/Cookies**	Specify Type		(See Manual)						

Cakes & Tarts	Specify Type										
Pancakes/Crumpets	Specify Type										
Rusks	Specify Types										
Scones	White, WM	**3237**	6cm diam=35g; 8cm diam=60g								
Muffins	Plain	**3408**									
	Bran	**3407**									
Koeksisters		**3231**	100 x 35 = 60g								
Savouries	Sausage Rolls	**2939**	Roll x 135mm = 165g								
	Samoosas (Meat)	**3355**	S/s = 42g								
	Biscuits e.g. Bacon Kips	**3331**	4g								
	Other										

	FOOD	**DESCRIPTION**	**CODE**	**QUANTITY (g/ml)**	**AMOUNT USUALLY EATEN (HHM)**	**AMOUNT USUALLY EATEN (g)**	**P/D**	**D/W**	**P/M**	**S E L/ N E V**
	How many times a week do you eat cakes/cookies? _____ less than 1/week _____									
PUDDINGS	**Jelly**		**3983**	1T=35g; 1SP=75g; ½ c = 110g						
	Baked Puddings	Specify Types		Med serving = 30g 30 x 65 x 65 = 50g						
	Instant Puddings	Specify Types		1T = 45g; SP = 95g; ½ c =145g						
	Infant Deserts	Specify Types		Jar=200g; 1t = 11g; ½ c = 125g						

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/NEV
	Ice Cream	Commercial Regular Commercial Rich	**3483 3519**	Scoop = 40g; 1SP=65g; ½ c = 75g						
		Soft serve	**3518**	Plain = 135g; + flake = 155g						
		Sorbet Ice Lollies Chocolate Coated Individual Ice Creams (E.g. Magnum)	**3491 3982 P0036**	Scoop = 40g; 1SP=65g; ½ c = 75g						
	Custard	Home Made (WM) (SM)	**2716 2717**	T=13g; SP = 40g						
	Other Puddings Specify									
	How many times a week do you eat pudding? _____ less than 1/week _____									
SAUCES, GRAVIES, CONDIMENTS	**Tomato Sauce**		3139	1t = 6g; 1T = 25g						
	Worcester Sauce		P0037							
	Chutney	Fruit Tomato	3168 3114	1t = 14g; 1T = 60g						
	Pickles		3866	1 = 10g						
	Packet Soups		3165	½ c = 125g						
	Others									

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/NEV
	Wild birds, animals, insects or fruits and berries (hunted or collected in rural areas or on farms): Specify									

	FOOD	DESCRIPTION	CODE	QUANTITY (g/ml)	AMOUNT USUALLY EATEN (HHM)	AMOUNT USUALLY EATEN (g)	P/D	D/W	P/M	SEL/NEV
	Please mention any other foods you eat more than once every two weeks which we have not talked about or foods eaten in other homes or places during the past week.									

16. Are there any foods that you do not eat? Please list them and give reasons why don't you eat them (e.g. because of religious beliefs).

FOODS NOT EATEN	CODES	REASON

17. For the main meal, do you buy, and/or cook and/or serve food separately from that of others in the house?	YES	NO	DON'T KNOW
	1	2	3
18. EATING PATTERNS: (FREQUENCY OF EATING) Please indicate which of the following best describes the eating pattern the child			

usually follows **(mark only one)**	
More than three meals with eating between meals	1
Three meals with eating between meals	2
Three meals with no eating between meals	3
Two meals with eating between meals	4
Two meals with no eating between meals	5
One meal with eating between meals	6
One meal with no eating between meals	7
Nibble the whole day, no specific meals	8
Others (Please specify):	9

19. Are there any foods that you eat which we haven't talked about? Please list them.

FOODS	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE
			Per day	Per week	Per month	Seldom/ Never	

1. 20. Do you sometimes eat elsewhere?

NO YES

Thank you for your co-operation. We appreciate your contribution.

APPENDIX VIII

24-hour recall questionnaire

INTRODUCTION

Greetings

Thank you for availing yourself to participate in this study. We would like to find out what types of food you eat and drink. This information will help us to know if you are eating adequately and are well nourished and healthy.

Please think carefully about the food and drinks you consumed during the past twenty-four hours. I will go through the past day starting from early morning and you tell me what the child ate at the various points of the day,

THERE ARE NO RIGHT OR WRONG ANSWERS. EVERYTHING YOU TELL ME IS CONFIDENTIAL.

IS THERE ANYTHING YOU WANT TO ASK ME NOW?

ARE YOU WILLNG TO GO ON WITH THE QUESTIONS?

DATE ……. /……../…….

ID NUMBER: _____

SURNAME: _____

PARTICIPNANT'S NAME _____

1. Name of							
2. Day of the week recalled	1 Mon	2 Tue	3 Wed	4 Thu	5 Fri	6 Sat	7 Sun
3. Was yesterday typical/routine for the child?	1 YES	2 NO IF NOT, WHY?					

INSTRUCTIONS

Now I want you to tell me everything what you ate and drank yesterday. Let's start with when you woke up.
Did you have anything to eat or drink? Proceed through the day following your activities. When you have finished, summarise it. Any forgotten items can then be added.

X Enter each item eaten in grams under the correct interval of the day eaten.
X Make sure that the code is circled.
X Items not on the questionnaire should be looked up in the Quantity Manual or list of food codes.
X Specify fully when new items are entered and look up the code later.
X Recipes should be added on page 15.

ABBREVIATIONS

Measures 1t = 1 rounded teaspoon 1T = 1 rounded tablespoon (15ml) 1SP = 1 rounded serving spoon (30ml) c = measuring cup (250ml) s/s = small size m/s medium L/s = large E = enriched P = plain

Milk: SM = skim milk WM = whole milk BL = blend CON = condensed milk ND = non-dairy
Bread:
Wh = white
Br = brown
Ww = wholewheat

Meat:
F = with fat
FT = fat trimmed

Oil/Fat

B = butter

HM = hard margarine

Med = medium fat/light

PM =- polyunsaturated

SO = sunflower oil

WF = white fat

PB = peanut butter

BR = breakfast (Up to 09h00) IS = in-between snack L = lunch (midday (12h00-14h00) D = dinner (evening) (17h00 – 20h00) AD = after dinner Comm = commercial Home = homemade Pot = potato Cab = cabbage Carr = carrot Fill = filling

Usually = at least 4x/week

	FOOD ITEMS	QUANTITY (g/ml)	BR	IS	L	IS	D	AD
TEA & COFFEE	**Tea: 4038; Rooibos 4054**	teacup = 180ml; mug = 250ml						
	Coffee 4037	cup = 180ml; mug = 250ml						
	+ Sugar White-3989; Brown-4005;Syrup-3988;Honey-3984	1 t sugar = 6g1 t honey/syrup = 15g						
	+ Condensed Milk: WM -2714; Cond Milk: SM-2744; Condensed Milk, Non-Dairy-P0042	1t = 10g						
	+ Evaporated WM-2715; Evaporated SM-2827; Lite-P0043	1t = 3g						
	+ Non-Dairy Creamer-2751	1t = 4g						
	+ WM Powder-2831	1t = 4g						
	+ Milk: SM-2719; WM-2718	MEDIUM PORTIONS: 20ml - tea in cup 35ml - tea in mug 40ml - coffee in cup 75ml - coffee in mug						
	BL-2771; 2%-2772							
	Soy-2737; Breast-2741; Goat-2738							
	Formula (Specify):_____							
	No of Scoops/Bottle:_____							
	Other (Specify)							
	Buttermilk - 2713	s/s = 175ml l/s = 500ml 2c = 125g						
	Maas/Amazi/Sourmilk - 2787							
	Custard: SM-2717; WM-2716	s/s = 350 ml						
	Milk: SM-2719; WM-2718	to drink 2c = 125ml						
	BL-2771; 2%-2772	baby bottle = 250ml						
	Soy-2737; Breast-2741; Goat-2738							
	Formula (Specify):_____							
MILK & MILKDRINKS	**No of Scoops/Bottle:_____**							
	+ Sugar White-3989;Brown-4005; Syrup-3988;Honey-3984	1 t sugar = 6g1 t honey/syrup = 15g						
	+ Ice Cream-3519; Sorbet-3491	1 scoop = 40g						
	+ Sustagen-4079; Complan-4082	2 scoops = 25g; 1T = 15g						
	+ Milo/Cocoa/Horlicks/Ovaltine-2736;	1t = 5g						
	Drinking Chocolate-4287							
	Yoghurt: Plain SM-2734; WM-2757	s/s = 175ml Yogisip = 350ml 2c = 125g						
	Flav-2756; Fruit-2732							
	Flavoured milk - 2774	carton = 250ml						
		s/s plastic = 350ml						
	Other (Specify) _____							
/JUICE	**Apple Juice – No Sugar – 3606**	Liquifruit s/s = 250ml L/s = 500ml Ceres s/s = 200ml cartons/bottles s/s = 350ml L/s = 500ml						
	Apricot: + Sugar-3539; No Sugar-3610							
	Mango-3683; Granadilla-3680; Grape-3690							
	Orange: +Sugar-3562; No sugar-3638							

	Guava: +Sugar-3554; No Sugar-3629							
	Peach-3642; Pear-3645; Naartjie-3682							
COLDDRINKS	**Cold drinks: Squash-3982**							
	Mageu-4056							
	Carbonated-3981	s/s bottle = 350ml L/s bottle = 500ml						
		s/s can = 340ml						

Diet Cold. & Low-Cal - 3990

∗ Infasoy-2808; Isomil-2796; Lactogen 1-2821; Lactogen 2-2822; Nan-2819; Pelargon-2820; Portagen-2799; Pregestimil-2800; Prosobee-2795; S26-2806; S26 Infagro-2810; SMA-2814; Similac-2797; Similac PM-2817

	Dairy Fruit Mix - 2791							
	FOOD ITEMS	**QUANTITY (g/ml)**	**BR**	**IS**	**L**	**IS**	**D**	**AD**
	+ Sugar White-3989;Brown-4005;Syrup-3988; Honey3984	1t = 6g						
	Syrup (undiluted)-2865; Guava Syrup-2864	1t = 5g						
	Other (Specify)							
	Maltabella: Soft-3241; Mabella: Soft-3437	2c = 125g						
	M/Meal: Soft: Plain-3399; Enrich-4277	1c soft = 250g						
	Stiff: Plain-3400; Enrich-4278	1c stiff = 250g						
	Crumbly: Plain-3401; Enrich-4279	1c crumbly = 140g						
	Sour Porridge: Maize with Vinegar-P0001, Maize Fermented- P0002 Mabella with Vinegar-P0003; Mabella Fermented-P0004	½c = 125g 1c = 250g						
	Oats-3239; Tastee Wheat-3240	2c = 125g						
	Corn Flakes-3243; Sugar Frosted-3374	1c = 40g						
	Honey Crunch and Muesli - 3303	2c = 65g						
BREAKFAST CEREALS	**Pronutro: Great Start-3438; High Energy-3245; Wholewheat-3436**	2c = 50g						
	Puffed Wheat-3325; Sweetened-3376 (Honey Smacks)	2c = 12g						
	Raisin Bran-3373; Fruit Loops-3425	Raisin Bran 2c = 45g Fruit Loops 2c = 18g						
	Special K-3322; All Bran-3242	2c = 25g						
	Rice Crispies-3252; Cocopops-3372	2c = 20g						
	Weetbix – 3244	1 = 25g						
	+ Fat: B -3479; HM-3484; Med-3531; PM-3496; WF-3516	1 t PB = 12g; 1 t marg/oil = 5g						
	Ghee-3525; PB-3485; Butro-3523; SO-3507							
	+ Sugar White-3989;Brown-4005; Syrup-3988;Honey-3984	1 t sugar = 6g 1 t honey/syrup = 15g						
	+ Cond Milk:SM-2744; Cond WM-2714;Cond ND-P0042	1t = 10g						

BREAD & ROLLS	**+ Evap WM-2715; Evap SM-2827; Evap Light-P0043**	1t = 3g						
	+ Non-Dairy Creamer-2751	1t = 4g						
	+ WM Powder-2831	1t = 4g						
	+ Milk: SM-2719; WM-2718	125g – instant cereal 60g – porridge 180g – Pro Nutro						
	BL-2771; 2%-2772							
	Soy-2737; Breast-2741; Goat-2738							
	Formula (Specify):_____							
	No of Scoops/Bottle:_							
	Other (Specify) _____							
	Bread: Comm & Home: Wh-3210	Wh + Br 10mm = 30g Ww 10mm = 35g Wh + Br 20mm = 60g Ww 20mm = 70g						
	Br-3211							
	Ww-3212							
	Cream Crackers-3230; Provita-3235; Tuc 3331; Crackers Ww-3391	Cr Cracker = 8g; Tuc = 4g; Provita = 6g						
	Maize Meal Bread - 3278	m/s = 30g; L/s = 50g						
	Muffins: Plain-3408; Bran-3407	6cm diam = 35g 8cm diam = 60g						
	Rolls: Wh-3210; Br-3211; Ww-3212	Wh round (10cm) = 30g Wh l (16) 40						

	Roti: SO-3358; HM-3357							
	FOOD ITEMS	**QUANTITY (g/ml)**	**BR**	**IS**	**L**	**IS**	**D**	**AD**
	Rusks: Comm Wh-3364; Bran-3330	Outspan = 15g; All Bran = 30g						
	Comm Buttermilk: Wh-3329;	Wh = 35g; Ww = 30g						
	Home Buttermilk: Wh-3215; Ww-3255; Bran & Raisins-3380	Wh = 30g; Ww = 30g						
	Scones: (Wh) SM-3411; WM-3237 (Ww) SM-3412; WM-3320	6cm diam = 35g 8cm diam = 60g						
	Vetkoek: Wh-3257; Ww –3324; Dumpling-3210 (no yeast)	8cm diam = 60g						
	Other (Specify) _____							
SPREADS ON BREAD	**Beef Fat-3494; Mutton Fat-3497; Lard-3495**	Thin Med Thick 5 10 15						
	Butter-3479; Butro-3523							
	Ghee-3525; WF-3516;							
	Fishpaste-3109; Liver Spread-2922; Meat Paste-2917	5 7 10						
	Jam-3985; Honey-3984; Syrup-3988	10 20 35						
	Marg: H-3484	5 7 10						
	Med-3531							
	PM-3496							
	Marmite-4030; Meat Spread (Bovril)-4029	2 4 7						

	Peanut Butter-3485; Sandwich Spread-3522; ChSpread-P0005	5 10 20						
	Other (Specify) _____							
EGGS	**Eggs: Boiled/Poached - 2867**	1 egg = 50g						
	Curried - 2902	1 egg + sauce (IT) = 75g						
	Fried: B-2868; HM-2877; PM-2878	1 egg = 52g						
	SO-2869; Bacon Fat-2870							
	Scrambled/Omelette: SM + B-2886; SM + HM-2887	IT = 35g; 1SP = 80g 2c = 115g (+ 2 eggs) omelette = 60g egg (med) 120g (L/s)						
	SM+PM-2888; SM+SO-2889; WM+B-2874							
	WM+HM-2890; WM+PM-2891; WM+SO-2873							
	Other (Specify) _____							
CHEESE	**Cheddar-2722;**	grated: med = 10g Thick = 15g						
	Gouda/Sweetmilk-2723	1 cheezi = 20g; cubes = 30g 1 slice = 8g						
	Cheese Spread-2730	med = 12g; thick = 25g						
	Cottage Cheese; Creamed-2759; Cream Cheese-2725	thin = 10g med = 20						
	Cottage Cheese: Fat Free-2729; Low Fat-2760	med = 20g; thick = 30g						
	Macaroni Cheese: SM-3343; WM-3301	1T = 45g; 1 SP = 90g; 2c = 115g						
	Pizza (Cheese + Tomato)-3353	S/s = 90g; L/s = 340g						
	Savoury Tart+Asparagus-3367;+Vienna-3326;+Tuna-366	wedge: small = 65g; med = 75g ; large = 110g						
	Other (Specify)							
MEAT	**Bacon: Fried: Lean-2915 F-2906**	1 rasher = 10g						
	Beef:Corned/Silverside/Cold cuts:F-2924;BullyBeef-2940	138 x 85 x 3 = 20g 2c = 100g						
	Lean-2962; Curry Beef-P0006							

	Fillet: F-2933; FT-2929	100 x 70 x 10 = 90g						
	FOOD ITEMS	**QUANTITY (g/ml)**	**BR**	**IS**	**L**	**IS**	**D**	**AD**
	Mince: Pan Fried F-2910; Lean-2961; Curry-3015	T = 40; SP = 85g 2c = 100g						
	-Savoury (Tomato + Onion)-2987							
	-Cottage Pie: WM + HM-3009							
	Roast: F-2944; FT-2960	120 x 60 x 5 = 35g 120 x 60 x 10 = 70g						

MEAT	**Rump: Fried: F-2908; FT-2959**	S/s 130 x 70 x 15 = 125g L/s 165 x 70 x 30 = 270g						
	Sirlion/T-Bone: Grilled: F-2946; FT-2907							
	Stew: Vegetables (Fat Meat)-3006	1 SP = 105g; 2c = 125g						
	: Pot + Carrots + Peas + Onions (Lean Meat)-2909							
	Biltong: Beef-2911; Game-2912	grated 1SP = 10g beefeater = 18g sliced 1SP = 35g						
	Bobotie: Lean, SM, SO-3013; F, WM, S0-2986	1SP = 85g; 2c = 115g						
	Chicken: Boiled + Skin-2926; No Skin-2963; Curry-P0007	breast + skin = 125g thigh = 80g drumstick = 42g foot = 30g wing = 30g pie(comm)=150g home = 90g liver = 30g; stomach = 20g						
	Feet-2997; Giblets-2998; Heads-2999							
	Pie (Comm)-2954							
	Roast + Skin-2925; No Skin-2950; Fried-2925							
	Stew: Vegetables-3005	1SP = 90g; 2c = 125g						
	Tomato + Onion – 2985							
	Batter Dipped-Fried eg. Kentucky-3018	1SP = 105g; 2c = 125g						
	Burger Pattie –2950	1 pattie = 80g						
	+ Bun (4 cm diam)-3210	1 bun = 60g						
	Cornish Pie: (Comm) - 2953	med = 150g						
	Frankfurter-2937	155 x 20 = 45g 168 x 21 = 60g						
	+ Roll (16 cm long)-3210	1 roll = 40g						
	Goat meat: Stewed (plain)-4281; (+ Veg)-4282	120 x 60 x 5 = 35g 120 x 60 x 10 = 70g						
	Fried F-P0008; Fried FT-P0009							
	Grilled F-P0010; Grilled FT-P0011							
	Ham-2967; Ham & Tongue loaf-2990	med slice = 25g						
	Heart: Beef-2968; Sheep-2969	sheep heart = 60g sheep kidney = 30g beef kidney = 85g						
	Kidney: Beef-2923; Sheep-2956							
	Lung: Beef-3019							
	Lasagne: SM-3440; WM-3261	T = 40g; SP = 75g; 2c = 120g						
	Liver: Fried : Beef-2920; Sheep-2955; Patty (Fried) - 2971	sheep = 55g chicken = 30g beef = 80g						
	Cooked: Chicken-2970							
	Meat Ball: F + Egg-2965; F-No Egg-2966	50mm = 60; 75mm = 120g						
	Lean + Egg-3033; Lean, No Egg-3034							
	Meat Loaf: F-3035; Lean-3002	80 x 85 x 15mm slice = 80g						
	Meat Patty: (Hamburger)-2984	s/s = 50g; m/s = 100g						

	+ Bun (4 cm diam)-3210	1 bun = 60g						
	Mutton: Chop (grilled) F-2927; FT-2934	loin chop = 60g rib chop = 40g						

	Roast: F-2947; FT-2973	s/s slice = 30g med = 70g						
	FOOD ITEMS	**QUANTITY (g/ml)**	**BR**	**IS**	**L**	**IS**	**D**	**AD**
	Stew: Plain-2974; Irish-2916 (Vegetables) Curry-3039; Greenbean-3040	1SP = 105g; 2c = 125g						
	Offal: Cooked-Tripe(Pens&Pootjies)-2951;Vetderm-P0023 **(Specify): _____**	1SP = 105g; 2c = 125g						
	Oxtail: Stewed-2976							
	Polony-2919	slice 5mm thick = 8g comm slice = 16g						
	Pork: Chop (Grilled) F-2930; FT-2977	chop: 115 x 80 x 20 = 100g schnitzel: 115 x 80 x 20 =110g roast: 110 x 65 x 5 = 30g 1SP = 105g; 2c = 125g						
	Crumbed-2992; Spareribs-3010							
	Rib, Braised: F-3046; FT-3045	3 ribs = 130g						
	Roast: F-2958; FT-2978							
MEAT	**Salami and Russians-2948**	slice 5mm thick = 12g 1 Russian = 50g						
	+ Roll-3210	1 roll = 40g						
	Samoosa: with Veg-3414; Meat-3355	s/s = 42g						
	Sausage: Beef: Dry-2949; Cooked-2931 (Boerewors)	thin x 200mm = 45g thick x 165mm = 90g						
	+ Roll-3210	1 roll = 40g						
	Pork: Cooked-2932	med = 55g						
	+ Roll-3210	1 roll = 40g						
	Roll/Meat Pie (Comm)-2939	25mm pie = 120g roll x 135mm = 165g						
	Spaghetti Bolognaise: Lean-3388; F-3260	T=40g; SP = 75g; 2c = 100g						
	Steak & Kidney: Pie-2957; Stew-2979	comm pie = 120g (30mm) 1SP = 100g; 2c = 135g						
	Tongue: Ox-2935; Sheep:2980	slice 75 x 45 x 10 = 40g						
	Toppers/Imana: Cooked-3196	SP = 85g; 2c = 120g						
	Veal: Cutlet (Fried): Plain-3049; Crumbed-2983	1 chop = 90g						
	Vienna Sausage/Canned Sausage-2936	100mm = 30g; 150mm = 40g						
	+ Roll-3210	1 roll = 40g						
	Worms/Insects:Mopani,Dried-4250;Mopani,Canned-							

	4284;							
	Wild Birds, Animals; Specify:							
	Other (Specify) _____							
FISH	**Bokkems (Dry Fish)-3097**	1 s/s = 25g (120mm) L/s = 40g (135mm)						
	Fatty Fish: Kipper; Galjoen; Snoek; Shad: Fried (SO)-3084; Batter-3094; Grill-3082	small 50 x 55 x 30 = 60g med 100 x 55 x 30 = 120g stew 1 SP = 95; 2c = 140g						
	Salted-3097; Steam-3103; Smoked-3112							
	Stew-3076 (Tomato and Onion) / Pickled /Curried							
	Fish Cakes: (Fried): Home-3098; Comm-3080	65 x 15mm = 50g						
	Fish Fingers: (Fried)-3081	85mm = 35g						
	Haddock: Smoked (Boiled)-3061	70 x 70 x 15 = 65g						

	Mackerel Canned-3113	1 = 80g (15 mm)						
	Pilchards: Tomato Sauce-3102; Brine-3055	1 = 75g						
	FOOD ITEMS	**QUANTITY (g/ml)**	**BR**	**IS**	**L**	**IS**	**D**	**AD**
	Sardines: + Sauce-3087; + Oil-3104	s/s = 7g; L/s = 25g						
	Smoorsnoek-3074	1SP = 55g; 2c = 80g						
	Sole: Fried-3090; Grilled-3073	baby sole: 180mm = 70g						
	Tuna: Oil Pack-3093; Tuna: Water-3054; Salmon-3058	3c = 50g						
	White Fish: Hake, Haddock, Kingklip; Cod : Stew-3076 (Tom + On); Baked+Fat-3092; No Fat3089	s/s piece 50 x 55 x 30 = 60g med 100 x 55 x 30 = 120g stew 1 SP = 95g; 2c = 140g						
	: Grilled-3079; Batter-3072; Fried-3060							
	Other: eg Fresh Water Fish; Specify:_____ P0012							
	Other (Specify) _____							
STARCH	**M/Meal: Soft: Plain-3399; Enrich-4277**	**T SP ½c** stiff 75 120 125 crum 30 75 70 soft 75 120 125						
	Stiff: Plain-3400; Enrich-4278							
	Crumbly: Plain-3401; Enrich-4279							
	Mabella Cornrice/Sorghum cooked (soft or stiff)-3437							
	Sour Porridge: Maize & Vinegar-P0001, FermentedP0002							
	Mabella with Vinegar-P0003; Fermented-P0004							
	Maize Rice (Mealie Rice)-3250	25 45 65						
	Samp: (Cooked) –3250; Fresh Mealies-3725	55 125 125						
	Rice: Wh-3247; Br-3315	25 60 65						
	Spaghetti/Macaroni: (Cooked)-3262	35 70 90						

	Spaghetti + Tomato Sauce -3258	45 80 125						
	Stamped Wheat/Wheat Rice-3249	30 80 80						
	+ Fat: B -3479; HM-3484; Med-3531; PM-3496; WF-3516	1 t PB = 12g; 1 t marg/oil = 5g						
	Ghee-3525; PB-3485; Butro-3523; SO-3507							
	Other (Specify) ___________________________							
LEGUMES SOUPS AND	Baked Beans-3176	T SP ½ c 50 105 135						
	Beans: (Cooked) Haricot-3185; Sugar-3205; Kidney-3183	50 85 135						
	Breyani: Rice + Lentils + Ghee-3194; +SO-3193	40 80 85						
	Lentils: Cooked/curried-3179	40 80 90						
	Samp and Beans (1:1)-3402; Comm-P0045 (No fat added)	50 125 125						
	Samp & Peanuts (80:20) P0013							
	Soup: Comm (Packets)-3165	125						
	Split Pea-3157; Lentil-3153; Beef + Veg-3159; Bean-3145	35 80 130						
	Sousboontjies (Dried Bean Salad)-3174	40 105 135						
	Stew: Bean + Potato + Onion-3178	60 120 125						
	Other (Specify) ___________________							

		Boil	Fat Added (or Fried)												
		NF	B	HM	PM	SO	T	SP	½C						
COOKEDVEGETA	Gr Beans	3696		3788	3789		25	60	80						

	FOOD ITEMS						QUANTITY (g/ml)			BR	IS	L	IS	D	AD
		Boil	Fat Added (or Fried)												
		NF	B	HM	PM	SO	T	SP	½c						
	Pumpkin (Yellow)	4164					45	85	105						
	Butternut	3759													
	Pump + Sugar	3728		3893											
	Pump Fritter					3784	75 x 50 x 9 = 25g								
	Spinach	3913		3898	3899		40	105	90						
	Spinach + Peanuts Ratio: 80:20	P0015					55	120	105						

COOKED VEGETABLES	**Spin + Pot + Onion**			**3901**		**3786**	50	105	110							
	Squash –Gem	**3760**					2 gem = 45g 1 SP marrow = 85g									
	Gem Squash + Sugar	**3754**														
	Squash – Marrow	**4179**														
	Marrow + Sugar			**3885**												
	Sw Potato:without skin	**3903**					50	110	145							
	Sw Potato with Skin	**3748**														
	Sw Pot + Sugar			**3749**												
	Tomato + Onion	**3925**					35	75	140							
	Tom + Onion +Sugar	**3910**														
	Tomato			**3908**		**3767**	1 slice 5mm = 15g (thin); med = 25g									
	Turnips	**3911**					25	45	90							
	Other (Specify)															
SALAD	**Asparagus-3695**						med asparagus = 15g									
	Avocado-3656						3 avo (80 x 50mm) = 40g									
	Beetroot (Grated) + Sugar-3699						1T = 25g; SP = 65g									
	Carrot: (Grated)+ Sugar-3721						1T = 25g;									
	+ Pine + Orange - 3710; + Orange Juice = 3711						1T = 35g; 1SP = 60g									
	Coleslaw + Mayonnaise-3705						T = 20g; SP = 40g; 2c = 50g									
	Cucumber Raw/Pickled-3718						med slice = 10g; thick = 15g									
	Lettuce-3723						1 med leaf = 30g									
	Mixed (Tom + Cucum + Lett) - No Dressing-3921						1T = 40g; 1SP = 85g									
	Mixed Green - No Dressing-3927															
	Potato Salad + Mayonnaise (Comm), Egg-3928						T = 45g; 1SP = 105g; 2c = 120g									

	Tomato (Raw)-3750	med = 120g; slice = 15g						
	Other (Specify) _____							

	FOOD ITEMS					QUANTITY (g/ml)	BR	IS	L	IS	D	A D
DRESSINGS	**French Dressing-3487**					1t = 5g; 1T = 15g						
	Mayonnaise: Home-3506; Comm-3488; Low Fat- 3489					1t = 10g 1T = 40g						
	Oil: Olive-3509; Sunflower-3507; Canola-4280					1t = 5g; 1T = 15g						
	Salad Dressing: Cooked-3503; Low-Oil-3505											
FRUIT		**Canned + Sugar**	**Raw**	**Dry**	**Stewed**							
	Apple	**3599**	**3532**	**3600**	**3603**	1T = 60g; 2c = 120g; 1 med = 150g (52 x 66)						
	Apricot	**3535**	**3534**	**3536**	**3537**	1 med = 35g						
	Banana		**3540**			1 med = 75g						
	Dates		**3543**			1 med = 10g						
	Figs		**3544**	**3557**		1 med = 40g (45 x 44) 1 dry = 20g						
	Fruit Salad	**3580**	**3605**	**3593**	**3590**	2c = 110g (med)						
	Granadilla		**3545**			1 med = 22g						
	Grape Fruit	**3547**	**3546**			2 med = 125g						
	Grapes	**3623**	**3550**			med bunch = 230g; 2c =90g						
	Guava	**3553**	**3551**			med (6cm) = 95g						
	Litchi	**3631**	**3632**			med (3cm) = 8g						
	Mango	**3633**	**3556**			135mm = 350g						
	Naartjie	**3635**	**3558**			med = (5cm) = 75g						
	Orange		**3560**			med (7cm) = 180g						
	Pawpaw		**3563**			wedge 165 x 26 x 27 = 90g						
	Peach	**3567**	**3565**	**3568**	**3569**	1 med = 150g (60 x 65)						
	Pear	**3583**	**3582**	**3585**	**3586**	1 med (80 x 65mm) = 165g						
	Pineapple	**3648**	**3581**			1 slice (85 x 10mm) = 40g						
	Plum		**3570**			1 med = 50g (45 x 40)						
	Prunes	**3676**	**4230**	**3596**	**3564**	1T = 50g; 2c = 110g; 1 = 12g						
	Raisins		**3552**			handfull = 27g						
	Strawberries	**3653**	**3573**			1 med = 12g; 2c = 80g						
	Sweetmelon, Green		**3575**			1 wedge (145 x 31 x 20mm) = 60g; 3 = 110g						

	Sweetmelon, Yellow		**3541**									
	Watermelon		**3576**			slice (330 x 70mm) = 220g						

	Wild Fruit, Berries: Specify:											
	Other Fruit:											

	FOOD ITEMS			QUANTITY (g/ml)	BR	IS	L	IS	D	AD
PUDDINGS		**SM**	**WM**							
	Apple + Batter	**3345**	**3327**	med serving = 70g						
	Apple Crumble		**3334**	med serving = 70g						
	Baked Pudd + Syrup	**3348**	**3312**	med serving = 30g 30 x 65 x 65 = 50g						
	- No Syrup	**3347**	**3221**							
	Blancmange	**3282**	**3281**	SP = 75; 2c = 95g						
	Egg Type eg. Bread, Sago	**3346**	**3263**	1T = 50g; 2c = 140g; SP = 100g						
	Ice Cream: Commercial Regular-3483			scoop = 40g; 1SP = 65g; 2c = 75g						
	Commercial Rich-3519									
	Ice Lollies-3982									
	Soft Serve-3518			plain = 135g; + flake = 155g						
	Sorbet-3491			1SP = 65g; 2c = 75g						
	Instant Pudding	**3314**	**3266**	T = 45g; SP = 95g; 2c = 145g						
	Jelly-3983			1T = 35g; 1SP = 75g; 2c = 110g						
	Jelly + Fruit-4006			1T = 40g; 1SP = 90g; 2c = 125g						
	Jelly Whip	**2749**	**2750**	1T = 55g; SP = 95g; 2c = 120g						
	Pancake/Crumpets	**3344**	**3238**	1 crumpet = 25g pancake = 70g						
	Trifle-3311; Vermicelli Pudding-3385			2c = 130g (med)						

	Other Puddings (Specify) _____							
SAUCES	**Cream: Plant-3492; Canned-3499**	1T = 13g (not whipped) 1T = 30g (whipped)						
	- Fresh (12%) -3481; Heavy (dessert, 20%)-3480							
	Chocolate Sauce-3129	T = 15g						
	Custard: SM-2717; WM-2716	T = 13g; SP = 40g						
	Sugar-3989	1t = 6g						
	Other (Specify) _____							
CAKE	**Banana Loaf: WM + HM-3333; SM + PM-3370**	slice = 45g; 90 x 80 x 10mm						

	Cake –Carrot-3392	80 x 40 x 40 = 50g						
	-Plain: SM + HM-3286; PM-3287	single slice = 50g (75 x 75 x 20mm) double slice = 100g (plain) icing = 10g per slice						
	WM + B-3218; HM-3288; SO-3290							
	Cake Icing: HM-4014; PM-4015							
	- Chocolate (No Icing) WM-3289; SM-3339							
	FOOD ITEMS	**QUANTITY (g/ml)**	**BR**	**IS**	**L**	**IS**	**D**	**AD**
CAKE	**- Fruit: Comm-3291; Home-3427**	home: 70 x 85 x 15mm = 70g comm: 90 x 70 x 15mm = 35g						
	- Sponge (Plain)-3219	100 x 50 x 50 = 40g						
	- Swiss Roll-3292	slice = 60g; 15cm thick						
	Cheese Cake: Baked-3293; Unbaked-3294	slice 95 x 50 x 30mm = 70g						
	Other (Specify) _____							
COOKIES & SPECIAL BREADS	**Comm + Fill-3217; Plain-3216; Shortbread-3296**	plain = 10g + fill = 15g						
	Home: Plain HM-3233; PM-3341	plain = 15g + fill = 20g hertzog = 50g; cupcake = 35g shortbread = 12g						
	Jam-3295; Oats-3265							
	Custard Slice-3338	110 x 45 x 35mm = 250g						
	Date Loaf; HM-3256; PM-3340	slice 90 x 75 x 10mm = 40g						
	Doughnuts: Jam-3423; Plain-3232	med round = 45g med long = 90g						
	Eclairs + Cream + Chocolate-3268	1 = 120g (160mm)						
	Gingerbread: HM-3253; PM-3371	90 x 75 x 15 = 70g						

	Koeksister-3231	100 x 35 = 60g						
	Pumpernickel Bread-3283	slice 85 x 100 x 10mm = 30g						
	Raisin Bread-3214	slice 85 x 100 x 10mm = 30g						
	Rye Bread-3213	slice 85 x 100 x 10mm = 30g						
	Sweetcorn Bread-3379	slice 85 x 100 x 10mm = 30g						
	Other (Specify) _____							
TARTS	**Apple: HM-3224; PM-3352**	50 x 50 x 50mm = 70g (med)						
	Coconut-3228	wedge 50 x 100 x 30mm= 55g						
	Condensed: HM-3294; PM-3439	95 x 70 x 30mm = 90g						
	Fridge (Fruit): HM-3394; PM-3434							
	Lemon Meringue: HM-3226; PM-3349	100 x 70 x 35mm = 75g						
	Milk (Short) WM + HM-3360; SM + PM-3351	120 x 70 x25mm = 75g						
	Milk (Flaky) WM + B-3443; WM + HM-3229							
	Savoury: Aspar-3367; Tuna-3366; Vienna-3326	120 x 50 x 25 = 75g						
	Tipsy: HM-3323; Jam-3225	87 x 70 x 50mm = 90g						

Other (Specify) _____

	FOOD ITEMS	**QUANTITY (g/ml)**	**BR**	**I S**	**L**	**I S**	**D**	**A D**
SWEETS	**Bubble/Chewing gum-3993**	**See Manual**						
	Chocolates: Assorted-3992							
	Coated Bars eg. Tex, Lunch, Chomp-3997							
	Milk (White Chocolate)-3987							
	Nuts/Raisins-3994							
	Plain eg Smarties, Flake, Aero-4003							
	Dry Fruit Sweets-3995							

	Fruit Gums-4000								
	Hard/Jelly Sweets eg. Sugus, Jelly Tots, Fruit Drops-3986								
	Ice Lollies-3982								
	Marshmallows-4001								
	Meringues-4008								
	Peanuts: Raw-4285; Peanut Brittle-4002;								
	Roasted, Salted-3458; Roasted Unsalted-3452								
	Peppermints-4004								
	Popcorn: Plain-3332; Sugar Coated-3359								
	Potato Crisps eg. Simba, O=Gradys-3417								
	Raisins, Seedless-4232								
	Snacks – Fritos, Niknaks, Cheese Curls-3267								
	Soft Sweets - Fudge, Toffees, Caramel-3991								
	Other (Specify) _____								
OTHER	**Cheese Sauce: WM + HM-3125; SM + PM-3128**	SP = 65g; 1T = 25g							
	Curry Sauce-3130	1T = 25g							
	Chutney-3168; Atjar-3117; Tomato Chutney-3114	1T = 14g; 1T = 60g							
	Gravy: Comm-3119; Meat-3122; NF-3121	1T = 15g; SP = 35g							
	Mustard-4034	1t = 6g							
	Pickles-3866	1 = 10g							
	Tomato Sauce (Comm)-3139	1t = 6g; 1T = 25g							
	White Sauce: WM + HM-3142; SM + PM = 3141								

	FOOD ITEMS	**QUANTITY (g/ml)**	**BR**	**IS**	**L**	**IS**	**D**	**AD**
	Baby Cereals (dry): Nestum 1-2832; Nestum 2-2834	1t = 2g 1T = 8g 2c = 20g						
	Purity:Mixed-2842;Wholewheat-2861; Rice-2862							
	Cerelac-2836; Nestum Rice & Maize-2835							
	Junior-2833							
	Milk: SM-2719; WM-2718	to drink 2c = 125ml baby bottle = 250ml						
	BL-2771; 2%-2772							

	Soy-2737; Breast-2741; Goat-2738							
	Formula (Specify):_____ **No of Scoops/Bottle: _____**							
	+ Sugar, White-3989;Brown-4005;Syrup-3988;Honey-3984	1t = 6g						
	First Food Fruit-2852; First Food Veg-2851	jar = 80g; 1t = 11g						
INFANT FOODS	**Fruit Juice (Strained)-2860; Fruit Juice-2866**	2c = 125ml						
	Infant Dinners (Dry): Beef + Veg-2841; Chicken+Veg-2840 **Guava + Custard-2837; Mix Veg-2839**	1t = 5g 1T = 15g 2c = 47g						
	Orange + Banana-2838							
	Junior Food (Jar): Veg + Meat-2848; Mix Veg-2849; Pasta + Beef-2850	jar = 200g 1t = 11g 2c = 125g						
	Junior Fruit (Jar): Fruit-2863; Guava-2855 **Junior Pudding: Fruit+Yog-2858; Vanilla Cust-2859**							
	Strained Food (Jar): Macaroni Beef-2845; Veg+Meat-2846	jar = 125g 1t = 11g 2c = 125g						
	Fruit + Yog-2857; Fruit-2854; **Av. Pudding-2844; Meat Soup-2847;** **Veg Soup-2843; Vegetables-2853;** **Junior Fruit Guava-2856**							
OTHER								

9. Did this child go to bed hungry last night?	1 Yes	2 No	3 Don't Know
10. Did this child eat from the same pot as the rest of the family at the main meal yesterday?	1 Yes	2 No	3 Don't Know

11. Did this child eat from the same plate as the siblings, at the main meal yesterday?	1 Yes	2 No	3 Don't Know

. ADD ADDITIONAL ITEMS EATEN TO THE 24-HR RECALL QUESTIONNAIRE RECIPES

NAME OF DISH	INGREDIENTS	CODES	AMOUNT (g) OR (mg)

If this space is not sufficient, write overleaf

APPENDIX IX

FIELD WORKERS TRAINING MANUAL

[A] FOOD FREQUENCY QUESTIONNAIRE

Procedures followed for training were similar to those outlined in the National Food Consumption Survey (NFCS) South Africa (Training Manual).

Cover page (page1).

Always start by filling in the date of the interview.

The participant's information:

1. Fill in the first name and the last name
2. Date of birth
3. Fill in the identity number given to the participant
4. Write your name as the interviewer.

N.B The field worker must not do the coding or fill in the quantities in g/ mL. This will be done coordinator or data analyst.

Introduction

The QFFQ consists of a list of foods and drinks that are most commonly used in South Africa. The purpose of the QFFQ is to find out from the participant whether:

• She eats these foods

• How the foods are prepared and cooked

• How much of the food is eaten at a time

• How many times a day does she eat the particular food; does she eat it every day, and if not, how many times a week or a month the food is eaten;

• Whether there are any foods, which the participant eats more than once a month, which are not on the QFFQ.

The questionnaire consists of 2 types of questions:

Information on food patterns and habits, e.g. breast feeding; supplement-use; meals away from home: these answers will be coded with numbers yes, no, and options 1 -10 etc. Information on foods **USUALLY** eaten: these answers are coded with **FOOD CODES** and **AMOUNTS** (gram, mL, 1T, cups).

The QFFQ is about **USUAL** intake; therefore, the concept of usual must first be explained to interviewee. Eventually, we want to know the amount of a particular food/dish/product eaten per day. Therefore, the amount **usually** eaten, (cups, etc grams/mL) either per day, week, month or seldom (only one of these), should be obtained from the interviewee. MODELS, PICTURES, REAL FOODS, etc., must be shown to assess portion sizes as accurately as possible. It is of course important to have as good as possible description of a particular food / dish / product: e.g. Meat: beef; fat removed before cooking; type: rump steak; cooking method: fried in ±1 tablespoon of Sunflower Oil.

The QFFQ is a tool to assess **WHAT** is eaten and how often it is eaten. To get quality information, one should have a **DISCUSSION** about foods with the interviewee - listen carefully. Information may pop-up during a particular question, which is actually relevant to another question. For example: vegetable intakes may be described in the meat section as part of stews, pies, etc. **PATIENCE** is of prime importance.

The questions at the end of each section about how often or how many times meat and related products or fruits and vegetables etc., are eaten, is **NOT FOR CODING.** It is there to help you to **DOUBLE CHECK** that, when broken-down into the different types in a particular category, the total amounts are more or less similar.

While you are filling in the form make sure that the **CODER** will eventually be able to decide on a particular **FOOD CODE** and **AMOUNT**. This will depend on the quality of information you get about the description of the food/dish/product and the portion size.

To get quality information (real, actual intake, the TRUTH) one must NEVER make moral or other judgments about foods or eating pattern during the interview. Be friendly and interested. Do not show disapproval or surprise. Remember, while interviewing your subject must be invited to tell all; he/she must feel it is **important** to be **truthful**; he/she must **TRUST** you, feel that you are really interested and that you will regard all information as confidential, worthwhile and important.

Detailed discussion of Quantitative Food Frequency Interview

Introduction

Follow the introduction on the questionnaire. Do not read it, but speak to the interviewee. Make sure that the interviewee understands what the interview involves and answer any questions she has. If you have any problems, or the interviewee wants to know something you are unable to answer, call the coordinator.

Quantitative Food frequency questionnaire

We now come to the main part of the QFFQ. It is very important that this information be filled in as accurately as possible. All that the interviewee tells you will be put onto a computer and analyzed to tell us how much energy, protein, fat, vitamins and minerals the participant is eating and whether it is too little or too much to be healthy or whether it is the correct amount.

The interviewee must answer about what she has eaten or drunk in the last six months. Anything which she has not eaten in this time must be marked with an X under 'Seldom/Never'

You may have to remind the interviewee during the interview that we are only interested in the last six months.

Samples and food models

We will be using the same food models and samples that are used for the 24-H Recall Questionnaire to help the interviewee describe and estimate the types and amounts of food the participant. We will also be using the same measurements. Whenever possible, let the interviewee show you what she is actually using at the moment.

Overview of the QFFQ

The aim of the FFQ is to find out the types of foods eaten, the amount of these foods usually eaten and how often the food is eaten by the participant. The QFFQ is divided into columns and rows, which the interviewer fills in as the interviewee answers the questions.

The first column gives the type of food; e.g. maize meal porridge, sour porridge, *Mabella* porridge. The foods are listed in groups according to the types.

The following main groups of foods are used:

- Porridges
- Breakfast cereals
- Starches
- Bread and spreads
- Chicken
- Red meat

- Meat, general
- Fish
- Eggs
- Vegetables
- Fruit
- Drinks
- Snacks and sweets
- Cakes, biscuits and cookies
- Puddings
- Sauces, gravies and condiment

It would be impossible to list all foods possibly eaten on the QFFQ. Therefore, at the end of each section there is space with the heading 'Other'. Any food not listed on the QFFQ, but eaten more than once a month is written in this block.

Description: The description column describes the different ways in which the food can be cooked or prepared. One food may have several descriptions. For example, maize-meal porridge may be cooked as soft porridge (eaten with a spoon), stiff porridge (eaten with the hands), or as crumbly porridge. Some maize-meals are enriched (that is, some vitamins are added to the meal by the manufacturer) while others are not. The different cooking methods and whether the maize-meal is enriched or not has a large effect on the energy and vitamin intake of the participant. It is therefore very important that the correct descriptions of all the foods are used.

Some foods can be cooked in several different ways or a participant may have her own way of cooking the food that is not listed on the QFFQ. For these cases, no description is typed into the Description column. Instead you will see 'Specify the preparation method' as for sour porridge or 'specify types usually eaten' as for breakfast cereals. For example, the following could be the exchange between the interviewer and the interviewee about sour porridge:

I: Do you eat sour porridge?

P: Yes, I do.

I: How do you cook the sour porridge?

P: I mix maize-meal with vinegar before I cook it

Action: Write maize-meal with vinegar in the description column.

Code: The code column gives the computer code of the particular food and is only used by the staff, who analyzes the QFFQ. The interviewers must ignore this column.

Quantity: The quantity column gives a guide to the amounts of the food commonly eaten. The interviewer uses this information to help the interviewee explain the amount eaten.

Amount usually eaten: This is the amount of the food usually eaten by the participant. The interviewer will write in the amounts as 1T, 1t, 1 cup, 1 med., etc. The interviewer **MUST NOT** write in the weights (g) or volumes (mL). These calculations will be done by the data analyst. If the interviewee describes the amount as spoons or teaspoons, ask her which size of spoon. For

example: If she takes one teaspoon of sugar in a cup of tea write *1t* under AMOUNT or if he takes 2 teaspoons of sugar per cup of tea write *2t* under AMOUNT. Study page 20 of the QFFQ for the correct abbreviations to use.

P/D, D/W, P/M, SELDOM/NEVER: The last four columns are used to give the number of times per day (P/D), days per week (D/W) and per month (P/M). If a particular food is eaten less than once per month or is never eaten, then put an X under the last column indicating Seldom/ Never.

To fill in the frequency, ask the interviewee how many times the participant has the food per day, then how many days he has it per week. If she has the food less than once a week, ask how many times per month. Write the number under the column P/D, D/W or P/M.

Filling in Amounts and Frequencies

Filling in the amounts and frequencies may not always be as straight forward as in the above example. The following examples show how to record a variety of responses to the same question. Please work through the QFFQ to familiarize yourself. Also refer to examples 1 and 2 below.

Example 1:

I: How do you cook maize-meal porridge? Is it soft, stiff, or crumbly?

P: Sometimes I make it soft and sometimes stiff Action: Circle stiff and soft

I: What type of maize-meal do you use?

P: I use *Iwisa*

Action: Tick Enrich next to stiff and soft

I: Let us talk about the stiff porridge. How much stiff porridge do you usually eat at a time?

P: I usually eat about a cupful

Action: Fill in 1 cup under AMOUNT USUALLY EATEN

I: How many times a day do you eat stiff porridge?

P: I eat stiff porridge in the evening

I: So, you eat stiff porridge in the evening. Do you eat stiff porridge at any other time of day?

P: No, only in the evening

Action: Write 1 under P/D

I: How many times a week do you eat stiff porridge?

P: We have stiff porridge in the evening everyday except Saturday and Sunday

I: So, you have 3 cup of stiff porridge on 5 days a week?

P: Yes

I: You said you also make soft maize-meal porridge. How much soft porridge do you take at a time?

P: About half a cup

Action: Write the amount under AMOUNT PER DAY

I: How many times a day do you eat soft maize-meal porridge?

P: Every morning

I: Do you eat soft porridge at any other time of day?

P: No, only in the morning

Action: Write 1 under P/D

I: How many times a week do you eat soft porridge in the morning?

P: One morning I eat soft maize-meal, the next soft *Mabella* and so on.

I: Do you mean you use soft maize-meal porridge one day, Mabella the next, maize meal the next.

P: Yes. So you have soft maize-meal porridge 4 days week.

Action: As soon as the P mentions soft *Mabella*, circle soft *Mabella*. Write 4 next to soft, enrich under P/W for maize-meal.

I: How much soft Mabella do you eat at a time?

P: The same as soft maize-meal

Action: Write the amount under AMOUNT PER DAY

I: How many times a week do you eat soft Mabella?

P: On the days you do not have maize-meal porridge.

I: Is that three times a week?

P: Yes

Action: Write 3 next to soft *Mabella* under P/W

Example 2:

The participant cooks sour porridge twice a month

I: Do you eat sour porridge?

P: Yes

I: How do you cook sour porridge?

P: I mix *Mabella* with vinegar

Action: Write *Mabella* and vinegar under description

I: How much do you eat at a time?

P: About the same as stiff porridge

I: You said you eat 3 cups of stiff porridge at a time. Do you also eat 3 cups of sour porridge at a time?

P: That's right

I: How many times a day do you eat sour porridge?

P: Twice a day when I cook it

I: How many times a week do you cook sour porridge?

P: I don't cook it every week

I: How many times a month do you cook it?

P: I cook it twice a month

Action: Write 2 under the P/M column

Summary of filling in amounts and frequencies

The above examples have used porridge to show how to fill in the amount and frequencies of the foods eaten. The same procedure applies to all the other foods listed on the QFFQ. To summarize:

1. Ask whether the food under the FOOD column is eaten. If the answer is NO, write an X under SELDOM/NEVER and go on to the next food on list.
2. Ask how the food is cooked or for the description of the food.
3. Mark the interviewee's answer. She may give more than one description, in which case mark all her answers.
4. Ask about each food description on its own.
5. First find out the amount eaten and write it down.
6. Ask how many times a day it is eaten and fill in the number.

7. Then ask how many days a week it is eaten. Ask if this is number of times eaten every week. If not eaten every week, ask how many times a month it is eaten.
8. If a food is eaten less than once a month, put an X under SELDOM / NEVER.
9. At the end of each section there is a checking or control question, which asks the number of times a week the previous group of foods, is used. (For example: the middle of page 4.) Ask the question and fill in the answer. This should correspond to the total times per week of the individual foods in the section.

Notes on Specific Foods

Brand names

In some sections such as breakfast cereals, infant cereals and soy products, the interviewee is asked what brand she uses. This is so that we can be sure to use the correct item for nutrient analyses. For example, some maize-meals have vitamins added, others do not. The interviewee may not know whether the maize-meal she uses has added vitamins or not, but she should know the brand name. We can then check if that brand has added vitamins or not, the same applies to margarine, milk powders, fruit juices, breakfast cereals.

Milk

Note that milk appears several times on the FFQ - after *porridge* on page 3; after *breakfast cereals* on page 4; and *with drinks* on page 14. Each time it refers specifically to that particular description. For example, on page 3, the question refers only to *milk used on porridge*. The same applies to *sugar and fat.*

Dumpling and vetkoek (page 5)

Dumpling and vetkoek are prepared in different ways in different areas. For example for some people dumpling is a bread dough mixture, which is steamed in a large pot and pieces are broken or cut off (like a round loaf of bread) (Note for coding: use white bread code for this). Other people cook balls of dough (usually made with baking powder) together with stew or soup and call these dumplings. Vetkoek may be made with baking powder or yeast, the size of vetkoek varies a lot and they may be fried in deep or shallow fat. It is therefore very important to obtain the correct description from the interviewee.

Vegetables and fruit

Ask the interviewee which vegetables and fruits does she eat and mark them on the questionnaire. Then go back to each answer and ask about the preparation, amount and frequency. Do not read the list to the interviewee.

Preparation methods/descriptions

Do not read out the list of all the possible preparation methods to the interviewee. Ask: *How do you prepare your beef?* Then circle the option closest to the interviewee's answer. If the answer does not fit one of the options, circle other and write in the description. Also, check if the interviewee cooks the food in more than one way.

Drinks, snacks, cakes and puddings

Ask the interviewee what she eats or drinks and mark them on the questionnaire. Then go back to each answer and ask about the preparation or type, amount and frequency. Do not read the list to the interviewee.

Additional protein-rich foods

On page 9 there is space for describing protein-rich foods which have not been covered by the QFFQ. Please only fill in foods here that have not already been mentioned and which are eaten once a month or more. Give a full description of the type and preparation method of the food. Do not write anything in the code column. The analyzers will do this.

Wild foods (page 18)

Obtain as much information about wild foods as possible. For example, the type of animal or insect, at what time of year are they hunted or collected, the indigenous name, how they are prepared and eaten.

Conclusion

Pages 19 and 20 of the QFFQ contain some general questions, which must be treated, as were the questions on page 1 and 2. That is, tick the option corresponding to the participant's answer. There is also space to list any foods eaten more than once a month that have not already been listed.

Training the field worker: Important points to stress or clarify

1. Explain the **difference** between the 24-H Recall and the QFFQ. The 24-H Recall only asked about what the participants ate or drank the previous day. With the QFFQ, we want to find out what the participant's intake is over a period of time that is his or her usual intake. It is important that the interviewee understands the idea of usual that is normal, common. Although the 24-H Recall and QFFQ may appear very similar and the same visual aids are used, they are actually obtaining very different information. It is extremely important that the field workers understand this difference and can explain it to the interviewees.

2. The field worker must understand the **differences** between the 24- H Recall and the QFFQ and the **different techniques** used. They must follow the instructions for the specific questionnaire, even if these are different for each questionnaire (especially coding and quantities). The **time covered** by the QFFQ is the last six months for participants. For example, if the interview is conducted at the beginning of March, the 6-month period would be from the beginning of September last. Actually tell the interviewee the months covered rather than just the last six months. The period must be kept in mind throughout the QFFQ to make sure that foods and quantities are realistic. For example, the period September to February, falls outside the peak orange season. It is therefore unlikely that oranges would be eaten every day. On the other hand, it covers the peak maize season, so a high intake of fresh maize, especially in rural and farm areas, could be possible.

3. The QFFQ includes ALL food eaten by the participant including food eaten other instances away from home. Getting accurate information about foods consumed away from home gets more difficult as the participant might not remember well. Remind the participant that we need to know what she eats away from home as far as possible.

4. Remind the interviewee that this is not a test and that there are no right or wrong answers. Encourage him/her to answer to the best of her ability. The QFFQ requires much patience from both the interviewer and interviewee to get good quality information.

Filling in the QFFQ

1. The sequence of questions must be followed exactly as on the QFFQ.
2. Follow the instructions for filling in the quantities and amounts, as explained in the manual.
3. Make sure that you fill in the correct row and column.
4. We want to know what the participant actually eats.
5. Continually ask yourself whether it is reasonable for her to consume the quantities of foods given. For example, could an adult consume 3 cups of stiff porridge, 3 cups of samp and beans and 3 cups of rice on one day?
6. Do not do mental arithmetic to calculate quantities while conducting the interview. Do not convert amounts from household measures to g or ml. Also, do not mark the codes. You have enough other things to be thinking of!

7. The quantities given on the QFFQ are average or most commonly used amounts. Write down the exact amount the caregiver tells you. This could be 1c, 1c, 3, etc. The quantities on the QFFQ are a guide. Do not think that these are the only amounts that could be consumed.
8. When writing descriptions, keep them brief, clear and legible. Remember that the coder will only have your description to go by when selecting the appropriate code or codes to enter into the computer.

Checking questions

1. The purpose of the questions at the end of each section (How many times a week do you eat starches?) is to check that the frequencies given for each food in that group are reasonable when the group is considered as a whole, as so try to limit over or underestimation.
2. Ideally, the frequency given in the check question should equal the sum of the frequencies per week including frequency per month converted to D/W. The field worker should add frequencies, without obviously doing so and compare this to the answer given by the interviewee.
3. If there is a small difference (1 or 2 portions) between the total frequency and the interviewee's answer, continue with the questionnaire. If the difference is larger than 2, go back and double check the section with the interviewee.
4. The field worker must ask these questions; the field worker must not add the frequencies and fill the answer in him/herself.

Frequencies

1. P/D = per day, that is the number of times that particular food is eaten per day.
2. D/W = days per week, that is the number of days a week the number of portions given P/D are eaten. This frequency MUST NOT be more than 7.
3. Different numbers of portions P/D may be eaten at different frequencies P/W. For example, a participant may eat 4 slices of bread on five days a week, 2 slices on Saturday and none on Sunday. These differences must be very clear on the QFFQ (see examples).
4. PM = per month. PM is the number of weeks in the month the food is eaten. If the D/W is completed P/M must not be >4. If D/W columns filled in, P/M is the number of times the food is eaten per month.
5. Seldom = less than once a month.
6. Different quantities of the same food may be eaten with different frequencies. These differences must be very clear on the QFFQ (see examples).
7. Watch out for answers like now and then, we eat it when we have it, at month end; insist on getting definite frequencies.

Specific food items

1. Go through the points made in the manual with QFFQ to make sure that field worker understand how to deal with each food group.
2. Emphasize the importance of knowing the food availability, customs and preparation methods of the community.
3. Quantities for meat: if the food models of meat (30g, 60 and 100g) are used to quantify amounts, the quantity must be given as g (an exception to the rule that only household

measures should be used for the QFFQ) but do not do any calculations with the quantity. For example, if the participant eats half the 30g portion write ½ x 30 and not 15g. Make sure that you write in the g.

4. Quantities for bread slices: give the thickness of the bread in mm according to the slice thickness shown by the interviewee. Do not do any calculations with the quantity. For example, write 2 x 20 mm and not 40 mm. Make sure that you write in the mm.
5. Make sure that you write in the correct unit for all quantities.

Question 16 - 34

1. All these questions must be asked.
2. Question 16 refers to foods that are available, but are not eaten by the participant, because of religious or traditional restrictions, allergies, she does not eat them or think they are harmful to her etc. Describe the food and preparation method and reason. Do not write in the code.
3. Question 18: mark the option that is closest to the participant's eating pattern.
4. Question 19 must be asked even if field worker thinks all food have been dealt with. Do not fill in codes. Remember that we only want to know about foods eaten in the last six (or one) month and that are eaten at least once a month.
5. Question 20: Elsewhere refers to any place away from the home. If the answer is no, go on to question 24.
6. There can be more than one type of supplement specified for questions 30.
7. Questions 31, 32, 34: mark the responses closest to the interviewee's answer.

[B] 24-HOUR RECALL QUESTIONNAIRE

Procedures followed for training were similar to those outlined in the National Food Consumption Survey (NFCS) in South Africa (Training Manual).

Introduction

The objective of this questionnaire is to record all food items and drinks, which the participant consumed during the previous 24 hours.

Overview of the layout of the questionnaire. It consists of the following:

On page 1:

- An identification (demographic) section at the top of the page
- Questions 1-3
- Specific instructions on how to complete the questionnaire, and
- A list of abbreviations

From page 2 onwards you will find the following:

- A column with food items grouped together in specific food groups, e.g. tea and coffee or milk and milk drinks. Food items in each section are generally in alphabetical order
- A column containing food codes (**N.B** *the field worker must do the coding as well as writing in the quantities*).
- A column containing the weight or volume of common portion sizes

- A section with the 6 intervals of the day for you to indicate when the food was eaten. The following abbreviations are used:
    - BR stands for Breakfast: (the interval up to 09:00)
    - IS stands for In-between Snack
    - L stands for Lunch: (the interval between12:00-14:00)
    - IS stands for In-between Snack
    - D stands for Dinner: (the interval between 17:00 - 20:00)
    - AD stands for After Dinner

Refer to Questionnaire per se

Questions 1 - 3

The demographic section you should complete as explained earlier:

Question 1

Fill in the name and surname of the participant.

Question 2

Requires you to fill in the day of the week recalled. This means that you should make a cross or a tick in the block indicating the previous day. For example, if you are conducting the interview on Wednesday, then the previous day you are recording the recall is Tuesday, so mark the Tuesday box.

Question 3

Requires you to determine whether the previous day was a typical eating day for the participant. You need to find out if the participant's eating pattern was different. If for example the she attended a wedding or a party and ate many different food items, the day would not have been a typical one, and you should tick no and describe the reason why the day was not a typical one, i.e. went to a wedding.

24-H Recall of Items per Specific Food Group

The following check list can be used with all food items consumed:

- What was consumed?
- How much was consumed?
- Which food models can be used to estimate the portion size?
- When was the food consumed?
- Was anything extra added?
- How much of the extra food item was added?
- Where would I find the addition?
- Have I circled the correct food codes?

Tea and coffee

Using this procedure you should determine how much tea or coffee was consumed. Was sweetener such as sugar, or milk added? If so, what type and how much was added? Please note that if milk formula was used you need to specify the type of formula as well as the number of scoops used per bottle. Formula milks are found at the footnote of page 2. To determine the

amount of tea and coffee drunk by the participant you should use the following food models: a mug and a teaspoon. Please note that the volume of milk added to tea and coffee differs depending on whether tea or coffee was drunk, and whether a mug or a cup was used.

Milk and milk drinks

This comprises:

- sour milk products such as buttermilk and *maas*
- custard (made from SM or WM)
- milk to drink : specify the type used
- yoghurt : can be plain, flavoured or contain fruit
- flavoured milk is a low fat milk which has been sweetened. Please note that additions might have been made to the milk drink: Sweeteners such as sugar, syrup or honey
- Ice cream or sorbet
- Flavourants such as Milo or Drinking Chocolate

In this section you need to know the different container sizes. For this purpose, collect an empty container of each size for the kit. You can use the glass, mug and teaspoon provided in the kit, to assist you in determining portion sizes.

Fruit juice and cold drinks

Fruit juice can be sweetened or be **WITHOUT** sugar. Examples of juice without sugar are *Liquifruit* and *Ceres*. Sweetened fruit juice is generally in a plastic bottle. Note the different container sizes of the fruit juices. Carbonated (fizzy) cold drinks purchased from the shop are available either in a can or in a bottle. A squash, such as *Oros*, is made by adding water to a sweetened concentrate. When the concentrate is artificially sweetened, it can be classified as *Sweeto* or *Low-cal*. A dairy-fruit mix is a mixture between milk and fruit juice (*Tropica*is a typical example). *Mageu* is a drink made from fermented maize, which is purchased in a carton.

Breakfast cereals

This includes cooked porridge and instant cereals. Cooked porridge includes Maltabella, Mabella, Maize-meal, Oats and Tasty wheat. Please pay special attention to maize porridge. You need to determine the texture and consistency, i.e. whether it was soft, stiff or crumbly. You also need to determine whether the brand used was plain or enriched with vitamins and minerals. Some examples include:

- Enriched: *Impala, Iwisa, Ace*
- Plain: *Super Sun* and *White Diamond*

If the porridge eaten was sour you need to indicate whether it was soured with vinegar or by fermentation. Instant cereals are given alphabetically from Corn flakes to Weet-Bix. You should use the following food models to determine portion sizes:

- wax maize models ½ 1cup and 2cups
- measuring cups

- raw maize-meal
- puffed wheat
- the plate

Do not forget any additions such as milk, sugar or fat made to the porridge and cereals:

- Fat may be added: check the type of fat
- Sugar or other sweetener (e.g. honey/syrup) may be added
- Raisins, bananas, etc., may be added
- Milk may be added: check the type of milk and the quantity.

On the questionnaire medium portions of milk are indicated. If a small or a large amount of milk was added, you need to indicate this according to the quantities. Notice that the amount of milk added differs for instant cereal, porridge and for *Pronutro*.

Bread and rolls

The most important consideration here is the type of bread eaten and the thickness of the slices eaten. Use the 3 slices in your kit to determine the thickness of the bread. The thinnest slice is a machine sliced one, measuring 10mm, the next is a 20mm slice and the thickest one measures 30mm. The slices can also be put together to create a specific thickness.

Note that this group also includes snack breads, such as cream crackers and *Provita*. If rolls are eaten, it should be specified whether they are long ones, '*hot dog'* rolls or round '*bun'* types.

The long rolls are used for hot dogs and the round ones for hamburgers. If rusks are eaten, distinguish whether they are commercial or homemade; white, whole wheat, buttermilk or bran. If scones are eaten determine whether they were made from white or whole wheat flour. Also distinguish whether whole or skim milk was used. The same applies to vetkoek. Vetkoek is made with yeast, and dumplings are vetkoek made without yeast or baking powder. Use the ruler and scone model in your kit to determine the portion size of muffins, scones and vetkoek.

Spreads used on bread and rolls

Please note that it is common to use fat, such as margarine with an additional spread such as jam or meat spread (*e.g. Bovril, Oxo*). Note the addition of beef fat, mutton fat and lard (pork fat) to the list of fats eaten. Meat spreads include *Oxo, Bovril* and *Fray Bentos*. Also note the inclusion of chocolate spread *(Nuttelo).*You need to determine how the spread was applied to the bread, i.e. thinly, medium or thickly spread, in order to record the portion size.

Egg group

It is important to determine what type of cooking method was used to cook the egg, namely, whether it was boiled, poached, curried, fried or scrambled. You also need to determine whether the type of fat and milk, that was used where appropriate. For example, if the egg was fried, you need to determine whether it was fried in butter, HM, PM, SO or in bacon fat.

Cheese group

The most commonly used cheeses are Cheddar/Colchester and Gouda.

- Cheddar can be distinguished by its strong taste
- Gouda is also known as Sweetmilk. It has a milder sweetish taste and can be distinguished by its red skin. *Tussers* is also classified under Gouda. Cottage cheese comes in 3 types:
- fat free
- low fat, and
- creamed cottage cheese

Other cheese dishes have been included in this group, e.g. macaroni cheese, pizza, and savoury tart. You need to use your tablespoon, serving spoon (also for grated cheese), ruler and match box in your kit to determine portion sizes.

Meats

This group includes:

- Beef/veal
- Chicken
- Goat
- Mutton/lamb
- Pork
- Processed meats, such as polony

The meat group has been listed alphabetically and you should go through all the items to make sure that you understand what they mean. If the participant has eaten a meat dish not listed in the questionnaire, please write the recipe down in detail in Section B of page 15.

When you are asking about meat always check the following:

- The type of meat and the cut of meat eaten
- The cooking method used
- Whether fat was added in cooking
- Whether the fat was trimmed off the meat (FT) or retained (F). (If the participant is unsure, use the code *with fat*)
- Whether the skin was removed from the chicken
- Whether traditional meat products were eaten, e.g. mopani worms or locusts.

You also need to specify the cooking method used to prepare the meat:

- Whether it was boiled
- Roasted in the oven or in a pot
- Fried in deep or shallow fat
- Fried with a batter
- What type of fat was used?
- Grilled or *braaied* on coals
- Stewed, in which case what was added?
- If a burger or hot dog was made, what was added?

Please use the following food models to determine meat portion sizes:

- 30g, 60g, 100g foam meat models
- The tablespoon and serving spoon
- The ½ cup and 1cup measure
- The ruler

Fish

This includes:

- Fatty fish, such as kipper and snoek
- Tinned fish, such as pilchards, and
- White fish, such as hake and kingklip

As for the Meat group, the cooking method needs to be determined.

Use the same food models as for the meat group.

Starch group

This group includes the following items:

- Maize porridge
- *Mabella*
- Mealie rice and samp
- Brown rice and white rice
- Pasta, such as spaghetti and macaroni
- Wheat rice

Please note that the quantities are given in a tablespoon, serving spoon and cup format. You should use your maize food models in the kit to determine portion sizes. Make sure that you specify which type of maize was used and what the texture of it was i.e. soft, stiff or crumbly. You also need to enquire whether fat was added to the starch and to specify what type of fat it was.

Soup and legumes

This group includes:

- Dry beans (cooked) (e.g. sugar beans)
- Lentils
- Samp and beans (Commercial 1:10; homemade 2:1 check by asking)
- Samp and peanuts
- Soups (If homemade, record recipe)
- Dry bean stew

The following food models can be used to estimate portion sizes:

- Tablespoon, serving spoon and ½ cup measure
- Plate
- Dry beans

Cooked Vegetables

These are easily identified and are presented in alphabetical order. You will notice 5 specific columns that present you with instructions on cooking methods:

- The first column indicates Boil - NF. This means that the vegetable was simply boiled and no fat was added.
- The next 4 columns indicate that the vegetable was cooked and fat was added, or the vegetable was fried in fat. You need to determine whether B, HM, PM or SO was used.
- Always circle the relevant code and enter the correct portion size.

Salads and raw vegetables

You should use your tablespoon or serving spoon in your kit to estimate the portion size. Any unusual salads eaten should be included on the other line. The salad section is followed by Dressings which include:

- French dressing (vinegar and oil)
- Mayonnaise (homemade or commercial)
- Mayonnaise low in fat e.g. Trim
- Oil
- Salad dressing

Fruit group (listed in alphabetical order)

Fruits have been divided into 4 columns:

- A column with sweetened, canned fruit
- A column with the raw / fresh fruit
- A column with the dry fruit
- A column with cooked, stewed fruit

Once again, you should circle the correct code and enter the portion size. Fruit portions given are always for a medium fruit. If the fruit is very large or very small, please record it as such. Use the tennis ball in your kit to indicate a medium fruit portion size.

Pudding and sauces

Puddings include:

- Baked puddings
- Ice cream and sorbet
- Instant pudding
- Jelly
- Pancakes/crumpets

You need to select whether baked puddings were made with SM or with WM. When unsure, take the WM code. Use the tablespoon, serving spoon and the cup measure in your kit to determine portion sizes. If a different pudding was eaten that is not listed in the questionnaire, please write down the recipe in detail in Section B of page 15. Sauces include:

- Cream
- Chocolate sauce
- Custard

If cream was eaten, please specify the type, e.g. plant: *Orley Whip*, canned or fresh. If custard was eaten, then specify the type of milk used and whether sugar was added. Different sauces can be entered under other.

Cake, cookies and special bread and tarts

All three sections require you to determine whether these products were made with:

- SM or WM
- HM or PM
- Whether icing was added
- Whether a filling was added

Portion sizes can be estimated using:

- The ruler
- The bread slices

Sweets

The weight of individual sweets and savoury snacks should be recorded:

- Chewing gum
- Chocolates
- Hard sweets
- Peanuts
- Crisps
- Snacks
- Soft sweets

After the 24-H Recall has been completed, you should specifically ask the participant ate any sweets on the previous day. Adults do not always associate sweets with foods eaten.

“Other” group

This group includes:

- Sauces, such as cheese sauce
- Condiments, such as atchaar and chutney; and
- Gravy

Infant foods

This group includes:

- Infant cereals
- Milk
- First foods (small jar)
- Fruit juices
- Infant dinners (dry)
- Junior foods (big jar)
- Strained foods (small jar)

Completion of Interview

Complete the interview by asking questions 10 to 12 on page 14, and to record a yes or no answer to each of these questions.

APPENDIX X

GREEN LEAFY VEGETABLE (GLV), MOROGO QUESTIONNAIRE

Interviewer_____ Date _____

Name of a participant_____Age _____

Area _____ Duration of stay in the area _____

KINDLY ANSWER THE FOLLOWING QUESTIONS IN RELATION TO THE MOROGO SAMPLE YOU HAVE GIVEN US:

1. What is the name of morogo (type) given?.....
2. How was it cooked?.....
3. Approximately how long was it cooked?.....
4. How long did you dry it on the sun?.....
5. Are the are ingredients you have mix it with and if yes, what they are?.....
6. Any information you would like us to know about the samlple you have given?

APPENDIX XI

PRINCIPLES OF BIOCHEMICAL PARAMETERS

DETERMINATION OF BIOCHEMICAL PARAMETERS

1] DETERMINATION OF FOLATE AND RED CELL FOLATE

Folate and red cell folate concentrations will be determined by using BECKMAN ACCESS IMMUNOASSAY system.

Principle of procedure

The Access folate is a competitive-binding receptor assay. For the assay of folate in the serum or plasma (heparin), no pre-treatment is required. For the assay of folate in red blood cells, a whole blood sample is first treated off-line with a lysing agent composed of ascorbic acid and folate free HSA (Human serum albumin). This pre-treatment haemolyses the red cells and converts the folate polyglutamic acid forms present in red cells to the monoglutamic acid form predominate in serum. The sample from the pre-treatment of whole blood is defined as a hemolysate.

A serum, plasma (heparin), or hemolysate sample is treated to release folate from endogenous binding proteins. After neutralization of the reaction mixture, folate binding protein, mouse anti-folate binding protein, folic acid-alkaline phosphate conjugate, and goat anti-mouse capture antibody coupled to paramagnetic particles are added to the reaction vessel. Folate in the sample competes with the folic acid-alkaline phophatase conjugate for binding sites on a limited amount of folate binding protein. Resulting complexes bind to the solid phase via mouse anti-folate binding protein. Separation in a magnetic field and washing remove materials not bound to the

solid phase. A chemiluminescent substrate, Lum-Phos* 530, is added to the reaction vessel and light generated by reaction is measured with a luminometer. The light production is inversely proportional to the concentration of folate in the sample. The amount of analyte in the sample is determined by means of a stored, multi-point calibration curve.

To determine red cell (RBC) folate the following formula will be used:

RBC folate (ng/ml) = (haemolysed folate X 21)/ (haematocrit/100)

2] DETERMINATION OF VITAMIN B12 CONCENTRATION

Vitamin B12 concentration will be determined by BECKMAN ACCESS IMMUNOASSAY system.

Principle of procedure

The access vitamin B12 is a competitive-binding immunoenzymatic assay. A sample is added to a reaction vessel along with alkaline potassium cyanide and dithiothreitol. This treatment denatures B12 binding proteins and converts all forms of vitamin B12 to the cyanocobalamin form. After neutralization, intrinsic factor-alkaline phosphate conjugate and paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-intrinsic factors are added to the sample. Vitamin B12 in the sample binds to the intrinsic factor conjugate, preventing the conjugate from binding to the solid phase anti-intrinsic factor. Separation in a magnetic field and washing remove materials not bound to the solid phase. A chemiluminescent substrate, Lumi-Phos* 530, is added to the reaction vessel and light generated by the reaction is measured with

luminometer. The photon production in inversely proportional to the concentration of vitamin B12 in the sample. The amount of analyte in the sample is determined by means of stored, multi-point calibration curve.

3] DETERMINATION OF VITAMIN B6 CONCENTRATION

Vitamin B6 concentration will be determined by High Performance Liquid Chromatography (HPLC) 1100 using a kit from Chromsystems Instruments and Chemicals GmbH.

Principle of procedure

Reverse phase chromatography differs from most of other chromatographic techniques in which the attractive forces between the stationary phase and mobile phase are dominant. The carbon 18 column and the mobile phase are supplied with the kit. Retention of an analyte on the column depends on the relative amount of polar and non-polar characters of the analyte. Retention is also favoured by increased non-polar content of the analyte, whereas residence in the mobile phase leading to early elution from the column is favoured by an increase content of polar functionalities present on the analyte. The organic modifier component of the mobile phase competes with the stationary phase for the non-polar part of the analyte molecules, and thus retention is decreased with increased organic modifier in the mobile phase.

Instrument settings

Sampler: Injection volume was 50μl; analytical run-time will be 15 minutes

HPLC pump: Flow rate 1.3 ml/min.

Column temperature: Room temperature (20-25 ^{0}C)

Fluorescence detector: Excitation 370 nm and Emission 470 nm

4] DETERMINATION OF HOMOCYSTEINE CONCENTRATION

Homocysteine concentration will be determined by HPLC 1100 using a kit supplied by Chromsystems Instruments and Chemical GMbH.

Principle of procedure

Reverse phase HPLC will be used to determine homocysteine. Carbon 18 column, mobile phase, precipitation reagent, derivatisation reagent 1, internal standard, reducing reagent controls I and II and a calibrator will be used.

Instrument settings

Sampler: Injection volume of 20 μl, analytical run-time 7 minutes

HPLC pump: Flow rate of 1.3 to 1.7 ml/min

Column temperature: Room temperature (20-25 ^{0} C)

Fluorescence detector: Excitation of 358 nm and Emission of 515 nm

5] DETERMINATION OF FERRITIN CONCENTRATION

Ferritin concentration will be determined by using BECKMAN ACCESS IMMUNOASSAY system.

Principle of procedure

The Access ferritin is a two-site immunoenzymatic (sandwich) assay. The sample is added to a reaction vessel with goat anti-ferritin-alkaline phosphatase conjugate, and paramagnetic particles coated with goat anti-mouse: mouse anti-ferritin complexes. Serum ferritin binds to the immobilized monoclonal anti-ferritin on the solid phase, while the goat anti-ferritin enzyme conjugate reacts with different antigenic sites on the ferritin molecules. Separation in a magnetic field and washing removes materials not bound to the solid phase. A chemilumniscent substrate, Lumi-Phos* 530, is added to the reaction vessel and light generated by the reaction is measured with a luminometer. The photon production is proportional to the amount of ferritin in the sample. The amount of the analyte is determined by means of a stored, multi-point calibration curve.

6] DETERMINATION OF IRON CONCENTRATION

ILAB 300 plus kit was used to measure serum iron concentration

Principle

End point analysis, colorimetric methodology based on the formation of a blue coloured complex between iron and ferene-S in acidic solution. The increase in absorbance due to the blue complex

is proportional to the iron concentration in the sample. Absorbance measurements are taken at 620nm.

7] DETERMINATION OF TRANSFERRIN

Quantex transferrin ILAB 300 plus kit was used to determine transferrin

Principle of procedure

The reagent is a goat serum anti-human transferrin which reacts specifically with the transferrin of the sample to yield an insoluble aggregate which can be measured by turbidimetry.

8] DETERMINATION OF C-REACTIVE PROTEIN

Quantex CRP ILAB 300 plus kit was used to determine C-reactive protein

Principle of procedure

A sample containing CRP will agglutinate when mixed with the latex reagent. The degree of turbidity is directly proportional to the CRP concentration in the sample and can be quantitated by measuring the increase of absorbance at 578nm.

9] DETERMINATION OF TOTAL IRON BINDING CAPACITY

Quantex TIBC ILAB 300 plus kit was used to determine transferrin

Principle of procedure

TIBC is a measurement of the maximum concentration of iron that transferrin can bind. Specimens are treated with a Fe (III) solution to saturate the unbound iron-binding sites of transferrin and the excess of iron is removed by precipitation with magnesium carbonate hydroxide. The total iron present in the supernatant, which is measured with IL Test™ iron reagent, represents TIBC

10] DETERMINATION OF ALBUMIN CONCENTRATION

Albumin concentration will be determined by using DIMENSION ES SAMPLE auto analyzer

Principle of procedure

In the presence of a solubilizing agent, bromocresol purple (BCP) binds to albumin at pH 4.9. The amount of albumin-BCP complex is directly proportional to the albumin concentration. The complex absorbs at (600, 540, 700 nm) endpoint technique.

Albumin + BCP dye (nonabsorbing at 600 nm) ⟶ Albumin-BCP-complex (Absorbs at 600 nm)

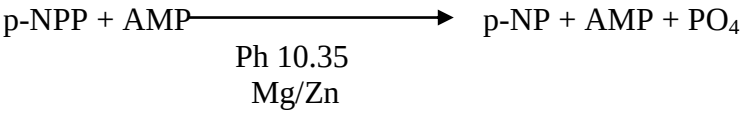
11] DETERMINATION OF ALKALINE PHOSPHATASE CONCENTRATION

Alkaline phosphatase will be determined by using DIMENSION ES SAMPLE auto analyzer

Principle of procedure

Alkaline phosphatase catalyses the transphosphorylation of p-nitrophenylphosphate (p-NPP) to p-nitrophenol (p-NP) in the presence of the transphosphorylating buffer, 2- amino-2-methyl-1-

propanol (AMP). The reaction is enhanced through the use of magnesium and zinc ions. The change in absorbance at 450 nm due to the formation of p-NP is directly proportional to the ALP activity, since other reactants are present in non-rate limiting quantities and is measured using a bichromatric (405, 510 nm) rate technique.

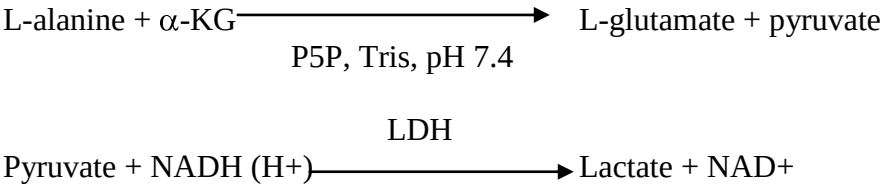


12] DETERMINATION OF ALANINE AMINOTRANSFERASE CONCENTRATION

Alanine aminotransferase will be determined by using DIMENSION ES SAMPLE auto analyzer

Principle of procedure

Alanine aminotransferase catalyses the transamination of L-alanine to α-ketoglutarate (α-KG), forming L-glutamate and pyruvate. The pyruvate formed is reduced to lactate by lactate dehydrogenase (LDH) with simultaneous oxidation of reduced nicotinamide-adenine dinucleotide (NADH). The change in absorbance is directly proportional to the ALT activity and is measured using a bichromatic (340, 700 nm) rate technique.



13] DETERMINATION OF γ-GLUTAMYL TRANSFERASE CONCENTRATION

γ-glutamyl transferase will be determined by using DIMENSION ES SAMPLE auto analyzer

Principle of procedure

γ-glutamyl transferase catalyses the transfer of the glutamyl moiety from γ-glutamyl-3-carboxy-4-nitranilide (GCNA) to glycylglycine thereby releasing 5-amino-2-nitrbenzoate which absorbs at 450 nm. This change is proportional to the γ-glutamyl transferase activity and is measured using a bichromatic (405, 600 nm) rate technique.

GCNA + glycylglycine ⟶ L-γ-glutamyl- glycylglycine + 5-amino-2-nitrobenzoate

14] DETERMINATION OF ASPARTATE AMINOTRANSFERASE

Aspartate aminotransferase will be determined by using DIMENSION ES SAMPLE auto analyzer

Principle of procedure

Aspartate aminotransferase catalyses L-aspartate to 2-oxoglutarate to form oxaloacetate and L-Glutamate. Pyridoxal-5-phosphate bound to the apoenzyme accepts the amino group from the first substrate aspartate to form enzyme-bound pyridoxamine-5-phosphate and the first reaction product is oxaloacetate. The co-enzyme in amino form then transfers its amino group to the

second substrate, 2-oxoglutarate, to form the second product, glutamate. Pyridoxal-5-phophate is the generated.

L-aspartate + 2-oxogluatarate $\Longleftrightarrow$ oxaloacetate + L-glutamate

15] DETERMINATION OF FULL BLOOD COUNT (HAEMATOLOGICAL INDICES)

Full blood count will be determined by using BECKMAN COULTER.

Principle of procedure

The A^c.T 5diff analyzer is a fully automated haematology analyzer providing a complete red cell count, haemoglobin and white blood cell five-part differential.