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# **DEVELOPMENT AND VALIDATION OF A DIETARY ASSESSMENT TOOL TO DETERMINE DIETARY INTAKE OF PEOPLE LIVING IN OESOPHAGEAL CANCER RISK AREAS IN THE RURAL EASTERN CAPE OF SOUTH AFRICA**

BY

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*Thesis Presented for the Degree of DOCTOR OF PHILOSOPHY in the Division of Nutrition and Dietetics.*

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## ABSTRACT

Rural areas in the Eastern Cape (EC) Province of South Africa have a high incidence of oesophageal cancer (OC). Amongst the known risk factors associated with the cancer is fumonisin exposure (a mycotoxin growing on maize), poor dietary habits and nutrient deficiencies. Little is known about the current dietary habits and nutrient intake of these people, and therefore maize consumption and fumonisins exposure. Because of large cultural differences a novel dietary assessment tool had to be developed. The dietary assessment tool was developed with interviews, focus groups and dishing up sessions. The tool comprises a quantitative food frequency questionnaire (QFFQ) and a set of food photographs (portion sizes and ratios). Although the validity of the photographs was tested, results indicated that the photographs mostly had poor validity and general over-reported, especially the large portion. This however, provided valuable information regarding the size and direction of the incorporated errors. The tool was further validated by comparing it with four 24-hour recalls energy expenditure (EE), blood and urinary biomarkers. Results indicated that the assessment method was valid for all food groups except bread and beverages and was therefore valid to measure fumonisin exposure. Furthermore, results indicated that the tool was only partially able to measure nutrient intake. The tool compared poorly with EE measured with a physical activity questionnaire, as well as with Schofield's EE equations. The use of urinary biomarkers was not very successful as very few complete samples were collected. The assessment method had also poor validity results when compared against blood biomarkers. Reliability was determined with a test-retest method and it was concluded that the tool was reliable when measuring dietary intake but is only partially capable of measuring nutrient intake. A cross sectional study was conducted to determine the dietary intake of the people living in this area. Results showed a higher than recommended fumonisin intake per person as well as nutrient deficiencies associated with a risk of developing OC. Also, a low consumption of fruit has been identified. Many of the dietary risk factors identified in the area are modifiable and appropriate prevention campaigns should be implemented by the appropriate health authorities.

**To my beloved .....**

**Without you I am but a shell.**

University of Cape Town

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Hester Burger; whom I met at the beginning of the study. The journey was long and we had a lot to learn in an environment where our aims and visions were not always understood. Not only did I gain a tremendous amount of knowledge, but rather unexpectedly I gained a friend. The trips to the Eastern Cape were not only work, we had a lot of fun together, and I would not trade that for anything. I sincerely hope that we will continue to work together, but more importantly that our friendship will continue to grow in size and in depth.

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## LIST OF ABBREVIATIONS

|                    |  |
|--------------------|--|
| AICR               | American Institute for Cancer Research   |
| ANOVA              | Analyses of variance   |
| B                  | Mean value of the biomarker  |
| BMI                | Body mass index  |
| BMR                | Basal metabolic rate   |
| BMR <sub>est</sub> | Estimated basal metabolic rate   |
| Ca                 | Calcium  |
| CCHIP              | Community Childhood Hunger Identification Project                              |
| CD                 | Compact Disk   |
| CHO                | Carbohydrates  |
| CI                 | Confidence intervals   |
| cm                 | Centimeter   |
| DHA                | Docosahexaenoic acid   |
| DLW                | Double labelled water  |
| EC                 | Eastern Cape   |
| EDTA               | Ethylenediaminetetraacetic acid  |
| EE                 | Energy expenditure   |
| EE <sub>calc</sub> | Calculated Energy Expenditure  |
| EI                 | Energy Intake  |
| EI <sub>rep</sub>  | Reported Energy Intake   |
| EPA                | Eicosapentaenoic acid  |
| EPIC               | European Prospective Investigation into Cancer and Nutrition                   |
| EPIC PA            | European Prospective Investigation into Cancer and Nutrition Physical Activity |
| ER                 | Endoplasmic Reticulum  |
| <i>et al.</i>      | And other people   |
| FAO                | Food and Agriculture Organisation  |
| FB <sub>1</sub>    | Fumonisin B <sub>1</sub>   |
| FB <sub>2</sub>    | Fumonisin B <sub>2</sub>   |
| FB <sub>3</sub>    | Fumonisin B <sub>3</sub>   |
| Fe                 | Iron   |
| FFQ                | Food Frequency Questionnaire   |

|           |   |
|-----------|---|
| FGDs      | Focus Group Discussions   |
| FPPB      | Food Portion Photograph Book                                      |
| FR        | Free Radicals   |
| g         | Gram  |
| GPAQ      | Global Physical Activity Questionnaire                            |
| GPS       | Global Positioning System   |
| HPLS      | High Performance Liquid Chromatography                            |
| Group 2B  | Possibly carcinogenic to humans                                   |
| ht        | Height  |
| IARC      | International Agency for Research on Cancer                       |
| IPAQ      | International Physical Activity Questionnaire                     |
| IQ range  | Inter Quartile range  |
| ISE       | Ion Selective Electrode   |
| JEFCA     | Joint Expert Committee on Food Additives                          |
| K         | Potassium   |
| Kg        | Kilogram  |
| L         | Large   |
| LOA       | Limits Of Agreement   |
| M         | Medium  |
| mcg       | Microgram   |
| MEOS      | Microsomal Ethano-Oxidizing System                                |
| MET score | Metabolic equivalent score  |
| Mg        | Magnesium   |
| MJ        | Millijoule  |
| mℓ        | Millileter  |
| MRC       | Medical Research Council  |
| MTHFR     | Methylenetetrahydrofolate Reductase                               |
| MTTR      | 5-methyltetrahydrofolate-homocysteine methyltransferase reductase |
| MUFA      | Mono-Unsaturated Fatty Acids                                      |
| Na        | Sodium  |
| NCI       | National Cancer Institute   |
| NFCS      | National Food Consumption Survey                                  |
| NFCS-FB-I | National Food Consumption Survey – Fortification Baseline         |

|             |  |
|-------------|--|
| NMBzA       | N-nitroso-N-methyl-N-benzylamine   |
| NMPA        | N-nitroso-N_methyl-N-methylamine   |
| NOAEL       | No Observed Adverse Effect Level   |
| Nu          | Urinary nitrogen   |
| OC          | Oesophageal Cancer   |
| $\rho$      | validity coefficient   |
| PABA        | Para-Amino Benzoic Acid  |
| PAL         | Physical Activity Level  |
| PD          | Percentage Difference  |
| PDI         | Probable Daily Intake  |
| PMTDI       | Provisional Maximum Tolerable Daily Intake                                       |
| PROMEC      | Program For Mycotoxins and Experimental Carcinogens                              |
| $\rho_{TB}$ | Validation coefficient between the true intake and the biomarker                 |
| $\rho_{TQ}$ | Validation coefficient between the true intake and the test questionnaire        |
| $\rho_{TR}$ | Validation coefficient between the true intake and the reference method          |
| PUFA        | Poly-Unsaturated Fatty Acids   |
| Q           | Test questionnaire   |
| R           | Mean values of the reference measure   |
| $r_{BR}$    | Correlation coefficient between the biomarker and the reference method           |
| RDA         | Recommended Daily Allowance  |
| REE         | Resting Energy Expenditure   |
| rmp         | Revolutions per minute   |
| $r_{QB}$    | Correlation coefficient between the test questionnaire and the biomarker         |
| $r_{QR}$    | Correlation coefficient between the test questionnaire and the reference measure |
| S           | Small  |
| SA          | South Africa   |
| SAVACG      | South African Vitamin A Consultative Group                                       |
| SD          | Standard Deviation   |
| Se          | Selenium   |
| SEM         | Structural Equation Modelling  |
| SFA         | Saturated Fatty Acids  |
| SFFQ        | Semi-quantitative Food Frequency Questionnaire                                   |

|                   |   |
|-------------------|---|
| SFFQ <sub>1</sub> | Semi-quantitative Food Frequency Questionnaire conducted in March during the reliability testing of the assessment method |
| SFFQ <sub>2</sub> | Semi-quantitative Food Frequency Questionnaire conducted in June during the reliability testing of the assessment method  |
| SSAAQ             | Sub-Saharan Africa Activity Questionnaire   |
| T                 | Participants' actual or true intake   |
| THUSA             | Transition, Health and Urbanization in South Africa   |
| UCT               | University of Cape Town   |
| UWC               | University of the Western Cape  |
| UK                | United Kingdom  |
| UNU               | United Nations University   |
| USA               | United States of America  |
| VC                | Validation Coefficient  |
| vit               | Vitamin   |
| wt                | Weight  |
| WCRF              | World Cancer Research Fund  |
| WHO               | World Health Organisation   |
| Zn                | Zinc  |

## DEFINITION OF TERMS

|                           |  |
|---------------------------|--|
| <i>Amagewu</i>            | A beverage made from cooked maize meal and white bread flour that is left overnight <sup>1</sup>                   |
| <i>Bisto</i>              | A stew made from tomato, onion and potato*   |
| Dietary assessment method | A method used to determine dietary intake, such as 24-hour recalls, food frequency questionnaires and food records |
| Dietary assessment tool   | A tool used to assist in dietary intake, such as portion size photographs, food models, food pictures              |
| Dumplings                 | Bread dough (maize or wheat) fried in oil*   |
| Eastern Cape Province     | Formerly known as the Transkei and Ciskei homelands  |
| Food groups               | Food items and dishes grouped together based on their basic nutrient composition                                   |
| Fumonisin                 | A group of mycotoxins produced by <i>Fusarium</i>  |
| <i>Imifino</i>            | A spinach-like plant that grows wild in the Eastern Cape*  |
| <i>Inqodi</i>             | A beverage similar to <i>amagewu</i> , but fermented over a longer period*.  |
| Maize                     | Also known as corn or mealies  |
| Mycotoxin                 | A mycotoxin is a toxic secondary metabolite produced by a fungus (Turner <i>et al.</i> , 2009)                     |
| <i>Pap</i>                | Maize meal porridge (the consistency varies depending on the dish)*  |
| Porridge                  | A traditional word used for maize meal porridge with a thin consistency*   |
| Reference method          | Mean 24-hour recalls   |
| Reference weight          | The actual portion size weight dished up by participants   |
| Samp                      | Broken, dried maize kernels*   |
| Samp and beans            | Broken, dried maize kernels mixed with dried sugar beans*  |
| <i>Sangoma</i>            | A spiritual person that can interact with both the physical and spiritual world                                    |
| Stiff <i>pap</i>          | A traditional word used for maize meal porridge with a stiff, thick consistency*                                   |
| Soup                      | A soup made with whole kernels and dried sugar beans*  |
| Test method               | Newly developed dietary assessment method combined with photograph series  |
| Test weight               | The portion size weight represented on the portion size photographs  |
| <i>Umngqusho</i>          | Samp and bean dish   |
| <i>Umphokoqo</i>          | Crumbly pap  |

---

<sup>1</sup> As defined by the population in focus group discussions.

*Unonca*

A traditional bread made from maize meal\*

*Vetkoek*

Bread dough (maize or wheat based) fried in oil\*

## Chapter 1

### INTRODUCTION

---



**A typical mud hut used to live in by the people living in rural Eastern Cape areas  
(Photo by H.J. Lombard)**

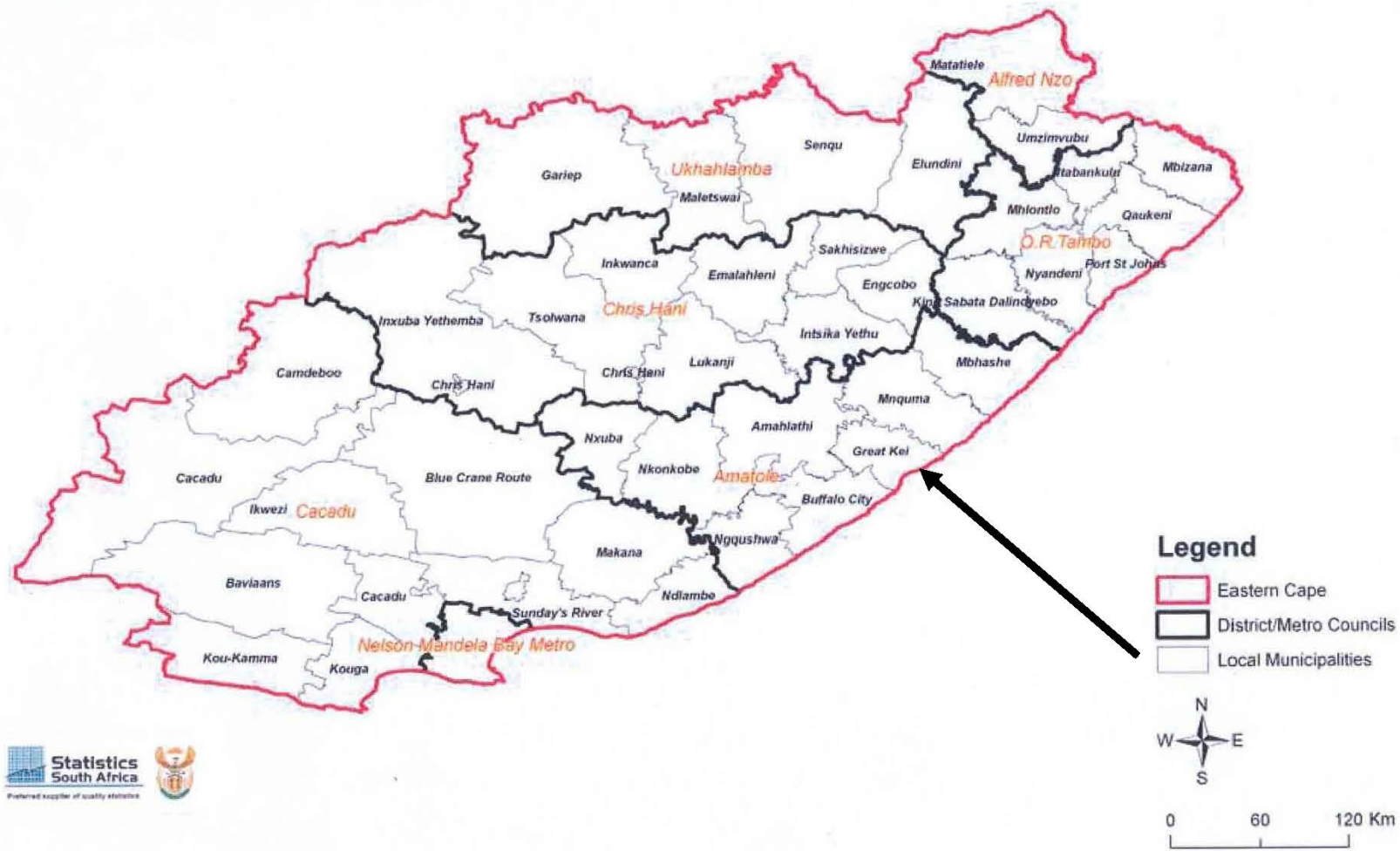
## 1. INTRODUCTION AND PROBLEM IDENTIFICATION

The former Transkei region of the Eastern Cape (EC) Province (Figure 1.1) in South Africa is known for its high incidence of squamous cell oesophageal cancer (OC) (Somdyala *et al.*, 2003:10, Somdyala *et al.*, 2008:6). Squamous cell OC has been described as a cancer with an highly irregular geographical distribution, with a high incidence rate in very specific areas and a low incidence rate in nearby areas, including China, India, Northern Italy and rural areas of the EC Province in South Africa, occupied by the Xhosa people (Day, 1975:3306, Rose, 1979:30, Gabriel *et al.*, 1982:788, Li *et al.*, 1989:758, Negri *et al.*, 1992:1170, Guar *et al.*, 1997:2129, White *et al.*, 2002:462, Abnet *et al.*, 2007:1889). The incidence in the EC is 49.0 / 100 000 compared with 20.2 / 100 000 for South Africa as a whole (Norman *et al.*, 2005:149, Somdyala *et al.*, 2008:6). The high OC areas in the EC Province include amongst others the Great Kei local municipality in the Amatole District (Figure 1.1).

Various factors are associated with squamous cell OC. Amongst the well known risk factors are alcohol consumption and tobacco use (McGlashan *et al.*, 1982:256, Graham *et al.*, 1990:464, Craddock, 1992:91, Tavani *et al.*, 1993:2534, Eskelson *et al.*, 1993:117, Castelletto *et al.*, 1994:563, Srivastava *et al.*, 1997:96, Yokoyama *et al.*, 2006:2213, Freedman *et al.*, 2007:1431, Ma *et al.*, 2010:644) as well as a low body mass index (BMI) (Gallus *et al.*, 2001:977, Yokoyama *et al.*, 2006:2213). *Fusarium* mycotoxins, *N*-nitrosamines and tannins have also been identified as potential risk factors for OC (Marasas *et al.*, 1988:112, Craddock, 1992:94, 96) as have other dietary components such as poor fruit and vegetable intake and deficiencies of certain micronutrients.

In the EC Province little data are available regarding dietary factors and OC. The few studies on dietary intake were done in the sixties and seventies. However, little is known about the traditional and habitual dietary intake patterns of Xhosa people living in this region. Information available is outdated, especially seen in the light of urbanisation during the last decade (Rose, 1972:1353, Byarugaba, 1991:444).

In the eighties, a microbiological study conducted in the EC, found significantly higher levels of mycotoxins in maize consumed by households with OC-affected persons compared to those with people not affected. This was directly related to exposure to food borne *Fusarium* mycotoxins, produced by a mould growing on maize (Van Rensburg, 1985:30, Marasas *et al.*, 1988:112, Craddock, 1992:94).



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Figure 1.1. Eastern Cape districts / metros and local municipalities (December 2005)

The Xhosa people living in these high OC areas in the EC are mostly poor subsistence farmers consuming a staple diet of home-grown maize. As poverty increases, the possibility of energy and micronutrient deficiencies increase which then lead to an increased OC risk. The home-grown maize also have much higher levels of fumonisin (a specific mycotoxin), compared to commercially available maize (Shephard *et al.*, 2007:622). Currently, fumonisin contamination of this food source is a major health concern, however to date the amount of fumonisin consumed by people (on average per day) is not available. This is mainly due to the fact that maize intake, particularly home-grown maize, has not been determined by means of a dietary assessment (Shephard *et al.*, 2007:622).

The focus of the current study was to develop and validate dietary methodology to firstly assess maize (fumonisins) intake and secondly to assess the adequacy of OC risk related nutrient intake of Xhosa speaking people living in a high risk OC area of the EC.

The development of the questionnaire and photograph series was based on information collected in both Bizana (low OC risk area) and Centane (high OC risk area) because this study was part of a larger study conducted by the Program for Mycotoxins and Experimental Carcinogens (RPOMEC unit) of the Medical Research Council (MRC) that included research in both areas. This also ensured that dietary choices of people living in both high risk and low risk areas were considered in the development of the dietary assessment questionnaire and photograph series. Because of logistics determined by the parent protocol and funding limitations, the dietary assessment method was validated in a single high OC risk area (Centane).

## **2. AIMS AND OBJECTIVES**

The overall aim of the study was to develop and validate a culturally specific dietary method to assess habitual dietary intake of Xhosa people living in high-risk OC areas of the EC that would accurately reflect maize intake as a proxy of fumonisins intake (primary aim) as well OC risk related nutrients (secondary aim).

In order to achieve these aims, the following specific objectives were formulated:

Objective 1: To develop a novel culturally specific dietary assessment method consisting of a food photograph series and quantitative food frequency questionnaire (QFFQ)(RAPP method<sup>1.1</sup>) to obtain dietary information from Xhosa people in high-risk OC areas;

Objective 2: To assess the validity of the food photograph series in estimation of portion sizes;

Objective 3: To assess the reliability of the RAPP method for food groups<sup>1.2</sup> and OC risk related nutrients<sup>1.3</sup>;

Objective 4: To assess the validity of the RAPP method for food groups and OC risk related nutrients;

Objective 5: To determine habitual dietary intake of Xhosa people living in a high risk OC area in the EC Province of South Africa with a focus on maize consumption and subsequent fumonisin exposure.

### **3. OUTLINE OF THE STUDY**

Chapter 2 of the thesis is an overview of literature regarding the current known risk factors associated with OC and present information available on the habitual dietary intake of the Xhosa people living in these areas. The literature review also includes information on the different dietary methods and statistical analyses available to develop dietary assessment methods and to determine reliability and validity of dietary methods. Chapter 3 focuses on the over-arching methodology used in the different parts of the study. Chapter 4 presents the steps taken to develop the culturally specific dietary (RAPP) method and training manual. Chapter 5 presents the methods used to validate the photograph series used for portion size estimation. Reliability testing of the RAPP method (test-retest design) is presented in Chapter 6 and the validation in Chapters 7 (against repeated 24-hour recalls) and 8 (against biomarkers). Chapter 9 focuses on data from a cross sectional study undertaken to determine nutrient intake and fumonisin (mycotoxin) exposure of the target population using the newly developed RAPP method. Chapter 10 provides the final conclusions and recommendations regarding the use of the RAPP method.

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<sup>1.1</sup> The RAPP method includes a Quantitative Food Frequency Questionnaire and food photograph series.

<sup>1.2</sup> Food groups include mostly maize that reflect fumonisins exposure.

<sup>1.3</sup> Oesophageal risk related nutrients include all macro- and micronutrients that have been associated with OC in the past.

## 4. ETHICAL CONSIDERATIONS

Ethical approval was obtained from the Research Ethics Committee of the University of Cape Town (Ref nr. 123/2003) (Addendum 1), as well as from the MRC's ethical committee. Each participant received detailed, easy to understand information (both orally and written) regarding the study, and consent was obtained in the participant's first language (isiXhosa) (see details regarding specific information and consent in Chapter 3) (Addendum 2).

The principles of ethics were adhered to according to the Declaration of Helsinki that was published by the World Medical Association in 1964 (World Medical Association, 1964) and the Belmont Report published in 1979 by the National Commission for the Protection of Human Subjects (National Commission for the Protection of Human Subjects, 1979).

### 4.1. The principle of respect

Before the onset of fieldwork consent was obtained from the local chief, headman, *sangoma* (traditional healer) (Figure 1.2) and other local leaders (Figure 1.3). This was an open meeting and anybody from the community was allowed to listen to the negotiations. Subsequently, the purpose of the study and methods were explained in detail, in the participant's first language (isiXhosa). Participation was voluntary, and participants could withdraw at any stage. All information was confidential. Consent was obtained from both the participant and where possible the participant's employer.



**Figure 1.2.** The research team provided information and obtained permission from the chief, headman and local *sangoma* (traditional healer)



**Figure 1.3.** The research team provided information on the study and obtained consent from other local leaders in the area.

## 4.2 The principle of beneficence

The participants enjoyed participating in the development of the dietary assessment dietary method and the validation of the food photograph series as it provided an opportunity for social events. The research team provided all food items and ingredients for dishes used in the development of the dietary method and the validation of the photograph series. Volunteers (usually the hosts and neighbours) prepared the dishes themselves.

### **4.3 The principle of justice**

Although participants did not benefit directly from the study, it has the potential to improve the accuracy of future nutritional and / or oesophageal cancer-related studies in the area. Information collected during the cross-sectional study may lead to the development of public health campaigns from which participants and their relatives may benefit.

University of Cape Town

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## Chapter 2

# LITERATURE REVIEW ON OESOPHAGEAL CANCER AND THE DEVELOPMENT AND VALIDATION OF DIETARY ASSESSMENT METHODS AND TOOLS

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Three women in rural Centane doing traditional dances in traditional dress clothes

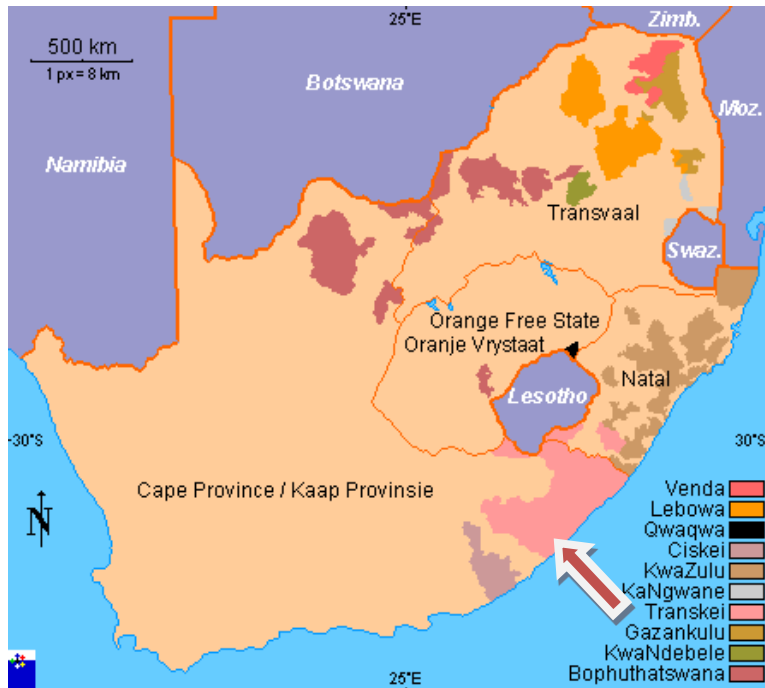
## 1. INTRODUCTION

The aim of this chapter is to present a review of literature on oesophageal cancer (OC) and related factors regarding the target population. The chapter also describes steps in the development of dietary assessment methods and tools, as well as factors that influence their development. The chapter further provides a summary of the different dietary assessment methods currently in use, as well as each method's strengths and limitations. This is followed by a discussion on portion size estimation and the development and validation of portion size photographs. The chapter further explains different methods used to determine validity and reliability of dietary assessment methods and tools as well as strengths and limitations of each method.

## 2. OESOPHAGEAL CANCER IN SOUTH AFRICA

The black people in South Africa are mostly divided into four major ethnic groups, including the Nguni, Sotho, Shangaan-Tsonga and the Venda. The Nguni group (the largest black ethnic group in South Africa) can be subdivided into four smaller groups: the Northern and Central Nguni (Zulu-speaking people), the Southern Nguni (Xhosa-speaking people), the Swazi (from Swaziland) as well as the Ndebele people (situated in the Northern Province and Mpumalanga) (South African history online, [s.a.]).

The South African Government proclaimed the homelands (Bantustans) in 1953, and the Transkei and Ciskei were allocated to the Xhosa people [South African history online, (s.a.)] (Figure 2.1). These regions were declared independent countries by the apartheid government. The Xhosas were denied South African citizenship and was forcibly relocated to these rural areas (Transkei and Ciskei). In 1976, the Transkei became the first independent homeland and broke all diplomatic relations with South Africa in 1980. However, the international arena did not recognize the homeland as an independent state. In 1994, with the end of apartheid, the Transkei and the Ciskei, (along with all the other homelands) was once again incorporated in South Africa, as the Eastern Cape Province [South African history online, (s.a.)].



**Figure 2.1. Former homelands in South Africa [Maps of the world, (net)].**

As mentioned earlier, squamous cell OC is particularly prevalent among Xhosa people living in specific rural areas of the Eastern Cape (EC) (formerly known as the Transkei), who have a low education and income, and are males (Craddock, 1992:92, Castelletto *et al.*, 1994:558, Blot, 1994:403, Somdyala *et al.*, 2003:5, Somdyala *et al.*, 2008:6, Wild & Gong, 2010:78). Black South Africans are affected more frequently and the diagnosis is frequently made at a late stage in the development of the tumour, decreasing the prognosis significantly (Jaskiewicz *et al.*, 1987:3, Sumeruk *et al.*, 1992:92, Blot, 1994:403, Mqoqi *et al.*, 2004:17).

As early as 1965 it was concluded that this form of OC is exceptionally geographic specific and that the total figures of a country does not provide true information because of the highly specific distribution (Rose 1965:1098). A study conducted by Rose (1965:1099) concluded that this was also the case for the rural areas of the Eastern Cape (then known as the Transkei). She reported that according to household survey data, the population groups who stayed the longest in the “Transkei” were more susceptible to the disease (Rose 1965:1099). This was however recorded in the mid 1960’s and very little is known about the current situation in these areas.

Specific rural areas in the EC have the highest OC incidence rates in South Africa (Bradshaw & Harington, 1986:57, Somdyala *et al.*, 2003:10, Somdyala *et al.*, 2008:6) (Table 2.1). These incidence rates are among the highest in the world (Thompson, 1987:20, World

Cancer Research Fund [WCRF] / American Institute Cancer Research [AICR], 2007:680, Wild & Gong, 2010:78). In two specific areas in the EC (Bizana and Centane) the reported incidence of cancer among males and females reached 63% (males) and 44% (females) of all diagnosed cancers (Somdyala *et al.*, 2003:10).

**Table 2.1. Mean age-standardised incidence rates (ASIR) for oesophageal cancer in males and females in three districts of the Eastern Cape during the period between 1995 and 2002**

| <b>Period</b>      | <b>Males<br/>Rate / 100 000</b> | <b>Females<br/>Rate / 100 000</b> |
|--------------------|---------------------------------|-----------------------------------|
| <b>Bizana</b>      |                                 |                                   |
| 1955 – 1959        | 2.6                             | 1.8                               |
| 1965 – 1969        | 10.5                            | 4.4                               |
| 1981 – 1984        | 19.5                            | 15.0                              |
| 1985 – 1990        | 37.0                            | 11.7                              |
| 1991 – 1995        | 33.6                            | 28.8                              |
| <b>1998- 2002</b>  | <b>37.2</b>                     | <b>14.4</b>                       |
| <b>Centane</b>     |                                 |                                   |
| 1955 – 1959        | 54.2                            | 30.3                              |
| 1965 – 1969        | 39.7                            | 16.1                              |
| 1981 – 1984        | 45.0                            | 29.3                              |
| 1985 – 1990        | 55.6                            | 22.1                              |
| 1991 – 1995        | 89.2                            | 32.0                              |
| <b>1998-2002</b>   | <b>48.3</b>                     | <b>19.2</b>                       |
| <b>Butterworth</b> |                                 |                                   |
| 1955 – 1959        | 103.1                           | 45.6                              |
| 1965 – 1969        | 73.4                            | 27.5                              |
| 1981 – 1984        | 31.5                            | 19.0                              |
| 1985 – 1990        | 42.8                            | 20.6                              |
| 1991 – 1995        | 33.8                            | 10.6                              |
| <b>1998-2002</b>   | <b>32.1</b>                     | <b>22.6</b>                       |
| <b>RSA 1999</b>    | <b>11.3</b>                     | <b>5.5</b>                        |

1955 - 1959 (Rose, 1973);  
 1965 - 1969 (Rose & McGlashan, 1975);  
 1981 - 1984 (Jaskiewicz *et al.*, 1987);  
 1985 - 1990 (Makaula *et al.*, 1996);  
 1991 – 1995, 1998-2002 (Somdyala *et al.*, 2003, 2008)  
 RSA 1999 (Mqoqi *et al.*, 2004 :18)

## 2.1. RISK FACTORS ASSOCIATED WITH OESOPHAGEAL CANCER

### 2.1.1. Non-dietary risk factors

Alcohol consumption and tobacco use have long been identified as specific risk factors for OC (McGlashan *et al.*, 1982:256, Graham *et al.*, 1990:464, Craddock, 1992:91, Tavani *et al.*, 1993:2534, Eskelson *et al.*, 1993:117, Castelletto *et al.*, 1994:563, Srivastava *et al.*, 1997:96, Danaei, *et al.*, 2005:1787, Garavello *et al.*, 2005:1392, Freedman *et al.*, 2007:1431, Ma *et al.*, 2010:644).

Chronic alcohol consumption has been identified as one of the major modifiable risk factors associated with OC (Eskelson *et al.*, 1993:117, Garavello *et al.*, 2005:1392, Freedman *et al.*, 2007:1431). Chronic alcohol consumption (> 5 times / week) increases the risk of OC 3 times more than those who do not consume alcohol (Srivastava *et al.*, 1997:97). Furthermore, more than three drinks per day of especially beer or liquor (Freedman *et al.*, 2007:1431) increases the risk of OC. Apart from an increased risk among those who recently stopped drinking, there is a clear decrease in risk among those with long periods of abstinence (Cheng *et al.*, 1995:1095). Although the exact mechanism has not been identified yet, various hypotheses exist regarding the association between alcohol and OC. It has been hypothesised that it is not ethanol *per se* that promotes carcinogenesis, but rather the increased free radicals formed during ethanol metabolism (Eskelson *et al.*, 1993:124, Mufti *et al.*, 1997:222). Also, it has been hypothesised that an additional risk factor of alcohol consumption lies in the tannin content of spirits, wine and beer. Most of these drinks are matured in wooden casks which increase the tannin content (Craddock, 1992:92). In the rural areas of the EC province the majority of alcohol is consumed as home brewed beer (McGlashan *et al.*, 1982:254, Odhav & Naicker, 2002:55). This beer is brewed from fermented maize and sorghum, cooked in iron pots and brewed in large plastic containers / drums (Rose, 1979:36, Odhav & Naicker, 2002:55).

Tobacco smoking also increases the risk of OC (McGlashan *et al.*, 1982:253, WHO, 2003:96, Gallus *et al.*, 2003:210, Garavello *et al.*, 2005:1392, Freedman *et al.*, 2007:1431). A study done by Castelletto *et al.*, (1994:558) found that smokers as well as those who smoked in the past, have an increased risk of developing OC and that the time elapsed since cessation did not influence risk. Risk increased significantly with duration of tobacco use, and there is no dose-response between the number of cigarettes smoked per day and OC risk. They also found that smokers of black tobacco (air-cured) had a higher risk for

developing OC than those smoking blond tobacco (flue-cured) (De Stefani *et al.*, 1993:763, Castelletto *et al.*, 1994:558, Launoy *et al.*, 2000:41).

McGlashan *et al.*, (1982:251) found that less tobacco was used in low-incidence risk areas and that pipe smoking was more prevalent in the high-risk areas (McGlashan, *et al.*, 1982:253). According to Rose (1979:37) the tobacco used for pipe smoking is mostly home-grown. The Xhosa people in these areas insert a substance from the stem of the pipe (*Injonga*) between the gums and the lips as a substitute to tobacco. Women and young girls also used this *Injonga* as “make-up”, as this blackens the lips. Analysis of this substance has indicated that *Injonga* is high in nitrosamines (Rose, 1979:37).

In the EC a traditional Xhosa cleansing ritual is practiced, which includes self-induced vomiting. It is believed that this ritual might also be a risk factor for OC, because acid from the stomach causes chronic oesophagitis, damaging the alkaline oesophagus cells (Sammon, 1992:864).

### **2.1.2. Dietary risk factors (excluding mouldy maize)**

*N*-nitrosamines are produced when amines and nitrates come together at a specific pH and are formed during the storage and preparation of food. Smoked meat or fish often contain high levels of nitrosamines, which are formed from amines in the meat / fish and nitrous fumes in the smoke. *N*-nitroso-*N*-methyl-*N*-benzylamine (NMBzA) and *N*-nitroso-*N*-methyl-*N*-methylamine (NMPA) are known carcinogens of the oesophagus (Craddock, 1992:91).

Tannins have been identified as yet another risk factor for OC. Tannins are comprised of two chemical compounds, the hydrolysable (black tea) and the non-hydrolysable (wood) types. Both are strong protein precipitants and therefore damage the surface of the oesophagus. During the preparation of black tea, tannins are formed by the fermentation of the leaves and when the tea is brewed, the tannins are extracted (Craddock, 1992:96). Tannins are also present in alcoholic beverages that have been stored or aged, in wooden barrels. The longer the alcohol is exposed to the wood, the higher the tannin content will be (Craddock, 1992:97).

Along with the above-mentioned risk factors, dietary intake and specific nutrient deficiencies have also been identified as risks for OC. Various researchers reported that a low body mass index (BMI) was an indicator of OC risk (Gallus *et al.*, 2001:977, Yokoyama *et al.*, 2006:2213). One of the prominent dietary patterns is the consumption of a maize staple diet

and that of mouldy, pickled vegetables (Guo *et al.*, 1990:125, Craddock, 1992:93, Cheng, 1992:1317, Franceschi, 1993:616). Other identified habitual dietary intake patterns include those of high meat consumption, especially that of lamb and salted meat, as well as a low consumption of fruit and vegetables (Guo *et al.*, 1990:124, Tzonou *et al.*, 1996:302, Launoy *et al.*, 1998:9, De Stefani *et al.*, 1999:35, Danaei *et al.*, 2005:1787).

Nutrient deficiencies that have been associated with OC include folic acid (Brown *et al.*, 1988:1620, Mayne *et al.*, 2001:1055, Wei *et al.*, 2004:83, Yang *et al.*, 2005:1285, Larsson *et al.*, 2006:1276), nicotinamide (Siassi *et al.*, 2000:300), vitamin C (Guo *et al.*, 1990:124, Muñoz & Castellsagué, 1994:653), vitamin A (van Rensburg, 1987:10), magnesium (Mg) (Craddock, 1992:93), vitamin D (Abnet *et al.*, 2007:1889), selenium (Se) (Guo *et al.*, 1990:125, Mark *et al.*, 2000:1753), zinc (Zn) (Chen *et al.*, 1992:405, Fong *et al.*, 1998:1595) and riboflavin (Guo *et al.*, 1990:125). The possible biochemical mechanisms resulting from nutrient deficiencies are shown in Table 2.2

Nutrients that have a protective effect include carotenoids (Nomura *et al.*, 1997:411), retinol (Zheng *et al.*, 1995:958), vitamin C (Franceschi, 1993:616, Tzonou *et al.*, 1996:302), vitamin E (Franceschi, 1993:616, Eskelson *et al.*, 1993:123) and folic acid (Galeone *et al.*, 2006:523). The combined intake of fruit and vegetables has also been identified as having a protective effect (Franceschi, 1993:616, Guo *et al.*, 1994:449, Albanes *et al.*, 1995:1429S, Levi *et al.*, 2000:259, WHO, 2003:95).

However, long-term vitamin and mineral supplementation seem to have no real protective benefit (Li *et al.*, 1993:1497, Dawsey *et al.*, 1994:172). The results of a large randomised trial in Linxian (China) where 29 584 participants received vitamin and mineral supplementation for five years showed little effect on OC (Blot *et al.*, 1993:1490). This might be because of the relatively short period (5 years) of supplementation (Blot *et al.*, 1993:1490, Wang *et al.*, 1994:165). The researchers concluded that vitamin and mineral intervention may not prevent OC, or influence the rate of progression once the cells have been transformed (Mark *et al.*, 1994:166, Rao *et al.*, 1994:279). Supplementation did influence the area where cell proliferation was found, suggesting that there may be some benefits in supplementation (Rao *et al.*, 1994:279, Taylor *et al.*, 1995:1423S). However, ten years after the supplementation study results indicated a protective effect for participants (receiving a mixture of selenium, vitamin E and beta-carotene) younger than 55 years and a harmful effect for those older than 55 years of age (Qiao *et al.*, 2009:513).

In 2000, Levi *et al.*, (2000:259) suggested a synergistic effect among OC risk factors, claiming that no single factor puts people at risk for developing OC.

**Table 2.2. Potential biochemical mechanisms of nutrient deficiencies associated with oesophageal cancer**

| Deficiency                      | Description  |
|---------------------------------|--|
| Folic acid                      | A folate deficiency causes polymorphisms on methylenetetrahydrofolate reductase (MTHFR) C677T and 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (MTRR) A66G genes which increase the risk of OC (Stolzenberg-Solomon <i>et al.</i> , 2003:1225). Also, folate deficiency decreases the repair activity of DNA because of a lack of methyl donors that are essential for DNA synthesis (Wei <i>et al.</i> , 2003:967, Pelucchi <i>et al.</i> , 2003:1680).  |
| Nicotinamide                    | Niacin deficiency results in widespread inflammation of mucous surfaces, cause dysphagia as well as oesophageal lesions (Franceschi <i>et al.</i> , 2000:6230).  |
| Vitamin A                       | There is controversy regarding the role of vitamin A and thus retinoid deficiency in OC. Some believe that vitamin A deficiency contributes to the process of atrophy of the oesophageal epithelium (Van Rensburg, 1987:10) while others believe that vitamin A enhances carcinogenesis (Groenewald <i>et al.</i> , 1981:967). According to the South African vitamin A consultative group (SAVACG) there is evidence of widespread vitamin A deficiency in the country (Coutsoudis <i>et al.</i> , 1996:1).                           |
| Vitamin E and $\beta$ -Carotene | Vitamin E and $\beta$ -carotene are antioxidants that protect the cell membrane against the oxidation of free radicals (Odeleye <i>et al.</i> , 1992:1815, Eskelson <i>et al.</i> , 1993:122).   |
| Riboflavin                      | Riboflavin deficiency may cause an increase in the cell replication rate, increasing the carcinogenicity of nitrosamines (Craddock, 1992:94). Riboflavin deficiencies have been reported in the EC region in the past (Jaskiewicz <i>et al.</i> , 1987:3).   |
| Magnesium                       | Magnesium plays an important role in some enzyme reactions and a deficiency may affect the integrity of the oesophagus (Craddock, 1992:93).  |
| Selenium                        | Selenium is a part of the formation and function of certain proteins. These proteins and their non-enzymatic activity are important in tissue repair and cell-regulatory pathways in carcinogenesis (Mark <i>et al.</i> , 2000:1753).  |
| Zinc                            | In a study done on Zn-deficient rats, it was found that the replenishment of Zn leads to an increase in the apoptosis process (Fong <i>et al.</i> , 1998:5387, Fong <i>et al.</i> , 2001:1531.). The study therefore suggests that Zn replenishment might play a role in the prevention of OC (Fong <i>et al.</i> , 1998:5387, Fong <i>et al.</i> , 2001:1531). <i>In-vivo</i> studies indicated that nutritional Zn deficiency increases cell proliferation and can be reversed by Zn replenishment (Fong <i>et al.</i> , 1998:5384). |

### 2.1.3. Maize contaminated with fumonisins

In many areas worldwide maize is grown as an animal feed, however, in the rural areas of the EC (and other areas in South Africa), maize is the primary staple food (Nel & Steyn, 2002). In the rural area where the current study took place, participants were mostly subsistence farmers and maize is grown to the exclusion of other cereals. According to the Food and Agriculture Organization (FAO), maize production increased during 1961 to 2004 from 9.08 million to 27.62 million metric tonnes in Sub-Saharan African. This is much more than other cereals, including sorghum and millet, which reached 19.39 million and 14.23 million metric tonnes in 2004, respectively (FAO, 2006). Because of this increase in maize consumption exposure to food-borne mycotoxins from maize consumption has increased the risk of a variety of serious chronic diseases.

A study conducted by Marasas *et al.*, (1988:113) found that maize from households in high OC rate areas in South Africa had significantly higher levels of *F. moniliforme* in maize than those from non-affected households in low OC rate areas (Marasas *et al.*, 1988:112). People in the EC rural areas mainly grow maize and to small extent vegetables. When maize supplies are low, the contaminated maize is used for human consumption.

Contaminated maize is also preferred for home brewed beer (Rose, 1972:1354, Craddock, 1992:95, Shephard *et al.*, 2005:9635). Beer is brewed at least once a week, depending on the availability of maize (Rose, 1972:1355) (Figure 2.2).

Interestingly, OC rates increased rapidly in the 1950s to 1960s and it is during this time that the staple food changed from sorghum to mostly maize (Rose, 1979:38, Sammon, 1992:864). Maize is either home-grown and processed at home, or commercially obtained. The home-grown maize kernels (removed from the cob) are either used as whole kernels, stamped, ground or milled at the local shop (Figure 2.3, 2.4 and 2.5). The maize is consumed in various consistencies, from a watery fermented beverage to a dry crumbly porridge (Rose, 1972:1353,1355, Beyers *et al.*, 1979:96). Maize meal dishes are also mixed with vegetables (including spinach, pumpkin and cabbage) or beans (Beyers *et al.*, 1979:96).



Figure 2.2. Traditional home brewed maize beer, fermented from mouldy maize



Figure 2.3. Home-grown maize being stamped Figure 2.4. Home-grown maize being ground



**Figure 2.5. Home-grown maize milled at the local shop**

The preferred ratio is two parts of vegetables to one part of maize, but this depends on the availability of vegetables (Rose, 1972:1358). Samp (dry maize kernels) is also used regularly (Beyers *et al.*, 1979:96).

Fumonisin is largely produced in maize by fungi, *Fusarium verticillioides* (Sacc.) Nirenberg (formerly *F. moniliforme* Sheldon) and *F. proliferatum* (Matsushima) Nirenberg (Marasas, 1996:11, Stockmann-Juvala & Savolainen, 2008:799, Wild & Gong, 2010:77) (Figure 2.6). Gelderblom *et al.*, (1988:1810) first described Fumonisin mycotoxins in 1988. It is produced by a mould that flourishes in a cool environment, and the strain most often associated with OC is *F. moniliforme* (Craddock, 1992:95). *Fusaria* produce two types of toxins, the zearalenones, which are estrogenic (will cause hyperestrogenism in swine), and trichothecenes, which are associated with haemorrhagic disease and carcinogenicity (Craddock, 1992:95). Previous animal research studies (on rats) indicated that these mycotoxins are not only hepatocarcinogenic, but also nephrocarcinogenic (Gelderblom *et al.*, 1991:1250, Howard *et al.*, 2001:2780, Wild & Gong, 2010:78). These fumonisins have also been associated with the high incidence of human squamous cell OC, especially in the rural EC Province of South Africa (Rheeder *et al.*, 1992:356, Wild & Gong, 2010:77) and certain geographic areas in China (Chu & Li 1994:850, Zhang *et al.*, 1997:33, Wang *et al.*, 2000:140, Wild & Gong, 2010:77).



**Figure 2.6. Mouldy maize consumed in the rural Eastern Cape areas**

Food safety authorities around the world have undertaken various international consultations and risk assessments on the consumption of contaminated maize. The International Agency for Research on Cancer (IARC) declared the toxins produced by *Fusarium moniliforme* as being potentially carcinogenic to humans (Vainio *et al.*, 1993:537). Then in 2002, the IARC declared fumonisin B<sub>1</sub> (FB<sub>1</sub>), (which are the most abundant strain), as a group 2B carcinogen (possibly carcinogenic to humans) (IARC, 2002). A risk assessment conducted by the Nordic Council of Ministers recommended an upper tolerable intake for fumonisins of 1 µgkg<sup>-1</sup> body weight day<sup>-1</sup>. This was based on the lowest observed effect level for toxic effects in animals (Eriksen & Alexander, 1998). The Scientific Committee of the European Commission recommended a tolerable daily intake of 2 µgkg<sup>-1</sup> body weight day<sup>-1</sup>, based on toxicity studies on FB<sub>1</sub>. This was mostly based on a no observed adverse effect level (NOAEL) conducted in animal (rodent) studies (European Commission, 2000:83). Subsequently, the 56<sup>th</sup> meeting of the Joint Food and Agricultural Organisation and the World Health Organisation (FAO/WHO) Expert Committee on Food Additives (JECFA), provided a NOAEL of 0.2 mgkg<sup>-1</sup> body weight day<sup>-1</sup> and a safety factor of 100, as a group provisional maximum tolerable daily intake (PMTDI) for FB<sub>1</sub>, fumonisin B<sub>2</sub> (FB<sub>2</sub>) and fumonisin B<sub>3</sub> (FB<sub>3</sub>), alone or in combination, of 2 µgkg<sup>-1</sup> body weight day<sup>-1</sup> (Bolger *et al.*, 2001:103). The Scientific Committee of the European Commission also extended their recommendation on FB<sub>1</sub> alone to include FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub> alone or in combination (European Commission, 2003:83).

Fumonisin risk assessment is conducted by four different methods, including hazard identification, hazard characterisation, exposure assessment and risk characterisation (Kuiper-Goodman, 1999:390). Internationally, risk assessments of fumonisins have been conducted with hazard identification and risk characterisation (Bolger *et al.*, 2001:250). Hazard identification and risk characterisation are qualitative or quantitative methods used to estimate the likely amount of an identified microbiological hazard present in a food item and

to provide information on the actual amount of the hazard consumed (WHO, 2008:2). However, fumonisin exposure assessments require comprehensive knowledge of the food consumption of the target population. Therefore, exposure assessments have been conducted in many developed countries, including Canada (Kuiper-Goodman *et al.*, 1998:369), Denmark (Petersen & Thorup, 2001:221), France (Leblanc *et al.*, 2005:652), the Netherlands (De Nijs *et al.*, 1998:879) and the United States of America (USA) (Humphreys *et al.*, 2001:211).

On the other hand, exposure assessments in developing countries, including Brazil (Machinski & Soares, 2000:875), Argentina (Solovey *et al.*, 1999:325), Iran (Yazdanpanah *et al.*, 2006:395) and South Africa (Gelderblom *et al.*, 1996:279) have been impossible to execute because of a lack of adequate and accurate food consumption data. Previously, exposure assessments were conducted in the rural areas of the EC Province of South Africa (Marasas, 1997:403) based on estimated maize consumption. These estimations were published by Thiel *et al.*, (1992:8), and were mostly based on published carbohydrate intakes obtained from 24-hour recall questionnaires. The questionnaires were administered in 1977 to lactating mothers in both urban and rural areas of the Ciskei (now part of the EC), which is adjacent to the high OC risk areas. The dietary habits of both populations (Transkei and Ciskei) were similar and data could be extrapolated to the Transkei (Shephard *et al.*, 2002:394).

## **2.2. DIETARY INTAKE AND NUTRITIONAL DEFICIENCIES IN RESIDENTS LIVING IN THE EASTERN CAPE**

As mentioned earlier, very little is known about the cultural and habitual dietary intake of residents in the EC, however the high rates of OC in these areas are suggestive of some dietary components being implicated in the aetiology of OC (Van Rensburg, 1987:9, McGlashan, 1988:98, Muñoz & Castellsagué, 1994:653, Blot, 1994:407, Levi *et al.*, 2000:259, Danaei *et al.*, 2005:1787).

Vegetables, including pumpkin and cabbage are grown in some home gardens, although these are dependent on the weather and rainfall. Some wild plants (*imifino*) are also picked in the veldt and consumed as a vegetable. Children pick wild fruit in the veldt when available, but bananas, oranges, guavas and peaches are sometimes home-grown (Rose, 1972:1354).

Meat (mostly chicken) and eggs are consumed weekly (Beyers *et al.*, 1979:97, Bembridge, 1987:425). Large portions of red meat are only consumed during traditional feasts and

celebrations when an animal is slaughtered (Rose, 1972:1354). Fresh milk is rarely consumed and given only to babies (Rose, 1972:1354). Milk is largely left over-night to become sour, and is a favourite drink among the Xhosas (Rose, 1972:1353).

Bread (mostly home-made from maize meal or from wheat flour) (Figure 2.7.) is consumed daily and predominantly without fat or margarine (Beyers *et al.*, 1979:97).



**Figure 2.7. Traditional home-made maize bread**

Micronutrient deficiencies in the EC that may contribute to the development of OC have been identified and include: riboflavin, niacin, folic acid, vitamin A, vitamin E, Zn, Se and Mg (Groenewald *et al.*, 1981:967, Van Rensburg, 1987:10, Craddock, 1992:93, 94, Eskelson *et al.*, 1993:122, Mark *et al.*, 2000:1753, Fong *et al.*, 2001:1531). These specific nutrient deficiencies can lead to chronic oesophagitis, atrophy of the mucosa, dysplasia, and ultimately to the development of OC (Jaskiewicz *et al.*, 1987:3).

### **3. DEVELOPMENT OF CULTURALLY SENSITIVE DIETARY ASSESSMENT METHODOLOGY**

When developing dietary assessment methodology, understanding the culture of the target population increases the level of communication and therefore, the accuracy of the data collected (Cassidy, 1994:190S, Teufel, 1997:1174S, Jerome, 1997:1166S, Strolla *et al.*, 2006:474). Because of this, a combination of qualitative and quantitative research methods should be used when a new culturally sensitive dietary assessment method is developed (Cassidy, 1994:192S, Kigutha, 1997:1169S, Satia *et al.*, 2000:940, Strolla *et al.*, 2006:474, Pierce *et al.*, 2007:501).

A review of previous dietary surveys conducted in the target population should provide important information regarding the types of food and average portion sizes that are consumed (Teufel, 1997:1175S, Cade *et al.*, 2002:569). Once this background information is collected, focus group discussions (FGD's) can provide additional information (Teufel, 1997:1175S, Strolla *et al.*, 2006:474, Pierce *et al.*, 2007:501).

Being aware of the knowledge, beliefs and attitudes of participants regarding food and dietary practices helps the researcher understand their food habits and traditions (Teufel, 1997:1175S). Focus group discussions are a useful way of learning about local customs, beliefs and practices (Kigutha, 1997:1169S, Leshabari *et al.*, 2006:27). This information will make the interviewers aware of the local taboos, and rules associated with dietary practices. Information regarding the acquisition, processing and preparation of food is also important. (Kigutha, 1997:1169S, Jerome, 1997:1166S). Other factors that can be discussed at FGD's are the way food is served and distributed among the family (Kigutha, 1997:1169S, Jerome, 1997:1166S, Taren *et al.*, 2002:1001).

Cooking sessions (where somebody from the area prepares the culturally specific dishes) provide valuable information regarding specific food preparation techniques, recipes, ingredients and preparation of dishes. From this information, a culture-specific database with the nutrient content of mixed dishes can be developed (Teufel, 1997:1177S).

### **3.1. FACTORS AFFECTING THE CHOICE OF DIETARY ASSESSMENT METHODS**

Various factors affect the choice of the dietary assessment method to be used. The disease under investigation and the characteristics of the target population will mostly determine the type of dietary assessment method that should be used. The disease being studied will also determine whether dietary patterns or exact nutrient intake is required and whether this should be at a group or individual level. Information acquired about dietary patterns, for instance, cannot provide information regarding the exact nutrient intake. Dietary patterns will take the effect of the overall habitual diet into account. (Hu *et al.*, 1999:249).

Specific characteristics of the target population will also influence the choice of the selected dietary assessment methods and tools (Zulkifli & Yu, 1992:681). These characteristics, presented in Table 2.3, will determine the way in which questions are asked (Buzzard & Sievert, 1994:276S). The findings summarized in Table 2.3 emphasize the importance of collecting the right information from people in the target area.

**Table 2.3. Factors influencing type of dietary assessment methods and tools to be used in nutrition studies**

| Factors                         | Choice of dietary assessment method and tool  |
|---------------------------------|---|
| Logistics                       | Time availability and costs will determine which type of dietary assessment method to use (Kigutha, 1997:1169S).  |
| Level of education              | Rural people might not have the knowledge of measuring units but be more accurate with local measures (cups, glasses, bundles, etc.) (Kigutha, 1997:1170S).<br><br>If participants are largely illiterate or semi-literate, face-to-face interviews will be more appropriate than self-administered questionnaires (Kigutha, 1997:1169S). |
| Language                        | Translating questionnaires can cause misconception since words and meaning might differ from one language to another (Cassidy, 1994:191S). Different dialects in one language might also change the meaning of questions (Cassidy, 1994:191S).  |
| Age                             | If the target population includes older people, the use of interviewers might be more effective, since older people have more trouble completing questionnaires, mostly because of sickness, poor sight or shorter memory (Goldbohm <i>et al.</i> , 1995:426).  |
| Gender                          | Females are more aware of their food intake because they are mainly responsible for food purchasing and preparation (Bazzarre <i>et al.</i> , 1983:211, James, 2004:363).   |
| Food availability (procurement) | Rural people are more dependent on home-produced food, making their diets monotonous and simple (Kigutha, 1997:1169S).  |
| Seasonal availability           | Data should be collected during more than one season, especially in rural or farming communities, where participants are more dependent on the weather and season for the availability of food (Kigutha, 1997:1172S, Romieu <i>et al.</i> , 1997:1160S).  |

### 3.2. EXISTING DIETARY ASSESSMENT METHODS

Various methods are available to determine dietary patterns and nutrient intake. The choice of method depends mostly on the level of precision required, available resources and the characteristics of the study population (Zulkifli & Yu, 1992:681, Hu *et al.*, 1999:249, Wrieden *et al.*, 2003:3). Because each dietary assessment method has its own advantages and

disadvantages (Addendum 3), the type of method that is chosen, must be considered with care.

### 3.2.1. 24-Hour recall method

When using 24-hour recalls as a dietary assessment method, participants are expected to recall the precise food items and dishes consumed within the previous 24-hours (Gibson, 2005:42). Four steps are required when conducting a 24-hour recall; initially a participant is expected to provide a list of food items and dishes consumed during the previous 24-hours. Participants then provide detailed information about the mentioned food items and dishes, including cooking methods and recipes (where possible). Afterwards participants provide portion sizes of each food item or dish and lastly the list is reviewed to determine if anything is forgotten (Wrieden *et al.*, 2003:4, Gibson, 2005:42.). Probing questions can be used to remind the participant of his day and food models, utensils or photographs should be used to improve portion size estimation (Gibson, 2005:42).

The 24-hour recall method is often used in dietary studies because it is quick, inexpensive and easy to determine the mean consumption of the population (Bingham *et al.*, 1988:86, Charzewska, 1994:157S). This method can be regarded as a culturally sensitive dietary assessment method, since it does not make assumptions about the participants' consumption, and no prior knowledge of the food consumption of participants is required (Cassidy, 1994:193S). The method therefore requires highly trained interviewers, making it impractical to use in large studies (Church, 2006:264). It is however imperative that the 24-hour interview and coding process are standardised and pretested to prevent errors (Gibson, 2005:43).

Nevertheless, because of the low reliability of the data and the large within-person variation, nutrient estimates obtained from single 24-hour recalls cannot be regarded as accurate when measuring usual intake (Bazzarre & Yuhas, 1983:212, Gibson, 2005:42, NCI, 2010). For the same reason data obtained by single 24-hour recalls should not be used for ranking participants (Bingham *et al.*, 1988:86, Briefel *et al.*, 1992:960, Smith, 1993:S7). Addendum 3 provides further information regarding the advantages and disadvantages of 24-hour recalls.

When repeated 24-hour recalls are used in large dietary surveys in order to determine the proportion of the population at risk for certain nutrition deficiencies [comparison with recommended dietary allowances (RDA) or the Estimate Average Requirements (EAR)] it is

suggested that the data be adjusted for within-person variation (Mackerras & Rutishauser, 2005:657).

In order to improve within-person variation, repeated 24-hour recalls can be conducted to provide an average food intake of individuals over a pre-selected period (Gibson, 2005:44). The actual number of recalls required to determine an individual's usual intake will depend on the extent of dietary variation. The more variety in the diet, the more 24-hour recalls will be required (Basiotis *et al.*, 1987:1638, Gibson, 2005:44, Ma *et al.*, 2009:557). This in turn is also determined by the type of nutrient being investigated, the seasonal variability and the study population (Gibson, 2005:44). It is further recommended that non-consecutive days are used where possible (Gibson, 2005:44).

### 3.2.2. Food frequency questionnaires

The food frequency questionnaire (FFQ) is the most frequently used method in dietary assessment, especially in studies that focus on chronic diseases (Teufel, 1997:1173S, 1174S, Nelson *et al.*, 1997:134). This type of questionnaire consists of a pre-determined list of frequently consumed food items (Teufel, 1997:1173S, Flegal, 1999:1340S, Wrieden *et al.*, 2003:5). Participants provide information on how frequently (daily, weekly, monthly, etc.) a food item is consumed. A set of pre-determined portion sizes may accompany the frequencies of food consumption (Flegal, 1999:1340S, Wierden *et al.*, 2003:5). This is then called a quantitative food frequency questionnaire (QFFQ) (Briefel *et al.*, 1992:960, Teufel, 1997:1174S, Wierden *et al.*, 2003:5).

The frequency of intake of a food item is summed (in grams) over the intake of a month, then divided by 28 / 30 days to provide food consumption per day. However, this method makes assumptions regarding the accuracy of the recording, the storage, preparation, bioavailability of food, as well as the food composition tables that are used for analyses (Romieu *et al.*, 1990:864).

Using the FFQ has various disadvantages (Addendum 3) including the overestimation of food intake (Kushi, 1994:181S, Rothenberg, 1994:733, Erkkola *et al.*, 2001:473). It includes only the most frequently consumed mixed dishes, and expects participants to report separately on the main ingredients of the other mixed dishes (Briefel *et al.*, 1992:960, Teufel, 1997:1174S, Flegal, 1999:1341S, Schaefer *et al.*, 2000:750, Cade *et al.*, 2002:568). Details of food preparation, recipe and brand names are not included (Briefel *et al.*, 1992:960, Teufel, 1997:1174S). This decreases the level of accuracy, since these factors are important

when nutrient intake is estimated (Briefel *et al.*, 1992:960, Schaefer *et al.*, 2000:750, Wierden *et al.*, 2003:5).

Because of these disadvantages, the use of FFQs in diet and cancer related studies has become the subject of intense debate among nutrition epidemiologists (Kristal *et al.*, 2005:2826, Willett & Hu, 2006:1757). In one such a debate, Willett & Hu (2006:1757) concluded that no single FFQ is appropriate for all participants and all studies. They emphasise the importance of the use of specific detailed FFQs in cancer related studies, which for instance include information such as cooking methods (Willett & Hu, 2006:1758).

However, questionnaire length remains another heated debate among researchers. According to Subar *et al.*, (2001:408) questionnaire length does not necessarily imply accuracy, and a simple, easy-to-administer questionnaire may compensate for the length (Subar *et al.*, 2001: 408,409). On the other hand, a meta analyses conducted by Molag *et al.*, (2007:1476) indicated that longer FFQs are better able to rank participants according to their nutrient intake than shorter FFQs. It can therefore be concluded that evidence points to the fact that questionnaire length does not have an impact on accuracy of the information and the length can therefore be either long or short. The quality of food-frequency data can also be improved in the following ways: 1) by increasing the number of food items on the food list, 2) by using trained interviewers, 3) by providing more detail on quantities of portion sizes, or 4) by providing more detail on consumption frequency (Willett, 1994:172S, Kumanyika *et al.*, 1997:1123S). Additionally, interviewers can provide assistance with complicated aspects such as portion size determination (Kumanyika *et al.*, 1997:1123S).

It is also important to bear in mind that because the FFQ is based on the recall of consumption, the response of participants may be biased by their food preferences. This means that participants might report a higher frequency of consumption of a food item they like, and less frequently for one they dislike (Drewnowski & Hann, 1999:35). A review by Molag *et al.*, (2007:1476) concluded that the use of portion size questions does not necessarily improve the precision of the FFQ. They also concluded that FFQs are generally able to differentiate between sub-groups in a population (such as gender and age), although the extent of this may be underestimated (Molag *et al.*, 2007:1476).

The picture-sort food frequency method entails the use of picture cards depicting various food and beverage items. Participants sort these cards into various piles, reflecting the frequency of consumption of each food item (Kumanyika *et al.*, 1997:1124S) (Addendum 3).

### 3.2.3. Weighed diet record

The weighed diet record is considered to be the only fully quantified dietary assessment method (Black *et al.*, 1991:583). Participants weigh food prior to consumption, as well as the leftovers. The total food intake is calculated by subtracting the leftovers from the initial dished up amount. The estimated diet record is used to estimate portion sizes by using household measures instead of scales (Wrieden *et al.*, 2003:3).

The number of records collected per person is once again determined by the dietary variability of the participants' usual diet, as well as the nutrient under investigation, the seasonal variability of the foods consumed and the study population (Gibson, 2005:45). Although this method is regarded as the closest to a gold standard, it has some disadvantages (Black *et al.*, 1991:583) (Addendum 2). For instance, the burden is on the participant to keep an accurate record, therefore this method can only be used in people who are highly motivated and literate (Bingham *et al.*, 1988:59, Hankin & Wilkens, 1994:199S). It is also known that participants may change their daily dietary habits to make the recording of their intake easier or to avoid embarrassment regarding their eating habits (Hankin & Wilkens, 1994:200S, Macdiarmid & Blundell, 1997:200).

### 3.2.4. Diet history

A diet history is a dietary assessment method that determines food intake and meal patterns over a longer period, such as a month (Gibson, 2005:45). The method is used during two different phases. Initially the participant provides a detailed overall description of his eating pattern, including usual portion sizes and frequency of consumption. The second phase of the method serves as a cross check of the first phase and consists of a frequency questionnaire of specific food items and dishes (Gibson, 2005:46).

The time period covered by the diet history may vary. Reliability and validity results indicate that this method is more reliable and valid for periods shorter than a month, although a maximum period has not been established (Gibson, 2005:46). Respondent burden is large, as interviews can take as long as 2 hours per participant (Slattery *et al.*, 2000:580).

### 3.3. PORTION SIZE ESTIMATION

Portion sizes can be determined in various ways, including those of direct weighing of food, visually estimating weights or by estimating portion sizes with aids such as food models or household measures (Young & Nestle, 1995:152, Wierden *et al.*, 2003:7). The ideal method of portion size determination is to allow the participant to provide the portion size. However, this will increase time and cost in completing the questionnaire and entering the data.

Numerous errors occur when participants have to estimate portion sizes of food items consumed. The accuracy thereof depends on the participants' ability to describe portion sizes (Lucas *et al.*, 1995:65, Nelson *et al.*, 1996:32, Robinson *et al.*, 1997:118, Taren *et al.*, 2002:1002, Noethlings *et al.*, 2003:514). It is therefore the researchers' responsibility to assist participants with this complicated task (Chambers *et al.*, 2000:892).

Noethlings *et al.*, (2007:2782) suggested the use of "fitted" portion sizes. This is a combination of pre-determined portion sizes and portion sizes obtained from keeping a 24-hour dietary record. However, this method is not totally satisfactory. The difficulty lies in obtaining enough 24-hour dietary records before the onset of the study. To circumvent this barrier, researchers often provide a set of pre-determined portion sizes from earlier dietary studies (Hunter *et al.*, 1988:1246, Wierden *et al.*, 2003:7). Between-person and within-person variation makes the use of pre-determined portion sizes difficult and provides less accurate data (Hunter *et al.*, 1988:1241), especially because this method is based on the assumption that participants have a usual portion size (Noethlings *et al.*, 2003:514). However, there are some advantages in the use of a QFFQ especially in large epidemiological studies where the questionnaire should ideally be kept simple (Noethlings *et al.*, 2003:514).

A selection of visual aids can be used to help participants determine usual portion sizes. These include the use of food models (replicas), bowls, plates, measuring cups, spoons, drawings and food photographs (Hankin & Wilkens, 1994:199S, Lee & Nieman, 2007:104). According to Posner *et al.*, (1992:740) two-dimensional methods are just as effective as three-dimensional methods in determining portion sizes.

The type of visual aid to be used should be determined by the population to be studied. In a study on the accuracy of portion sizes as reported by South African teenagers, it was found that 58% of black children and 54% of white children scored correctly when using three-

dimensional models. When two-dimensional models (drawings) were used, 57% of black and only 38% white children scored correctly (Steyn *et al.*, 2006:31).

Chambers *et al.*, (2000:896) recommend that portion size estimation aids must be flexible to allow the participants to resize and shape the specific portion size consumed. By using something like clay or multiple size pictures or photographs, participants do not have to reshape the portion sizes mentally. It has been suggested that portion sizes should be stratified according to gender and age (Clapp *et al.*, 1991:319, Subar *et al.*, 2000:283) although a study conducted by Noethlings *et al.*, (2003:514) indicated very little benefit in grouping specific portion sizes.

### **3.4. DEVELOPMENT OF A PORTION SIZE PHOTOGRAPH SERIES**

Three cognitive tasks are involved when photographs are used in a series for portion size estimation.<sup>2.1</sup> Perception is a participant's ability to report the amount of food, which is present to the amount depicted in the photograph (Nelson *et al.*, 1994:649). Conceptualisation is the participant's ability to construct a mental picture of a portion size that is not present, and then to be able to relate this to a photograph (Nelson *et al.*, 1994:649). The last cognitive task is that of memory. Participants must be able to recall the actual amount of food they consumed accurately. The above-mentioned three tasks will influence the accuracy of each other. For instance, memory will influence the accuracy of conceptualisation, which is important, since this will be expected of participants when a dietary recall is conducted (Nelson *et al.*, 1994:649, Nelson *et al.*, 1996:32).

Photographs are easy to use in dietary surveys, because they can be copied, carried easily, and can visually represent various portion sizes (Lucas *et al.*, 1995:65,66, Nelson *et al.*, 1996:32 Robinson *et al.*, 1997:123) and participants find it easy and enjoyable (Small *et al.*, 2009:33). However, this method is not without errors, which include those of recall and perception (Lucas *et al.*, 1995:66). For instance, not everybody's perception of a small size is the same (Robinson *et al.*, 1997:118).

Data on portion size estimation with the use of photographs indicate that food photographs provide more reliable data at group level than studies without portion size photographs (Foster *et al.*, 2006:513, Small *et al.*, 2009:33).

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<sup>2.1</sup> A photograph series is a set of photographs (in this case three) depicting different portion sizes of a specific food item (Nelson & Haraldsdóttir, 1998b:231).

Captions on photos may improve recognition of food items. Although photographs help participants to visualize foods consumed the captions may cause participants to focus only on foods shown on the photographs and thus omit other foods consumed on a habitual basis (Kumanyika *et al.*, 1997:1128S).

Commercially available dietary aids (food models) are difficult to use in the South African context (Steyn *et al.*, 2006:29). These aids are generally imported, expensive and do not represent the variety of cultural dishes and recipes consumed in South Africa (Steyn *et al.*, 2006:29). Furthermore, because of the high levels of illiteracy in rural South Africans, it is important that dietary assessment methods be visual and specific to the culture under investigation (De Souza, 2001:242, Steyn *et al.*, 2006:29). A good example of such a portion size tool developed in South Africa is the Food Portion Photograph Book (FPPB) developed for the Transition, Health and Urbanisation in South Africa (THUSA) study (Venter *et al.*, 2000:205).

#### **3.4.1. Factors influencing the design of a portion size photograph series**

When a portion size photograph series is developed, consultation with the study population is crucial, in order to obtain reliable information of different foods consumed and their portion sizes. Published data based on the target population's eating habits will also provide information on specific portion sizes of food items consumed (Nelson & Haraldsdóttir, 1998b:231).

An advisory group, comprising representatives of those participating in collecting the dietary data, should be involved in developing the photograph series (Nelson & Haraldsdóttir, 1998b:231). Once a portion size photographic series has been developed, it is important to return to the population to determine its practical use (Nelson & Haraldsdóttir, 1998b:231). A focus-group discussion can provide valuable information on how the population will perceive the newly developed series. Goodwin *et al.*, (2001:926) described the development of a portion size model booklet for 6 - 17-year-olds to be used in conjunction with an activity record. After its development, focus-group discussions indicated that the participants were enthusiastic and able to use the tool (photograph series with food and activity record). However, participants between the ages of 6 and 11 years old (and their parents) had difficulty with the tool and changes had to be made to both before the onset of the pilot phase (Goodwin *et al.*, 2001:928). The portion size booklet was eventually discarded because children could not use it by themselves (Goodwin *et al.*, 2001:928).

### 3.4.2. The format of the portion size photographs

The main purpose of a portion size photographic series is to decrease the size of error during the estimation of portion sizes. This error usually depends on the participants' ability to describe usual portions consumed (Nelson & Haraldsdóttir, 1998b:232). Factors that influence the participants' ability to report accurate portion sizes relate to the format of the photographs. The size of the actual photograph can vary, although Nelson and Haraldsdóttir (1998b:232) suggested a minimum size of 6 x 8 centimetre (cm). The number of portion sizes depicted per dish can vary from single portions, three (small (S), medium (M) and large (L)) portions, to four, six or eight portions (Kuehneman *et al.*, 1994:550, Nelson & Haraldsdóttir, 1998b:231, Robson & Livingstone, 2000:192, Chambers *et al.*, 2000:896).

The range of weights depicted must be determined with care and must include intervals consumed by the majority of the population (Nelson & Haraldsdóttir, 1998b:231). Addendum 4 describes various factors that influence the format of portion size photographs.

## 4. VALIDATION OF A PORTION SIZE PHOTOGRAPHIC SERIES

Validation of portion size photographs is as essential as that of questionnaires. Validation provides information on how the participants perceive the portion sizes depicted on the photographs (Lucas *et al.*, 1995:73).

The researcher can test the visual aids depicting portion sizes by allowing respondents to interpret them in terms of the usual portion sizes consumed by them. It is therefore imperative to ensure that the portion sizes depicted represent "usual" portions (Hunter *et al.*, 1988:1248, Lillegaard *et al.*, 2005:612).

There are three different, basic psychological skills needed for the validation of portion size photographs namely, the skill of perception,<sup>2.2</sup> conceptualisation<sup>2.3</sup> and memory<sup>2.4</sup>. This type of validation will be used if the portion size photographs are used along with 24-hour recalls (Nelson & Haraldsdóttir, 1998a:222).

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<sup>2.2</sup> Perception: is the ability to recount the amount of food present to the amount depicted on the photographs (Nelson *et al.*, 1994:649).

<sup>2.3</sup> Conceptualisation: is the ability to develop a mental picture of a portion size that is not present and to be able to relate this to a photograph (Nelson *et al.*, 1994:649).

<sup>2.4</sup> Memory: is the ability to recall the actual amount of food consumed (Nelson *et al.*, 1994:649).

When planning the validation of photographs, it is important to keep the above-mentioned contribution of each mental construct in mind and to know what type of errors will contribute to the validation of the portion size photographic series. These constructs should then be related to the type of dietary method for which the photographs will be used (Nelson & Haraldsdóttir, 1998a:223). By knowing the psychological components that contribute to estimation errors, improvements can be made to the tool (Nelson & Haraldsdóttir, 1998a:219).

If a detailed validation study is not possible, the photographs should be tested in the context that they are to be used in the main study (Nelson & Haraldsdóttir, 1998a:223).

Visual characteristics of a food item can lead to over- or under-reporting, and unusual shapes and sizes should thus be avoided (Lucas *et al.*, 1995:73). The manner of presenting food may also influence validation outcomes. For instance, if there is a difference between the shape and size of a real food item and the portion size depicted on the photograph, participants may over- or under-estimate the portion size. During validation, foods should be prepared in exactly the same way as those depicted on the photographs (Lucas *et al.*, 1995:73, Venter *et al.*, 2000:212).

If the purpose of the study is to determine the type of errors related to assessment of specific food items and their portion sizes the reference<sup>2.5</sup> measure should be actual food items that are weighed (Nelson & Haraldsdóttir, 1998a:226). Either researchers pre-determine portion sizes and participants have to identify the portions (covert), or else participants can be asked to dish up their own estimated usual portion size. If validation studies are conducted in the above-mentioned way, the largest errors may be technical errors occurring during the weighing process (Nelson & Haraldsdóttir, 1998a:219).

#### **4.1. FACTORS INFLUENCING THE VALIDATION OF A PORTION SIZE PHOTOGRAPHIC SERIES**

From the results of previous photographic portion size validation studies, it was found that people are biased towards the medium portion size, and tend to over-estimate small portions and under-estimate larger portion sizes (the flat slope syndrome) (Nelson *et al.*, 1994:660, Lucas *et al.*, 1995:73). This phenomenon was observed in a study done in South Africa when portion size photographs were validated for the THUSA project (Venter *et al.*,

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<sup>2.5</sup> In this case, the reference measure is the actual food, while the test measure is the photographs.

2000:212). This has also been observed in studies done abroad (Nelson *et al.*, 1994:660, Lucas *et al.*, 1995:72, Robinson *et al.*, 1997:122).

Lucas *et al.*, (1995:73) found that participants consistently over- or under-estimated portion sizes. The authors suggested that this may have been related to their visual perception. Various factors appear to play a role in the way participants perceive portion sizes depicted on photographs. Because of this, the validation of portion size photographs should be carefully planned. The literature indicates that numerous factors influence the validity of food photographs. These include characteristics of the participants, the photographs themselves, and characteristics of the food items depicted in the photographs.

#### **4.1.1. Characteristics of the participants**

- Gender of participants

Some studies have found that females report more accurately than males, while others reported no differences between genders (Yuhas *et al.*, 1980:1475, Robinson *et al.*, 1997:122, Venter *et al.*, 2000:214). Males tend to under-estimate portion sizes (Nelson *et al.*, 1994:661, Venter *et al.*, 2000:214). This is particularly the case between males and females with the same level of exposure to food (chefs for instance) (Young & Nestle, 1995:154). Females generally have a better concept of their dietary intake than males, and are usually more familiar with portion sizes expressed in household measures or grams (g) (Pietinen *et al.*, 1988a:663, James, 2004:363).

- Age of participants

People older than 65 years and young children may over-estimate portion sizes (Nelson *et al.*, 1994:661; Robinson *et al.*, 1994:122, Young & Nestle, 1995:154, Nelson *et al.*, 1998:22).

In a study by Foster *et al.*, (2006:509), it was found that children in primary school estimated portion sizes more accurately from age-appropriate food photographs than from photographs designed for adults. Children may be misclassified (for instance classified as having a low intake of a food / nutrient when in fact they are not), because of their inability to utilize specific mental skills required when photographs are used. This may cause a systematic bias especially when single portions (versus a series) are used (Nelson & Haraldsdóttir, 1998a:221, Robson & Livingstone, 2000:192).

- Body mass index of participants

Participants with a body mass index (BMI) > 30 kg/m<sup>2</sup> tend to under-estimate portion sizes, possibly because they consume larger portions than those with normal BMIs (Nelson *et al.*, 1994:661, Nelson & Haraldsdóttir, 1998a:219).

- Education level of participants

Different levels of education of participants may lead to different abilities regarding perception and conceptualisation of photographs (Nelson & Haraldsdóttir, 1998a:219). However, results of the study done by Venter *et al.*, (2000:215) in black adults showed no significant differences between level of education and portion size estimation.

- Occupation of participants

Participants working in food-related environments (such as restaurants) may have a better perception of portion sizes (Nelson & Haraldsdóttir, 1998a:219).

- Training of participants in portion size estimation prior to validation of the photographic series

Training participants in portion size estimation before conducting such a study may decrease estimation error, especially for amorphous<sup>2.6</sup> foods (Yuhas *et al.*, 1989:1475, Weber *et al.*, 1997:177).

#### 4.1.2. Characteristics of the photographs

- Size of photographs

Photograph size does not appear to influence accuracy, although the minimum acceptable size is 75 x 100 mm (Nelson *et al.*, 1994:662). Life-size photographs can be used, but are difficult to transport and expensive to reproduce (Venter *et al.*, 2000:215).

- Colour of photographs

Quality black-and-white photographs can render the same results as colour photographs; however, colour photographs tend to hold peoples' attention longer (because they are attractive). This may be beneficial in long, tedious interviews (Nelson *et al.*, 1994:661).

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<sup>2.6</sup> Amorphous foods are food items that do not have their own shape, but take on the shape of the container, for instance spaghetti (Yuhas *et al.*, 1989:1475).

- Number of portion sizes

Photographs depicting single portions were associated with larger errors of estimation than the use of photographs depicting more than one portion size (Nelson *et al.*, 1994:659). The range of portion sizes should cover the full range of food quantities that may be consumed (Nelson & Haraldsdóttir, 1998b:232).

Volume should be depicted with care on photographs, as well as shapes and sizes of certain food items, because people tend to use visual cues as indicators for portion size (Lucas *et al.*, 1995:66, 73).

- The order of presentation of portion sizes

Presenting the photographs in their natural order (small to large) may cause a reporting bias, depending on how the participants perceive themselves (i.e., whether they see themselves as small or big eaters) or how they think the interviewers perceive them. On the other hand, showing the participant the photographs at random will increase the burden on the participant to choose the correct portion size. (Nelson & Haraldsdóttir, 1998b:233).

#### **4.1.3. Food items depicted in a photographic series**

- Frequency of consumption

Foods consumed as relishes and those eaten in small or infrequent amounts may be estimated less accurately than frequently consumed food (Venter *et al.*, 2000:216).

- Preparation of food items depicted in the photographs

The actual food must be prepared in exactly the same way as depicted by the photographs to prevent recall bias (Lucas *et al.*, 1995:73). Visual clues also influence estimation because participants match shape rather than size, for instance, the number of pieces or thickness of the pieces (Lucas *et al.*, 1995:66, Venter *et al.*, 2000:214). If participants cannot determine depth on the photographs, they use the surface area of the plate as an indicator of portion size (Lucas *et al.*, 1995:73). This type of problem can be resolved by reducing the camera angle to make the depth of the food items more clear (Nelson *et al.*, 1994:660).

- Type of food depicted in photographs

It is easier to determine the portion sizes of some specific food items from photographs (Robson & Livingstone, 2000:190). In their study, Robson and Livingstone (2000:190) found that their participants (thirty adults aged 18 - 38 years) had difficulty estimating portion sizes of cheese, while the portion sizes of orange juice were more accurate.

It is easier to estimate solid food accurately compared to amorphous foods (Venter *et al.*, 2000:213). Because amorphous foods do not hold their shape, it is difficult to find visual cues on the photographs to match with the actual food (Venter *et al.*, 2000:214). In the study by Venter *et al.*, (2000:207), it was observed that solid pieces of food (for instance fried fish, apples etc.) were easier to identify than amorphous foods such as soft porridge, samp and beans (*Umngqusho*) and margarine spread on bread.

- The size of containers used in photographs

People tend to estimate the contents on a photograph better if the dishes are depicted in smaller rather than in larger containers (Yuhua *et al.*, 1989:1475).

Regardless of the afore-mentioned factors influencing accuracy of estimating portion sizes from photographs; it is possible that the portion size errors obtained might be from different factors other than those described. Additional factors may include the motivation of the participant, the participant's mood or the degree of cooperation (Robson & Livingstone, 2000:183).

## 5. VALIDATION OF DIETARY ASSESSMENT METHODS

Validation of a dietary method / tool is the process of determining the accuracy with which a dietary assessment method measures what it should measure (Block & Hartman, 1989:1135, Nelson, 1997:241, Katzenellenbogen *et al.*, 2001:90, Masson *et al.*, 2003:313). Validating dietary assessment methods or tools is imperative as it provides an understanding of the measurement error, the causes of the measurement error and can be used to find ways to improve these errors (Bellach, 1993:S42). Validation of dietary assessment methods and tools are crucial since it provides information on possible misclassification, especially in studies where diet-disease association is studied (Nelson, 1997:241).

Validating dietary assessment methods and tools are difficult because existing methods depend on the ability of participants to provide accurate dietary information (Bingham *et al.*,

1994:227S). Relative or criterion validity is mostly used in dietary assessment validation studies by comparing the test method against another, more valid method (the gold standard) (Lee & Nieman, 2007:98). The gold standard is used as a measure against which to determine the amount of correctly identified values (Katzenellenbogen *et al.*, 2001:92). Because of the lack of “gold standards” in dietary assessment, the degree of measurement error during the estimation of habitual dietary patterns cannot truly be determined (Sempos, 1992:1131, Kohlmeier, 1993:S2, Bingham & Day, 1997:1130S, Nelson, 1997:242, Taren *et al.*, 2002:1002).

Validation studies can therefore not compare a test method with the absolute truth, but only against a better-quality method. Because both methods are inaccurate, measurement errors must not correlate with each other (to avoid high estimates of validity) (Willet *et al.*, 1985:52, Gibson, 2005:152). Poor validity is not necessarily attributable to errors associated with the test measure, but might be errors that occur while conducting the reference method (Cade *et al.*, 2002:575, Lee & Nieman, 2007:98). To prevent this from occurring, a third criterion method (such as biomarkers) could be introduced (Lee & Nieman, 2007:98).

## **5.1. APPROACHES USED TO VALIDATE DIETARY ASSESSMENT METHODS**

Various approaches have been used to validate dietary assessment methods. Because of the lack of a gold standard, it is preferable to test the dietary assessment method against more than one measure. Addendum 5 shows the different methods used to determine the validity of dietary assessment methods, as well as the advantages and disadvantages of each method.

### **5.1.1. Comparison of a dietary assessment method against another**

A newly developed FFQ can be tested either against multiple food records or against multiple 24-hour recalls (Pietinen *et al.*, 1988b:670, Block & Hartman, 1989:1135, Cade *et al.*, 2002:575, Wrieden *et al.*, 2003:3, NCI, 2010). Both these methods provide detailed, quantitative estimates of dietary intake over a selected period, including weekdays and weekends (Pietinen *et al.*, 1988a:656, Block & Hartman, 1989, Teufel, 1997:1177S). Weighed records used as the reference method may under-estimate the validity of the test method (the FFQ being tested), if it only provides information on a single day of consumption (Block & Hartman, 1989:1135).

Using 24-hour recalls as the reference method is regarded as not being as accurate as using weighed records, because like the FFQ, these recalls rely on memory and portion size estimation and therefore may introduce correlated errors (Taren *et al.*, 2002:1002, Kipnis *et al.*, 2003:22). However, if participants in the target population are mostly illiterate, or if collecting weighed records are too demanding, several 24-hour recalls can be used as the reference method (Kaaks, 1997:1233S, Nelson, 1997:247, Cade *et al.*, 2002:575, Gibson, 2005:151, NCI, 2010).

A sub-study of the European Prospective Investigation into Cancer and Nutrition (EPIC) study compared a FFQ with 16 days of weighed records over one year (Bingham *et al.*, 1997:S141). Spearman correlations ranged from 0.39 [potassium (K) to 0.55 (fat and carbohydrates (CHO)] and implied that the FFQ over-estimated the weighed records significantly (Bingham *et al.*, 1997:S141) (Addendum 6).

In a study conducted by Kroke *et al.*, (1999:442) (part of the German side of the EPIC study) the FFQ was compared with 12 24-hour recalls. Pearson correlations coefficients ranged between 0.47 (for polyunsaturated fatty acids and fibre) and 0.83 for alcohol (unadjusted) (Addendum 6).

Researchers validating the Block 98 FFQ for Canadian females, compared FFQ data with only two 24-hour recalls, and Pearson correlations coefficients ranged between 0.07 (cholesterol) and 0.68 (thiamine and vitamin E) (Boucher *et al.*, 2006:88). Interestingly, the correlations for macronutrients were smaller than those for micronutrients (Addendum 6), which they attributed to the small sample size ( $n = 166$ ) and the fact that only two recalls were included in the validation study (Boucher *et al.*, 2006:89).

Past validation studies (Addendum 6) compared FFQs with weighed records or 24-hour recalls (one month to a year) as reference methods (Katsouyanni *et al.*, 1997:S122, Bingham *et al.*, 1997:S141, Kroke *et al.*, 1999:442, MacIntyre *et al.*, 2001b:64, Parr *et al.*, 2002:775, Shu *et al.*, 2004:20, Boucher *et al.*, 2006:85). Correlation coefficients found in these studies for macronutrients using weighed records as reference method ranged between 0.17 (fat) and 0.64 (also for fat), and using repeated 24-hour recalls as reference method ranged between -0.14 (protein) and 0.87 (energy) (Parr *et al.*, 2002:775, Slater *et al.*, 2003:633, Masson *et al.*, 2003:319, Mouratidou *et al.*, 2006:519).

Correlation coefficients for vitamins using either records or 24-hour recalls as reference method, range between 0.12 (vitamin C) and 0.69 (vitamin B<sub>2</sub>), while those for minerals

range from -0.12 (Ca) to 0.75 (also Ca) (Addendum 6) (Rodriguez *et al.*, 2002:694, Masson *et al.*, 2003:319, Mouratidou *et al.*, 2006:519). From the data presented in Addendum 6, it appears that the number of 24-hour recalls / recording days of the reference method does not always predict outcome. Shu *et al.*, (2004:20) tested twenty-four 24-hour recalls and found correlations ranging between 0.48 and 0.55 (macro and micronutrients) and Bingham *et al.*, (1997:S141) tested 16 recalls with correlations ranging between 0.43 and 0.54, while those with less repetitions had similar correlation coefficients. Slater *et al.*, (2003:633) for instance, used three 24-hour recalls with correlation coefficients ranging from 0.42 - 0.87.

Masson *et al.*, (2003:319) found correlation coefficients ranging between 0.5 and 0.8 for most nutrients using four weighed records as reference method (n = 81). A FFQ validation study conducted on 22 postmenopausal females in China resulted in Pearson correlation coefficients ranging between 0.36 and 0.69 for their estimated weight FFQ and between 0.37 - 0.56 for their volume estimated FFQ. Both FFQs were compared against 4 day food records (Xu *et al.*, 2004:95) (Addendum 6).

Correlation coefficients for FFQ reliability studies (Addendum 7) have ranged between 0.14 (Ca) and 0.88 (vitamin C) (MacIntyre *et al.*, 2001a:57, Boucher *et al.*, 2006:85). Periods for repeated reliability tests usually ranged between six weeks and 12 months (Bohlscheid-Thomas *et al.*, 1997:S75, Pisani *et al.*, 1997:157, MacIntyre *et al.*, 2001:55, Ogawa *et al.*, 2003:150, Shu *et al.*, 2004:20). Correlation coefficients in another black South African population (n = 144) were generally poor, ranging between 0.14 (Ca) and 0.75 (alcohol) (MacIntyre *et al.*, 2001a:55), while the reliability of the FFQ used by Boucher *et al.*, (2006:85) ranged between 0.65 [iron (Fe)] and 0.88 (vitamin C) (n = 93) (Addendum 7).

Results for validation and reliability provided by Addendum 6 and 7 suggest that either 24-hour repeated recalls or weighed records can be used as a reference method (Parr *et al.*, 2002:775, Slater *et al.*, 2003:633, Masson *et al.*, 2003:319, Mouratidou *et al.*, 2006:519) and that the number of repeats of the reference method does not always influence the results (Slater *et al.*, 2003:633, Shu *et al.*, 2004:20), although the greater number of studies showed best results with at least two to four repeated 24-hour recalls.

### 5.1.2. The use of double-labelled water as validation method for energy consumption

Reported energy intake ( $EI_{rep}$ ) can be effectively compared with energy expenditure (EE) estimated using the double-labelled water (DLW) method [Gibson, 2005:164, Church, 2006:265, National Cancer Institute (NCI), 2010]. After six hours of fasting, participants received DLW ( $^2H_2^{18}O$ ). This water is labelled with deuterium (an isotope of hydrogen) and the isotope  $^{18}O$  (Nelson, 1997:246, Gibson 2005:165). The deuterium is excreted through the urine, while the  $^{18}O$  is eliminated through the urine and the lungs in the form of carbon dioxide.

The excretion of deuterium provides an estimate of water loss, while the excretion of the  $^{18}O$  provides a measure of the amount of water loss as well as carbon dioxide production over a pre-determined period. The total amount of energy spent, can then be determined from the production of carbon dioxide with the use of indirect calorimetry (Gibson 2005:166).

A validation study conducted by Kroke *et al.*, (1999:443) in Germany, (part of the European Prospective Investigation into Cancer and Nutrition (EPIC) study), found that the FFQ under-reported energy expenditure (EE) compared with the DLW method of EE. The Observing Protein and Energy Nutrition (OPEN) study reported a 49% under-reporting of their FFQ compared to the DLW method to determine EE (Subar *et al.*, 2003:9, Schatzkin *et al.*, 2003:1058) compared to the 21% of under-reporting using two 24-hour recalls. Results also showed that under reporting increased as BMI increased (Subar *et al.*, 2003:12). Results from the Women's Health Initiative Dietary Modification Trial (WHI-DM) also reported under-reporting of energy (27 - 32%) when DLW were used (Neuhouser *et al.*, 2007:1256), while results from a Block '98 questionnaire which was compared against the DLW ( $n = 12$ ) also indicated an under-reporting of the FFQ (Paul *et al.*, 2005:810).

### 5.1.3. The use of accelerometers as a validation method for energy consumption

Energy expenditure can be predicted with either pedometers or accelerometers (Nichols *et al.*, 1999:908, Hendelman *et al.*, 2000:S442, Church, 2006:265, NCI, 2010). Accelerometers are sensors that measure movement (either forward and backward, up and down, or left and right) (Nichols *et al.*, 1999:908, Jakicic *et al.*, 1999:747). A variety of motion sensors have been developed and validated in the past in an attempt to measure physical activity and therefore, EE in different populations (Jakicic *et al.*, 1999:747, Iltis & Givens, 2000, Hendelman *et al.*, 2000:S442, Sirard *et al.*, 2000:695).

Accelerometers measure EE in such a way that they also allow researchers to determine the intensity of activity (Freedson *et al.*, 1998:777). They are small, convenient, and easy to use and participants can wear them for long periods without interference with their normal daily activities (Fehling *et al.*, 1999:171, Hendelman *et al.*, 2000:S442, Welk *et al.*, 2000:S489). The same is true for pedometers.

Pedometers are small sensors that can be tied around a participant's waist or ankle. The main purpose of this mechanism is to count the number of steps taken by a person, which can be translated into daily EE (Kumahara *et al.*, 2009:1427). Although pedometers are considered to be a practical measure to determine EE in large populations, a recent study suggested that recording the actual number of daily steps taken by participants is only a crude predictor of EE (Kumahara *et al.*, 2009:1427). This is probably because of the fact that pedometers are not able to measure physical activity intensity (Marshall *et al.*, 2009:410).

#### **5.1.4. Calculated energy expenditure as validation method for energy consumption**

If a person's weight is stable, calculated energy expenditure ( $EE_{calc}$ ) should equal  $EI_{rep}$  ( $EE_{calc} = EI_{rep}$ ) (Livingstone & Black, 2003:900S). Therefore, if the EE is known the validity of the  $EI_{rep}$  of a dietary assessment method can be tested. The ratio of energy intake (EI) to EE should therefore equal 1 ( $EI: EE = 1$ ) (Black *et al.*, 1997:410). Either EE can be calculated or it can be determined with the use of a physical activity questionnaire [for instance, the International Physical Activity Questionnaire (IPAQ)].

To calculate theoretical EE, the physical activity level (PAL) [the ratio of EE to basal metabolic rate (BMR)] is multiplied by the BMR (Black *et al.*, 1996:72, Vasconcellos & Anjos, 2003:1025). Therefore, the equation would be  $EE_{calc} = PAL \times BMR$ , or in other words,  $EI_{rep} = PAL \times BMR$  (Livingstone & Black, 2003:897S). Basal metabolic rate is the resting EE of an individual, in a thermo-neutral environment and fasting state (Gibson, 2005:168).

If the PAL for the population is not known, the WHO recommend using a level of 1.55 indicative of light physical activity [FAO / WHO / United Nations University (UNU), 1985:78, Goldberg *et al.*, 1991:571]. This is a conservative value at population level, and DLW studies indicated that most population groups have theoretically a higher EE than this (Black *et al.*, 1996:83, Black, 2000:1120). In a study conducted by Vasconcellos and Anjos, (2003:1031) a PAL of 1.9 and 1.68 was found for rural and urban living males, respectively. The assumption is that in rural areas or developing countries, people start working at a younger

age and work for longer periods, causing an increase in the average PAL (Vasconcellos & Anjos, 2003:1031).

Expressing EE as a multiple of BMR, provides a guide by which the activity level of individuals can be compared, since most of the between-person variance arising from differences in weight, height, age and gender are automatically removed with the use of BMR (Goldberg *et al.*, 1991:571).

The BMR can be estimated from calculations determined by Schofield (1985:19). The formula takes the participant's weight, height and age into account (Schofield, 1985:19). Schofield's calculations for adult males and females between 18 and 30 years are as follows:

- Male =  $0.063 (\text{weight}) - 0.042 (\text{height}) + 2.953$
- Female =  $0.057 (\text{weight}) + 1.184 (\text{height}) + 0.411$

The calculations for adult males and females between 30 and 60 years are as follows:

- Male =  $0.048 (\text{weight}) - 0.011 (\text{height}) + 3.670$
- Female =  $0.034 (\text{weight}) + 0.006 (\text{height}) + 3.530$

[BMR is given in MJ/24 hours, weight in kilogram and height in metres (Schofield, 1985:19)].

In the past it has been found that participants tend to under-report habitual intake. Because of this, Goldberg and colleagues developed the Goldberg cut-off values to enable researchers to identify these under-reporters (Goldberg *et al.*, 1991:570).

- Classifying under-reporters with the use of the Goldberg cut-off values

The Goldberg cut-off values are based on the principle that all individuals need a minimum EI to stay alive (Gibson, 2005:167). Any reported intake lower than this minimum is considered too low for any person to survive (Goldberg *et al.*, 1991:574, Gibson, 2005:167).

The Goldberg cut-off method compares the mean daily  $EI_{\text{rep}}$  with recommended EI for a sedentary lifestyle of  $1.55 \times \text{BMR}$  (as determined by the FAO/WHO/UNU work group) (FAO/WHO/UNU 1985:78, Goldberg *et al.*, 1991:569, Black *et al.*, 1997:407, Livingstone & Black, 2003:910S, Gibson, 2005:168).

Under-reporters can be identified individually by calculating the EI (reported) to BMR (estimated) ( $EI_{\text{rep}} : \text{BMR}_{\text{est}}$ ) ratio. This ratio is then compared against pre-determined cut-off

points, calculated by the number of days of dietary assessment used to calculate the  $EI_{rep}$  (Gibson, 2005:168).

These pre-determined cut-off points represent the minimum amount of energy that is statistically likely for any  $EI_{rep} : BMR_{est}$  ratio to reflect long-term EI (Gibson, 2005:168). The cut-off points are set at the 95% confidence limit, based on an energy requirement of 1.55 X BMR (Goldberg *et al.*, 1991:573, Gibson, 2005:168).

The more days used to determine the EE, the higher these cut-off values will be. Under-reporters at group level can also be identified with Goldberg's cut-off values. This can be done by determining the mean  $BMR_{est}$  for each individual. The mean EI for the group is then divided by the BMR for the group. The results can be compared with the pre-determined cut-off point for the population, based on the sample size and number of dietary assessment repeats (Gibson, 2005:168).

- Limitations of the Goldberg cut-off points

The use of the Goldberg cut-off method is limited for various reasons, which include the following:

Not everybody in the population may follow a sedentary lifestyle. Therefore, using a PAL of 1.55 may not be appropriate for everyone in the population (Gibson, 2005:168).

Participants with a high EE and high EI may, even if they under-report, have values that are not lower than the cut-off values. Therefore, not all the participants with values lower than the cut-off points are under-reporters and not all under-reporters are going to be identified (Ballard-Barbash *et al.*, 1996:104, Black, 2000:1128, Gibson, 2005:168).

The equations for BMR are applied to those with a maximum body weight of 84 kilogram (kg). If most of the population is heavier than this, the cut-off points cannot be applied at individual level (Gibson, 2005:168).

Participants must be in energy balance for the cut-off points to be applied. Therefore, these cut-off points cannot apply to children, or to adults on a weight loss diet (Gibson, 2005:169).

The cut-off points only identify under-reporters and make no assumptions regarding over-reporters (Gibson, 2005:169).

Because the sensitivity of the Goldberg cut-off points is poor at the individual level, only extreme under-reporters will be identified (Black, 2000:1127). However, more under-reporters will be identified if PALs that are more appropriate are used. It is ideal if every participant's own PAL (determined by physical activity questionnaires) can be used (Black *et al.*, 1997:412).

Results from a study conducted by Ballard-Barbash *et al.*, (1996:104) indicated that more females than males under-report. Those people with low education levels, who are inactive, smoke, and who have a poor self-reported health status underestimate their EI. These people should not be excluded from the data, as this will cause a differential exclusion bias (Ballard-Barbash *et al.*, 1996:104).

#### **5.1.5. Energy expenditure determined with the use of physical activity questionnaires as validation method for energy consumption**

Energy expenditure can also be calculated with physical activity questionnaires or activity diaries (Bull, 2003). Various physical activity questionnaires have been developed, including those of the EPIC study (EPIC PA) (Pols *et al.*, 1997:S181), the International Physical Activity Questionnaire (IPAQ), the sub-Saharan Africa Activity Questionnaire (SSAAQ) (Sobngwi *et al.*, 2001:1361) and the Global Physical Activity Questionnaire (GPAQ) (Bull, 2003). The latter questionnaire is most appropriate for application in a developing country.

The WHO developed the GPAQ to be able to determine the physical activity levels of people in developing countries accurately (valid and reliable). In these countries the energy expenditure patterns differ from developed countries and therefore a cultural specific questionnaire was needed. An expert working group on the measurement of physical activity measurement developed a draft GPAQ for global advice, which was validated in nine different countries. Results from the validation studies were reported at another expert committee meeting on Global Physical Activity measurement. Afterwards another round of global advice led to the necessary changes and the final GPAQ was developed (Armstrong & Bull, 2006:66).

The GPAQ is used to describe a population's physical activity level by determining an estimation of the mean and median of the physical activity as a continuous indicator. The metabolic equivalent (MET)-minutes per week is used for these purposes. Participants are expected to report their physical activities and the amount of time and frequency they spend doing these (Warenham, 2001:1369). The time spent on physical activity is then multiplied with the appropriate MET (metabolic equivalent) scores (Warenham, 2001:1369). One MET

equals 4.2 kJ per kilogram of body weight per hour ( $\text{kcal}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ) (Ainsworth *et al.*, 2000:S498).

#### **5.1.6. Urinary biochemical markers of selected nutrients as validation method for nutrient consumption**

Biomarkers are usually parts of body tissue or fluids that have a strong association with dietary consumption (Gibson, 2005:161). For a biomarker to be selected, it must be closely associated with dietary intake and be assessed in a way other than by dietary intake (Potischman *et al.*, 2003:873S). Addendum 8 describes the different factors to take into consideration when using biomarkers, and Addendum 9 describes the different biomarkers used to validate dietary assessment methods, with their advantages and disadvantages.

When two dietary assessment methods are compared, it is possible that the errors included are similar, but because biomarkers are collected independently, errors may not correlate with those included in dietary assessment (Van't Veer *et al.*, 1993:S59, S60, Ocké & Kaaks, 1997:1240S, Lee & Nieman, 2007:98).

Although the use of biomarkers in validation studies provides uncorrelated errors, other factors may influence the results obtained from these markers (Van't Veer *et al.*, 1993:S60, Taren *et al.*, 2002:1002, Potischman, 2003:876S). Between-person variations, especially in the form of absorption, digestion and nutrient distribution will influence results (Van't Veer *et al.*, 1993:S62). The stability of nutrients during cooking, sample collection, transportation and storage of samples will differ from nutrient to nutrient and will influence which biomarkers to use (Bates *et al.*, 1997:171, Potischman, 2003:876S, Gibson, 2005:163). Based on these factors, the biomarkers to use should be considered with care (Addendum 8 & 9 provides more detail).

Poor correlations between biomarkers and dietary questionnaires may not necessarily be because of errors in dietary assessment methods (Van't Veer *et al.*, 1993:S62, Nelson, 1997:259) but may be because of increased within person variability of the biomarkers (Willett & Hu, 2006:1757). The biomarkers that are mostly used for validation of dietary assessment methods include 24-hour urinary nitrogen, sodium or potassium excretion.

Twenty-four-hour nitrogen excretion is frequently used as a biomarker in validation studies, because there is a high correlation between nitrogen (thus protein) intake and urine nitrogen excretion (Bingham, 1994:228S, Bates *et al.*, 1997:219, Bingham, 2003:922S, Lee & Nieman, 2007:99). Therefore, it can be concluded that because agreement is good between

nitrogen (converted to protein) dietary intake and urinary excretion, it will also be good for other nutrients (Lee & Nieman, 2007:99).

Using 24-hour urine nitrogen excretion as a biomarker, however, depends on two assumptions. Firstly, that the participants have a nitrogen balance and secondly, no nitrogen changes are caused by growth, muscle repair, starvation, diet or injury (Bingham, 1994:228S, Bates *et al.*, 1997:219, Black *et al.*, 1997:410, Bingham, 2003:922S, Gibson, 2005:170). Not all nitrogen is excreted through the kidneys; some is excreted through the skin and faeces. These losses are not calculated into the renal losses, but are addressed by adding an extra 2 g of nitrogen to the 24-hour urinary nitrogen excretion (Gibson, 2005:170).

Because no person's nitrogen intake and output are always in balance, it is important to obtain enough urine samples (several days of measurement) to include possible within-person variation (NCI, 2010). It is also imperative to ensure a complete 24-hour sample (Bingham, 1994:228S, Bates *et al.*, 1997:219, Dyer *et al.*, 1997:1247S, Bingham, 2003:924, Gibson, 2005:170, NCI, 2010). Participant burden is less if at least four to eight single urine collections are taken, compared to those of four consecutive collections (Rothenberg, 1994:733, NCI, 2010).

Urinary nitrogen was used as a biomarker in the validation study conducted by Kroke *et al.*, (1999:443) (the German part of the EPIC study). They found a Pearson correlation coefficient of 0.41 (crude) and 0.46 (de-attenuated) between the urinary biomarker and the FFQ intake (Kroke *et al.*, 1999:443). It was found that the FFQ under-reported protein intake (Kroke *et al.*, 1999:444). Results from the OPEN study also indicated an under-reporting of protein intake from the FFQ compared to 24-hour recalls. They reported unadjusted correlation coefficients of 0.26 (females) to 0.41 (males) when they compared single 24-hour recalls against urinary nitrogen and 0.22 (females) to 0.33 (males) when they compared single FFQs against urinary nitrogen (Subar *et al.*, 2003:9).

Alternatively, sodium (Na) excretion can also effectively be used as a biomarker of intake because it is mostly excreted through the kidneys (95 - 98%), with small amounts (2 - 4 mmol per day) excreted through faeces and skin (Bates *et al.*, 1997:188). Although the measurement of excreted Na can effectively be used as biomarker, poor validation results should be expected as dietary measurement of Na is difficult, mainly due to salt being added at the table (Bentley, 2006:63). Liu *et al.*, (1979:529) concluded that because of large intra-individual differences at least 14 or more collections of 24 hour urine is necessary to determine average daily Na excretion, to avoid the large intra-individual variation (Kawasaki

*et al.*, 1982:952, Gibson, 2005:173). Urinary sodium excretion depends largely on sodium consumption (Elliott & Brown, 2007:18). Sodium excretion reported by the INTERSALT study varied dramatically between different countries, ranging between 0.8 mmol/d (Brazil) and 258 mmol/d (China) (INTERSALT Co-operative research group, 1988:324). In a study by Williams & Bingham, (1986:21) the average Na excreted by males was 172 mmol / 24 hours, and that of females was 128 mmol / 24 hour. On the other hand, Maseko *et al.*, (2006:186) reported an average of 117 mmol/d ( $\pm$  54) amongst 291 normotensive participants in an urban area in South Africa, while Charlton *et al.*, (2005:355) reported ethnic differences in sodium urinary excretion. Urinary excretion for black participants were 135 mmol/d, participants with mixed ancestry excreted 147.5 mmol/d and white participants excreted 164.8 mmol/d (Charlton *et al.*, 2005:357).

Another biomarker that can be used is 24-hour urinary potassium (K) excretion compared to K intake (Church, 2006:265). However, more K is lost through faecal excretion than with Na. The amount excreted includes up to 5 - 13 mmol/d or 11 - 30% of that taken in through the diet and this varies among different population groups (Bates *et al.*, 1997:189). Maseko *et al.*, (2006:186) found an average K excretion of 33 mmol/d ( $\pm$  17) amongst 291 normotensive participants in Johannesburg. Charlton *et al.*, (2005:357) reported higher K excretion levels amongst 325 participants in Cape Town than the participants in Johannesburg (Maseko *et al.*, 2006:186). An average K excretion of 61.9 mmol/d for white participants, 55.6 mmol/d for black participants and 54.3 mmol/d for participants of mixed ancestry was reported by Charlton *et al.*, in Cape Town (Charlton *et al.*, 2005:357).

The basic principle of using the above mentioned urinary biomarkers (nitrogen, Na and K) is that the amount of a nutrient that is absorbed and taken up in the tissues is the same as that excreted by the body (through the urine, faeces, skin or other routes) (Bates *et al.*, 1997:220). It is imperative that the participant provides a complete 24-hour sample and the selected nutrient be chosen carefully and preserved and stored in the required way (Bingham, 1994:926S, Bates *et al.*, 1997:221, Lee & Nieman, 2007:99).

Various ways are used to determine the completeness of urinary collections, including para-amino benzoic acid, creatinine excretion and lithium excretion. Para-amino benzoic acid (PABA) is currently the most frequently used biomarker to determine completeness of urine samples (NCI, 2010). Participants receive three oral tablets (80 mg each) of PABA to take with meals (Williams & Bingham, 1986:20, Bingham *et al.*, 1997, Gibson, 2005:171). PABA is then absorbed and excreted through the kidneys within 24-hours (intake should therefore equal output) (Bates *et al.*, 1997:220). A sample is considered complete if up to 85% or more

of the PABA is excreted (Williams & Bingham, 1986:20, Bingham *et al.*, 1997:S142, Gibson, 2005:171).

In the past creatinine was the standard method used to determine completeness of 24-hour urinary excretion, based on the principle that a person's creatinine levels balance (Gibson, 2005:171). However, creatinine excretion is not only dependent on intake (mostly from meat), but is also dependent on creatinine production, making it a less reliable biomarker for a complete 24-hour sample (Bates *et al.*, 1997:219, Bingham, 2003:924S).

Lithium can also be used to determine the completeness of a urine sample (Bingham, 2003:925S, Gibson, 2005:174). However, using Lithium can be problematic (similar to PABA), because participants must remember to take it daily before collecting the 24-hour urine sample (Bingham, 2003:925S).

#### **5.1.7. Blood biomarkers of selected nutrients as validation method for nutrient consumption**

Various blood biomarkers can be used to determine the validity of a FFQ. In a study done by Boeing *et al.*, (1997:S85) as part of the EPIC study (Germany), serum and plasma  $\alpha$ -carotenes,  $\beta$ -carotenes, retinol,  $\alpha$ -tocopherols,  $\gamma$ -tocopherols and ascorbic acid were collected and correlations varying between 0.16 (tocopherols) and 0.35 (carotenoids) were found. They concluded that the FFQ successfully ranked participants for carotenoids and ascorbic acid intake, but not for tocopherol and retinol compared to the blood biomarkers (Boeing *et al.*, 1997:S89).

Other biomarkers can also be used. Bogers *et al.*, (2004:906) used plasma concentrations of carotenoids and vitamin C as biomarkers. However, the use of plasma concentrations of carotenoids and vitamin C is limited as it is not only influenced by nutrition but also by other biological factors such as absorption and metabolism (Mayne, 2003:938).

Bogers *et al.*, (2004:906) used plasma concentrations of carotenoids and vitamin C as biomarkers. However, the use of plasma concentrations of carotenoids and vitamin C is limited as they are not only influenced by dietary intake but also by other biological factors such as absorption and metabolism (Mayne, 2003:938). Toft *et al.*, (2008:1039) also used plasma carotenoids as validation method of a FFQ in the Inter99 study and reported unadjusted Spearman rank correlation coefficients of  $\alpha$ -carotenoids between 0.41 (males) and 0.44 (females) and  $\beta$ -carotenoids between 0.29 (males) and 0.38 (females) (Toft *et al.*, 2008:1044).

Owens *et al.*, (2007:3737) used red blood cell folate to validate a FFQ and reported correlations of 0.28 (Owens *et al.*, 2007:3739). Verkleij-Hagoor *et al.*, (2007:612) used both red blood cell folate and serum folate to validate the folate intake of a FFQ used in a case-control study which studied the association between maternal dietary intake and the risk of having a child with congenital heart defects. They reported correlation coefficients between the FFQ and red blood cell folate of 0.28 and between the FFQ and serum folate of 0.20 (Verkleij-Hagoor *et al.*, 2007:612).

Verkleij-Hagoor *et al.*, (2007:610) used serum Vitamin B<sub>12</sub> to validate their FFQ used in a case-control study of women in their reproductive years (Verkleij-Hagoor *et al.*, 2007:612) and reported a correlation of 0.21. Mina *et al.*, (2007:1025) used erythrocyte membrane omega-3 fatty acid [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] as biomarkers to validate their FFQ which was designed to measure habitual fish and seafood intake. Spearman correlation results for the EPA was 0.33 and 0.28 for the DHA of all fresh fish and seafood intake (Mina *et al.*, 2007:1027).

Sullivan *et al.*, (2006:846) developed a FFQ to measure long-chain omega-3 polyunsaturated fatty acids (PUFA). The FFQ was validated against long-chain omega-3 PUFA content of red blood cells and plasma and reported correlations of 0.50 for total PUFA, 0.39 for EPA and 0.40 for DHA (Sullivan *et al.*, 2006:847). Adipose tissue has also been shown to be a good biomarker for validating dietary assessment methods. Adipose tissue can be used to compare long term intake of linoleic acid, long-chain n-3 fatty acids as well as *trans*-fatty acids (Willett, 1998).

In the Nurses' Health Study II, acrylamide (a human carcinogen formed during the frying of potatoes [De Wilde *et al.*, 2005:6550]) intake was measured with a FFQ which was validated with haemoglobin and adducts of acrylamide as well as glycidamide (the metabolite of acrylamide) (Wilson *et al.*, 2009:269). The adjusted correlation between the acrylamide intake and the sum of acrylamide and glycidamide adducts was 0.31 (Wilson *et al.*, 2009:269).

## 5.2. FACTORS AFFECTING VALIDATION OF DIETARY ASSESSMENT METHODS AND TOOLS

### 5.2.1. Design of the validation study

- Population used in the validation of a dietary assessment methods and tools

Participants in a validation study should either be from the target population or similar to the target population (Ambrosini *et al.*, 2001:263, Cade *et al.*, 2002:574, Gibson, 2005:150, Marks *et al.*, 2006:465). This is particularly important in large studies conducted among different population groups (Gibson, 2005:150). Using volunteers in a study may improve the results, as volunteers are more likely to be aware of their eating habits than those who do not volunteer (Kroke *et al.*, 1999:445).

- Time frame used in a validation study

The test method and the reference method must cover the same time interval, and the reference method must preferably be scattered over the test period to avoid seasonal differences (Willett *et al.*, 1985:54, Nelson, 1997:250, Cade *et al.*, 2002:574, Gibson, 2005:150).

- Sequence of data collection during the validation of dietary assessment methods and tools

The test method must be conducted first, to mimic the main study where only this method will be administered (Nelson, 1997:249, Cade *et al.*, 2002:574, Gibson, 2005:150). The method used first will draw the participant's attention to their diet. If the reference method is completed first, participants might change their dietary responses based on information given during the reference method (Nelson, 1997:250).

- Sample size

Ideally, a sample size of 100 to 200 participants is recommended for a validation study. If this is not possible, many repeated measurements should be conducted on each subject (Cade *et al.*, 2002:574). To rank participants optimally, at least 15 days of recordings are necessary (Nelson *et al.*, 1989:164).

- Repeated 24-hour recalls as reference method

The type of dietary assessment method used as the gold standard plays a significant role in the validation results. Limitations of retrospective methods such as 24-hour recalls involve forgetting dietary intake and may be selective (Addendum 3 gives the different advantages and disadvantages of the different dietary assessment methods) (Lee & Nieman, 2007:101). On the other hand, prospective methods, such as weighed records, may lead to unconscious or conscious alterations of the usual diet (Lee & Nieman, 2007: 101).

Most validation studies conducted within the last couple of years that made use of repeated 24-hour recalls as reference method corrected results for within- and between-person variation by de-attenuating correlations (Segovia-Siapco *et al.*, 2007:179, Wong *et al.*, 2008:541, Cheng *et al.*, 2008:168, Satia *et al.*, 2009:505, Carithers *et al.*, 2009:1186, Lora *et al.*, 2010:553) while a study conducted by Osowski *et al.* (2007:1353) reduced between-person variation by calculating nutrient intakes per 1000 kcal.

- *Within-person variation*

Within-person variance reflects the actual day-to-day variation in dietary intake of an individual and therefore represents the sum of true variation in the day-to-day intake of an individual, as well as all random variation such as measurement error (Cole, 2007:71).

Using unadjusted data will lead to an overestimation of the proportion of participants in the population who are below a certain cut-off point, regardless of whether this cut-off point is the RDA or EAR (Mackerras & Rutishauser, 2005:662). Adjusting for within-person variation involves lowering the distribution of 24-hour intakes to a level that better reflects the distribution of habitual intakes by using the analysis of variance to remove the effect of day-to-day variation on the distribution. However, in order to adjust for within-person variation the data needs to be normally distributed and the between-person standard deviation (SD) must be calculated. These adjusted values do not provide accurate values on individual level and should not be used in individual-based analysis (Mackerras & Rutishauser, 2005:659).

Within-person variance ( $S_w^2$ ) is expressed as a standard deviation ( $S_w$ ) or as a coefficient of variation ( $CV_w$ ), where  $CV_w = S_w / \text{mean level of intake}$  (Gibson, 2005:137).

Assessing within-person variation is mostly important for determining the prevalence of inadequate intakes in a population group, for ranking within a group, or when data on usual intakes of individuals are required for correlation or regression analysis with biochemical or

clinical parameters at the individual level (Gibson, 2005:136). This is measured by calculating  $S_w^2$  (Cole, 2007:69).

One way of dealing with within-person variation is to take several measurements of each individual and to calculate the mean (Cole, 2007:70). An increased number of measurement days per individual will reduce the effect of within-person variance (Cole, 2007:70, Gibson, 2005:138). The number of measurement days required depends on the required precision of the estimate. Measurement days may be closely spaced (to reduce bias introduced from seasonal changes), and should include weekend and week days. Ideally nonadjacent days should be included to avoid the effects of autocorrelation of consecutive daily intakes (Gibson, 2005:138).

The number of repeated measures used depends on the reason for validation of a method (Carroll *et al.*, 1997:1189S). If the reason / purpose is to estimate the correlations between a FFQ and a participant's true habitual intake, more participants and fewer repeat measures will provide accurate data (Carroll *et al.*, 1997:1189S). If the reason for the validation is to determine whether a bias is present when comparing the FFQ and the participants' true habitual diet, fewer participants and more repeated measurements per participant are needed (Carroll *et al.*, 1997:1189S).

Furthermore, to correct for within-person variation in situations where multiple dietary intake measurements (such as 24-hour recalls) are used as the reference method in validation, the following formula can be used:

$$r_c = \frac{r_o \sqrt{1 + S_w^2 / S_b^2}}{n}$$

where  $r_c$  = corrected /de-attenuated correlation coefficient;  $r_o$  = uncorrected / attenuated correlation between the FFQ and the multiple 24-hour recalls;  $S_w^2$  = within-person variance of the multiple 24-hour recalls;  $S_b^2$  = between-person estimate of variance in the reference method (24-hour recalls) and  $n$  = number of repeated measures of the dietary recalls (Segovia-Siapco *et al.*, 2007:179).

If the number of measures (days) is high the coefficient can be made close to unity. Yet, the ratio within- and between-person variation is important (Cole, 2007:71). If the ratio is small, the attenuation is not important; however, if the ratio is large, the effect of within-person

variation can be large (Cole, 2007:71). This will weaken correlations and could result in misclassification when participants are divided into quantiles (Cole, 2007:71).

In a validation study conducted by Segovia-Siapco *et al.* (2007:179) where six 24-hour recalls were used as reference method among 87 adults in Southern California, it was found that correcting for random errors associated with within-person variation increased correlation values as well as for de-attenuated correlations (Segovia-Siapco *et al.*, 2007:182). However, they reported that high within-to-between person variances resulted in very high correction factors for some of the nutrients. Hence the decision was made to report uncorrected correlations for these nutrients. It is therefore suggested that to successfully adjust for within-person variation, large sample sizes are a requirement (Segovia-Siapco *et al.*, 2007:182, Gibson, 2005:137).

The extent of within-person variation recorded during dietary assessment depends on various factors:

- Variety versus monotony in the food choices of a participant. In a population where a limited number of foods are consumed because consumption is associated with income and not food availability, within-person variation should be less than between-person variation. In a population such as subsistence farmers, higher within- than between-person variance ratios will be reported because of the occasional and irregular use of certain food sources such as animal products (Gibson, 2005:138).
- Physiological factors such as the menstrual cycle may also influence the within-person variation (Gibson, 2005:138).
- Within-person variation is further determined by the nutrient of interest. Nutrients found in high concentrations, in a limited amount of foods and are occasionally consumed will show a high within-person variation. This makes it more difficult to obtain accurate estimates of the usual intakes of these nutrients for individuals; conversely within-person variation is lower for nutrients found in many foods such as carbohydrate and protein (Gibson, 2005:138).

- *Between-person variation*

Between-person variation assumes that every individual in the population has a fixed, unknown value for their usual intake. The between-person variation can then be considered as the population variance of each of these true means (Cole, 2007:69).

This fixed, unknown value or usual intake varies from person to person and between-person variation measures these differences. The between-person variation depends on the nutrient being studied, as well as the characteristics of the study population. Also, for the majority of nutrients, there is more variability in intakes within persons than between. Therefore, the within- to between-person variance ratio is at times greater than 1.0. Because of this, it is easier to use the mean intake of a group than that of an individual (Gibson, 2005:136). Between-person variation is important when calculating sample size and power (Cole, 2007:70). To allow for the effect of between-person variation on group mean nutrient intakes, the sample size should be as large as possible, and representative of the study population (Gibson, 2005:136).

### **5.2.2. Participant characteristics**

The age and gender of participants, their BMIs, possible medical conditions they might have, the day of the week or the season when the questionnaires was administered, and the use of multivitamin supplements can all influence the results of a validation study (Gibson, 2005:152, Marks *et al.*, 2006:399). Furthermore, psychological factors as well as the socio-cultural environment might influence validation results. In cultures where obesity is not acceptable, under reporting might occur, where in areas with high levels of hunger and food insecurity, over reporting may occur (Taren *et al.*, 2002:1002), especially if participants are embarrassed about their insufficient intakes (Lee & Nieman, 2007:101). Under reporting is also expected in individuals consuming large quantities of alcohol (Lee & Nieman, 2007:101).

## 6. RELIABILITY OF DIETARY ASSESSMENT METHODS

Reliability<sup>2.7</sup> is defined as the amount of variation in data obtained when the same questionnaire is repeated (Bates *et al.*, 1997:172). Therefore, reliability indicates the extent to which a method is able to provide similar results every time it is used (Nelson, 1997:242). Reliability provides information on the accuracy of classifying participants over a longer period, especially when determining diet-disease associations in diseases with long-term exposure, such as cancer (Goldbohm *et al.*, 1995:427).

The extent of reliability is determined with a test and retest design (Gibson, 2005:129). The period between the two repeat measurements depends on the time interval of the method and needs to be conducted on the same participants every time (Gibson, 2005:129).

The time interval between the repeated measurements should not be too short so that memory of the first measurement influences reporting of the second measurement. Neither should it be so long apart that seasonal changes, random errors, within-person variation and other changes cause differences in reporting (Gibson, 2005:129).

Reliability is also influenced by the amount of variability the method being tested allows. If the method has a tool with a variety of portion sizes, reliability will be poor; while a method with no or single portion sizes will have better reliability (Barret-Connor 1991:185S). Other factors, such as the properties of the method itself, the accuracy of respondents' memories and actual changes in food intake also influence the reliability of a dietary assessment method (Bogers *et al.*, 2004:906).

Furthermore, interviewers can also influence the level of reliability obtained. Reliability is influenced by the results that different interviewers obtain when they use the same method (inter-interviewer reliability), as well as by the results the same interviewer obtains when repeating the same method that is administered to the same participant (intra-interviewer reliability) (Cade *et al.*, 2002:573).

Within-person and between-person variation also play a role in the results of reliability studies. Within-person variation can be addressed by more repetitions of the questionnaire. Between-person variability, on the other hand, will depend on the population as well as the nutrients under investigation, but can be reduced by having a large and representative sample (Gibson 2005:138).

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<sup>2.7</sup> The term reliability and reproducibility can be used interchangeably (Cade *et al.*, 2002:573).

## 7. STATISTICAL ANALYSES USED IN VALIDATION AND RELIABILITY STUDIES

Various statistical approaches can be applied in the assessment of the validation and reliability of dietary intake data.<sup>2.8</sup> Each statistical method provides information on different facets of the validity or reliability of the dietary assessment method and ideally all statistical methods should show acceptable validity or reliability.

### 7.1. PAIRED T-TEST OR WILCOXON SIGNED RANK TEST

A paired t-test (for normally distributed data) or the nonparametric Wilcoxon matched-pairs signed-rank test (for skewed data) indicates agreement between two measures at group level (Gibson, 2005:142). Significance levels are mostly set at  $p = 0.05$  (Nelson *et al.*, 1994:653). P-values larger than 0.05 are considered acceptable (no significant difference between the test weight and the reference weight), p values between 0.05 and 0.001 are considered poor and p values less than 0.001 are considered exceptionally poor. The Wilcoxon signed rank test indicates agreement between the two measures, but does not say anything about the strength of the association or the presence of bias.

### 7.2. PERCENTAGE DIFFERENCE BETWEEN THE REFERENCE METHOD AND THE TEST METHOD

The mean difference between the reference measure and the test measure is used as a measure of accuracy (Kuehneman *et al.*, 1994:548). For each assessment, the reference measure is subtracted from the test measure (Nelson *et al.*, 1994:653, Robinson *et al.*, 1997:120). Positive values, therefore, indicate over-estimation, while negative values indicate under-estimation (Nelson *et al.*, 1994:653). Ideally, one would want the difference to be as close to zero as possible. Estimation is considered “accurate” if the percentage difference (PD) is smaller than 10% (Venter *et al.*, 2000:209), acceptable if PD is between 11% and 20% and poor if larger than 20% percentage difference.

By subtracting the reference measure<sup>2.9</sup> from the test measure<sup>2.10</sup> for each individual and then dividing this by the reference measure of the individual provides the PD. This is expressed as a percentage. A mean percentage difference for the group is then calculated from each individual's percentage difference.

<sup>2.8</sup> The same statistical methods can be used for validity and reliability (Cade *et al.*, 2002:567).

<sup>2.9</sup> The reference measure is the known measure (from the gold standard).

<sup>2.10</sup> Test measure is the newly developed dietary assessment measure that is being developed and validated.

$$\% \text{ Difference} = \frac{\text{test measure} - \text{reference measure}}{\text{reference measure}} \times 100$$

### 7.3. CORRELATION COEFFICIENTS BETWEEN THE REFERENCE METHOD AND THE TEST METHOD

Correlation coefficients are widely used statistical measures in validation and reliability studies (Borrelli *et al.*, 1989:459, Gibson, 2005:183). Correlation coefficients measure the strength of the relationship between two different measurements (the test and reference measurements) at individual level. The correlation coefficient is used to determine if there is a linear association between two measures. Correlation coefficient values can range between -1 and 1. A correlation coefficient of 1 indicates perfect linear association, meaning that when one measure is high, so is the other. A perfect, negative linear association ( $r = -1$ ) indicates that when one measure is high, the other is low. A correlation coefficient of 0 indicates no linear relationship between the two methods (Easton & McColl, 2010). However, correlation coefficients do not provide any information regarding the agreement between the two measurements (Bland & Altman, 1986:307, Borrelli *et al.*, 1989:459, Gibson, 2005:185). Thus, the correlations are not appropriate to use as the sole determinant of validation because no gold standard exist and because there is always bias involved between two dietary assessment methods (Taren *et al.*, 2002:1002).

Correlations less than 0.2 are considered exceptionally poor, correlations between 0.21 and 0.50 are acceptable while correlations above 0.51 are considered to be good (Masson *et al.*, 2003:320).

Pearson product-moment correlation coefficients are used on normally distributed data, while Spearman rank correlations are used on skewed (nonparametric) data (Cade *et al.*, 2002:573, Flood *et al.*, 2004:753, Gibson, 2005:144). Intra-class correlation can also be used, especially because it takes chance agreement into consideration (Gibson, 2005:144).

A recent study conducted by Masson *et al.* (2003:320), compared results from Pearson and Spearman correlation coefficients and found that these measures can be used successfully. On average, it seems as if associations found with Pearson correlation coefficients are higher than those of Spearman correlation coefficients, although it is possible that log transformations used in calculated Pearson correlation coefficients do not always remove the effect of outliers in the data (Masson *et al.*, 2003:319). Because Spearman correlation coefficients make use of rank order, they provide accurate information on skewed datasets (Masson *et al.*, 2003:319).

Confidence intervals for Pearson correlation coefficients (normally distributed data) can be calculated according to Fisher's z transformation (Dawson and Trapp 2004:195, Steyn *et al.*, 1995:494).

$$\left[ z(r) - \frac{1.96}{\sqrt{n-3}}, z(r) + \frac{1.96}{\sqrt{n-3}} \right]$$

A Z- transformation table for the correlation coefficient determine r values (Dawson and Trapp 2004:195, Steyn *et al.*, 1995:494).

$$Z(r) = \frac{1}{2} \ln \left[ \frac{1+r}{1-r} \right]$$

#### 7.4. CLASSIFYING PARTICIPANTS INTO QUANTILES

Continuous data can be classified into different groups (quantiles) to categorise data (Altman & Bland, 1994:996). Classifying nutrition data is useful in dietary validation studies, especially concerning FFQs, since this dietary assessment method ranks participants into categories for diet-disease associations (Flood *et al.*, 2004:754, Gibson, 2005:142). Quartiles are most often used as it ensures an equal number of participants in each group. When dividing participants into groups of four the three cut-off points are referred to as quartiles, but other groupings (tertiles, deciles or centiles) can also be used (Altman & Bland, 1994:996, Masson *et al.*, 2003:320). Although information may be lost if divided into too few groups, for instance when classified into tertiles, it is acceptable in small sample sizes. The mean and standard deviation (SD) can be used to classify participants according to intake, or if the data are skewed, the median and the 10<sup>th</sup> and 90<sup>th</sup> centiles (Altman & Bland, 1994:996).

Ideally, more than 50% of the participants should be classified in the same category with less than 10% of participants classified in the opposite category (Masson *et al.*, 2003:320). Accurate classification is important and indicates to what extent the dietary assessment method is able to rank participants correctly (Flood *et al.*, 2004:755). Unfortunately, classifying data into quantiles does not account for chance agreement (Gibson, 2005:143).

## 7.5. WEIGHTED KAPPA STATISTICS

Kappa statistics do not take into account the degree of disagreement between methods and all disagreement are treated equally as total disagreement. Therefore, when categories are ordered it is preferable to use weighted Kappa statistics. Weighted Kappa statistics are used for data that are ranked into categories or groups (Cade *et al.*, 2002:573, Masson *et al.*, 2003:320). The advantage of using weighted Kappa statistics is that they present agreement, but exclude chance agreement (Masson *et al.*, 2003:319, McGinn *et al.*, 2004:1370, Gibson, 2005:143).

According to Masson *et al.* (2003:319) weighted Kappa values may generally be lower than corresponding correlation coefficients. Kappa values can range from -1 to 1 although values between 0 and 1 are generally expected (Sim & Wright, 2005:259). A value of zero or close to it implies agreement “*no more than is expected from pure chance*”, while a negative value indicates agreement “*worse*” than that expected by chance alone (Barkto, & Carpenter, 1976:315, Sim & Wright, 2005:259, Viera & Garrett, 2005:361). However, it must be kept in mind that the Kappa value does not indicate if agreement, or lack thereof, is because of a systematic difference between the two methods, or because of random differences (error because of chance) (Hartman, 1977:110).

The magnitude of kappa values are mostly determined by factors such as the weighting applied, as well as the number of categories included in the scale (Haas, 1991:125, Brenner & Klibsch, 1996:200).

In this study, weighted Kappa values, between test and reference methods, higher than 0.8 reflect very good agreement, 0.61 - 0.80 good agreement, 0.41 - 0.60 moderate agreement, 0.21 - 0.40 fair agreement, while less than 0.20 is poor agreement (Landis & Koch, 1977:170, Masson *et al.*, 2003:315, McGinn *et al.*, 2004:1370, Viera & Garrett, 2005:362).

## 7.6. BLAND-ALTMAN PLOTS

Bland-Altman plots are used to determine the presence and the extent of bias in a validity study, as well as the level of agreement between two measures (Cade *et al.*, 2002:573).

For these purposes two plots are constructed. Firstly, the results from the one method are plotted against the results from the other measure. A line of equality (not a regression line) is then drawn across the plot. If the results are generally either above or under the line, bias may be present. These graphs will also identify the outliers (Gibson, 2005:186).

A second graph plots the difference between the two measurements against the mean of the two measures (Bland & Altman, 1986:308, Gibson, 2005:186). Spearman correlation coefficients can be calculated for the mean of the two methods and the difference between the two methods to establish a relationship between the size of the error (or difference between the two methods) and the mean intake of these methods. Such a relationship would mostly be because of an increase between method differences and the increase of intake, in other words proportional or reporting bias (Bland & Altman, 1986:307, Gibson, 2005:187).

Spearman correlation coefficients reaching statistical significance ( $p < 0.05$ ) are indicators of the presence of proportional bias and the direction thereof (MacIntyre *et al.*, 2001b:65, Gibson, 2005:144). Two lines [the limits of agreement (LOA)] are calculated at  $\pm 2$  SDs from the mean difference (Bland & Altman, 1986:310, Gibson, 2005:187). Bland and Altman (1986:308) indicated that the width of the LOA should be judged according to the clinical importance of the differences between the test and reference methods. However, no formal criteria against which the clinical importance of the width of the LOA can be judged have yet been published.

Data values are expected to lie between the LOAs and the mean difference must be close to zero (Gibson, 2005:186). At least 95% of the difference values between the two methods should lie within two SDs of the mean difference (Bland & Altman, 1986:310). Satisfactory Bland-Altman plots should include narrow LOAs; mean differences close to zero; data points close to the mean difference and no bias (Bland & Altman, 1986:310, Bakker *et al.*, 2003:402).

## 7.7. METHOD OF TRIADS

When two dietary intake methods are compared, the errors in both the test method (FFQ) and the reference method (weighed records or 24-hour recalls) are correlated (such as memory) and dependent on each other, making it impossible to determine the true intake (Nelson, 1997:260). Because of this, it becomes difficult to determine the level of correlation between the test method and the reference method accurately. Poor correlations between the test and reference method may be because of errors associated with the reference method and not because of the test method (Nelson, 1997:260).

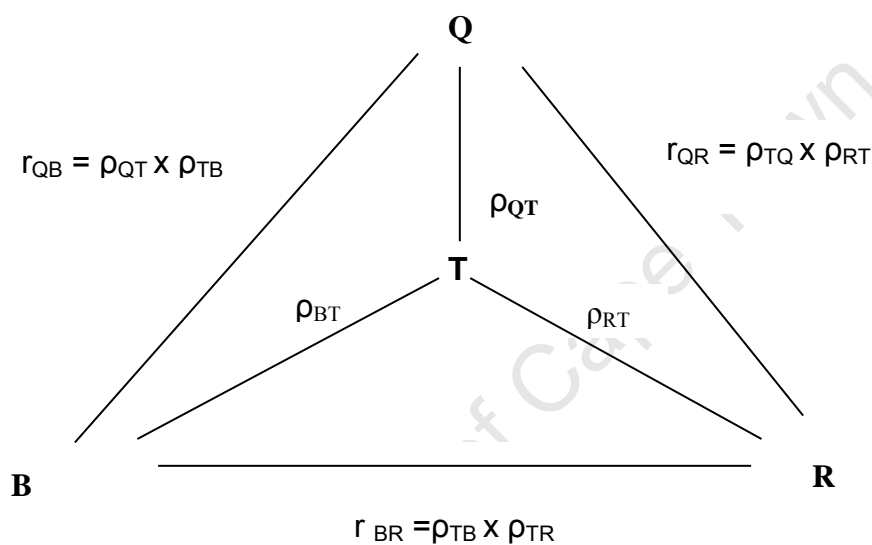
Errors occurring at random, with the use of biomarkers during validation studies will generally be different from those occurring during the application of the FFQ (memory compared to biomarker collection processing and analyses) (Kaaks, 1997:1237S, Bogers *et al.*, 2004:906). Even with different random errors, correlation coefficients from biomarkers and dietary assessment methods are often poor. Therefore, biomarkers should be used with other methods (preferably weighed records or 24-hour recalls). Because no statistical method provides clear-cut answers regarding actual intake and validity of the test method, a triangular comparison between test method, reference method and biomarker should be conducted to determine the true intake of participants (Figure 2.8.) (Bellach, 1993:S45, Kaaks, 1997:1237S, Masson *et al.*, 2003:320). Structural equation modelling can be used to further describe the validity of a dietary assessment method. The method of triads has been developed / constructed to determine the validity coefficient (VC), which is a correlation between the observed and true intake for each of the different validation methods (Kaaks, 1997:1237S).

The method of triads is a triangular comparison between the test method, the reference method, and biomarker and provides a hypothetical estimate of the validity coefficient ( $\rho$ ) of the test method (Figure 2.8.) (Kaaks 1997:1236S).

The method of triads depends on two assumptions: 1) the errors between the test measure and the reference measure are independent from each other, and 2) there is a linear association between the actual intake of a participant and the correlations of the three methods (test, reference and biomarker methods) (Kaaks, 1997:1237S).

Figure 2.8 illustrates the hypothesis regarding the method of triads. The correlation coefficient between the test questionnaire and the reference measure ( $r_{QR}$ ) will be equal to the VC ( $\rho$ ) between the true intake and the test questionnaire ( $\rho_{TQ}$ ), multiplied by the VC ( $\rho$ )

between the true intake and the reference method ( $\rho_{TR}$ ), therefore  $r_{QR} = \rho_{TQ} \times \rho_{TR}$ . The same will be true for the correlation coefficient between the test questionnaire and the biomarker ( $r_{QB}$ ), which would be equal to the VC ( $\rho$ ) between the true intake and the test method (FFQ) ( $\rho_{TQ}$ ), multiplied by the VC ( $\rho$ ) of the true intake and the biomarker ( $\rho_{TB}$ ), therefore  $r_{QB} = \rho_{TQ} \times \rho_{TB}$ . The correlation coefficient between the biomarker and the reference measure ( $r_{BR}$ ) and the true intake and the reference method ( $\rho_{TR}$ ) therefore  $r_{BR} = \rho_{TB} \times \rho_{TR}$ . (Ocké & Kaaks, 1997:1241S). Validation coefficients ( $\rho_{TQ}$ ,  $\rho_{TB}$  and  $\rho_{TR}$ ) can be calculated with the same reasoning (Figure 2.8) (Ocké & Kaaks, 1997:1241S).



$\rho$  = validation coefficient

R = Reference method (24H recall or food record)

B = Biomarker (urinary or blood biomarker)

T = True validation

Q = The test method (FFQ)

Validation coefficients are estimated as follows:

$$\rho_{QT} = \sqrt{r_{BQ} \cdot r_{QR} / r_{RB}}$$

$$\rho_{MT} = \sqrt{r_{RB} \cdot r_{BQ} / r_{QR}}$$

$$\rho_{RT} = \sqrt{r_{QR} \cdot r_{RB} / r_{BQ}}$$

**Figure 2.8. A schematic explanation of the method of triads (Kaaks, 1997:1236S, Ocké & Kaaks, 1997:1241S)**

If all three measurements have high correlation coefficients (test, reference and biomarker methods), then VCs ( $\rho_{TQ}$ ,  $\rho_{TB}$  and  $\rho_{TR}$ ) will be relatively high. If one of the three correlation coefficients (test, reference or biomarker methods) is lower, then two of the three VCs will be low ( $\rho_{TQ}$ ,  $\rho_{TB}$  or  $\rho_{TR}$ ). If two or all three of the correlation coefficients are low (test, reference and biomarker methods), all three VCs will be low ( $\rho_{TQ}$ ,  $\rho_{TB}$  and  $\rho_{TR}$ ) (Ocke & Kaaks, 1997:1241S).

A mathematical complication may arise when computing VC. If the product of two of the three correlation coefficients is larger than the third, it will result in a VC that is larger than 1. This is mathematically not possible, as correlation coefficients are always between -1 and 1. This occurrence is referred to as Haywood cases and may arise in the following situations (Ocke & Kaaks, 1997:1241S): In the presence of random sampling fluctuations, correlation coefficient larger than 1 result. An increased sample size may decrease this effect (Ocké & Kaaks, 1997:1241S). Haywood cases may also appear if one of the mathematical assumptions for the method of triads were violated. In such situations, the estimated validity coefficients are biased (Ocké & Kaaks, 1997:1241S). Finally, the method of triads cannot be applied if one of the correlations is negative as calculation of the square root of a negative value is impossible. Once again, sampling fluctuations might be the cause and, therefore, a larger sample size would avoid the situation (Ocké & Kaaks, 1997:1242S).

In a study conducted by Kabagambe *et al.*, (2001:1127) a FFQ among adults was validated in Costa Rica. Seven recalls and blood biomarkers for carotenoids, tocopherols and fatty acids were compared against the FFQ (Kabagambe *et al.*, 2001:1132). The results indicated good correlations with a median correlation of 0.55 between the FFQ and the recalls (Kabagambe *et al.*, 2001:1134). The validity coefficient was then estimated for the FFQ, the recalls and the biomarkers. The VCs for the FFQ (0.76) and the biomarkers (0.71) were good, while that for the recalls was lower (0.50) indicating that the FFQ reflex the true intake the best of the three methods. However, it was suggested that errors between the FFQ and the recalls might correlate (Kabagambe *et al.*, 2001:1134).

The Dietary Evaluation and Attenuation of Relative Risks (DEARR) study used 3 FFQs, 6 24-hour recalls, 2 x blood samples to determine which is a more valid dietary assessment method to use when measuring macro and micronutrient intake in that specific population (Shai *et al.*, 2005:576). Results also indicated better agreement between the FFQ and urinary biomarkers (nitrogen) compared to the recalls, while the blood biomarkers ( $\alpha$ -tocopherol) compared better to the recalls than the FFQ (Shai *et al.*, 2005:576).

Verkleij-Hagoor *et al.*, (2007:610) used the method of triads to validate a FFQ used in a case-control study. Their validation included the measurement of a FFQ, 3 24-hour recalls and a single sample of serum folate, red blood cell folate and vitamin B<sub>12</sub>. The validity coefficients for the FFQ were 0.94 for serum folate, 0.75 for red blood cell folate and 1.66 for vitamin B<sub>12</sub> (Verkleij-Hagoor *et al.*, 2007:614).

## 8. SUMMARY AND CONCLUSION

Squamous cell OC is prevalent among Xhosa people living in specific rural areas of the EC, especially amongst those with a low education and income, and males (Wild & Gong, 2010:78). Various risk factors have been associated with OC, including alcohol consumption, tobacco use (Ma *et al.*, 2010:644), *N*-nitrosamines (Craddock, 1992:91) and tannins (Craddock, 1992:97). Dietary patterns such as pickled vegetables (Franceschi, 1993:616), high meat consumption, low fruit and vegetables consumption (Danaei *et al.*, 2005:1787) and a maize staple diet have been identified as further risk factors. Fumonisin, (a mycotoxin produced by a fungus growing on maize) has been associated with a high OC (Craddock, 1992:95), especially in the rural EC (Wild & Gong, 2010:77). Fumonisin exposure assessments in South Africa (Gelderblom *et al.*, 1996:279) have been difficult to execute because of a lack of adequate food intake data.

Nutrient deficiencies associated with OC include folic acid (Larsson *et al.*, 2006:1276), nicotinamide (Siassi *et al.*, 2000:300), vitamin C (Muñoz & Castellsagué, 1994:653), vitamin A (van Rensburg, 1987:10), nicotinamide, Mg (Craddock, 1992:93), vitamin D (Abnet *et al.*, 2007:1889), Se (Mark *et al.*, 2000:1753), Zn (Fong *et al.*, 1998:1595) and riboflavin (Guo *et al.*, 1990:125).

Nutrients identified as having a protective effect include carotenoids (Nomura *et al.*, 1997:411), retinol (Zheng *et al.*, 1995:958), vitamin C (Tzonou *et al.*, 1996:302), vitamin E (Eskelson *et al.*, 1993:123) and folic acid (Galeone *et al.*, 2006:523). In addition, an increased intake of fruit and vegetables has also been identified as having a possible protective effect (WHO, 2003:95).

Currently very little is known about the dietary intake of residents in the high OC areas in the EC, however, as study conducted by Marasas *et al.*, (1988:113) reported higher fumonisin levels in maize from households in high OC rate areas compared to those in low OC areas (Marasas *et al.*, 1988:112). Vegetables are only consumed as part of mixed maize meal dishes (such as spinach, pumpkin and cabbage) (Beyers *et al.*, 1979:96) and the ratio maize

to vegetable depends on the available vegetables (Rose, 1972:1358) (vegetables are home grown). Chicken and eggs are only consumed weekly (Beyers *et al.*, 1979:97, Bembridge, 1987:425), although large portions of red meat are occasionally consumed (Rose, 1972:1354). Because of the highly culturally specific dietary patterns associated with the residents in the EC, a dietary assessment method had to be developed to determine dietary intake and therefore fumonisins exposure.

When developing a dietary assessment method, an in-depth understanding of the culture of the target population is important (Strolla *et al.*, 2006:474) and therefore a combination of qualitative and quantitative research methods should be used (Pierce *et al.*, 2007:501). A review of previous dietary surveys provides the necessary background while FGDs provide in-depth information (Cade *et al.*, 2002:569, Pierce *et al.*, 2007:501). A variety of dietary assessment methods are available including 24-hour recalls, FFQs, weighed diet records and diet histories, however various factors influence the choice of dietary assessment method such as the logistics of the study, language, age, gender and level of education of the participants as well as food availability (Bazzarre *et al.*, 1983:211, Cassidy, 1994:191S, Goldbohm *et al.*, 1995:426, Romieu *et al.*, 1997:1160S, Kigutha, 1997:1169S, James, 2004:363).

To increase accuracy of portion size estimation, aids such as food replicas, (Wierden *et al.*, 2003:7), bowls and plates, measuring cups, spoons, drawings and food photographs (Lee & Nieman, 2007:104) can be used. Photographs are easy to use in dietary surveys, because they can be copied, carried easily, and can visually represent various portion sizes (Robinson *et al.*, 1997:123) while participants find it easy and enjoyable (Small *et al.*, 2009:33). However, various factors influence the design of a portion size photograph series, especially the format of the photographs.

These portion size photographs should be validated as it provides information on how the participants perceive the portion sizes depicted on the photographs (Lucas *et al.*, 1995:73). When validating such photographs, actual food should be prepared in exactly the same way as those depicted on the photographs (Lucas *et al.*, 1995:73, Venter *et al.*, 2000:212). Factors such as the characteristics of the participants (gender, age, BMI, education level, occupation), characteristics of the photographs (size, colour, number of portion sizes of photographs, order of presentation) and food items depicted on the photographs (frequency of consumption, preparation of food items, type of food, size of containers) influence the results of portion size photograph validation studies.

In addition to validating food photographs, dietary assessment methods should also be validated, as it provides information on possible misclassification, especially in studies where diet-disease association is studied (Nelson, 1997:241).

Validating dietary assessment methods is difficult because existing methods depend on the ability of participants to provide accurate dietary information (Bingham *et al.*, 1994:227S). Relative or criterion validity is mostly used in dietary assessment validation studies by comparing the test method against another dietary assessment method (Lee & Nieman, 2007:98). Validation studies can unfortunately not compare a test method with the absolute truth, but only against another method. Because both methods are inaccurate, measurement errors must not correlate with each other (to avoid high estimates of validity) (Willet *et al.*, 1985:52, Gibson, 2005:152). Poor validity can not necessarily be attributed to the test measure, as errors may be present in the reference method (Cade *et al.*, 2002:575, Lee & Nieman, 2007:98), and therefore using a third criterion method such as a biomarker is advised.

The third criterion method can include the double-labelled water method, while accelerometers, calculated energy expenditure (such as Schofield's equations) and physical activity questionnaires can be used to validate energy consumption. Furthermore, urinary biomarkers (such as nitrogen, Na or K) can be used, provided that a full 24-hour urinary sample is collected. Completeness of urinary samples should be determined with PABA, creatinine excretion or lithium excretion. In addition to urinary biomarkers various blood biomarkers can also be used.

Reliability (the amount of variation in data obtained when the same questionnaire is repeated) (Bates *et al.*, 1997:172) should be tested along with the validation. Reliability provides information on the accuracy of classifying participants over a longer period, especially when determining diet-disease associations in diseases with long-term exposure, such as cancer (Goldbohm *et al.*, 1995:427). Reliability is mostly tested with a test retest design (Gibson, 2005:129) and the period between the two repeat measurements depends on the time interval of the method (Gibson, 2005:129).

Various statistical approaches can be applied in the assessment of the validation and reliability of dietary intake data. Each statistical method provides information on different facets of the validity or reliability of the dietary assessment method and ideally all statistical methods should show acceptable validity or reliability. These methods include the paired t-test (Gibson, 2005:142), percentage difference between the reference and test method,

correlation coefficients, classifying participants into quantiles, weighted Kappa statistics, Bland-Altman plots and the method of triads can be used.

In view of the information provided in this section of Chapter 2, it is concluded that validity and reliability testing of dietary assessment methods should be based on multiple statistical assessments. The outcome of each statistical method provides perspectives on different aspects of reliability and validity. Final conclusions regarding the reliability and validity of a dietary assessment method should thus be drawn from a combination of the results of these different statistical methods.

University of Cape Town

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## Chapter 3

# OVER-ARCHING METHODOLOGY USED IN THE DEVELOPMENT AND ASSESSMENT OF RELIABILITY AND VALIDITY OF THE RAPP METHOD

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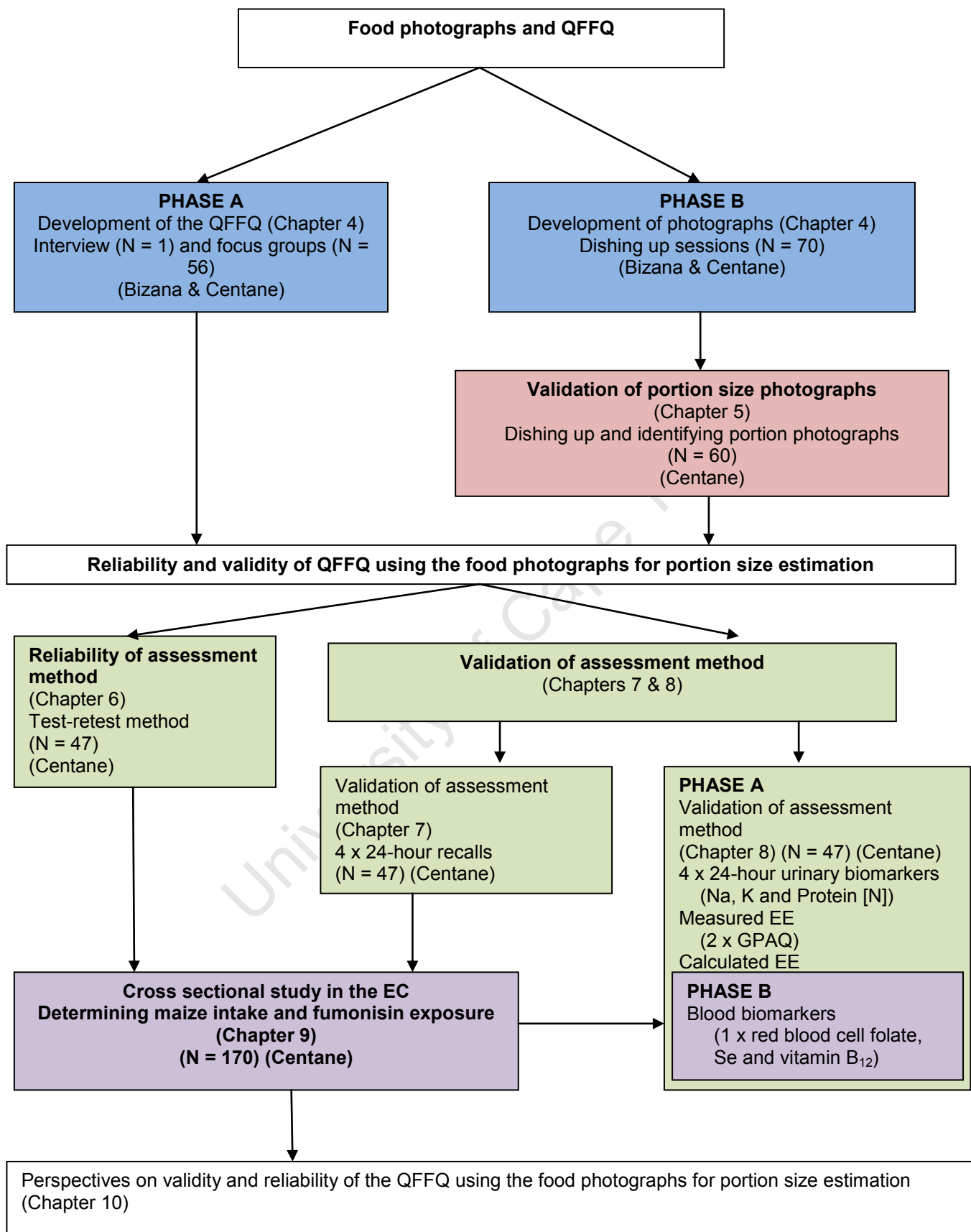


**An interviewer conducting a food frequency questionnaire with a rural participant**

## 1. INTRODUCTION

The aim of this chapter is to present a general layout of the study as well as the over-arching methodology used in the various sub-studies to develop and assess the reliability and validity of the developed RAPP method [food photograph series (FPS) and quantitative food frequency questionnaire (QFFQ)].

Chapter 4 describes the development of the QFFQ and the FPS. Chapter 5 describes the assessment of the validity of the FPS. The effect of different participant characteristics on validity of portion size estimation using the photographs, including gender, age, BMI and education is also explored. Chapter 6 presents the assessment of reliability of the RAPP method (QFFQ and FPS) with a test-retest design. The assessment of the validity of the QFFQ (using the FPS) against four 24-hour recalls is described in Chapter 7, while Chapter 8 describes the assessment of the QFFQ (using the FPS) against biomarkers. These biomarkers include 24-hour urinary biomarkers [Na, K and nitrogen (N)], EE [calculated with Schofield's equations and measured with the Global Physical Activity Questionnaire (GPAQ)], and blood biomarkers (Se, Vitamin B<sub>12</sub> and folic acid). Chapter 9 provides information on the current dietary intake and fumonisins exposure of the participants who participated in the cross sectional study. Chapter 10 presents an over-arching discussion and conclusions of the study. Figure 3.1 provides a schematic representation of the steps taken for the chapters as described above, with different colours indicating different samples from each areas.



**Figure 3.1.** The development and assessment of the reliability and validity of the RAPP method (food photograph series and QFFQ).

## 2. MATERIALS AND METHODS

### 2.1. STUDY POPULATIONS USED

The current study formed part of a larger parent study conducted by the Programme of Mycotoxins and Experimental Carcinogens (PROMEC) of Medical Research Council (MRC). Different study areas and populations were used in the execution of the research for this thesis for the following reasons:

- Research platform: The PROMEC unit has an established research platform in Bizana and Centane. Because of the remoteness of the areas it was decided to conduct the study in these areas where the research platform was already established;
- Logistics of the parent protocol: This study formed part of an over-arching protocol with a number of researchers conducting different research projects in the area (collecting maize samples, developing mycotoxins biomarkers etc.). Because of the distance from Cape Town ( $\pm 1\ 300$  km) all researchers conducted their research in the same geographical area for logistical reasons;
- Funding limitations: Conducting research 1 300 km from Cape Town is expensive. To reduce travel and accommodation expenses all researchers conducted research in pre-determined time slots in collective research areas.

The development (described in Chapter 4) of the RAPP method (FPS and QFFQ) was conducted in two areas: Bizana, a low oesophageal cancer (OC) area and Centane, a high OC area. Both areas are rural, although Centane is classified as a deep rural area. Both areas were included for the above mentioned reasons as well as the importance of ensuring the inclusion of dietary practices of both areas (low and high OC areas). An equal number of FGDs, interviews and dishing up sessions were held in each area.

Photograph validation (Chapter 5) and assessment of the reliability and validity of the RAPP method (FPS and QFFQ) was conducted in Centane only. The reasons for this decision was once again determined by the existing research platform (focussed mainly on Centane as it is a known high risk area for OC and was known that farmers in this area produced maize with a high fumonisins content) and time frame and funding limitations.

Five of the six villages in Centane were thus selected and an equal number of participants in each village identified using snowball sampling (Figure 3.2). Factors that influenced sampling drives include the following:

- Participants and local leaders from one of the villages refused to participate because of cultural beliefs, especially relating to taking physical measures (anthropometry);
- Safety of the research team;
- Accessibility; as villages are not contained living areas and cover large geographic areas with no or very poor accessibility via roads. Roads often do not reach individual houses, only foot paths do (see Figure 3.3).



**Figure 3.2. Selected villages in the high oesophageal cancer area (Centane).**



**Figure 3.3. A typical village in the high oesophageal cancer area (Centane)**

Two sets of participants were recruited from the five villages to prevent respondent “over burden” (equal number of participants from each village). A total of 60 were recruited for the validation of the FPS. A further 60 were recruited for the assessment of the reliability and validity of the QFFQ (using the FPS for portion size estimation).

## **2.2. ANTHROPOMETRIC MEASURES**

The height (measured with a metal *Panomedic* stadiometer) and weight (measured with a calibrated *AND* precision health scale: UC-300) of each participant in the various samples were taken. Fieldworkers were trained and standardized to perform these measures according to the procedures described by Lee and Nieman (1996) (see anthropometric training manual provided in Addendum 10 for detail). Each fieldworker was equipped with a portable, calibrated electronic scale, a stadiometer as well as a wooden platform to provide a level area for the measuring weight and height.

However, cultural perceptions necessitated changes in these standard procedures. Participants believed that weighing and measuring are for burial purposes and thus initially refused to participate. This was discussed with the local chief and *sangoma* who explained the situation to the researchers. In order to establish some trust, participants and the local leaders decided that they would be willing to participate in the weighing and measuring provided that they see the procedure and that nothing is conducted behind the participant's back. Measuring methods were therefore adapted to allow the participant to stand facing the

stadio meter. Also, female participants refused to remove the last layer of blankets. Within the Xhosa cultural context a “big” woman is a sign of having a wealthy husband. Women therefore use various additional layers of blankets to increase their waist circumferences. Due to the layout of the homes of participants, it was not possible to weigh the participants alone in a room (this also related to the trust issue regarding height and weight measures) and therefore privacy could not have been ensured. It was consequently decided to allow woman to wear the last layer of blankets if they wished to do so. A standard blanket weighs approximately 546 g (Figure 3.4) and the error introduced in measuring height facing the stadiometer was determined to be approximately 0.1 cm. It was estimated that these errors might result in a maximum error in BMI of 0.2 kg/m<sup>2</sup>. This difference was considered acceptable and that it would not result in a major systematic error.

The weight and height of each participant were measured twice and the average of the two measures was taken as the final value.

Participants were classified in three BMI categories for classification of weight, namely normal weight: BMI < 25 kg/m<sup>2</sup>, overweight: BMI 25 – 29.99 kg/m<sup>2</sup> and obese: BMI ≥ 30 kg/m<sup>2</sup> (WHO, 2006).



**Figure 3.4. Weight of an average blanket worn as outer clothing by the females of the population**

## 2.3. QUESTIONNAIRES ADMINISTERED

Questionnaires administered in various sub-studies included the following:

- Socio-demographic questionnaire

The socio-demographic questionnaire developed by the PROMEC Unit for use in the rural areas of the Eastern Cape (EC) was utilised (Addendum 12) to describe the socio-demographic characteristics of the population. This questionnaire elicited information regarding demographics and oesophageal cancer (OC) risk behaviours, including age, gender, language, location, medical history, cultural practices, smoking habits, alcohol consumption, occupation and education. Additional information was obtained from a questionnaire adapted from the National Food Consumption Survey (NFCS) conducted in South Africa (Labadarios *et al.*, 1999).

- QFFQ and using food photograph series

See Chapter 4 for detail on the QFFQ and the FPS.

- Four 24-hour recall questionnaires were collected per participant;
- Physical activity questionnaire (GPAQ)

The GPAQ was developed and validated by the World Health Organisation (WHO) (Armstrong & Bull, 2006:66) and standardised for use in South African adults by the University of Cape Town / Medical Research Council (UCT / MRC) Research Unit.

The training manuals provided information on all aspects of the different questionnaires used for each sub-study (Section 4.1 below and Addendums 10 & 11).

## 3. QUALITY CONTROL

### 3.1 FIELD MANAGEMENT

#### 3.1.1. Preliminary field work

Each area was visited before the onset of a research visit in order to liaise with community leaders such as tribal chiefs, headman and local *sangomas*. During the process of the study, these pre-identified leaders were constantly informed regarding the progress of the study. One of these leaders also accompanied the research team and conveyed important messages and information to the community. The Department of Health (DOH) and all

possible employers were also informed about the aims, objectives, ethical aspects and methods to be used during each visit.

### **3.1.2. Team composition**

The team mostly comprised two researchers and two trained, Xhosa speaking interviewers along with a driver / general assistant. During the procedures of the cross sectional study, an experienced phlebotomist was included.

### **3.1.3. Supervision**

After each data collection section, field workers were required to check questionnaires for completeness. A researcher also checked the completeness of the questionnaires after completion and looked for field errors and possible hand writing problems. Furthermore, regular quality evaluations of data in the field were conducted to ensure that all is progressing according to design.

A short meeting was daily conducted to discuss all problems that arose during the day.

## **3.2. TRAINING**

Interviewers received intense training before the onset of each sub-study to conduct effective focus group discussions (FGDs) and dishing up sessions, administer relevant questionnaires and facilitate urine collection. The training offered to the interviewers was in the form of workshops, lectures and practical sessions. Training was provided by the researchers and various specialists. Training was conducted until each individual interviewer reached the minimum standards set for the study. The fieldworkers who performed the best during these practical applications and tests were identified and were then appointed as primary field worker for the particular sub-study. The high standard set by this training program was maintained throughout the study and all interviewers were subjected to regular training sessions.

Furthermore, training manuals were compiled and provided to each researcher and interviewer before the onset of the different phases of the study. These manuals covered all aspects of the particular sub-study (where relevant) regarding the following:

- Introduction at the household;
- Interviewing techniques;
- Information and consent;
- Sampling methods;
- Required measurements such as anthropometric measures;
- Completing the required questionnaires;
- Required sample collection.

These manuals also described the functions and responsibilities of each team member during each particular visit (Addenda 10 and 11).

Training was conducted at the following times and was always conducted two weeks before the onset of the study:

- Development study (Chapter 4): January 2003;
- Validation of FPS (Chapter 5): January – February 2004;
- Assessment of reliability and validity (Phase A) of the RAPP method: January – February 2004 and May 2004;
- Cross sectional study: May 2005, October 2005 and January 2006.

### **3.2.1. Dishing up sessions**

In order to ensure accuracy, a researcher weighed the actual food, while an interviewer provided assistance. Fieldworkers received training on the different steps involved in the dishing up sessions. The plates used in the dishing up were washed, dried and weighed before each portion was dished up. The electronic, digital laboratory scale was set on a level surface during the weighing process. The weight of the plate was subtracted during data capturing.

### **3.3. PILOT STUDY**

After completion of the training for each field visit, a pilot study was conducted in Cape Town before the roll out in the field. As part of the pilot test, each interviewer completed all required measurements for five individual participants. After completion of the pilot study, the researchers and interviewers jointly addressed all questions and problems that arose.

## **4. STATISTICAL ANALYSES**

Data were captured by an experienced data capturer after which two researchers checked every entry of each questionnaire. All discrepancies were discussed with the person responsible for capturing the data. Where disagreement arose between the researchers and the data analyst, a third researcher or the interviewer was approached before the final decision was made.

All continuous data was tested for normality with the Shapiro Wilk test and found to be skewed.

Further statistical calculations were performed with the Software program Stata 6. A range of seven statistical tests (see Sections 5.1 – 5.7) were conducted for the validation of the photographs and the assessment of the reliability and validity of the QFFQ using the photographs for portion size estimation.

### **4.1. PAIRED T-TEST OR WILCOXON SIGNED RANK TEST**

The nonparametric Wilcoxon matched-pairs signed-rank test (for skewed data) was used to indicate the level of agreement between two measures at group level (Gibson, 2005:142). Significance levels were set at  $p = 0.05$  (Nelson *et al.*, 1994:653). P-values larger than 0.05 were considered acceptable (indicating no significant difference between the two measures), while P-values smaller than 0.05 were considered poor.

## 4.2. MEAN PERCENTAGE DIFFERENCE BETWEEN THE REFERENCE METHOD AND THE TEST METHOD

The mean percentage difference between the reference measure and the test measure is used as a measure of accuracy (Kuehneman *et al.*, 1994:548). For each assessment, the reference measure is subtracted from the test measure, divided by the reference measure and multiplied by 100 (Nelson *et al.*, 1994:653, Robinson *et al.*, 1997:120). A mean percentage difference is then calculated for the total sample.

$$\% \text{ Difference} = \frac{\text{test measure} - \text{reference measure}}{\text{reference measure}} \times 100$$

Values reported in the tables reflect the mean percentage difference for the group, which is then used to compare with the given cut-off values.

Positive values indicate over-estimation, while negative values indicate under-estimation (Nelson *et al.*, 1994:653). The difference should be as close to zero as possible. Estimation is considered “accurate” (good for the purpose of this research) if the percentage difference (PD) is smaller than 10% (Venter *et al.*, 2000:209), acceptable if PD is between 11% and 20% and poor if larger than 20% percentage difference.

## 4.3. CORRELATION COEFFICIENTS BETWEEN THE REFERENCE METHOD AND THE TEST METHOD

Spearman correlation coefficients were used to measure the strength of the relationship between two different measurements at individual level. Correlations indicate the level of linear association between two measures. A correlation coefficient of 1 indicates perfect linear association (when one measure is high, the other is also high), while a correlation coefficient of -1 indicates perfect negative linear association (when one measure is high, the other is low). Correlations of 0 indicate no linear association (Easton & McColl, 2010). Correlations less than 0.2 were considered poor, correlations between 0.20 and 0.50 were classified as acceptable while correlations above 0.50 were considered to be good (Masson *et al.*, 2003:320).

Confidence intervals for correlation coefficients of normally distributed data can be calculated with the Fisher's z transformation. However, an independent statistician recommended that this should not be calculated in the present study because of the

skewness of the data. Therefore the significance of the correlation coefficients was based on p-values.

#### **4.4. CLASSIFYING PARTICIPANTS INTO QUANTILES**

Because of the small sample size, all food group and nutrient intake data were classified into three tertiles and not in quartiles as is recommended (Altman & Bland, 1994:996, Masson *et al.*, 2003:320). As the data was found to be skewed, the median and the 10<sup>th</sup> and 90<sup>th</sup> centiles were used to determine the tertiles (Altman & Bland, 1994:996).

Ideally, (outcome denoted as good for the purposes of this study), more than 50% of the participants should be classified in the same tertile with less than 10% of participants classified in the opposite tertiles (Masson *et al.*, 2003:320). If the outcomes do not comply with these criteria, it is denoted as being poor.

#### **4.5. WEIGHTED KAPPA STATISTICS**

To exclude chance agreement, the food group and nutrient intake data were analysed with weighted Kappa statistics (Masson *et al.*, 2003:319, McGinn *et al.*, 2004:1370, Gibson, 2005:143).

Kappa values can range from -1 to 1 with values between 0 and 1 being generally expected (Sim & Wright, 2005:259). Values of zero or close to zero can be considered as an indication of “*no more than pure chance*”, while negative values indicate agreement “*worse*” than that expected by chance alone (Barkto, & Carpenter, 1976:315, Sim & Wright, 2005:259, Viera & Garrett, 2005:361).

For the purposes of this study, weighted Kappa values higher than 0.61 reflected good agreement, 0.21 - 0.60 acceptable agreement, while less than 0.20 reflecting poor agreement (Landis & Koch, 1977:170, Masson *et al.*, 2003:315, McGinn *et al.*, 2004:1370, Viera & Garrett, 2005:362).

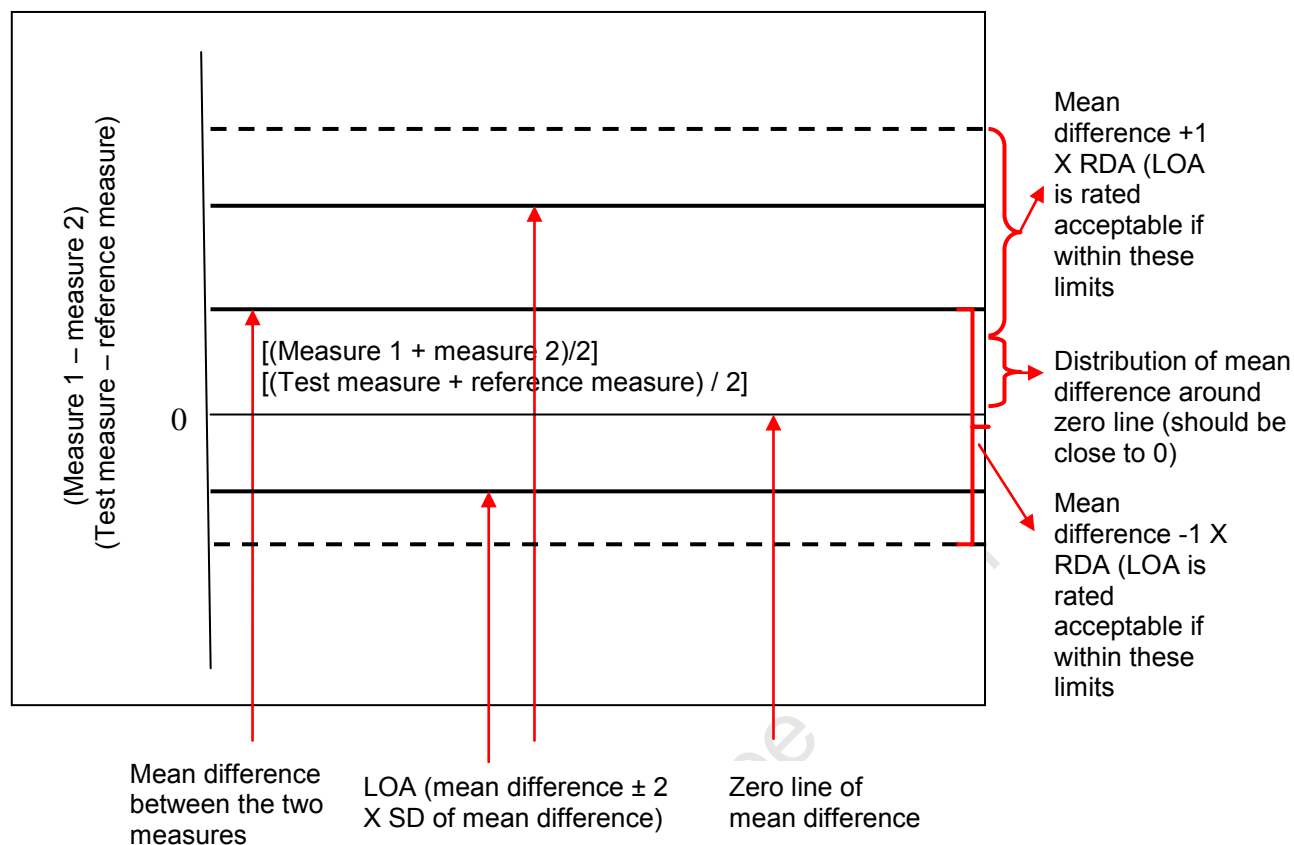
#### 4.6. BLAND-ALTMAN PLOTS

Bland-Altman plots were used to determine the presence and the extent of bias, as well as the level of agreement between two measures (Cade *et al.*, 2002:573).

Currently, the literature advises visual inspection of the plots to determine the presence of bias and the width of the LOA (Carithers *et al.*, 2009:1187).

The y-axis of the Bland-Altman plots indicates the mean difference between the two measurements (test - reference measures) while the x-axis indicates the mean of the two measures  $[(\text{test measure} + \text{reference measure} / 2)]$  (Bland & Altman, 1986:308, Gibson, 2005:186) (Figure 3.5).

Spearman correlation coefficients were calculated (between the mean of the two methods and the mean difference of the two methods) to establish a relationship between the size of the error (or difference between the two methods) and the mean intake of these methods. Such a relationship would indicate the presence of proportional or reporting bias (Bland & Altman, 1986:307, Gibson, 2005:187). A significant Spearman correlation coefficient ( $p < 0.05$ ) indicates the presence of proportional bias (poor outcome) as well as the direction thereof (MacIntyre *et al.*, 2001b:65, Gibson, 2005:144).



**Figure 3.5. Schematic interpretation of Bland-Altman plots of the current study**

Two lines [the limits of agreement (LOA) were calculated (mean difference between the two measures  $\pm$  2 SD of the mean difference)] (Bland & Altman, 1986:310, Gibson, 2005:187) (Figure 3.5). Data values are expected to lie between the LOAs and the mean difference must be close to zero (Gibson, 2005:186). At least 95% of the difference values between the two measures should be within two SDs of the mean difference (Carithers *et al.*, 2009:1193, Osowski *et al.*, 2007, 1352, Bland & Altman, 1986:310).

Bland and Altman (1986:308) indicated that the width of the LOA should be judged according to the clinical importance of the differences between the test and reference methods. However, no set criteria against which the clinical importance of the width of the LOA can be judged within the nutritional context have yet been published. For the purpose of assessing the clinical importance of the LOAs found in the portion size and energy and nutrient comparisons in this study, the following was proposed:

- For the purpose of portion size estimation the small portion size for photograph validation should be used as a standard against which to evaluate the width of the LOAs as this is the largest error that will be allowed. If the medium or large portion size is used to determine the width of the LOA a very large margin of error will be

allowed for. Therefore, the mean difference between the two methods  $\pm 1 \times$  small portion size (to minimize error introduced by the photographs);

- For food groups the average small portion size should be used as a standard against which to evaluate the width of the LOAs, (mean difference between the two methods  $\pm 1 \times$  average small portion size) (to minimize the size of the error);
- For energy and nutrients the recommended dietary allowances (RDAs) for females (Standing Committee on the Scientific Evaluation of Dietary Intake References, 1997, 1998, 2000, 2001, 2004),, should be used as a standard against which to evaluate the width of the LOAs (mean difference between the two methods  $\pm 1 \times$  RDA). If this is applied it would mean that if the difference between two reports is more than the RDA for that particular nutrient, the participant could potentially be classified as having a deficient intake using the one method and as having an adequate intake using the other method. Clinically this would be unacceptable. Using the RDA as a standard to evaluate the width of the LOAs can be effectively applied for most nutrients. The clinical significance of the LOAs is indicated in the text as either  $< \text{RDA} / \text{small portion size}$ ,  $> \text{RDA} / \text{small portion size}$  or  $\approx$  to indicate that it is very close if not equal to the  $\text{RDA} / \text{small portion size}$ .

Limits of agreement wider than the mean difference  $\pm 1 \times$  small portion size / RDA are therefore not acceptable (Seele, 2007:102).

Satisfactory Bland-Altman plots should thus include narrow LOAs; mean differences close to zero; data points close to the mean difference and no bias. Currently these guidelines are mostly based on visual inspection (Bland & Altman, 1986:310, Bakker *et al.*, 2003:402, Seele, 2007:102).

#### **4.7. METHOD OF TRIADS**

The methods of triads was used to determine the validation coefficient for urinary sodium, potassium, protein (urinary nitrogen converted to protein), as well as for blood folate, selenium and vitamin B<sub>12</sub>. Chapter 8 provides more detail on this method.

#### **4.8. STATISTICAL SUMMARY**

The outcome of all seven tests were summarized and compared to interpretation criteria in a comprehensive table included in each sub-section of the research and analyses. Table 3.1 presents a generic example of these tables.

For final interpretation of the reliability and validity results the research group decided after in-depth consultations that reliability and validity could be deemed acceptable if four or more of the seven tests indicated acceptable or good outcomes, based on the stated criteria.

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Table 3.1 Example of summary table of statistical test outcomes in terms of specified interpretation criteria

| Nutrient            | Agreement                 | Agreement             | Strength of association | Agreement Including chance | Agreement Excluding chance | Bias                                  | Agreement                   | Final Reliability                 |
|---------------------|---------------------------|-----------------------|-------------------------|----------------------------|----------------------------|---------------------------------------|-----------------------------|-----------------------------------|
|                     | Wilcoxon Signed rank test | Percentage difference | Spearman correlations   | Tertile classification     | Weighted Kappa statistics  | Bland – Altman (Spearman correlation) | Bland-Altman (Width of LOA) | > 3 acceptable validation results |
| Level of validation | Group                     | Group                 | Individual              | Individual                 | Individual                 | Individual                            | Individual                  |                                   |
| Energy (KJ)         | Poor                      | Poor                  | Good                    | Poor                       | Poor                       | Absent                                | Wide                        | Poor                              |
| Protein (g)         | Poor                      | Poor                  | Good                    | Poor                       | Poor                       | Absent                                | Wide                        | Poor                              |
| Fat (g)             | Poor                      | Acceptable            | Good                    | Acceptable                 | Good                       | Absent                                | ne                          | Good                              |
| Carbohydrates (g)   | Poor                      | Poor                  | Good                    | Poor                       | Poor                       | Absent                                | Wide                        | Good                              |

Wilcoxon signed rank test agreement at group level  $p < 0.05$  = Acceptable,  $p > 0.05$  Poor

Percentage difference:  $< 10\%$  = Good,  $11 - 20\%$  = Acceptable,  $> 20\%$  = Poor

Strength of association and correlation = Results from correlation coefficient (individual level)

Good =  $> 0.50$ , Acceptable  $0.20 - 0.50$  and Poor  $< 0.20$

Agreement = Results from tertile classification (include chance) and Kappa statistics (exclude chance)

Bias = Bland-Altman data indication of bias on individual level (if  $r_{BA}$  is significant),

$r_{BA}$  = Spearman correlation coefficient for Bland-Altman data (correlation between mean and difference of intake)

Agreement Bland-Altman:  $< 1 \times RDA$  = Narrow LOA,  $\approx 1 \times RDA$  = Acceptable,  $> 1 \times RDA$  = Wide LOA

ne = RDA not established

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## Chapter 4

# DEVELOPMENT OF THE QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE AND FOOD PHOTOGRAPHS

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**A rural woman in Centane strengthening her hut's wall with mud**

## 1. INTRODUCTION

In order to develop an appropriate dietary assessment methodology it was necessary to take the unique cultural, educational and environmental factors of the population into account. Various factors influenced the choice of dietary assessment method for this population, including:

- Logistics; i.e. distance to travel with equipment and questionnaires as well as time constraints indicated to the use of a single assessment reflecting habitual intake [such as a quantitative food frequency questionnaire (QFFQ) rather than various 24-hour recalls that does not necessarily reflect habitual intake];
- Education level of participants; the population are extremely rural and are largely semi-literate and face-to-face interviews would be required instead of self-administered questionnaires;
- Language; various dialects of isiXhosa exist, therefore English would need to be the language for written material (questionnaire), while it is administered by an isi Xhosa speaking field worker in the appropriate dialect;
- Food availability; the participants are subsistent farmers and although little is known about their dietary habits, it was expected that their diet would be monotonous;
- Cooking and eating practices; no measures are used in cooking and eating from a communal pot is not common;
- Oesophageal cancer; long-term habitual dietary intake was therefore required.

It was therefore decided to develop a culturally specific quantified food frequency questionnaire (QFFQ) that can be implemented as a single assessment that provides information on long term dietary intake and seasonal variability. To ease the administration process, to shorten the administration time and to increase accuracy of the QFFQ especially in the light of the low literacy levels of the participants, it was administered by isiXhosa speaking interviewers.

It is suggested that using a QFFQ for this population reduces respondent burden and increases accuracy as no record keeping is required. It captures dietary intake over a period of one month, which may reflect longer term dietary intake as the diet of the target population is believed to be monotonous. This type of dietary assessment method can be quick to administer, cheap and feasible for large studies.

A challenge that was identified in the development process is the fact that the Xhosa people either eat home-grown maize on its own or combine the maize with vegetables such as pumpkin and spinach. The ratio of maize to vegetables varies considerably and in order to assess maize intake accurately, it was necessary to develop a portion size estimation tool that would also display the ratios adequately. This led to the development of photographs comprising maize dishes in various ratios and portion sizes.

The aim of this chapter is therefore to describe the steps taken to develop the culturally specific dietary assessment questionnaire (QFFQ), as well as the development of a food photograph series (FPS) to be used in conjunction with the QFFQ.

## **2. DEVELOPMENT OF THE QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE AND SERIES OF PHOTOGRAPHS**

The development of the QFFQ and food photograph series (FPS) were mostly conducted throughout 2002 - 2003 and executed in three separate phases (Figure 4.1 and 4.2):

*Phase A:* The development of a culturally specific QFFQ.

*Phase B:* The development of three standard [small (S), medium (M) and large (L)] portion sizes and three different ratio photographs of maize and / or vegetables to accompany the QFFQ (FPS).

*Phase C:* Revision of the QFFQ and FPS developed during Phases 1 and 2.

### **2.1. PHASE A: DEVELOPMENT OF THE CULTURALLY SPECIFIC QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE**

To develop a culturally specific QFFQ, it is imperative to know what participants in the area under investigation consume. This includes knowledge of the recipes used for specific dishes. Phase A describes the steps taken to identify food items and dishes to be included in the QFFQ, as well as the recipes and ingredients used for these items and dishes.

#### **2.1.1. Development of a preliminary food frequency questionnaire**

A food frequency questionnaire (FFQ) obtained from the Nutrition and Dietetics Division at the University of Cape Town (UCT) (developed for urban Xhosas) was used as the basis for the development of the QFFQ. The original FFQ included 139 items, which were divided into ten food groups that included bread, porridge, vegetables, fruit, meat, dairy, snacks, condiments, beverages and fat (Addendum 14).

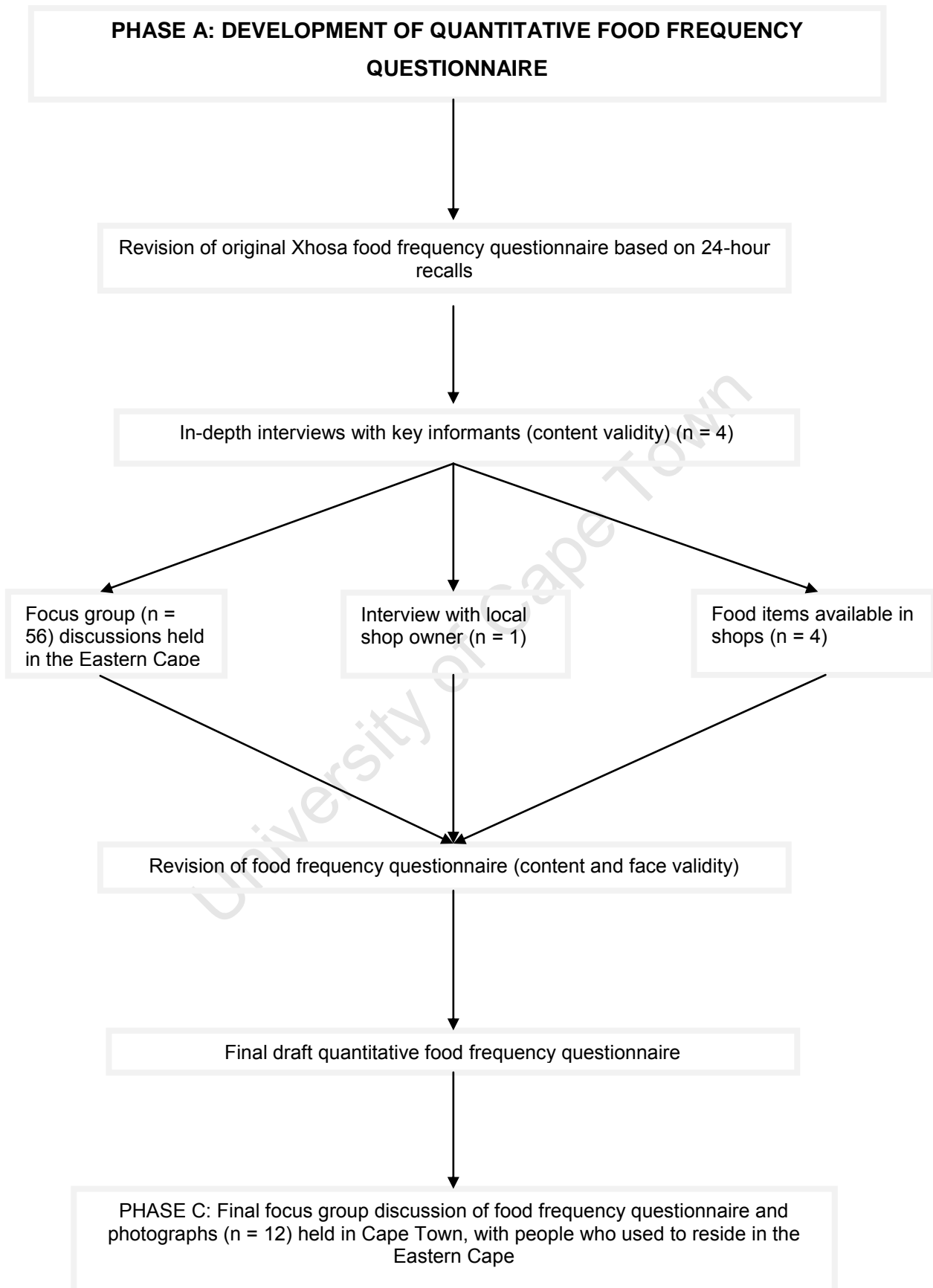
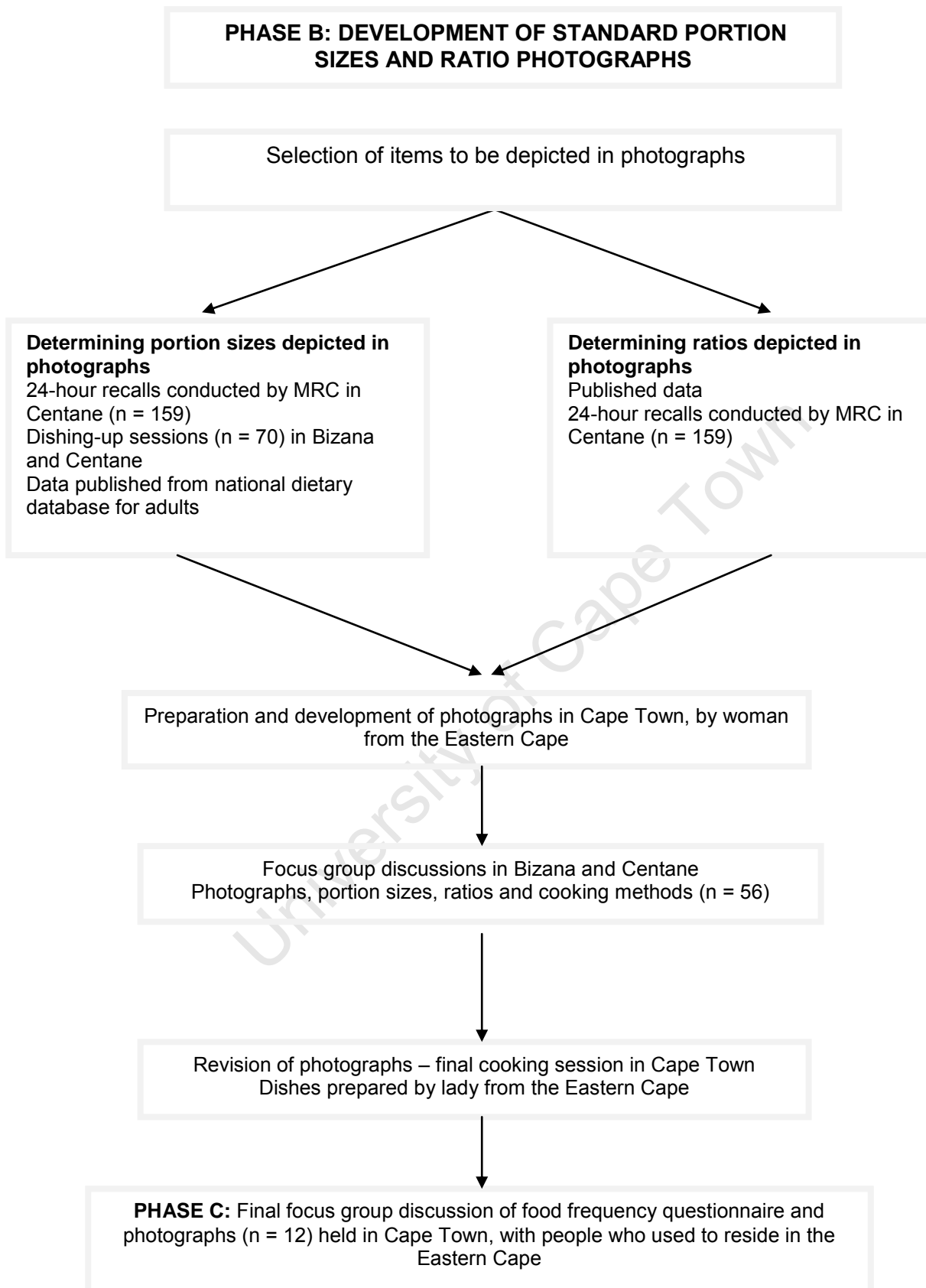


Figure 4.1. Process followed during the development of the quantitative food frequency questionnaire



**Figure 4.2. Process followed during the development of the portion size and ratio photographs**

The original FFQ from UCT was then checked against food items determined from previous 24-hour recall questionnaires completed in the Eastern Cape (EC) by the Program for Mycotoxins and Experimental Carcinogens (PROMEC Unit) of the Medical Research Council (MRC). The 24-hour recalls (n = 159) were conducted during June 2001, in the same rural areas under investigation for this study (Bizana and Centane). The following food items were subsequently added to the original FFQ: bread, samp, *umngqusho* (samp and beans), porridge, pumpkin, meat, *amagewu*, rice, eggs, vegetables (Addendum 15 shows food items included and excluded from this section). The original FFQ<sup>3.2</sup> was revised using the information obtained from the 24-hour recalls (Addendum 16) (August – October, 2002).

### **2.1.2. In-depth interviews with key informants and focus group discussions**

To ensure face and content validity of the QFFQ, in-depth interviews were conducted with four women who originated from the EC, but who were residing in Cape Town at the time of the study (November 2002). These interviews were conducted in the participants' first language, isiXhosa, with the help of two isiXhosa-speaking interviewers. This was done to update and improve the draft QFFQ. The women evaluated the draft QFFQ and identified the most commonly used food preparation methods and recipes used with specific reference to rural areas of the EC.

Following on from the in-depth interviews the list of food items used for the draft QFFQ was modified and evaluated by means of FGDs conducted in the EC (February, 2003). These sessions provided further information on the food items, cooking methods and recipes of the dishes consumed by the residents. To ensure that food items and dishes from all areas of the rural EC were included, the focus groups were conducted in peri-urban and rural areas, which included areas from the north-eastern and the south-western parts (Figure 1.1). Two focus group sessions were conducted in each of the two areas (Bizana and Centane) (Figure 4.3 & Figure 4.4).

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<sup>3.2</sup> The original urban FFQ was obtained from the Nutrition and Dietetics Division at UCT



**Figure 4.3. Focus group discussions in rural Centane**



**Figure 4.4. Focus group discussions in rural Bizana**

Women aged 18 to 55 year ( $n = 56$ ) were included in the discussions. Although the males were excluded from the FGDs, the most senior males from each village attended but did not participate in the discussions. In total, 30 participants from Bizana (15 per focus group) and another 26 participants from Centane (13 per focus group) participated in these sessions. Participants were residents of the above-mentioned areas who volunteered to participate. They were recruited on a door to door basis. Two nursing sisters from the EC rural areas (employed by the MRC) were trained as facilitators (January, 2003) and each received a flexible guide for conducting the FGDs.

Information and consent were obtained before participation, in isiXhosa, the participants' first language (Addendum 2).

Exclusion criteria used for the recruitment of volunteers were the following:

- a) Men, because traditionally they are not involved in the cooking process (Rose, 1972:1356);
- b) Women who did not prepare traditional Xhosa dishes;
- c) Women with hearing or speech impediments;
- d) Women who were not residing in the pre-selected areas (visitors).

Participants in the focus groups discussed the foods included on the draft QFFQ to ensure that all traditional and local (Xhosa) foods were included and the unnecessary food items were excluded. Discussions included information regarding bread, cereals, combined dishes (maize meal and vegetables) vegetables, fruit, meat, milk, eggs, snacks, herbs and spices and fat, as well as recipes and the ingredients that were used locally by the Xhosa people.

Focus group discussions were recorded and transcribed by hand and then translated to English by another Xhosa speaking interviewer (March – August 2003). The transcriptions of the different FGDs were compared (by an expert panel) and the most frequently mentioned food items, dishes and recipes were included on the revised QFFQ.

The following information that was generated during the FGDs contributed to the revisions of the draft QFFQ:

- Bread

Bread (mostly home-baked maize based) forms a large part of the staple diet. Commercially baked bread (wheat based) is regarded as expensive and only bought on special occasions. Home-baked maize bread includes a variety of recipes, including steamed bread, baked bread, corn bread and dumplings<sup>3.3</sup> (see training manual for recipes). *Vetkoek*<sup>3.4</sup> and *unonca*<sup>3.5</sup> are also consumed from time to time. Rainfall and maize yields determine the amount of home-made bread baked, especially in the more rural areas.

- Cereals

Corn on the cob is popular and is roasted on a fire. Whole corn kernels are mostly parboiled and consumed as a snack, especially in the more rural areas of the EC. Samp (dried whole maize kernels) is not frequently consumed as a dish on its own but as part of a combined (samp and beans) dish. Mealie rice (crushed dried maize kernels) is mostly consumed in rural areas like Centane and not in peri-urban areas, and can be used as the basis for combined dishes.

Maize meal is prepared in three different ways – as porridge, stiff *pap* and crumbly *pap* (*umphokoqo*). Porridge is soft and consumed with milk or fermented milk for breakfast. Stiff *pap* (maize meal with a thick consistency) and crumbly *pap* (*umphokoqo*) are consumed for lunch and dinner. Stiff *pap* also forms the basis of most combined dishes. Combined dishes that are consumed include: maize meal and *imifino*,<sup>3.6</sup> maize meal and spinach, maize meal and pumpkin, maize meal and dried sugar beans, samp and dried sugar beans, soup (kernels and dried sugar beans), mealie rice and *imifino*, mealie rice and spinach, mealie rice and pumpkin, cabbage (a combination of cabbage, tomato and potato) and *bisto*<sup>3.7</sup>.

Sorghum is never consumed as a dish, but is used for brewing beer. Oats is rarely consumed. Brown rice is never consumed and was therefore excluded from the FFQ.

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<sup>3.3</sup> Dumplings are home-baked maize bread dipped in a watery broth

<sup>3.4</sup> *Vetkoek* is bread dough fried in oil

<sup>3.5</sup> *Unonca* is a traditional bread made from maize and consumed by the older people

<sup>3.6</sup> *Imifino* is a spinach-like vegetable that grows wild in the EC

<sup>3.7</sup> *Bisto* is a combination of tomato, onion and potato stew

- Vegetables

Vegetables consumed are mostly home-grown. The following vegetables are commonly consumed: cabbage, spinach, pumpkin, onions and potatoes. Vegetables are generally cooked with maize meal porridge and not consumed as dishes on their own. Based on this it was decided to exclude vegetables as “single” dishes and to include them as part of combined dishes.

- Fruit

Fruit is very rarely consumed and if, then it would be bought on “pension day” (when grants are paid). Usually only children eat wild fruit. Commercially available fruit includes: apples, bananas, pears, peaches, oranges, grapes and to some degree strawberries although these are procured by tourists rather than the residents of the area. Dried fruit and fruit juice were not available. Based on the low frequency of fruit consumption (twice or thrice a year) it was decided to exclude fruit as a food group from the FFQ. Participants consuming fruit could report on this in the “other foods consumed” section.

- Meat

Chicken is by far the preferred choice of meat but pork, mutton and beef are at times also consumed. Cattle are slaughtered for special occasions like weddings and funerals and then consumed in huge amounts (because of the lack of cold storage). Tinned fish (mostly pilchards) is bought on special occasions. Although some residents consume corned meat “Bully beef”, it is expensive and only consumed on special occasions. Minced meat, sausages, pies and bacon are not consumed or procured at all (because of cost). Although liver (all kinds) is popular, it is not consumed regularly. However, when animals are slaughtered the men usually consume the liver.

Meat is boiled with salt as flavouring. When possible tomato and onion are added to the meat and no other spices are used.

- Eggs

Eggs are boiled and eaten with bread and tea.

- Beverages

Milk consumption is common in the EC. In Bizana (peri-urban) people do not necessarily own cattle and full cream long life milk is generally bought. In Centane (rural) people are able to have fresh cow’s milk. In both areas milk is left to turn sour, and is then consumed with dry crumbly porridge.

Tea and coffee are regularly drunk, also *amagewu*. *Amagewu* is a beverage made from cooked maize meal<sup>3.8</sup> and white bread flour that is left overnight. The following morning it is strained and drunk three or more times a day. *Inqodi* (similar to *amagewu*) is fermented over a longer period.

The most common cold drinks consumed are orange squash (Oros and Orocrush). Carbonated cold drinks are regarded as being too expensive.

- Fat

Fat consumed includes “Holsum” (commercial white animal fat), sunflower oil (mostly referred to as fish oil) and animal fat (derived from cooling meat). Brick margarine is popular and when available it is used on bread, while animal fat and sunflower oils are used in cooking. It was decided not to include margarine on the FFQ as this is not consumed on a regular basis, “*Holsum*”, animal fat and sunflower oil were included in specific recipes and not as single items on the QFFQ.

- Condiments

Spices are rarely used. The most commonly used condiments are sugar and salt, chilli Aromat (flavoured salt high in monosodium glutamate), and curry powder.

Honey is consumed if there is a hive in the vicinity, but it would not be procured because of its cost. Some people in the area would occasionally buy jam. Peanut butter and Marmite are also consumed infrequently (less than once a month) and therefore not included on the QFFQ.

- Sweets and desserts

Sweets, crisps and biscuits are rarely bought, and then mostly for children. Desserts are consumed at special occasions, such as weddings and funerals (maybe three or four times a year) and usually include tinned fruit, custard and jelly. Because of the infrequent consumption of sweets and desserts, it was decided not to include it on the QFFQ.

### 2.1.3. Interviews with local shop owners

An interview was conducted with one of the few local shop owners who had been in the Centane area for more than 50 years (February 2003). This was done since the only literature on food choices and consumption of traditional Xhosa dishes was considered to be outdated (Rose, 1972:1353, Beyers *et al.*, 1979:96). During this interview, the shop keeper described what people in Centane currently preferred to buy, and how their choices had

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<sup>3.8</sup> The terms maize, corn and mealies are used interchangeably

changed over the past decade. Local shops in Bizana were not included as they were more formal South African chain stores such as Checkers. A number of local shops (n = 4) were visited by the researcher in order to ensure that food items sold at local shops were included in the questionnaire. The local shops were visited and a list was compiled from the items available. These items are listed in Addendum 17.

#### **2.1.4. The final draft quantitative food frequency questionnaire**

All the information obtained during the various steps was integrated to provide a final draft food list for the QFFQ, comprising the most commonly consumed foods and beverages.

The final QFFQ (Addendum 18) comprises 33 items, subdivided into 6 food groups (based on the original FFQ); bread (mostly maize-based), cereals (main maize dishes), combined dishes (maize and vegetables), meat, condiments and beverages. The different food groups are colour coded on the questionnaire to coincide with colour codes on the back of the photographs. This colour coding assists in sorting the photographs.

The QFFQ was developed to determine habitual intake over a period of a month, and includes information such as: “home-grown / bought”, “portion size”, “portions at a time” (if they consume more than one portion at a single time), “less than once a month”, “amount per week”, “amount per day” (if they consume the dish more than once a day). If an item is consumed less than once a month, it is recorded as such and the rest of the information is not completed as consumption of an item less than once a month is not sufficient to make a difference to the nutrient intake.

The QFFQ also provides an opportunity for the participant to report the maize source used for the different maize-based dishes (commercially procured or home-grown). This provides crucial information regarding maize consumption (raw and cooked), and thus dietary fumonisins exposure; as well as nutrient intake resulting from mandatory fortification of commercially procured maize meal. Home-grown maize is not fortified while commercially obtained maize is. The QFFQ allows for ratios to be reported for combined dishes. If more than one ratio of a particular mixed dish was consumed during a particular month it can also be recorded.

The amount of each dish consumed in the period of a month is calculated and divided by 28 to provide a daily amount consumed. The amount of daily consumed cooked maize is then converted to the dry maize portion. (Ratios of dry to cooked maize were carefully calculated during the cooking sessions). Once the dry maize amount for home-grown and commercially

procured maize is calculated, the exact fumonisins exposure can be determined by multiplying the raw maize weight with the known fumonisins content and by summing the fumonisins exposure calculated for both home-grown and commercially procured maize.

Because of different dialects in the Xhosa language, the questionnaire is available only in English and interviewers were trained to translate it into isiXhosa as they explain the different foods and dishes.

## **2.2. PHASE B: DEVELOPMENT OF THE FOOD PHOTOGRAPH SERIES**

### **2.2.1. Determination of portion sizes**

Because portion size photographs can increase accuracy during reporting of dietary intake (Posner *et al.*, 1992:740) and since the population was mostly semi-literate (De Souza, 2001:242), it was decided to develop and use portion size photographs of the most frequently consumed dishes. Based on practicality and published literature (Beyers *et al.*, 1979:99) three portion sizes (S, M and L) were developed. Since local residents combine maize with vegetables (Beyers *et al.*, 1979:99) and the ratio of maize to vegetables varies according to availability, the FPS that was developed includes three<sup>3.9</sup> photographs depicting different ratios of maize and vegetable dishes (compact disc 1 [CD 1]).

The FPS was used in conjunction with the QFFQ during the dietary intake survey (Chapter 9). Each local dish was depicted by three photographs of three portion sizes (S, M and L). Coding on the reverse side of the photographs correspond with that on the QFFQ, so that the interviewer never saw the front of the photograph (i.e. blinded), but rather the coding on the reverse side. After indicating that they had consumed a specific dish, participants were shown the portion size photographs of that dish in order to assist them in selecting the amount consumed. In order to develop the photographs, standard portion sizes and ratios of combined dishes consumed in the area were first determined.

Three different methods were used to determine standard portion sizes of commonly consumed foods in the area studied:

- 1) Evaluation of previous 24-hour recalls (n = 159) (data collected by the PROMEC Unit, MRC during June, 2001 in Centane) (Table 4.1) was done as an initial step to determine standard portion sizes consumed by people living in rural areas of the EC (May –

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<sup>3.9</sup> Four different ratio photographs were originally developed but only the three most frequently consumed ratios (according to FGDs) were included in the final method

June, 2003). Ratios for the different combined dishes were identified from previously published data (Beyers *et al.*, 1979:99) as well as from these 24-hour recalls.

**Table 4.1. Portion sizes obtained from 24-hour recalls conducted in the Eastern Cape (2000 – 2001) (unpublished)**

| Dish<br>n = 159                         | Percentiles    |                 |                | Minimum | Maximum | Mean | SD  |
|---|----------------|-----------------|----------------|---------|---------|------|-----|
|   | 25%<br>(Small) | 50%<br>(Medium) | 75%<br>(Large) |         |         |      |     |
| Porridge (g)                            | 338            | 470             | 590            | 184     | 862     | 490  | 176 |
| Stiff <i>pap</i> (g)                    | 375            | 501             | 703            | 210     | 1290    | 555  | 234 |
| Stiff <i>pap</i> and cabbage (g)        | 364            | 526             | 707            | 255     | 930     | 547  | 193 |
| Stiff <i>pap</i> and pumpkin (g)        | 656            | 727             | 824            | 524     | 1250    | 771  | 227 |
| Stiff <i>pap</i> and <i>imifino</i> (g) | 448            | 588             | 770            | 330     | 985     | 600  | 204 |
| Stiff <i>pap</i> and spinach (g)        | 328            | 426             | 530            | 310     | 1170    | 531  | 323 |
| Stiff <i>pap</i> and beans (g)          | 445            | 544             | 765            | 376     | 935     | 613  | 232 |
| <i>Umngqusho</i> (g)                    | 328            | 468             | 646            | 150     | 1290    | 536  | 261 |
| <i>Amagewu</i> (ml)                     | 300            | 450             | 1000           | 200     | 1000    | 600  | 355 |

50% medium = median

SD = standard deviation

Porridge = maize meal porridge with a soft consistency

Stiff *pap* = maize meal porridge with a thick consistency

Stiff *pap* and cabbage = maize meal porridge with a thick consistency cooked with cabbage

Stiff *pap* and pumpkin = maize meal porridge with a thick consistency cooked with pumpkin

Stiff *pap* and *imifino* = maize meal porridge with a thick consistency cooked with a wild spinach like plant

Stiff *pap* and spinach = maize meal porridge with a thick consistency cooked with spinach

Stiff *pap* and beans = maize meal porridge with a thick consistency cooked with sugar beans

*Umngqusho* = Samp and beans (broken dried kernels cooked with sugar beans)

*Amagewu* = maize based drink

2) Dishing-up (serving) sessions were undertaken to determine the standard portion sizes of the main maize dishes consumed in the EC rural areas (February, 2003). The dishing-up sessions were conducted in two areas, Bizana and Centane. Thirty-five female volunteers (between ages 18 and 55 years) were recruited from each area. These volunteers were asked to dish up a "usual" portion for an adult male and female living in the household (n = 70 portion sizes) (Figures 4.5 and 4.6). Another six volunteers from the area were requested to prepare one of the six most commonly eaten dishes. Researchers provided the ingredients for these dishes, which included: stiff *pap*<sup>3.10</sup> porridge<sup>3.11</sup>, samp<sup>3.12</sup> and beans, spinach combined with *pap*, pumpkin combined with *pap*, and soup<sup>3.13</sup>.

<sup>3.10</sup> Stiff *pap* is a traditional word used for maize meal porridge with a stiff, thick consistency

<sup>3.11</sup> Porridge is a traditional word used for maize meal porridge with a thin consistency

<sup>3.12</sup> Dry maize kernels

<sup>3.13</sup> Soup is made with whole maize kernels and dried sugar beans



Figure 4.5. Dishing up session in Bizana



Figure 4.6. Dishing up session in rural Centane

The dished-up adult portions were weighed individually, and the weight of the plate subtracted to provide a portion size. Addendum 19 presents the data capturing sheet used for the dishing-up sessions. Participants were informed that they would not be eating the food so that their current state of satiety or the fact that the food was free would not influence the dishing up process (Robinson *et al.*, 1997:119). Amounts depicted in Table 4.2 include portion sizes of both men and women and these compare well with portions from previous 24-hour recalls (Table 4.1).

**Table 4.2. Portion sizes of six main maize dishes determined during the dishing-up session**

| Dish<br>n = 70                          | Inter quartile percentiles |                 |                | Minimum | Maximum | Mean | SD  |
|---|----------------------------|-----------------|----------------|---------|---------|------|-----|
|   | 25%<br>(Small)             | 50%<br>(Medium) | 75%<br>(Large) |         |         |      |     |
| Porridge (g)                            | 358                        | 557             | 630            | 146     | 760     | 497  | 174 |
| Stiff <i>pap</i> (g)                    | 308                        | 478             | 640            | 164     | 1204    | 505  | 232 |
| <i>Umngqusho</i> (g)                    | 592                        | 744             | 896            | 294     | 1396    | 743  | 217 |
| Stiff <i>pap</i> and pumpkin (g)        | 462                        | 592             | 794            | 196     | 1201    | 626  | 237 |
| Stiff <i>pap</i> and <i>imifino</i> (g) | 426                        | 512             | 710            | 102     | 1303    | 561  | 227 |
| Soup (g)                                | 466                        | 608             | 726            | 228     | 916     | 587  | 177 |

50% medium = Median

SD = standard deviation

Porridge = maize meal porridge with a soft consistency

Stiff *pap* = maize meal porridge with a thick consistency

*Umngqusho* = Samp and beans (broken dried kernels cooked with sugar beans)

Stiff *pap* and pumpkin = maize meal porridge with a thick consistency cooked with pumpkin

Stiff *pap* and *imifino* = maize meal porridge with a thick consistency cooked with a wild spinach like plant

Soup = watery soup made with whole maize kernels and sugar beans

The mean weight (including the smaller portion sizes for the females and the larger portion sizes for the males) was determined for each dish and the inter-quartile (IQ) range [25%, 50% (median) and 75% percentiles] were used to provide S, M, and L portions.

3) For items for which portion sizes could not be extrapolated by applying the former two methods (maize on the cob, whole kernels, rice, samp, tea and coffee), appropriate information was obtained from a national dietary database. This database comprises food portion sizes from different studies; reflecting different population groups in South Africa from 1983 to 2000 (Nel & Steyn, 2002).

Addendum 20 indicates the final portion sizes used for the QFFQ as well as the various sources of these portion sizes.

### 2.2.2. Determining the ratios depicted in photographs

The ratios depicted in the photographs were determined from the literature (Beyers *et al.*, 1979:99) and 24-hour recall data conducted by the MRC in Centane. Four different ratios were determined for each main maize combination dish of which the three most frequently consumed ratios were identified during the focus group discussions. Table 4.3 presents the different ratios for each dish, as well as the sources of each ratio.

Figure 4.7 shows an example of the portion size photographs used for stiff *pap*. Some of the photographs were used for more than one food item or dish (for instance, there is one photograph indicating the two different types of home-made bread (mostly maize-based) since they look alike but the recipes differ). A single photograph (different mug sizes) was used for all the different beverages that were consumed. For nine of the main maize combination dishes there are also three ratio photographs each, these include maize meal and *imifino*, maize meal and spinach, maize meal and pumpkin, maize meal and beans, *umngqusho* (samp and beans), soup (whole mealie kernels and beans), mealie rice and *imifino*, mealie rice and spinach and mealie rice and pumpkin (CD 1). Figure 4.8 shows an example of the ratio photographs used for maize and beans.

Table 4.3. Ratios depicted on the photographs

| Nr                     | Food type                    | Ratios                        |                               |                               |                               |
|------------------------|------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
|                        |                              | Cooked<br>maize:<br>Vegetable | Cooked<br>maize:<br>Vegetable | Cooked<br>maize:<br>Vegetable | Cooked<br>maize:<br>Vegetable |
| <b>Group 3</b>         |                              |                               |                               |                               |                               |
| <b>Combined dishes</b> |                              |                               |                               |                               |                               |
| 3.1                    | Maize meal + <i>imifino</i>  | 1:1#                          | 1:2# *                        | 1:5#                          | 2:1                           |
| 3.2                    | Maize meal + spinach         | 1:1#                          | 1:2▲                          | 2:1▲                          | 1:5▲                          |
| 3.3                    | Maize meal + pumpkin         | 1:3#                          | 3:1*                          | 1:2*                          | 2:1▲                          |
| 3.4                    | Maize meals + dried beans    | 1:2#                          | 2:1▲                          | 1:3▲                          | 3:1▲                          |
| 3.5                    | <i>Umngqusho</i>             | 2:1# *                        | 3:1#                          | 5:1# *                        | 1:2▲                          |
| 3.6                    | Soup                         | 1:1*                          | 2:1*                          | 1:2▲                          | 1:3▲                          |
| 3.7                    | Mealie rice + <i>imifino</i> | 1:3▲                          | 3:1▲                          | 1:2▲                          | 2:1▲                          |
| 3.8                    | Mealie rice + spinach        | 1:3▲                          | 3:1▲                          | 1:2▲                          | 2:1▲                          |
| 3.9                    | Mealie rice + pumpkin        | 1:3▲                          | 3:1▲                          | 1:2▲                          | 2:1▲                          |

# 24-hour recall, EC data

\* Published data (Beyers *et al.*, 1979:99)

▲ No information obtained for portion size. Portion size adapted from another dish

Maize meal and *imifino* = maize meal porridge with a thick consistency cooked with a wild spinach like plant

Maize meal and spinach = maize meal porridge with a thick consistency cooked with spinach

Maize meal and pumpkin = maize meal porridge with a thick consistency cooked with pumpkin

Maize meal and beans = maize meal porridge with a thick consistency cooked with sugar beans

*Umngqusho* = Samp and beans (broken dried kernels cooked with sugar beans)

Soup = watery soup made with whole maize kernels and sugar beans

Mealie rice and *imifino* = crushed maize kernels cooked with a wild spinach like plant

Mealie rice and spinach = crushed maize kernels cooked with spinach

Mealie rice and pumpkin = crushed maize kernels cooked with pumpkin



Stiff *pap*  
Small (308 g)

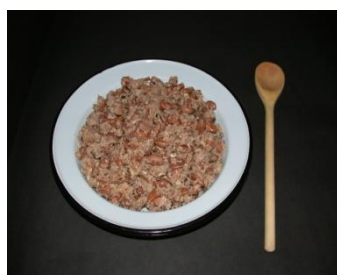


Stiff *pap*  
Medium (478 g)



Stiff *pap*  
Large (640 g)

**Figure 4.7. Portion size photographs for stiff *pap***



Samp and beans  
Ratio 1:2



Samp and beans  
Ratio 2:1



Samp and beans  
Ratio 3:1

**Figure 4.8. Ratio photographs for *umngqusho* (samp and beans)**

### 2.2.3. Development of photographs

The recipes used for the dishes depicted on the photographs were based on information received from 24-hour recalls conducted by the MRC in Centane and in-depth interviews.

- Initial photographs

A preliminary set of photographs was taken at the PROMEC laboratories by the researchers (November-December 2002). To ensure face and content validity, different backgrounds, scales (for dimension), types of plates as well as different colour plates were considered (Table 4.4). Initially it was decided to use a tablespoon to illustrate dimensions on each photograph.

- Second set of photographs

After the initial photographs were taken and extensively discussed with other nutrition professionals, some adaptations were made. A professional photographer took the second set of photographs in January 2003, using a white background. The food items, prepared by a Xhosa woman from the EC area, were dished up on a blue plate. Since a wooden spoon is

the utensil most frequently used in the EC (not a tablespoon), this was used to illustrate dimensions. A knife and fork were also included (as described in the literature) (Lucas *et al.*, 1995:66, Robinson *et al.*, 1997:119). These photographs were then tested in the EC by means of FGDs (February, 2003).

**Table 4.4. Different factors considered in making the food photograph series**

| Factors considered           | Various options for the photographs | Reasons for decisions taken   |
|------------------------------|-------------------------------------|---|
| Colour of the plate          | White                               | Maize dishes are mostly white and therefore there was little contrast between the food and the plate. |
|                              | Yellow or cream                     | Yellow or cream coloured plates influenced the colour of dishes containing pumpkin.                   |
|                              | Green                               | Green coloured plates influenced the colour of dishes containing spinach.                             |
|                              | Blue                                | Blue plates influenced some of the photographs containing spinach, but not as much as the green.      |
| Type of plate used           | Bowl                                | Determining the depth of the dish was difficult when it is presented in a bowl.                       |
|                              | Plate                               | Determining depth on a plate was easier than that of a bowl.  |
| Background of the photograph | Dark (navy or black)                | White maize dishes were more pronounced on a dark background.   |
| Scale                        | Match box                           | This was disregarded because smoking is a risk factor for cancer and would send mixed messages.       |
|                              | Ruler                               | A ruler is not a known or much used item in this rural area.  |
|                              | Knife and fork                      | These utensils are not used in the area.  |
|                              | Tablespoon                          | Tablespoons vary in size.   |

#### 2.2.4 Finalisation of the photographs

A female from the EC region prepared the dishes listed on the QFFQ according to recipes obtained in FGDs. The raw ingredients of recipes were weighed and cooking methods and preparation steps recorded for coding purposes. Photographs were taken at an angle of 42° above the horizon, (the average angle of viewing when a person is seated at a table) (November, 2003) (Nelson *et al.*, 1994:651).

Recipes were included in the South African Food Composition Tables database, Foodfinder III (Grant *et al.*, 1992), as well as in the training manual. Although the weight shown on the photographs (front and reverse side) is for cooked weight, the information regarding recipes

and ingredients presented on the photographs are based on raw ingredients. Foodfinder III generally includes cooked weights for food items. Since the intakes of raw portions are crucial for the development of the ratio photographs, the raw portions of single ingredients were converted back to individual cooked weights. This was mainly because of the fact that Foodfinder does not account for water loss and absorption. Recipes were adapted to account for fluid loss as far as possible. The QFFQ included salt as a separate item; therefore, no salt was included in the Foodfinder recipes.

Final photographs were taken using a black background to emphasise the mostly white foods. The plate used was blue and a wooden spoon (most common dishing-up utensil) was used as a scale to illustrate dimension. All utensils used in the photographs were procured in the EC.

### **2.3. PHASE C – RESULTS FROM FINAL FOCUS GROUP**

Participants in the final focus group (February, 2004) conducted in Cape Town (n = 12) agreed that normal post card size photographs (15 x 10 cm) were too small to be used. Participants had difficulty in recognising the different dishes and portion sizes depicted. It was thus decided to have life-sized photographs (42 x 30 cm) since they made identification easier. Unfortunately, life-size photographs are cumbersome and heavy to carry. Therefore, as a set they can be transported on a trolley and used in studies going from house to house. However, it is still easier if the QFFQ and FPS are used in one central location. Table 4.5 provides comments and final changes that were made to the portion size photographs after the final FGD were held.

Participants found it difficult to estimate the three-dimensional perspective of brown bread, cabbage, spinach and stiff *pap* in the photographs. This was resolved by reducing the camera angle to allow more depth (Nelson *et al.*, 1994:660) (March, 2004).

**Table 4.5. Comments received during the final focus group session**

| Nr.  | Dish                              | Comments  | Conclusions   |
|------|-----------------------------------|---|---|
| 1.2  | Brown bread                       | Could not recognise the difference between the medium and large portion.  | These photographs were taken again. The bread is repositioned on the plate.   |
| 1.7  | Dumplings (bread dipped in broth) | Requested not to cut them in the same shape as presented in the photographs. However, they did recognise the photographs and portion sizes. | It was decided to leave the photographs as they are, since they have been recognised. The way bread is cut differs from household to household. |
| 2.7  | Oats                              | Participants did not recognise the oats photographs. The oats in the photographs was too stiff.   | These photographs were taken again with a soft consistency.   |
| 2.8  | White rice                        | According to the participants the small white rice portions were too small.   | These photographs were taken again with adapted portion sizes.  |
| 3.1  | Cabbage and spinach               | They confused the medium and large portion size.  | The photographs were taken again. The portion sizes stayed the same but the way it is presented in the photographs is improved.                 |
| 9.11 | Traditional beer                  | Portion sizes were all too small. Requested to rather use a 5 l container.  | It was decided to leave the portion sizes as they were. Participants can provide the information on larger portions if needed.                  |

### 3. DISCUSSION

The final dietary assessment method (RAPP method) comprises a QFFQ and a FPS. The QFFQ includes 33 items, subdivided into six food groups, namely: bread, cereal, combined dishes, meat, condiments and beverages. The bread included into the QFFQ is mostly maize based (with the exception of white bread and brown bread). Cereals and combined dishes included are all maize-based dishes with the exception of rice. The above mentioned food groups will all provide detailed information on the daily amount of maize consumed (raw and cooked) as well as exposure to fumonisins. Fruit and vegetables (another risk factor associated with oesophageal cancer (OC) were rarely consumed and were not included on the QFFQ. Vegetables are however included in the combined dishes. Red meat (another risk factor identified with the development of OC) is also rarely consumed and not included in the QFFQ. Condiments include mainly salt and sugar as no other condiment was consumed frequently enough to justify inclusion. Beverages include mostly maize-based drinks which will once again provide information on maize consumption.

## Chapter 5

### VALIDATION OF THE PORTION SIZE PHOTOGRAPHS DEVELOPED FOR THE USE WITH THE QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE



Figure 5.1. An interviewer explaining the procedure for the photo validation to a participant

## 1. INTRODUCTION

The newly developed quantitative food frequency questionnaire (QFFQ) and food photograph series (FPS) were compiled (Chapter 4) using a combination of qualitative and quantitative research methods.

However, when using portion size photographs in a study it is essential that these photographs be validated. Validation results provide insight in how accurately participants perceive the portion sizes depicted on these photographs (Lucas *et al.*, 1995:73).

Previous studies have shown that a variety of factors influence the validity of portion size photographs. These factors include specific characteristics of participants namely gender, age, body mass index (BMI) (Gibson, 2005:262) and education (Nelson & Haraldsdóttir, 1998a:219), the photographs themselves (for instance size, colour and order of presentation) (Nelson *et al.*, 1994:662, Nelson & Haraldsdóttir, 1998b:233) and food items depicted on the photographs (for example the preparation method, type of food and containers used) (Venter *et al.*, 2000:216, Lucas *et al.*, 1995:73, Robson & Livingstone, 2000:190).

## 2. MATERIALS AND METHODS

### 2.1. STUDY POPULATION USED













Due to logistics of the parent protocol [the research conducted by the Programme of Mycotoxins and experimental carcinogens Unit (PROMEC) of the Medical Research Council (MRC)] and funding limitations, the validation study was conducted in only one area, Centane (June 2004). Sixty volunteers from five selected villages in the Centane area were recruited on a door-to-door basis to participate in this phase of the study (Figure 5.1). One person per household was recruited. These participants were a new group who was used to test the validity of the FPS. Similar studies in the past included sample sizes ranging from 51 to 270 participants (Nelson *et al.*, 1994:649; Lucas *et al.*, 1995:65, Nelson *et al.*, 1996:31; Venter *et al.*, 2000:205), respectively.

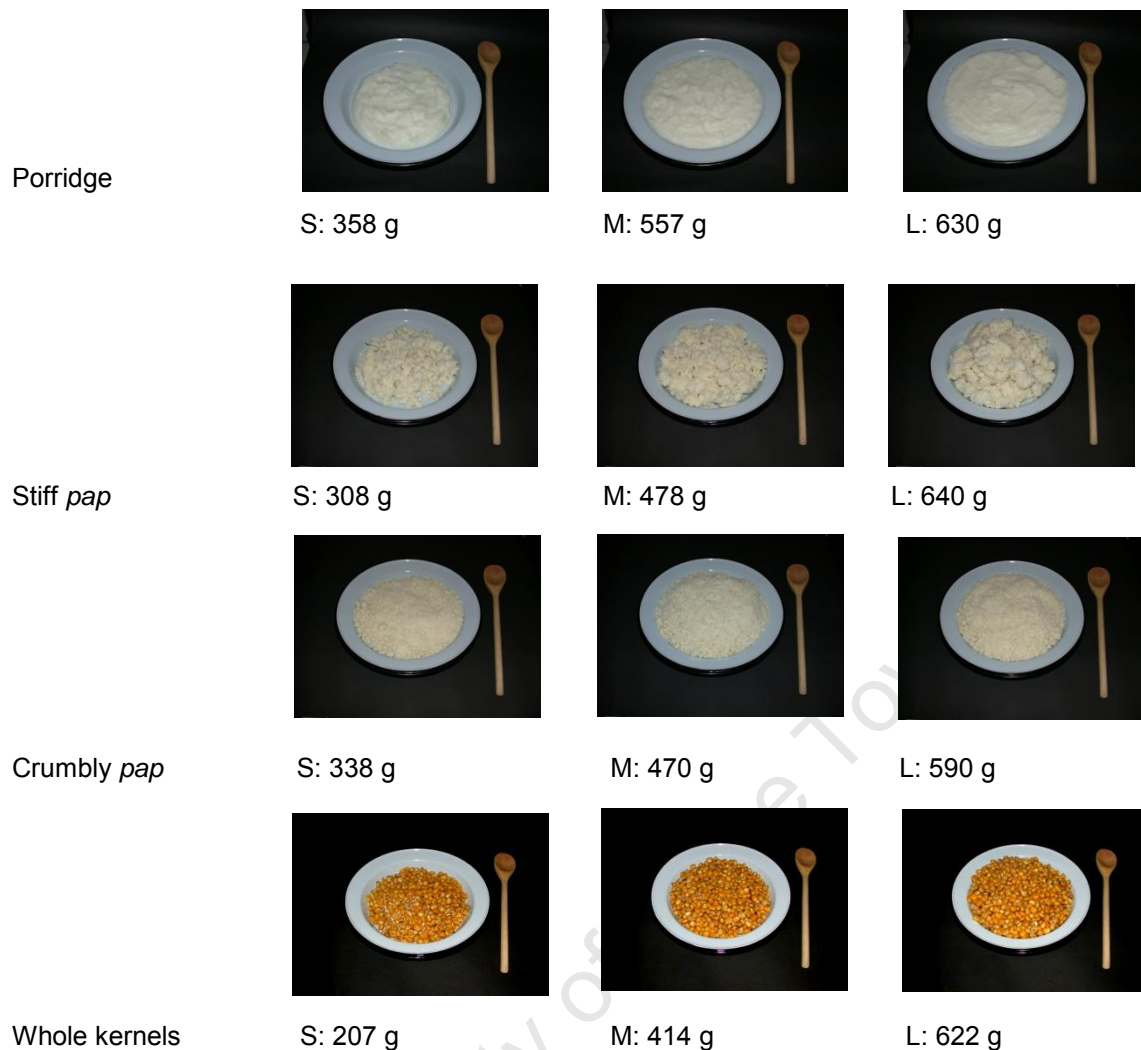
People were excluded from the study if they had problems with eyesight (Venter *et al.*, 2000:206) or were younger than 18 years.

## 2.2. PROCEDURES FOLLOWED DURING THE VALIDATION OF THE PHOTOGRAPHS

Photographs were tested in a situation where the influence of poor memory was minimized. The dishing up of portions of actual foods by participants and viewing of portion size photographs took place in the same session as recommended by Lucas *et al.*, (1995:66).

Photographs of the most frequently consumed maize dishes (n = 8) were selected for the validation procedure. The chosen dishes included *umngqusho* (samp and beans), soup, stiff *pap* and pumpkin, stiff *pap* and *imifino*, porridge, stiff *pap*, *umphokoqo*, and whole kernels. Figure 5.2 provides a summary of the different portion size photographs that were tested. These dishes were all amorphous which are known to provide additional challenges when estimating portion size.

|                                     |   |  |   |
|-------------------------------------|---|--|---|
|                                     |   |   |   |
| Samp and beans                      | S: 592 g  | M: 744 g   | L: 896 g  |
|                                     |  |  |  |
| Soup                                | S: 462 g  | M: 592 g   | L: 794 g  |
|                                     |  |  |  |
| Stiff <i>pap</i> and pumpkin        | S: 462 g  | M: 592 g   | L: 794 g  |
|                                     |  |  |  |
| Stiff <i>pap</i> and <i>imifino</i> | S: 426 g  | M: 512 g   | L: 710 g  |



**Figure 5.2. Portion size photographs of eight cooked maize dishes used in the validation of the photographs**

Two different stations were set up at one of the volunteers' houses. At the first station, participants were shown a set of photographs [small (S), medium (M) and large (L)] of the different maize dishes. Participants had an opportunity to identify which portion size they would usually consume of each specific dish. To avoid systematic bias, the order in which the photographs were presented (S, M & L) was not consecutive (Kuehneman *et al.*, 1994:548). In addition, to avoid respondent bias, participants were unaware of actual portion sizes of the photographs (Posner *et al.*, 1992:738). If a participant did not consume a specific dish (for whatever reason) she / he was not expected to dish it up. The portion sizes identified by each participant from the photographs were recorded by a field worker as being the **test weights** (Figure 5.3). Addendum 21 represents the data-capture sheet used during this part of the study.

At the second station, participants were asked to dish up the amount of food they would usually consume of each dish (once again, only for those dishes they consumed). For these

purposes eight volunteers from the area prepared the food that was depicted in the photographs with local ingredients provided by the Medical Research Council (MRC). The plates in which portions were dished up were those that appear on the photographs (Figure 5.2). After dishing up, the weight of the plate was subtracted, giving the actual weight of the portion usually consumed. The actual weight dished up by each participant was recorded by a researcher as being the **reference weight** (Figure 5.4). Dished up portions were weighed using a calibrated, Soehnle electronic, digital laboratory scale. Addendum 22 includes the data-capturing sheet used during this part of the study.



**Figure 5.3. An interviewer recording the portion size identified by a participant**



**Figure 5.4. A participant dishing up a usual portion size during the validation of the photographs**

### 2.3. ASSESSMENTS

Weight and height of each participant was taken (see Chapter 3, Section 3.2 for detail). Addendum 23 depicts the anthropometric data-capturing sheet used during this part of the study.

The socio-demographic questionnaire (see Chapter 3, Section 3.3 for detail) was completed for background information.

### 2.4. STATISTICAL ANALYSES

Data was tested for normality with the Shapiro Wilk test. Statistical tests that were conducted include the Wilcoxon signed rank test, Spearman correlation coefficients, classification into tertiles, Kappa statistics and Bland-Altman test (see Chapter 3, Section 5 for detail regarding the test as well as interpretation criteria).

The effect of different participant characteristics on portion size estimation was also investigated. The following characteristics were included:

- gender, [some studies indicated that females reported more accurately than males, while other studies indicated no differences between the two genders (Yuhás *et al.*, 1980:1475, Robinson *et al.*, 1997:122, Venter *et al.*, 2000:214)];
- age (18 - 44 years, 45 - 64 years and > 65 years) [the first two groups were distributed to provide near equal age ranges with near sample sizes per group, while the last age group was included because previous studies indicated perception differences between those younger than 65 years and those older than 65 years (Nelson *et al.*, 1994:661, Robinson *et al.*, 1994:122, Young & Nestle, 1995:154, Nelson *et al.*, 1998:22)].
- BMI (< 25 kg/m<sup>2</sup>, 26 – 30 kg/m<sup>2</sup> and > 30 kg/m<sup>2</sup>) [according to the literature those with a BMI > 30 kg/m<sup>2</sup> under-estimate portion sizes (Nelson *et al.*, 1994:661, Nelson & Haraldsdóttir, 1998a:219)].
- level of education (no schooling, Grade 1 – Grade 7 and Grade 8 – 12) [to determine the differences between those with no schooling, those with primary schooling and those with secondary schooling, a similar study conducted in South Africa indicated no differences between the level of schooling and portion size estimation (Venter *et al.*, 2000:215)].

The Wilcoxon signed rank test was conducted to assess differences between the males and females. For the other characteristics (age, BMI and education level) the analyses of variance test for non-normal data (Kruskal Wallis test) was used. A p-value of 0.05 denoted a significant difference between the designated groups. Data from the different characteristic groups were also compared for mean portion size, mean differences, percentage differences and spearman correlations, Bland-Altman analyses, tertiles classification and weighted Kappa statistics (as described in Chapter 3, Section 5).

### 3. RESULTS

#### 3.1. DEMOGRAPHIC INFORMATION OF PARTICIPANTS

All participants were born in the EC, except for one who was born in the Northern Cape. However, this person had been living in the study area for most of her life. All the participants were isiXhosa speaking.

More females (n = 50) than males (n = 10) participated in the validation study which took place in 2006. Males, in a rural traditional setting consider food and cooking as being a woman's domain, and therefore many refused to participate. The average age of males was 48.8 (15.0) years and for females 42.8 (15.5) years (Table 5.1).

Both genders, on average, had similar heights, while the females in general had a higher than average weight, with a higher than average BMI.

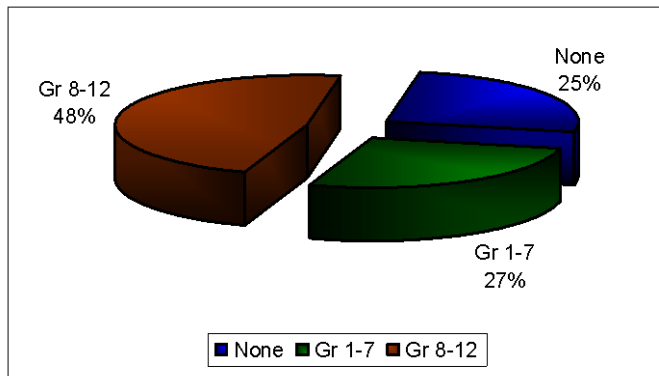
**Table 5.1. Mean ( $\pm$  SD) of the age and anthropometric measurements of participants**

| Measurements             | Males<br>n = 10 |        | Females<br>n = 50 |        | Total<br>n = 60 |        |
|--------------------------|-----------------|--------|-------------------|--------|-----------------|--------|
|                          | Mean            | (SD)   | Mean              | (SD)   | Mean            | (SD)   |
| Age (years)              | 48.8            | (15.0) | 42.8              | (15.5) | 43.8            | (15.5) |
| Height (cm)              | 160.0           | (10.0) | 160.0             | (10.0) | 160.0           | (10.0) |
| Weight (kg)              | 63.9            | (6.7)  | 70.6              | (15.9) | 69.5            | (14.9) |
| BMI (kg/m <sup>2</sup> ) | 24.0            | (3.0)  | 27.9              | (6.4)  | 27.2            | (6.2)  |

BMI = Body Mass Index  
n = sample size  
cm = centimetres  
kg = kilogram  
SD = Standard deviation

The majority of participants (52%) had primary school grades 1 - 7 (27%) or no (25%) formal education. The rest of the participants (48%) had a high school (grades 8 - 12) qualification.

Figure 5.5 illustrates the percentage distribution of the different education levels of the participants.



**Figure 5.5. Education levels of participants**

Fifty percent of the participants were unemployed. The largest group who had employment were employed to work at hotels in the province. A small percentage (28%) of participants lived in brick houses and 67% in traditional mud houses. The average household used river water (62%), 27% used a communal tap; only 12% have a tap in the home. Most of the participants (70%) did not have toilets in their homes. Cooking was mostly (63%) done outside on a fire using wood.

Most of the households (78%) had only one person contributing to the household income and only 7% had two people contributing. The average monthly income per family was between R 500 and R 1000, with only 13% claiming to earn more than R 1000 per month per family. Most of the income is derived from a pension or children's government grants. Of this income, about R 200 a month (for 60% of the participants) was spent on food.

Forty-two percent of females and 90% of males reported alcohol consumption. Home-made traditional beer was mainly consumed. Although smoking is a major risk factor for OC, only 7% of the participants claimed to have smoked in the past, while 17% reported to be current smokers. Few of the participants (only 4% of the females and no males) reported using snuff.

### 3.2. RESULTS OF THE PORTION SIZE PHOTOGRAPH VALIDATION STUDY

Since the objective of this study was to compare the photographs with habitual intake, the participants dished up the different dishes they consumed. Therefore, if a participant did not consume a specific dish (for whichever reason), the participant did not dish up that particular dish. This resulted in different sample sizes for each dished up dish. Also, one dish-up session did not have whole kernels as they forgot to prepare the dish, resulting in much smaller sample sizes for that dish compared to the other dishes.

The Wilcoxon signed rank paired t-test showed a highly significant difference ( $p < 0.001$ ) between the test weight and the reference weight for all dishes except for whole kernels (Table 5.2). Individual percentage differences calculated ranged between 4.2% and 44.4%. Individual mean percentage differences were acceptable ( $< 20\%$ ) for all dishes except for stiff *pap* and pumpkin, stiff *pap* and *imifino* and *umphokoqo*.

Spearman correlation coefficients between the test and reference weights were mostly poor ( $< 0.20$ ) (Table 5.3). Correlations ranged between -0.14 for both stiff *pap* and *imifino* and *umphokoqo*, and 0.38 for whole kernels. Only whole kernels' correlation coefficient reached statistical significance ( $p < 0.05$ ) although the small sample size influenced the results. Three dishes, namely, *umngqusho* (samp and beans), stiff *pap* and *imifino* and *umphokoqo* (crumbly *pap*) had negative correlations. Poor Spearman correlation coefficients overall indicate poor strength of association between the two weights (at individual level).

The only dish with acceptable classification in terms of tertiles was whole kernels, with up to 64.9% of both portion sizes (test and reference weights) classified into the same tertile (Table 5.3). None of the other dishes had acceptable agreement in this regard as the percentage classified in the same tertile ranged between 41% and 48%. Ideally, this should be  $> 50\%$ . Classification of dishes in opposite tertiles was acceptable for kernels ( $< 10\%$ ) and poor for the remainder of the dishes (ranging between 12.2% and 30.0%), indicating poor agreement between the test and reference weights.

When classification by chance was excluded, results were also poor (Table 5.3). The weighted Kappa statistics showed results ranging between -0.02 for *umphokoqo* and 0.34 for whole kernels. Statistical significance of the weighted Kappa statistic was not achieved for any of the dishes. The Kappa statistic for kernels was considered to be acceptable (0.21 -

0.60), while those of all the other dishes were considered to be poor ( $< 0.20$ ). Once again, results illustrated poor agreement between the test and reference weights.

**Table 5.2. The means, standard deviations, medians, inter-quartile ranges, differences and percentage differences between the test and reference weights**

| Dish                                    | Sample size | Reference weight (g) |                       | Test weight (g)  |                      | Difference (g)<br>(Test – reference weight) |                      |                          | Percentage difference (%)<br>[(Test – reference weight) /<br>reference weight * 100] |                      |                                |
|---|-------------|----------------------|-----------------------|------------------|----------------------|---|----------------------|--------------------------|--|----------------------|--------------------------------|
|   |             | Mean<br>(SD)         | Median<br>(IQ Range)  | Mean<br>(SD)     | Median<br>(IQ Range) | Mean<br>(SD)                                | Median<br>(IQ Range) | CI of<br>Difference<br>% | Mean<br>(SD)   | Median<br>(IQ Range) | CI of relative<br>difference % |
| <i>Umngqusho</i> (g)                    | 60          | 530.6<br>(170.0)     | 510.0***<br>(414-635) | 736.4<br>(132.5) | 744.0<br>(592-896)   | 205.8<br>(219.7)                            | 200.0<br>(27-368)    | -178-579                 | 18.8<br>(55.3)   | 28.3<br>(3-45)       | -32-68.2                       |
| Soup (g)                                | 57          | 495.1<br>(198.2)     | 484.0***<br>(384-580) | 610.6<br>(132.9) | 592.0<br>(462-794)   | 115.5<br>(213.0)                            | 88.0<br>(-2-270)     | -329-452                 | 16.5<br>(34.8)   | 16.8<br>(0-43)       | -48-65                         |
| Stiff <i>pap</i> and pumpkin (g)        | 56          | 425.4<br>(157.1)     | 405.0***<br>(294-553) | 583.2<br>(130.2) | 592.0<br>(462-592)   | 157.8<br>(198.7)                            | 149.0<br>(19-254)    | -184-556                 | 24.8<br>(30.9)   | 21.8<br>(3-46)       | -31-75                         |
| Stiff <i>pap</i> and <i>imifino</i> (g) | 56          | 399.7<br>(148.0)     | 396.0***<br>(291-507) | 540.6<br>(117.5) | 512.0<br>(426-710)   | 140.9<br>(202.9)                            | 139.0<br>(-12-306)   | -170-466                 | 21.9<br>(35.2)   | 26.8<br>(-2-45)      | -40-75                         |
| Porridge (g)                            | 45          | 373.1<br>(85.8)      | 372.0***<br>(312-420) | 475.9<br>(119.5) | 552.0<br>(358-557)   | 102.9<br>(129.9)                            | 90.0<br>(14-198)     | -148-298                 | 17.6<br>(25.5)   | 25.1<br>(3-36)       | -41-49                         |
| Stiff <i>pap</i> (g)                    | 57          | 375.5<br>(106.7)     | 376.0*<br>(312-450)   | 425.3<br>(140.5) | 308.0<br>(308-478)   | 49.8<br>(162.5)                             | 64.0<br>(-82-168)    | -228-318                 | 4.2<br>(38.8)  | 18.4<br>(-17-33)     | -74-54                         |
| <i>Umphokoqo</i> (g)                    | 58          | 229.6<br>(80.9)      | 226.0***<br>(166-284) | 439.8<br>(102.7) | 470<br>(338-470)     | 210.2<br>(137.7)                            | 220.0<br>(102-320)   | -6-444                   | 44.4<br>(25.6)   | 48.1<br>(29-64)      | -1-78                          |
| Kernels (g)                             | 37          | 246.8<br>(113.2)     | 238.0<br>(158-322)    | 341.5<br>(163.7) | 207.0<br>(207-414)   | 94.7<br>(156.8)                             | 67.0<br>(0-166)      | -147-426                 | 19.6<br>(39.8)   | 23.7<br>(3-42)       | -71-79                         |

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\* Wilcoxon signed rank t-test significant at  $p < 0.05$

\*\* Wilcoxon signed rank t-test significant at  $p < 0.01$

\*\*\* Wilcoxon signed rank t-test significant at  $p < 0.001$

Reference weight = dished up weight

Test weight = weight represented on the portion size photographs

*Umngqusho* = Samp and beans (Dried kernels and sugar beans)

Soup = Whole kernels and sugar beans

Stiff *pap* and pumpkin = Stiff maize meal porridge and pumpkin

Stiff *pap* and *imifino* = Stiff maize meal porridge and spinach

Porridge = Thin maize meal porridge

Stiff *pap* = Stiff maize meal porridge

*Umphokoqo* = Crumbly *pap* (Dry maize meal porridge)

Kernels = Boiled whole maize kernels

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**Table 5.3. Spearman correlation coefficients, tertile distribution and weighted Kappa statistics**

| Dish                                | n  | Spearman correlation<br>r | Percentage classified in tertiles |                   |                   | Weighted Kappa<br>K <sub>w</sub> |
|-------------------------------------|----|---------------------------|-----------------------------------|-------------------|-------------------|----------------------------------|
|                                     |    |                           | Same tertile                      | Adjacent tertiles | Opposite tertiles |                                  |
| <i>Umngqusho</i>                    | 60 | -0.07                     | 41.7                              | 28.3              | 30.0              | 0.07                             |
| Soup                                | 57 | 0.17                      | 42.1                              | 36.8              | 21.1              | 0.12                             |
| Stiff <i>pap</i> and pumpkin        | 56 | 0.13                      | 48.2                              | 35.7              | 16.1              | 0.13                             |
| Stiff <i>pap</i> and <i>imifino</i> | 56 | -0.14                     | 35.7                              | 37.5              | 26.8              | 0.02                             |
| Porridge                            | 45 | 0.26                      | 46.7                              | 33.3              | 20.0              | 0.02                             |
| Stiff <i>pap</i>                    | 57 | 0.16                      | 43.9                              | 43.9              | 12.2              | 0.09                             |
| <i>Umphokoqo</i>                    | 58 | -0.14                     | 43.1                              | 34.5              | 22.4              | -0.02                            |
| Kernels                             | 37 | 0.38**                    | 64.9                              | 27.0              | 8.1               | 0.34                             |

r = Spearman correlation coefficient

\* Spearman rank correlation coefficient significant at  $p < 0.05$

\*\* Spearman rank correlation significant at  $p < 0.01$

\*\*\* Spearman rank correlation significant at  $p < 0.001$

K<sub>w</sub> weighted Kappa statistics

*Umngqusho* = Samp and beans (Dried kernels and sugar beans)

Soup = Whole kernels and sugar beans

Stiff *pap* and pumpkin = Stiff maize meal porridge and pumpkin

Stiff *pap* and *imifino* = Stiff maize meal porridge and spinach

Porridge = Thin maize meal porridge

Stiff *pap* = Stiff maize meal porridge

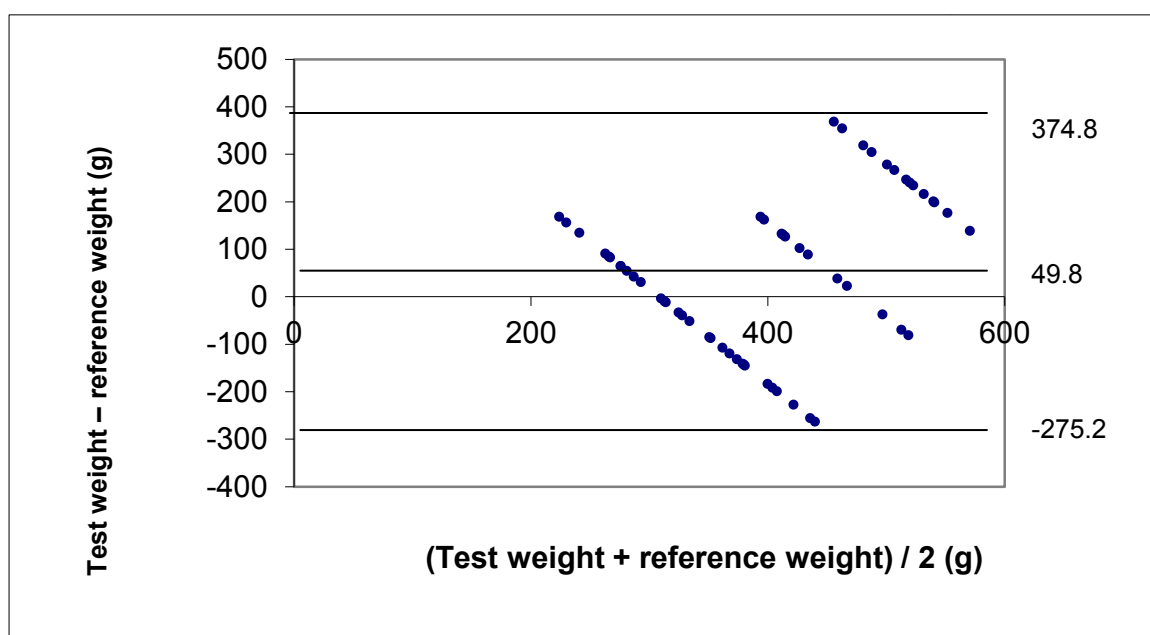
*Umphokoqo* = Crumbly *pap* (Dry maize meal porridge)

Kernels = Boiled whole maize kernels

Agreement in terms of falling between the limits of agreement (LOA) was good (> 95%) for five dishes and poor (< 95%) for three dishes (soup, stiff *pap* and pumpkin, and whole kernels) (Table 5.4). For the purpose of this study, the width of the LOA (mean difference between the two methods  $\pm 1$  SD of the mean) was considered acceptable, since the LOA were narrower than the mean difference  $\pm 1 \times$  small portion size. Spearman correlation coefficients between the mean of the portion sizes (test + reference weights) and the mean difference in portion sizes (test - reference weights) ranged between -0.31 (soup) and 0.35 (porridge). *Umngqusho* (samp and beans), soup, porridge, stiff *pap* and *umphokoqo* all reached statistical significance, suggesting the presence of proportional bias in the estimation of these dishes.

The Bland-Altman plot presented in Figure 5.6 is based on recommendations by Gibson, 2005:187. Bland-Altman plots are firstly visually inspected to assess the distance between the zero line and the mean difference, as well as the distance between the mean difference,

the zero line and the LOA. The Bland-Altman plot for stiff *pap* (Figure 5.6) illustrates a mean difference close to zero (49.8 g), with narrow ( $< 1 \times$  small portion size) LOA (mean difference  $\pm 2$  SD  $<$  small portion size). Therefore the distance from the mean to the upper limit was 324 g. The small portion size was 375 g. This means that every participant reported a portion size within 324 g of the mean difference. The agreement was 100%, with all participants falling between these LOA. Proportional bias can be observed as over estimation increases with portion size, which is verified by the significant Spearman correlation coefficient (Table 5.4).



**Figure 5.6.** Bland-Altman plot comparing the mean of the test and reference weight against the difference between the test and reference weight for stiff *pap*

**Table 5.4. Bland-Altman calculations for the test and reference weight**

| Dish                                    | Sample size | Percentage agreement <sup>‡</sup> | Limits of agreement |             | Spearman correlation | LOA vs portion size <sup>#</sup>      |
|---|-------------|-----------------------------------|---------------------|-------------|----------------------|---------------------------------------|
|   |             |                                   | Lower limit         | Upper limit |                      |                                       |
|   | n           |                                   |                     |             | r <sub>BA</sub>      | (mean difference ± 1 X small portion) |
| <i>Umngqusho</i> (g)                    | 60          | 96.6                              | -233.6              | 644.9       | 0.30*                | < 1 x Small portion                   |
| Soup (g)                                | 57          | 91.1                              | -310.5              | 541.5       | -0.31*               | < 1 x Small portion                   |
| Stiff <i>pap</i> and pumpkin (g)        | 56          | 94.6                              | -239.6              | 555.2       | -0.21                | < 1 x Small portion                   |
| Stiff <i>pap</i> and <i>imifino</i> (g) | 56          | 96.4                              | -264.9              | 546.7       | -0.21                | < 1 x Small portion                   |
| Porridge (g)                            | 45          | 95.4                              | -156.9              | 362.7       | 0.35*                | < 1 x Small portion                   |
| Stiff <i>pap</i> (g)                    | 57          | 100.0                             | -275.2              | 374.8       | 0.32**               | ≈ 1 x Small portion                   |
| <i>Umphokoqo</i> (g)                    | 58          | 100.0                             | -65.2               | 485.6       | 0.32*                | < 1 x Small portion                   |
| Kernels (g)                             | 37          | 91.2                              | -218.9              | 408.3       | 0.30                 | < 1 x Small portion                   |

<sup>‡</sup> Percentage of data points between the limits of agreement

Lower limit = mean difference between photograph weight and dished up weight – 2 standard deviations of the mean difference between the photograph weight and dished-up weight

Upper limit = mean difference between photograph weight and dished-up weight + 2 standard deviations of the mean difference between the photograph weight and dished-up weight

r<sub>BA</sub> = Spearman correlation between the mean of photographic weight and dished-up weight and the mean difference between the photograph weight and dished-up weight

Significance of the Spearman correlation between mean intake and mean difference

\* Spearman rank correlation coefficients significant at p < 0.05 level,

\*\* Spearman rank correlation coefficients significant at p < 0.01 level,

\*\*\* Spearman rank correlation coefficients significant at p < 0.001 level

# LOA is considered clinical acceptable if it is smaller than mean difference ± 1 X small portion

*Umngqusho* = Samp and beans (Dried kernels and sugar beans)

Soup = Whole kernels and sugar beans

Stiff *pap* and pumpkin = Stiff maize meal porridge and pumpkin

Stiff *pap* and *imifino* = Stiff maize meal porridge and spinach

Porridge = Thin maize meal porridge

Stiff *pap* = Stiff maize meal porridge

*Umphokoqo* = Crumbly *pap* (Dry maize meal porridge)

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Kernels = Boiled whole maize kernels

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The summary of the statistical results (Table 5.5) illustrate that the validity of the food photographs in portion size estimation was overall poor.

For samp and beans, soup, stiff *pap* and *umphokoqo* five of the seven statistical tests point to poor validity of the photographs in portion size estimation, while only two of the tests point to acceptable validity. The same is true for the photographs representing stiff *pap* and pumpkin, stiff *pap* and *imifino* with six of the seven statistical methods indicating poor validity and one acceptable validity. For maize porridge four of the statistical tests indicated poor validity.

The validity of the photographs for maize kernels in estimating portion sizes can be considered acceptable as all seven statistical tests showed acceptable to good validity (Table 5.5).

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**Table 5.5. Summary of statistical results for the validation of the portion size photographs**

| Dish                                | Agreement                 | Agreement             | Strength of association | Agreement (Including chance) | Agreement (Excluding chance) | Presence of bias | Limits of Agreement         | Final validity*                   |
|-------------------------------------|---------------------------|-----------------------|-------------------------|------------------------------|------------------------------|------------------|-----------------------------|-----------------------------------|
|                                     | Wilcoxon signed rank test | Percentage difference | Spearman correlations   | Tertile classification       | Kappa statistics             | Bland-Altman     | Bland-Altman (Width of LOA) | > 3 acceptable validation results |
| Level of validation                 | Group                     | Group                 | Individual              | Individual                   | Individual                   | Individual       | Individual                  |                                   |
| <i>Umngqusho</i>                    | Poor                      | Acceptable            | Poor                    | Poor                         | Poor                         | Present          | Acceptable                  | Poor                              |
| Soup                                | Poor                      | Acceptable            | Poor                    | Poor                         | Poor                         | Present          | Acceptable                  | Poor                              |
| Stiff <i>pap</i> and pumpkin        | Poor                      | Poor                  | Poor                    | Poor                         | Poor                         | Present          | Acceptable                  | Poor                              |
| Stiff <i>pap</i> and <i>imifino</i> | Poor                      | Poor                  | Poor                    | Poor                         | Poor                         | Present          | Acceptable                  | Poor                              |
| Porridge                            | Poor                      | Acceptable            | Acceptable              | Poor                         | Poor                         | Present          | Acceptable                  | Poor                              |
| Stiff <i>pap</i>                    | Poor                      | Good                  | Poor                    | Poor                         | Poor                         | Present          | Acceptable                  | Poor                              |
| <i>Umphokoqo</i>                    | Good                      | Poor                  | Poor                    | Poor                         | Poor                         | Present          | Acceptable                  | Poor                              |
| Kernels                             | Good                      | Good                  | Acceptable              | Good                         | Acceptable                   | Absent           | Acceptable                  | Acceptable                        |

Wilcoxon signed rank test agreement at group level  $p < 0.05$  = Good,  $p > 0.05$  Poor

Percentage difference:  $< 10\%$  = Good, 11 - 20% = Acceptable,  $> 20\%$  = Poor

Strength of association and correlation = Results from correlation coefficient (individual level)

Good =  $> 0.50$ , Acceptable 0.21 – 0.50 and Poor  $< 0.20$

Agreement = Results from tertile classification (include chance) and Kappa statistics (exclude chance)

Tertile classification:  $> 50\%$  is same group = Good

Kappa statistics:  $< 0.20$  = Poor, 0.21 – 0.60 = Acceptable, 0.61 – 0.80 = Good

Bias = Bland-Altman data indication of bias on individual level (if  $r_{BA}$  is significant),

$r_{BA}$  = Spearman correlation coefficient for Bland-Altman data (correlation between mean and mean difference of intake)

Agreement Bland-Altman:  $< 1$  x small portion = Narrow LOA,  $\approx 1$  x small portion = Acceptable,  $> 1$  small portion = Wide LOA

Final validity\* = Four or more of statistical methods indicate agreement

*Umngqusho* = Samp and beans (Dried kernels and sugar beans)

Soup = Whole kernels and sugar beans

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Stiff *pap* and pumpkin = Stiff maize meal porridge and pumpkin

Stiff *pap* and *imifino* = Stiff maize meal porridge and spinach

Porridge = Thin maize meal porridge

Stiff *pap* = Stiff maize meal porridge

*Umphokoqo* = Crumbly *pap* (Dry maize meal porridge)

Kernels = Boiled whole maize kernels

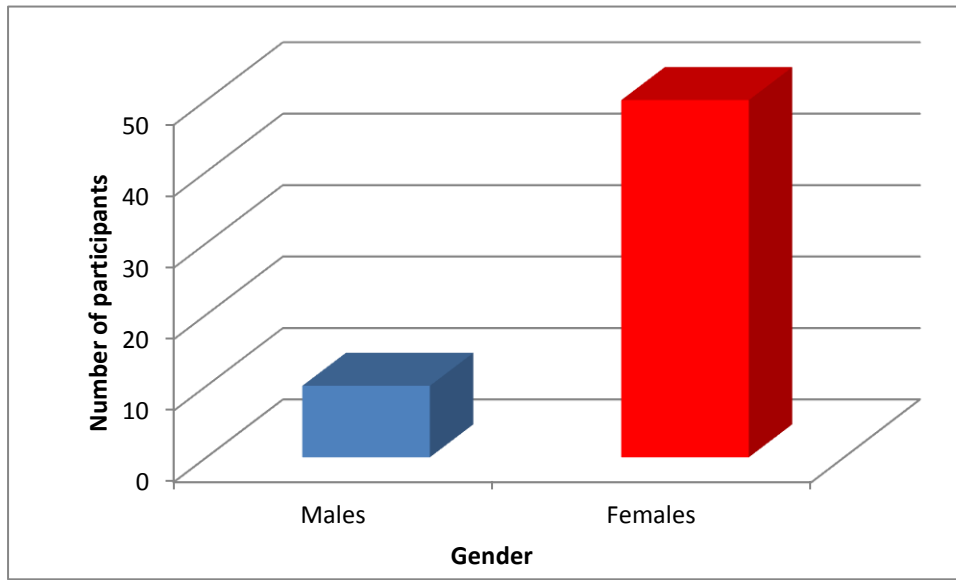
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### 3.3. EFFECT OF PARTICIPANT CHARACTERISTICS ON ACCURACY OF PORTION SIZE ESTIMATION

#### 3.3.1. Gender

Figure 5.7 shows the frequency distribution of the males and females included in the sample.



**Figure 5.7. Frequency distribution of both gender groups**

The mean portion sizes of the test weights of the males and females were generally similar for all dishes (Table 5.6) and Wilcoxon signed rank test indicated that there were no significant differences ( $p > 0.05$ ) in portion size estimation between the two genders. Overall, agreement between the test and reference weights was poor, regardless of gender. The mean of individual percentage differences ranged between -19.6% (stiff *pap*) and 36.7% (*umphokoqo*) for the males and between 8.6% (stiff *pap*) and 46.0% (*umphokoqo*) for the females.

Spearman correlation coefficients between the test and reference weights ranged between -0.29 (stiff *pap* and *imifino*) and 0.81 (kernels) for the males' estimation of portions (Table 5.6). Correlations for the females ranged between -0.20 for *umphokoqo* and 0.33 for porridge. Generally, strength of association for the different dishes was stronger in the male group than in the female group.

Agreement in terms of lying within the LOA was poor (< 95%) for soup, and stiff *pap* and *imifino*, in the male group while agreement for kernels were poor (< 95%) in the female

group (Table 5.7). Spearman correlation coefficients between the mean values of the test plus reference weights and the differences between the mean values of the test and reference weights, ranged between -0.77 and 0.59 in the male group. Results from the male group for soup, and stiff *pap* and pumpkin illustrated reporting bias ( $p < 0.05$ ). In the female group the correlation coefficients ranged between -0.18 and 0.41. Stiff *pap* and *umphokoqo* both illustrated the presence of reporting bias ( $p < 0.05$ ). The LOA were generally narrow and regarded as being acceptable ( $< 1 \times$  small portion), with the exception of soup, stiff *pap* and *imifino*, as well as stiff *pap*, which had wider LOA than acceptable ( $> 1 \times$  small portion) in the male group. Tertile classification into the same tertile was good ( $> 50\%$ ) for three dishes in the male group, while only one dish was classified as good in the female group (Table 5.8). Although poor in both genders, agreement (including chance) tended to be stronger for the males.

Kappa statistics in the male group were acceptable (0.21 - 0.60) for three dishes while in the female group only one dish showed acceptable agreement and the rest showed poor agreement. Therefore, it appears that agreement (with and without chance) tends to be better in the male group than in the female group (Table 5.8).

**Table 5.6. Means, differences, Wilcoxon signed rank, percentage differences and Spearman correlation coefficients for test and reference weights for each gender**

| Dish                                    | Mean portion size (g)<br>(Mean test weight) |                   | Difference (g)<br>(Test – reference weight) |                  | Wilcoxon signed rank<br><br>P value | Percentage difference (%)<br>[(Test – reference weight) / reference weight * 100] |                   | Spearman correlation |         |
|---|---|-------------------|---|------------------|-------------------------------------|---|-------------------|----------------------|---------|
|   | Males<br>n = 10                             | Females<br>n = 50 | Males                                       | Females          |                                     | Males<br>n = 10   | Females<br>n = 50 | Males                | Females |
|   | Mean<br>(SD)                                | Mean<br>(SD)      | Mean<br>(SD)                                | Mean<br>(SD)     |                                     | Mean<br>(SD)  | Mean<br>(SD)      | r                    | r       |
| <i>Umngqusho</i> (g)                    | 618.7<br>(89.4)                             | 636.5<br>(109.2)  | 268.4<br>(197.9)                            | 193.3<br>(223.6) | 0.37                                | 33.6<br>(23.4)  | 15.8<br>(59.4)    | 0.35                 | -0.17   |
| Soup (g)                                | 539.9<br>(121.7)                            | 555.6<br>(133.9)  | 56.6<br>(260.8)                             | 128.0<br>(202.5) | 0.31                                | 6.5<br>(44.6)   | 18.7<br>(32.6)    | 0.13                 | 0.15    |
| Stiff <i>pap</i> and pumpkin (g)        | 474.9<br>(64.7)                             | 509.9<br>(110.3)  | 57.4<br>(126.4)                             | 179.6<br>(205.7) | 0.06                                | 11.7<br>(27.2)  | 27.7<br>(31.3)    | 0.57                 | 0.08    |
| Stiff <i>pap</i> and <i>imifino</i> (g) | 439.4<br>(73.9)                             | 476.1<br>(88.8)   | 100.0<br>(238.0)                            | 148.8<br>(197.5) | 0.67                                | 13.1<br>(45.3)  | 23.7<br>(33.2)    | -0.29                | -0.11   |
| Porridge (g)                            | 391.6<br>(67.9)                             | 430.6<br>(82.9)   | 128.6<br>(157.7)                            | 97.3<br>(124.9)  | 0.55                                | 21.5<br>(30.3)  | 16.8<br>(24.8)    | 0.04                 | 0.33*   |
| Stiff <i>pap</i> (g)                    | 380.4<br>(122.9)                            | 404.7<br>(88.6)   | -47.1<br>(177.3)                            | 68.0<br>(154.8)  | 0.06                                | -19.6<br>(48.2)   | 8.6<br>(35.6)     | 0.07                 | 0.21    |
| <i>Umphokoqo</i> (g)                    | 334.8<br>(78.9)                             | 334.7<br>(58.6)   | 175.4<br>(130.1)                            | 217.4<br>(139.4) | 0.35                                | 36.7<br>(23.9)  | 46.0<br>(25.8)    | 0.22                 | -0.20   |

|             |                 |                  |                |                 |      |                |                |       |      |
|-------------|-----------------|------------------|----------------|-----------------|------|----------------|----------------|-------|------|
| Kernels (g) | 223.3<br>(79.3) | 307.8<br>(119.0) | 72.4<br>(89.6) | 99.9<br>(169.4) | 0.91 | 16.5<br>(25.9) | 20.3<br>(42.7) | 0.81* | 0.28 |
|-------------|-----------------|------------------|----------------|-----------------|------|----------------|----------------|-------|------|

Difference = test weight – reference weight

\* Spearman rank correlation coefficients significant at  $p < 0.05$  level,

\*\* Spearman rank correlation coefficients significant at  $p < 0.01$  level,

\*\*\* Spearman rank correlation coefficients significant at  $p < 0.001$  level

SD = standard deviation

g = gram

Wilcoxon signed rank test =  $p < 0.05$  = significant difference,  $p > 0.05$  = no significant difference

*Umngqusho* = Samp and beans (Dried kernels and sugar beans)

Soup = Whole kernels and sugar beans

Stiff *pap* and pumpkin = Stiff maize meal porridge and pumpkin

Stiff *pap* and *imifino* = Stiff maize meal porridge and spinach

Porridge = Thin maize meal porridge

Stiff *pap* = Stiff maize meal porridge

*Umphokoqo* = Crumbly *pap* (Dry maize meal porridge)

Kernels = Boiled whole maize kernels

**Table 5.7. Bland-Altman data for test and reference weights for each gender**

| Dish                                    | Agreement       |                   | LOA                    |                        | Spearman correlation |                 | Significance of LOA  |                     |
|---|-----------------|-------------------|------------------------|------------------------|----------------------|-----------------|----------------------|---------------------|
|   | Males<br>n = 10 | Females<br>n = 50 | Males                  | Females                | Males                | Females         | Males                | Females             |
|   | Mean<br>%       | Mean<br>%         | Lower – Upper<br>limit | Lower – Upper<br>limit | r <sub>BA</sub>      | r <sub>BA</sub> |                      |                     |
| <i>Umngqusho</i> (g)                    | 100.0           | 95.9              | -127.4 – 664.2         | -253.9 – 640.5         | -0.26                | -0.16           | < 1 x Small portion  | < 1 x Small portion |
| Soup (g)                                | 80.0            | 93.5              | -465.0 – 578.2         | -277.0 – 533.0         | -0.77*               | -0.16           | < 1 x Medium portion | < 1 X Small portion |
| Stiff <i>pap</i> and pumpkin (g)        | 100.0           | 93.3              | -195.4 – 310.2         | -231.8 – 591.0         | -0.34**              | -0.12           | < 1 x Small portion  | < 1 x Small portion |
| Stiff <i>pap</i> and <i>imifino</i> (g) | 88.9            | 97.8              | -376.0 – 576.0         | -246.2 – 543.8         | -0.38                | -0.18           | < 1 x Medium portion | < 1 x Small portion |
| Porridge (g)                            | 100.0           | 94.6              | -186.8 – 444.0         | -152.5 – 347.1         | 0.59                 | 0.31            | < 1 x Small portion  | < 1 x Small portion |
| Stiff <i>pap</i> (g)                    | 100.0           | 100.0             | -307.5 – 307.5         | -241.6 – 377.6         | -0.30                | 0.41**          | < 1 x Medium portion | ≈ 1 x Small portion |
| <i>Umphokoqo</i> (g)                    | 100.0           | 100.0             | -84.8 – 435.6          | -61.4 – 496.2          | 0.21                 | 0.35*           | < 1 x Small portion  | < 1 x Small portion |
| Kernels (g)                             | 100.0           | 89.7              | -106.8 – 251.6         | -238.9 – 438.7         | 0.39                 | 0.28            | < 1 x Small portion  | < 1 x Small portion |

Agreement = Percentage of responses falling within the limits of agreement

g = gram

SD = standard deviation

LOA = Limits of agreement

Lower limit = mean difference of photograph weight and dished up weight – 2 standard deviations of the mean difference between the photograph and dished-up weight

Upper limit = mean difference of photograph weight and dished up weight + 2 standard deviations of the mean difference between the photograph and dished-up weight

r<sub>BA</sub> = Spearman correlation between the mean of photographic weight plus dished-up weight and the mean difference between the photograph weight and dished-up weight

\* Spearman rank correlation coefficients significant at p < 0.05 level,

\*\* Spearman rank correlation coefficients significant at p < 0.01 level,

\*\*\* Spearman rank correlation coefficients significant at p < 0.001 level

Significance of LOA = mean difference ± 1 X small portion

*Umngqusho* = Samp and beans (Dried kernels and sugar beans)

Soup = Whole kernels and sugar beans

Stiff *pap* and pumpkin = Stiff maize meal porridge and pumpkin

Stiff *pap* and *imifino* = Stiff maize meal porridge and spinach

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Porridge = Thin maize meal porridge

Stiff *pap* = Stiff maize meal porridge

*Umphokoqo* = Crumbly *pap* (Dry maize meal porridge)

Kernels = Boiled whole maize kernels

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**Table 5.8. Tertile distribution and weighted Kappa statistics for the test and reference weights, for each gender**

| Dish                                | Tertiles        |                     |                   |                     | Kappa          |                |
|-------------------------------------|-----------------|---------------------|-------------------|---------------------|----------------|----------------|
|                                     | Males<br>n = 10 |                     | Females<br>n = 50 |                     | Males          | Females        |
| Dish                                | Same<br>tertile | Opposite<br>tertile | Same<br>tertile   | Opposite<br>tertile | K <sub>w</sub> | K <sub>w</sub> |
| <i>Umngqusho</i>                    | 40.0            | 40.0                | 42.0              | 28.0                | 0.21*          | 0.04           |
| Soup                                | 40.0            | 20.0                | 42.6              | 21.3                | 0.18           | 0.11           |
| Stiff <i>pap</i> and pumpkin        | 70.0            | 30.0                | 43.5              | 19.6                | 0.35           | 0.09           |
| Stiff <i>pap</i> and <i>imifino</i> | 22.2            | 22.2                | 38.3              | 27.7                | -0.24          | 0.04           |
| Porridge                            | 37.5            | 37.5                | 48.7              | 16.2                | -0.14          | 0.06           |
| Stiff <i>pap</i>                    | 44.4            | 22.2                | 43.8              | 10.4                | 0.06           | 0.11           |
| <i>Umphokoqo</i>                    | 50.0            | 20.0                | 41.7              | 22.9                | 0.06           | -0.03          |
| Kernels                             | 71.4            | 0.00                | 63.3              | 10.0                | 0.50*          | 0.29           |

K<sub>w</sub> = weighted Kappa statistic (Mason *et al.*, 2003:315)

*Umngqusho* = Samp and beans (Dried kernels and sugar beans)

Soup = Whole kernels and sugar beans

Stiff *pap* and pumpkin = Stiff maize meal porridge and pumpkin

Stiff *pap* and *imifino* = Stiff maize meal porridge and spinach

Porridge = Thin maize meal porridge

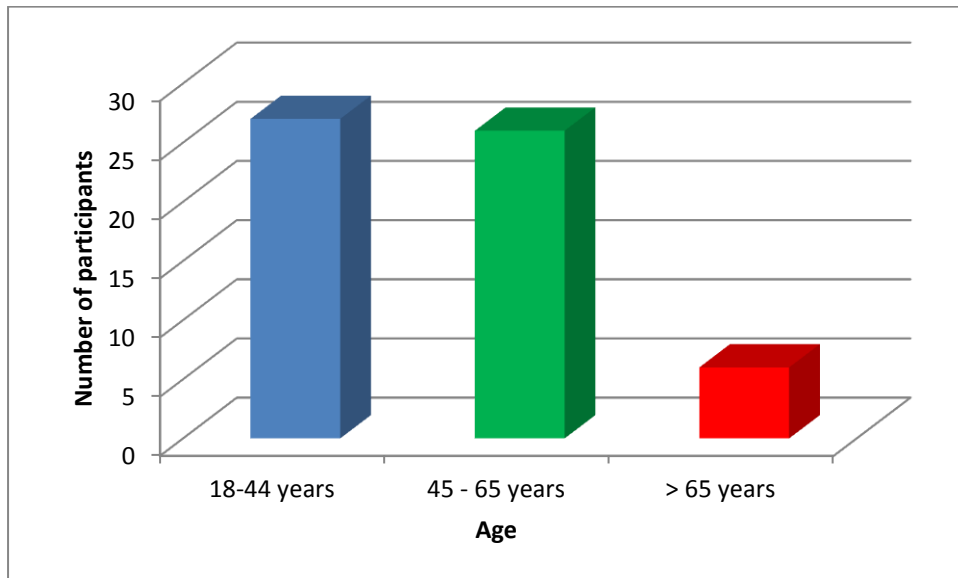
Stiff *pap* = Stiff maize meal porridge

*Umphokoqo* = Crumbly *pap* (Dry maize meal porridge)

Kernels = Boiled whole maize kernels

### 3.3.2. Age

Figure 5.8 shows the frequency distribution of the sample according to age group. The proportions of participants in the two younger groups were similar (46% and 44% respectively) while the proportion 65 years and older was smaller (10%). This age group was not collapsed with the 45-65 age group as the literature indicates that this age group should be analysed separately since their portion size perceptions may differ from that of the younger age groups, possible confounding the results of younger age groups if collapsed.



**Figure 5.8** Frequency distribution of the different age groups

Kruskal Wallis results showed no significant differences in mean weights (test and reference weights) of food items between the three age groups ( $p > 0.05$ ) (Table 5.9). Percentage differences between the individual test and reference weights in the first age group were good for one dish ( $< 10\%$ ), acceptable for five dishes ( $11\% - 20\%$ ) and poor for two ( $> 20\%$ ). The PDs between the test and reference weights for the second age group were good for one dish ( $< 10\%$ ), acceptable for four dishes ( $11\% - 20\%$ ) and poor for three dishes ( $> 20\%$ ). In the third age group there were no dishes that were considered to be good, one that was acceptable and seven that was considered to be poor.

Spearman correlation coefficients between the test and reference weights for the younger age group (18 - 44 years) ranged between  $-0.25$  (*umphokoqo*) and  $0.55$  (kernels) (Table 5.9). Five dishes had correlation coefficients that were considered to be poor ( $r < 0.2$ ), two dishes had correlations classified as being acceptable ( $r = 0.21 - 0.50$ ) and one dish had correlations that were good ( $r > 0.50$ ). Correlation coefficients for the second age group were lower, ranging from  $-0.28$  (*umphokoqo*) to  $0.36$  (soup). Correlation coefficients for the older age group ( $> 65$  years) were the best, ranging from  $0.00$  (*umphokoqo*) to  $1.0$  (whole kernels). Therefore, the strength of association at individual level appeared to be stronger for the older group than for the other age groups. This may be influenced by the small sample size.

In the youngest age group (18 - 44 years), Bland-Altman data showed that agreement was poor ( $< 95\%$ ) for soup, stiff *pap* and pumpkin, and for kernels (Table 5.10). Spearman

correlations between the mean and mean difference ( $r_{BA}$ ) were significant ( $p < 0.05$ ) for stiff *pap*, and *umphokoqo*, illustrating the presence of reporting bias. For the second age group (45 – 64 years) two dishes (porridge and kernels) showed poor (< 95%) agreement. Spearman correlation coefficients were significant ( $p < 0.05$ ) only for porridge. In the oldest age group (> 65 years) there were no dishes that showed poor agreement and no dishes with significant Spearman correlation coefficients ( $r_{BA}$ ). Limits of agreement were generally acceptable in all age groups, with the exception of stiff *pap* and kernels (18 – 44 years) and kernels (45 – 64 years). Therefore, the present of bias and individual agreement were stronger in the older group than in the two younger age groups.

Tertile classification for soup, stiff *pap*, *umphokoqo*, and kernels was good (> 50%) in the oldest group, while the youngest group showed good tertile classifications for stiff *pap* and pumpkin, stiff *pap* and *imifino*, porridge and kernels (Table 5.11). The middle age group performed the poorest on tertile classification with only two dishes meeting the criteria for good validity (> 50%). Therefore individual agreement were better for the younger and older group when chance agreement were included.

Kappa statistics for the first and second age groups were poor for all dishes except for whole kernels, which showed acceptable agreement for both age groups (Table 5.11). Kappa statistics for the older participants were good (> 0.61) for soup and kernels, and acceptable (0.21 – 0.60) for stiff *pap*. It is thus evident that with the exception of soup and stiff *pap* in the older group, results illustrating strong agreement between the two weights were mostly based on chance, especially in the younger group. Therefore, individual agreement were better for the older age group when chance agreement were excluded.

**Table 5.9. Mean, differences, Kruskal Wallis results, percentage differences and Spearman correlation coefficients for test and reference weights for each age group**

| Dish                                       | Mean portion size (g)<br>(Mean test weight) |                            |                        | Difference (g)<br>(Test – reference weight) |                            |                        | Kruskal<br>Wallis | Percentage difference (%)<br>[(Test – reference weight) /<br>reference weight * 100] |                            |                        | Spearman correlation |                  |               |
|--|---|----------------------------|------------------------|---|----------------------------|------------------------|-------------------|--|----------------------------|------------------------|----------------------|------------------|---------------|
|  | 18 - 44<br>years<br>n = 27                  | 45 - 64<br>years<br>n = 26 | > 65<br>years<br>n = 6 | 18 - 44<br>years<br>n = 27                  | 45 - 64<br>years<br>n = 26 | > 65<br>years<br>n = 6 |                   | 18 - 44<br>years<br>n = 27   | 45 - 64<br>years<br>n = 26 | > 65<br>years<br>n = 6 | 18 - 44<br>years     | 45 - 64<br>years | > 65<br>years |
|  | Mean<br>(SD)                                | Mean<br>(SD)               | Mean<br>(SD)           | Mean<br>(SD)                                | Mean<br>(SD)               | Mean<br>(SD)           | P value           | Mean<br>(SD)   | Mean<br>(SD)               | Mean<br>(SD)           | r                    | r                | r             |
| <i>Umngqusho</i><br>(g)                    | 625.6<br>(99.0)                             | 656.4<br>(102.1)           | 571.2<br>(137.8)       | 149.2<br>(224.2)                            | 233.7<br>(208.9)           | 345.7<br>(187.4)       | 0.10              | 17.6<br>(28.8)   | 13.8<br>(77.9)             | 46.5<br>(21.0)         | -0.24                | 0.01             | 0.12          |
| Soup (g)                                   | 555.8<br>(121.1)                            | 553.3<br>(136.4)           | 529.0<br>(195.3)       | 109.5<br>(232.7)                            | 114.8<br>(201.4)           | 162.0<br>(178.8)       | 0.90              | 13.0<br>(35.4)   | 18.5<br>(36.0)             | 29.7<br>(32.2)         | -0.11                | 0.36             | 0.63          |
| Stiff <i>pap</i> and<br>pumpkin (g)        | 521.4<br>(101.4)                            | 491.7<br>(107.0)           | 485.3<br>(115.9)       | 102.9<br>(187.9)                            | 203.9<br>(220.4)           | 194.0<br>(65.1)        | 0.18              | 16.2<br>(29.2)   | 27.3<br>(31.9)             | 38.7<br>(18.6)         | 0.26                 | -0.12            | 0.93**        |
| Stiff <i>pap</i> and<br><i>imifino</i> (g) | 459.6<br>(89.5)                             | 483.3<br>(77.5)            | 459.6<br>(126.5)       | 150.1<br>(211.1)                            | 107.0<br>(206.9)           | 263.2<br>(65.1)        | 0.28              | 24.6<br>(35.8)   | 13.7<br>(35.9)             | 46.4<br>(15.8)         | -0.13                | -0.27            | 0.87*         |
| Porridge (g)                               | 424.5<br>(92.8)                             | 425.2<br>(71.2)            | 422.3<br>(80.7)        | 84.3<br>(111.7)                             | 97.1<br>(150.2)            | 185.3<br>(109.8)       | 0.24              | 15.3<br>(22.0)   | 15.1<br>(30.0)             | 35.3<br>(16.8)         | 0.50*                | -0.03            | 0.48          |
| Stiff <i>pap</i> (g)                       | 426.0<br>(87.0)                             | 387.6<br>(89.7)            | 326.0<br>(125.2)       | 60.8<br>(175.4)                             | 28.6<br>(153.3)            | 96.8<br>(148.4)        | 0.62              | 3.5<br>(39.1)  | -0.2<br>(38.6)             | 23.3<br>(41.4)         | 0.01                 | 0.19             | 0.35          |
| <i>Umphokoqo</i><br>(g)                    | 337.8<br>(52.8)                             | 348.8<br>(63.3)            | 265.0<br>(55.3)        | 220.6<br>(140.3)                            | 202.5<br>(143.2)           | 192.0<br>(118.9)       | 0.85              | 44.1<br>(23.9)   | 43.0<br>(27.2)             | 52.5<br>(30.9)         | -0.25                | -0.28            | 0.00          |

|             |         |         |         |         |         |        |      |        |        |        |       |      |      |
|-------------|---------|---------|---------|---------|---------|--------|------|--------|--------|--------|-------|------|------|
| Kernels (g) | 270.6   | 310.3   | 288.3   | 80.1    | 109.2   | 44.5   | 0.79 | 17.5   | 23.1   | 18.7   | 0.55* | 0.33 | 1.00 |
|             | (115.5) | (118.1) | (162.3) | (146.1) | (172.3) | (31.8) |      | (30.6) | (47.3) | (19.4) |       |      |      |

Difference = test weight – reference weight

\* Spearman rank correlation coefficients significant at  $p < 0.05$

\*\* Spearman rank correlation coefficients significant at  $p < 0.01$

\*\*\* Spearman rank correlation coefficients significant at  $p < 0.001$

SD = standard deviation

Kruskal Wallis  $< 0.05$  = significant difference between two or more groups,  $p > 0.05$  = no significant difference between any groups

*Umgqusho* = Samp and beans (Dried kernels and sugar beans)

Soup = Whole kernels and sugar beans

Stiff *pap* and pumpkin = Stiff maize meal porridge and pumpkin

Stiff *pap* and *imifino* = Stiff maize meal porridge and spinach

Porridge = Thin maize meal porridge

Stiff *pap* = Stiff maize meal porridge

*Umphokoqo* = Crumbly *pap* (Dry maize meal porridge)

Kernels = Boiled whole maize kernels

**Table 5.10. Bland-Altman data for test and reference weights for each age group**

| Dish                                    | Agreement               |                         |                     | LOA                     |                         |                     | Spearman correlation    |                         |                     | Significance of LOA     |                         |                     |
|---|-------------------------|-------------------------|---------------------|-------------------------|-------------------------|---------------------|-------------------------|-------------------------|---------------------|-------------------------|-------------------------|---------------------|
|   | 18 - 44 years<br>n = 27 | 45 - 64 years<br>n = 26 | > 65 years<br>n = 6 | 18 - 44 years<br>n = 27 | 45 - 64 years<br>n = 26 | > 65 years<br>n = 6 | 18 - 44 years<br>n = 27 | 45 - 64 years<br>n = 26 | > 65 years<br>n = 6 | 18 - 44 years<br>n = 27 | 45 - 64 years<br>n = 26 | > 65 years<br>n = 6 |
|   | Mean %                  | Mean %                  | Mean %              | Lower - Upper           | Lower - Upper           | Lower - Upper       | r <sub>BA</sub>         | r <sub>BA</sub>         | r <sub>BA</sub>     |                         |                         |                     |
| <i>Umngqusho</i> (g)                    | 96.3                    | 96.2                    | 100.0               | -299.2 – 597.6          | -18.4 – 651.5           | -29.1 – 720.5       | -0.14                   | -0.19                   | -0.49               | < 1 x Small portion     | < 1 x Small portion     | < 1 x Small portion |
| Soup (g)                                | 85.2                    | 96.0                    | 100.0               | -355.9 – 574.9          | -288.0 – 517.6          | -195.6 – 519.6      | -0.11                   | -0.36                   | -0.60               | ≈ 1 x Small portion     | < 1 x Small portion     | < 1 x Small portion |
| Stiff <i>pap</i> and pumpkin (g)        | 91.7                    | 96.0                    | 100.0               | -272.9 – 478.7          | -236.9 – 644.7          | 63.8 – 324.2        | -0.02                   | -0.32                   | 0.03                | < 1 x Small portion     | < 1 x Small portion     | < 1 x Small portion |
| Stiff <i>pap</i> and <i>imifino</i> (g) | 96.0                    | 96.0                    | 100.0               | -272.1 – 572.3          | -306.8 – 520.8          | 133.0 – 393.4       | -0.26                   | -0.21                   | -0.70               | < 1 x Small portion     | < 1 x Small portion     | < 1 x Small portion |
| Porridge (g)                            | 95.2                    | 94.4                    | 100.0               | -139.1 – 307.7          | -203.3 – 397.5          | -34.3 – 404.9       | 0.21                    | 0.55*                   | 0.54                | < 1 x Small portion     | < 1 x Small portion     | < 1 x Small portion |
| Stiff <i>pap</i> (g)                    | 100.0                   | 100.0                   | 100.0               | -290.0 – 411.6          | -278.0 – 335.2          | -200.0 – 393.6      | 0.49*                   | 0.18                    | 0.00                | > 1 x Small portion     | < 1 x Small portion     | < 1 x Small portion |
| <i>Umphokoqo</i> (g)                    | 100.0                   | 100.0                   | 100.0               | -60.0 – 501.2           | -83.9 – 488.9           | -45.8 – 429.8       | 0.38*                   | 0.33                    | -0.43               | < 1 x Small portion     | < 1 x Small portion     | < 1 x Small portion |
| Kernels (g)                             | 92.9                    | 90.0                    | 100.0               | -212.1 – 372.3          | -235.4 – 453.8          | -19.1 – 108.1       | 0.26                    | 0.22                    | -1.00               | > 1 x Small portion     | > 1 x Small portion     | < 1 x Small portion |

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Agreement = Percentage of responses falling within the limits of agreement

g = gram

SD = standard deviation

LOA = Limits of agreement

Lower limit = mean difference between photograph weight and dished up weight – 2 standard deviations of the mean difference between the photograph weight and dished-up weight

Upper limit = mean difference between photograph weight and dished up weight + 2 standard deviations of the mean difference between the photograph weight and dished-up weight

$r_{BA}$  = Spearman correlation between the mean of photographic weight plus dished-up weight and the mean difference between the photograph weight and dished-up weight

\* Spearman rank correlation coefficients significant at  $p < 0.05$  level,

\*\* Spearman rank correlation coefficients significant at  $p < 0.01$  level,

\*\*\* Spearman rank correlation coefficients significant at  $p < 0.001$  level

Significance of LOA = mean difference  $\pm 1$  X small portion size

*Umngqusho* = Samp and beans (Dried kernels and sugar beans)

Soup = Whole kernels and sugar beans

Stiff *pap* and pumpkin = Stiff maize meal porridge and pumpkin

Stiff *pap* and *imifino* = Stiff maize meal porridge and spinach

Porridge = Thin maize meal porridge

Stiff *pap* = Stiff maize meal porridge

*Umphokoqo* = Crumbly *pap* (Dry maize meal porridge)

Kernels = Boiled whole maize kernels

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**Table 5.11. Tertile distribution and weighted Kappa statistics for the test and reference weights, for each age group**

| Dish                                | Tertiles      |                  |               |                  |              |                  | Kappa          |                |                |
|-------------------------------------|---------------|------------------|---------------|------------------|--------------|------------------|----------------|----------------|----------------|
|                                     | 18 - 44 years |                  | 45 - 64 years |                  | > 65 years   |                  | 18 - 44 years  | 45 - 64 years  | > 65 years     |
| Dish                                | Same tertile  | Opposite tertile | Same tertile  | Opposite tertile | Same tertile | Opposite tertile | K <sub>w</sub> | K <sub>w</sub> | K <sub>w</sub> |
| <i>Umngqusho</i>                    | 44.8          | 20.7             | 41.7          | 44.0             | 33.3         | 16.7             | -0.01          | 0.13           | 0.00           |
| Soup                                | 37.9          | 24.1             | 41.7          | 20.8             | 75.0         | 00.0             | 0.07           | 0.09           | 0.64*          |
| Stiff <i>pap</i> and pumpkin        | 50.0          | 11.5             | 50.0          | 25.0             | 33.6         | 00.0             | 0.16           | 0.18           | -0.04          |
| Stiff <i>pap</i> and <i>imifino</i> | 50.0          | 26.9             | 28.0          | 28.0             | 00.0         | 20.0             | 0.19           | -0.08          | -0.14          |
| Porridge                            | 57.1          | 14.3             | 38.9          | 22.2             | 33.3         | 33.3             | 0.18           | -0.11          | 0.00           |
| Stiff <i>pap</i>                    | 40.7          | 18.5             | 44.0          | 8.0              | 60.0         | 00.0             | 0.11           | 0.05           | 0.23           |
| <i>Umphokoqo</i>                    | 37.9          | 24.1             | 39.1          | 26.1             | 83.3         | 00.0             | 0.00           | -0.06          | 0.00           |
| Kernels                             | 71.4          | 7.1              | 57.1          | 9.5              | 100.0        | 00.0             | 0.24           | 0.26           | 1.0            |

K<sub>w</sub> weighted Kappa (Mason *et al.*, 2003:315)

*Umngqusho* = Samp and beans (Dried kernels and sugar beans)

Soup = Whole kernels and sugar beans

Stiff *pap* and pumpkin = Stiff maize meal porridge and pumpkin

Stiff *pap* and *imifino* = Stiff maize meal porridge and spinach

Porridge = Thin maize meal porridge

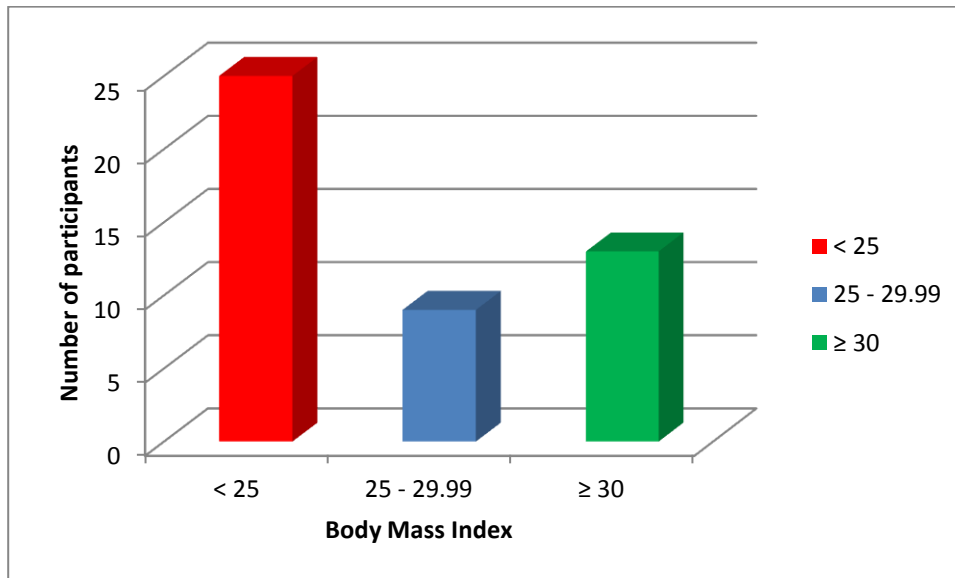
Stiff *pap* = Stiff maize meal porridge

*Umphokoqo* = Crumbly *pap* (Dry maize meal porridge)

Kernels = Boiled whole maize kernels

### 3.3.3. Body mass index

Figure 5.9 presents the frequency distribution of the different BMI groups. The majority of participants had a normal to low BMI (53%), while the smallest proportion was in the BMI 25 – 29.99 category (19%).



**Figure 5.9. Frequency distribution of body mass index groups**

Kruskal Wallis tests between the three BMI groups showed statistically significant ( $p < 0.05$ ) differences between the three groups for estimation of the test weight of *umngqusho* (samp and beans), stiff *pap* and *imifino*, and for kernels (Table 5.12). Ad hoc Bonferroni tests further showed that the differences in portion size estimation for *umngqusho* were between the normal weight and the obese weight group (group 1 and 3), while the differences for stiff *pap* and *imifino* were between the normal and the overweight group (group 1 and 2) and for kernels between the overweight and obese group (group 2 and 3).

The normal BMI group (BMI  $< 25 \text{ kg/m}^2$ ) overestimated portion sizes when comparing the test and reference weights. Three dishes, namely, soup, stiff *pap* and *imifino*, and stiff *pap* showed good PDs ( $< 10\%$  weight difference) and only *umphokoqo* had a PD above  $20\%$  (Table 5.12). The overweight group (BMI  $25 - 29.99 \text{ kg/m}^2$ ) also overestimated portion sizes when comparing the test and reference weights. Only stiff *pap* showed good PDs ( $< 10\%$  weight difference), while estimations for soup, porridge and whole kernels showed acceptable differences (PD =  $11 - 20\%$ ) between test and reference weights. The obese group (BMI  $\geq 30 \text{ kg/m}^2$ ) was the most likely to overestimate, with only the PD for stiff *pap* meeting the criterion of a PD less than  $10\%$ . Overall, it appears that agreement between the test and the reference weights became poorer as BMI increased.

Spearman correlation coefficients between the test and reference weights for the normal weight group (BMI  $< 25 \text{ kg/m}^2$ ) were generally poor, ranging from  $-0.28$  for *umphokoqo* (crumbly *pap*) to  $0.66$  for whole kernels (Table 5.12). Six dishes showed poor correlations ( $r$

< 0.20), with one dish (porridge) showing an acceptable correlation ( $r = 0.21 - 0.50$ ) and one dish (whole kernels) showing a good correlation ( $r > 0.50$ ). Correlation coefficients for the overweight group ( $25 - 29.99 \text{ kg/m}^2$ ) tended to be stronger, but were still poor, ranging from -0.16 for porridge to 0.39 for whole kernels. Correlations for the obese group ( $\geq 30 \text{ kg/m}^2$ ) ranged from -0.48 for *umngqusho*, to 0.34 for soup. For the obese five dishes showed poor correlation coefficients ( $r < 0.20$ ), three dishes acceptable correlations ( $r = 0.21 - 0.50$ ) while no dishes showed good correlation coefficients. Strength of association seems equally poor, for all three the weight groups.

Agreement (based on the LOA) in the normal weight group was poor (< 95%) for *umngqusho*, soup and kernels (Table 5.13). Furthermore, the LOA for soup, stiff *pap* and kernels were wide (> 1 X small portion). Spearman correlation coefficients between the mean and mean difference ( $r_{BA}$ ) showed the presence of reporting bias for four dishes namely soup, stiff *pap*, *umphokoqo* and kernels.

Bland-Altman statistics of the overweight group indicated poor agreement (< 95%) for stiff *pap* and pumpkin, stiff *pap* and *imifino*, and for porridge (Table 5.13). There were no dishes with wide LOA. Spearman correlation coefficients ( $r_{BA}$ ) showed that there were no significant coefficients ( $p < 0.05$ ) illustrating an absence of reporting bias. Results furthermore indicated that agreement for soup, stiff *pap* and pumpkin and kernels was poor (< 95%) for the obese group, although only kernels showed wide LOA (> 1 X small portion) and non-significant Spearman correlations indicated the absence of reporting bias for all dishes. These results suggest that the overweight and obese groups showed less or no reporting bias and narrower LOA compared with the normal weight group reflecting more acceptable validity with higher weight.

**Table 5.12. Mean, differences, Kruskal Wallis results, percentage differences and Spearman correlation coefficients for test and reference weights, for each body weight group**

| Dish                                    | Mean test weight (g) |                    |                  | Difference in weight (g)<br>(Test – reference weight) |                    |                  | Kruskal<br>Wallis<br><br>P value | Percentage difference (%)<br>[(Test – reference weight) /<br>reference weight * 100] |                    |                | Spearman correlation |                |           |
|---|----------------------|--------------------|------------------|---|--------------------|------------------|----------------------------------|--|--------------------|----------------|----------------------|----------------|-----------|
|   | < 25<br>n = 25       | 25 – 29.9<br>n = 9 | ≥ 30<br>n = 13   | < 25<br>n = 25  | 25 – 29.9<br>n = 9 | ≥ 30<br>n = 13   |                                  | < 25<br>n = 25   | 25 – 29.9<br>n = 9 | ≥ 30<br>n = 13 | < 25<br>r            | 25 – 29.9<br>r | ≥ 30<br>r |
| <i>Umngqusho</i> (g)                    | 665.3<br>(117.6)     | 605.3<br>(119.9)   | 614.4<br>(69.2)  | 133.0<br>(191.6)                                      | 168.9<br>(199.2)   | 317.0<br>(228.1) | 0.01 <sup>1-3</sup>              | 16.9<br>(26.4)   | 22.5<br>(27.0)     | 18.6<br>(87.8) | 0.19                 | 0.13           | -0.48*    |
| Soup (g)                                | 590.7<br>(139.6)     | 575.4<br>(126.0)   | 491.9<br>(102.8) | 59.1<br>(240.7)                                       | 136.5<br>(226.3)   | 173.4<br>(151.5) | 0.19                             | 6.6<br>(39.8)  | 17.5<br>(36.8)     | 28.4<br>(22.5) | 0.12                 | 0.05           | 0.34      |
| Stiff <i>pap</i> and pumpkin<br>(g)     | 519.2<br>(95.8)      | 494.1<br>(106.5)   | 495.1<br>(115.5) | 119.3<br>(209.0)                                      | 171.0<br>(176.4)   | 190.8<br>(204.1) | 0.50                             | 16.8<br>(31.9)   | 29.3<br>(30.1)     | 30.5<br>(30.4) | 0.04                 | 0.20           | 0.22      |
| Stiff <i>pap</i> and <i>imifino</i> (g) | 493.6<br>(84.2)      | 461.4<br>(100.5)   | 444.8<br>(77.6)  | 76.6<br>(215.3)                                       | 262.2<br>(198.6)   | 149.1<br>(155.8) | 0.03 <sup>1-2</sup>              | 9.6<br>(37.3)  | 41.4<br>(34.5)     | 26.1<br>(26.8) | -0.19                | -0.02          | 0.13      |
| Porridge (g)                            | 454.7<br>(87.1)      | 428.2<br>(60.9)    | 378.0<br>(68.4)  | 116.5<br>(123.3)                                      | 102.0<br>(172.6)   | 84.1<br>(106.1)  | 0.78                             | 19.6<br>(24.0)   | 14.7<br>(35.8)     | 17.1<br>(19.1) | 0.31                 | -0.16          | 0.17      |
| Stiff <i>pap</i> (g)                    | 433.0<br>(78.7)      | 353.9<br>(95.4)    | 389.3<br>(101.9) | 44.6<br>(175.9)                                       | 36.5<br>(154.9)    | 65.8<br>(156.3)  | 0.87                             | 0.6<br>(40.9)  | 4.1<br>(41.0)      | 8.9<br>(35.8)  | -0.14                | 0.10           | 0.31      |
| <i>Umphokoqo</i> (g)                    | 346.6<br>(55.6)      | 326.2<br>(77.7)    | 325.3<br>(57.1)  | 188.2<br>(140.5)                                      | 205.3<br>(140.7)   | 242.7<br>(132.7) | 0.43                             | 38.5<br>(24.5)   | 45.2<br>(27.5)     | 51.6<br>(24.9) | -0.28                | 0.05           | -0.02     |
| Kernels (g)                             | 343.7<br>(126.3)     | 267.9<br>(82.8)    | 237.5<br>(89.7)  | 95.2<br>(132.4)                                       | -52.8<br>(106.0)   | 162.2<br>(168.5) | 0.02 <sup>2-3</sup>              | 16.6<br>(28.7)   | -19.5<br>(54.1)    | 41.8<br>(32.2) | 0.66**               | 0.39           | 0.17      |

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Difference = test weight – reference weight

\* Spearman rank correlation coefficients significant at  $p < 0.05$

\*\* Spearman rank correlation coefficients significant at  $p < 0.01$

\*\*\* Spearman rank correlation coefficients significant at  $p < 0.001$

SD = standard deviation

Kruskal Wallis:  $p < 0.05$  = significant difference between two or more groups,  $p > 0.05$  = no significant difference between any groups

Kruskal Wallis 1 - 3 = significant difference between BMI group 1&3, 1 - 2 = significant difference between BMI group 1 & 2, 2 - 3 = significant difference between BMI group 2 & 3

*Umngqusho* = Samp and beans (Dried kernels and sugar beans)

Soup = Whole kernels and sugar beans

Stiff *pap* and pumpkin = Stiff maize meal porridge and pumpkin

Stiff *pap* and *imifino* = Stiff maize meal porridge and spinach

Porridge = Thin maize meal porridge

Stiff *pap* = Stiff maize meal porridge

*Umphokoqo* = Crumbly *pap* (Dry maize meal porridge)

Kernels = Boiled whole maize kernels

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Table 5.13. Bland-Altman data for test and reference weights for each body weight group

| BMI group (kg/m <sup>2</sup> )             | Agreement <sup>†</sup>              |                                       |                                     | LOA                                 |                                       |                                     | Spearman correlation                |                                       |                                     | Significance of LOA                 |                                       |                                     |
|--|-------------------------------------|---------------------------------------|-------------------------------------|-------------------------------------|---------------------------------------|-------------------------------------|-------------------------------------|---------------------------------------|-------------------------------------|-------------------------------------|---------------------------------------|-------------------------------------|
|  | < 25<br>kg/m <sup>2</sup><br>n = 25 | 25-29.9<br>kg/m <sup>2</sup><br>n = 9 | ≥ 30<br>kg/m <sup>2</sup><br>n = 13 | < 25<br>kg/m <sup>2</sup><br>n = 25 | 25-29.9<br>kg/m <sup>2</sup><br>n = 9 | ≥ 30<br>kg/m <sup>2</sup><br>n = 13 | < 25<br>kg/m <sup>2</sup><br>n = 25 | 25-29.9<br>kg/m <sup>2</sup><br>n = 9 | ≥ 30<br>kg/m <sup>2</sup><br>n = 13 | < 25<br>kg/m <sup>2</sup><br>n = 25 | 25-29.9<br>kg/m <sup>2</sup><br>n = 9 | ≥ 30<br>kg/m <sup>2</sup><br>n = 13 |
| Dish                                       | Mean<br>%                           | Mean<br>%                             | Mean<br>%                           | Lower -<br>Upper                    | Lower -<br>Upper                      | Lower -<br>Upper                    | r <sub>BA</sub>                     | r <sub>BA</sub>                       | r <sub>BA</sub>                     |                                     |                                       |                                     |
| <i>Umngqusho</i> (g)                       | 92.0                                | 100.0                                 | 100.0                               | -250.2 – 516.2                      | -229.5 –<br>567.3                     | -139.2 – 773.2                      | -0.30                               | -0.27                                 | 0.04                                | < 1 x Small<br>portion              | < 1 x Small<br>portion                | < 1 x Small<br>portion              |
| Soup (g)                                   | 84.0                                | 100.0                                 | 94.7                                | -422.3 – 540.5                      | -316.1 –<br>589.1                     | -129.6 – 476.2                      | -0.45*                              | -0.24                                 | -0.02                               | > 1 x Small<br>portion              | < 1 x Small<br>portion                | < 1 x Small<br>portion              |
| Stiff <i>pap</i> and<br>pumpkin (g)        | 100.0                               | 85.7                                  | 94.7                                | -298.7 – 537.3                      | -181.8 –<br>523.8                     | -217.4 – 599.0                      | -0.21                               | -0.06                                 | -0.24                               | < 1 x Small<br>portion              | < 1 x Small<br>portion                | < 1 x Small<br>portion              |
| Stiff <i>pap</i> and<br><i>imifino</i> (g) | 96.0                                | 91.7                                  | 100.0                               | -354.0 – 507.2                      | -135.0 –<br>659.4                     | -162.5 – 460.7                      | -0.30                               | -0.15                                 | -0.07                               | ≈ 1 x Small<br>portion              | < 1 x Small<br>portion                | < 1 x Small<br>portion              |
| Porridge (g)                               | 100.0                               | 81.8                                  | 100.0                               | -130.1 – 363.1                      | -243.2 –<br>447.2                     | -128.1 – 296.3                      | 0.29                                | 0.15                                  | 0.13                                | < 1 x Small<br>portion              | < 1 x Small<br>portion                | < 1 x Small<br>portion              |
| Stiff <i>pap</i> (g)                       | 100.0                               | 100.0                                 | 100.0                               | -307.2 – 396.4                      | -273.3 –<br>346.3                     | -246.8 – 378.4                      | 0.48*                               | -0.02                                 | 0.30                                | > 1 x Small<br>portion              | ≈ 1 x Small<br>portion                | ≈ 1 x small<br>portion              |
| <i>Umphokoqo</i> (g)                       | 100.0                               | 100.0                                 | 100.0                               | -92.8 – 469.2                       | -76.1 – 486.7                         | -227.0 – 508.1                      | 0.41*                               | 0.27                                  | 0.38                                | < 1 x Small<br>portion              | < 1 x Small<br>portion                | < 1 x Small<br>portion              |
| Kernels (g)                                | 94.4                                | 100.0                                 | 83.3                                | -169.6 – 360.0                      | -264.8 –<br>159.2                     | -174.8 – 499.2                      | 0.59*                               | -0.43                                 | 0.51                                | > 1 x Small<br>portion              | ≈ 1 x Small<br>portion                | > 1 x Small<br>portion              |

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‡ Percentage between the limits of agreement

g = gram

SD = standard deviation

LOA = Limits of agreement

Lower limit = mean difference between photograph weight and dished up weight – 2 standard deviations of the mean difference between the photograph weight and dished-up weight

Upper limit = mean difference between photograph weight and dished up weight + 2 standard deviations of the mean difference between the photograph weight and dished-up weight

$r_{BA}$  = Spearman correlation between the mean of photographic weight and dished-up weight and the mean difference between the photograph weight and dished-up weight

\* Spearman rank correlation coefficients significant at  $p < 0.05$  level,

\*\* Spearman rank correlation coefficients significant at  $p < 0.01$  level,

\*\*\* Spearman rank correlation coefficients significant at  $p < 0.001$  level

Significance of LOA = mean difference between the photograph weight and dished up weight  $\pm 1$  X small portion

*Umngqusho* = Samp and beans (Dried kernels and sugar beans)

Soup = Whole kernels and sugar beans

Stiff *pap* and pumpkin = Stiff maize meal porridge and pumpkin

Stiff *pap* and *imifino* = Stiff maize meal porridge and spinach

Porridge = Thin maize meal porridge

Stiff *pap* = Stiff maize meal porridge

*Umphokoqo* = Crumbly *pap* (Dry maize meal porridge)

Kernels = Boiled whole maize kernels

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Table 5.14 shows the tertile classification and weighted Kappa statistics for the test and reference weights according to BMI status of the participants. Classification of participants into the same tertile was generally poor for the normal body weight group, with only whole kernels showing more than 50% in the same tertile group. The tertile classification for the overweight group was better, although classification of stiff *pap* and *imifino*, stiff *pap* and pumpkin, porridge and soup were also poor. Tertile classification within the same group ranged from 28.6% to 78.6% for the obese group of participants. Portion sizes were correctly classified for five of the eight dishes, namely soup, stiff *pap* and pumpkin, stiff *pap* and *imifino*, porridge, and kernels. Agreement was generally poor for all three body weight groups, although that of the obese group tended to be better.

Except for whole kernels (0.48), the weighted Kappa statistic for portion size estimation of participants in the normal weight group was poor, ranging from 0.01 (stiff *pap* and *imifino*) to 0.18 (soup) (Table 5.14). The same was true for portion size estimation by those in the overweight group. Only kernels (0.33) showed an acceptable weighted Kappa value. Kappa statistics for portion size estimation of the obese group was better, with *umngqusho*, soup, stiff *pap*, kernels and *umphokoqo* showing poor values. Stiff *pap* and pumpkin, stiff *pap* and *imifino*, and porridge had acceptable Kappa values. These results support the tertile classification results namely that agreement is poor whether by chance or not, but that agreement in both cases may be better for the obese.

**Table 5.14. Tertile distribution and weighted Kappa statistics for the test and reference weights, for each body weight group**

| BMI group                              | Tertiles                         |                     |                                    |                     |                                  |                     | Weighted Kappa            |                              |                           |
|--|----------------------------------|---------------------|------------------------------------|---------------------|----------------------------------|---------------------|---------------------------|------------------------------|---------------------------|
|  | < 25 kg/m <sup>2</sup><br>n = 25 |                     | 25-29.9 kg/m <sup>2</sup><br>n = 9 |                     | ≥ 30 kg/m <sup>2</sup><br>n = 13 |                     | < 25<br>kg/m <sup>2</sup> | 25–29.9<br>kg/m <sup>2</sup> | ≥ 30<br>kg/m <sup>2</sup> |
| Dish                                   | Same<br>tertile                  | Opposite<br>tertile | Same<br>tertile                    | Opposite<br>tertile | Same<br>tertile                  | Opposite<br>tertile | K <sub>w</sub>            | K <sub>w</sub>               | K <sub>w</sub>            |
| <i>Umngqusho</i>                       | 44.0                             | 24.0                | 57.2                               | 14.3                | 28.6                             | 47.6                | 0.14                      | 0.11                         | 0.00                      |
| Soup                                   | 44.0                             | 28.0                | 16.7                               | 16.7                | 55.0                             | 15.0                | 0.18                      | -0.20                        | 0.19                      |
| Stiff <i>pap</i> and<br>pumpkin        | 45.5                             | 18.2                | 42.9                               | 7.1                 | 55.0                             | 20.0                | 0.09                      | 0.02                         | 0.26*                     |
| Stiff <i>pap</i> and<br><i>Imifino</i> | 36.0                             | 24.0                | 8.3                                | 41.7                | 52.6                             | 21.1                | 0.01                      | -0.22                        | 0.23                      |
| Porridge                               | 35.0                             | 30.0                | 27.3                               | 18.2                | 78.6                             | 7.1                 | 0.02                      | -0.29                        | 0.33*                     |
| Stiff <i>pap</i>                       | 36.0                             | 16.0                | 53.9                               | 7.7                 | 47.4                             | 10.5                | 0.02                      | -0.04                        | 0.14                      |
| <i>Umphokoqo</i>                       | 44.0                             | 20.0                | 57.1                               | 28.6                | 31.6                             | 21.1                | 0.09                      | 0.00                         | -0.09                     |
| Kernels                                | 66.7                             | 5.6                 | 66.7                               | 0.0                 | 61.5                             | 15.4                | 0.48**                    | 0.33                         | 0.00                      |

K<sub>w</sub> weighted Kappa (Mason *et al.*, 2003:315)

*Umngqusho* = Samp and beans (Dried kernels and sugar beans)

Soup = Whole kernels and sugar beans

Stiff *pap* and pumpkin = Stiff maize meal porridge and pumpkin

Stiff *pap* and *imifino* = Stiff maize meal porridge and spinach

Porridge = Thin maize meal porridge

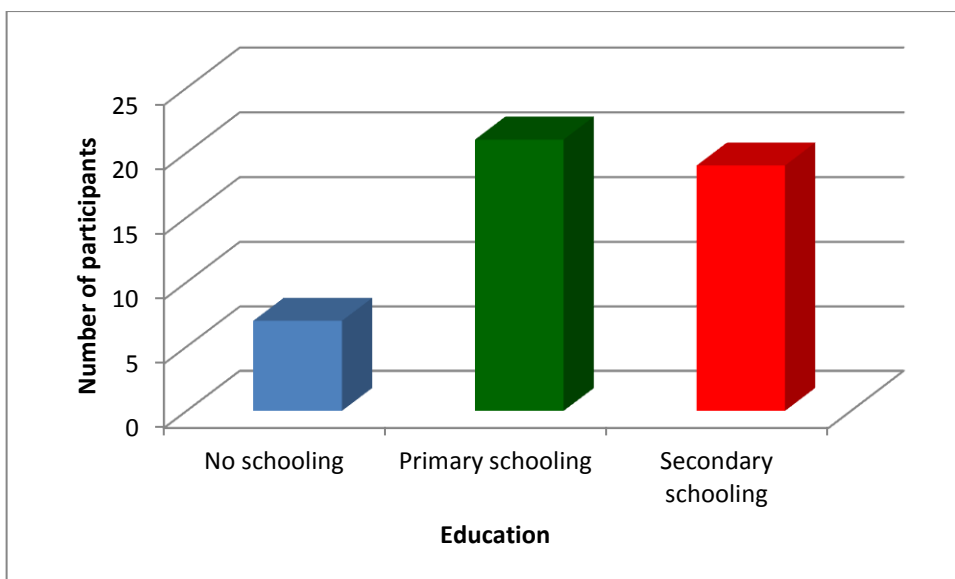
Stiff *pap* = Stiff maize meal porridge

*Umphokoqo* = Crumbly *pap* (Dry maize meal porridge)

Kernels = Boiled whole maize kernels

### 3.3.4. Education

Figure 5.10 presents the frequency distribution of the different age groups. The majority of the participants had some form of primary school education (Grade 1 – Grade 7) (45%), while 40% had a secondary level education and 15% had no formal education (schooling).



**Figure 5.10. Frequency distribution of the different education groups**

Kruskal Wallis results and PD of the three different education groups showed no significant differences between the groups when comparing test and reference weights ( $p > 0.05$ ) between the education groups (Table 5.15).

For those with “no schooling” it was found that the soup, stiff *pap* and *imifino*, and stiff *pap* and whole kernels dishes showed good PDs when comparing the test and reference weights ( $< 10\%$ ) while the PDs for stiff *pap* and pumpkin, porridge and *umphokoqo* were poor ( $> 20\%$ ). For those with a primary school education only stiff *pap* and porridge showed good ( $< 10\%$ ) and acceptable ( $< 20\%$ ) PDs respectively. For those with a high school education *umngqusho*, soup, stiff *pap* and pumpkin, porridge and stiff *pap*, showed acceptable PDs ( $< 20\%$ ). No clear trends regarding differences in agreement between the test and reference methods between education groups are thus evident (Table 5.15).

For participants with no schooling, Spearman correlation coefficients between the reference and test weights ranged between  $-0.17$  for stiff *pap* and *imifino* and  $0.68$  for whole kernels (Table 5.15). Six dishes showed poor correlations ( $r < 0.20$ ) while one dish (soup) had an acceptable correlation ( $0.21 - 0.50$ ) and one dish (kernels) had a good correlation ( $> 0.51$ ). Correlation coefficients for those in the primary education group ranged between  $-0.56$  (*umngqusho*) and  $0.63$  (soup). Once again, six dishes showed poor correlations ( $r < 0.20$ ), one dish (porridge) acceptable ( $0.21 - 0.50$ ) and one dish (soup) good ( $> 0.51$ ) correlations. For the high school education group correlations ranged between  $-0.33$  (soup) and  $0.36$  (porridge). Five dishes (*umngqusho*, soup, stiff

*pap* and pumpkin, stiff *pap* and *imifino* and *umphokoqo*) had correlations classified as poor ( $< 0.20$ ), and the remaining three dishes (porridge, stiff *pap* and kernels) had correlations classified as acceptable ( $0.21 - 0.50$ ). Thus, the results are indicative of a mostly poor association between the test weight and the reference weight, regardless of level of education.

Bland-Altman data showed poor agreement ( $< 95\%$ ) in terms of lying in the LOA in the group with no schooling for *umngqusho* (somp and beans), soup, stiff *pap* and pumpkin, and stiff *pap* and *imifino* (Table 5.16). For the primary education group poor agreement ( $< 95\%$ ) was only found for porridge and for the secondary education group poor agreement ( $< 95\%$ ) was showed for soup and stiff *pap* and pumpkin. The width of the LOA was generally regarded as being acceptable ( $< 1$  small portion) for those with no schooling with the exception of soup and kernels. For those with a primary schooling the width of the LOA was acceptable for all dishes except for stiff *pap* and kernels, while the width of the LOA for those with a secondary schooling was acceptable ( $< 1$  small portion size) for all dishes except for kernels.

Spearman correlation coefficients illustrate the absence of reporting bias for all dishes (with the exception of soup) for those in the “no schooling” group, while only stiff *pap* showed reporting bias for those in primary education group. None of the dishes for the participants in the secondary schooling group had significant Spearman correlations, indicating the absence of reporting bias for all these dishes.

Based on results overall, it can be concluded that the group with primary education had the best agreement; while the group with high school education had the narrowest LOA, also the least reporting bias.

Five dishes in the group with no schooling showed poor classification ( $< 50\%$ ) in the same tertiles (Table 5.17), including *umngqusho*, stiff *pap* and *imifino*, stiff *pap* and pumpkin, porridge, and stiff *pap*. In the group with primary education, seven dishes showed poor classification in the same tertile ( $< 50\%$ ). The only dish with acceptable classification in the same tertile was stiff *pap* and pumpkin. The high school education group showed the best results. For this group only three dishes, (soup, stiff *pap*, and *umphokoqo*) showed poor classification in the same tertile. Results overall therefore illustrate poor agreement between the test and reference weight for the group with no education and for those with a primary education thus improved estimation with higher education.

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Kappa statistics for those with no schooling showed poor agreement ( $< 0.20$ ) for *umngqusho* (*samp and beans*), stiff *pap* and pumpkin, stiff *pap* and *imifino*, porridge, stiff *pap* and *umphokoqo* (seven dishes) (Table 5.17). The same is true for the primary education and high school education groups with the value for only soup showing acceptable agreement ( $p < 0.05$ ) for the primary school group and stiff *pap* and *imifino* showing acceptable agreement for the high school group. Results therefore show poor agreement between the test and reference weight irrespective of level of education confirming the tertile classification results.

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**Table 5.15. Mean, differences, Kruskal Wallis results, percentage differences and Spearman correlation coefficients for test and reference weights, for each education level**

|  | Mean test weight (g) |                  |                     | Difference (g)<br>(Test – reference weight) |                  |                     | Kruskal<br>Wallis | Percentage difference (%)<br>[(Test – reference weight) /<br>reference weight * 100] |                |                     | Spearman correlation |        |           |
|--|----------------------|------------------|---------------------|---|------------------|---------------------|-------------------|--|----------------|---------------------|----------------------|--------|-----------|
|  | Education level      | None<br>n = 7    | Gr1 - Gr7<br>n = 21 | Gr8 - Gr12<br>n = 19                        | None<br>n = 7    | Gr1 - Gr7<br>n = 21 |                   | Gr8 - Gr12<br>n = 19   | None<br>n = 7  | Gr1 - Gr7<br>n = 21 | Gr8 - Gr12<br>n = 19 | None   | Gr1 - Gr7 |
| Dish                                       | Mean<br>(SD)         | Mean<br>(SD)     | Mean<br>(SD)        | Mean<br>(SD)                                | Mean<br>(SD)     | Mean<br>(SD)        | P value           | Mean<br>(SD)   | Mean<br>(SD)   | Mean<br>(SD)        | r                    | r      | r         |
| <i>Umngqusho</i> (g)                       | 703.5<br>(118.7)     | 650.6<br>(60.6)  | 583.7<br>(94.2)     | 176.0<br>(240.1)                            | 205.8<br>(229.2) | 222.9<br>(208.5)    | 0.80              | 19.9<br>(29.9)   | 23.6<br>(27.3) | 15.4<br>(75.9)      | -0.06                | -0.56* | -0.14     |
| Soup (g)                                   | 625.2<br>(175.5)     | 557.9<br>(125.4) | 507.9<br>(80.3)     | 25.9<br>(246.9)                             | 175.4<br>(125.9) | 130.3<br>(225.0)    | 0.13              | 3.0<br>(43.1)  | 26.3<br>(16.3) | 18.3<br>(32.2)      | 0.33                 | 0.63** | -0.33     |
| Stiff <i>pap</i> and<br>pumpkin (g)        | 496.6<br>(116.0)     | 486.0<br>(95.8)  | 519.7<br>(105.2)    | 203.0<br>(202.9)                            | 182.9<br>(195.9) | 117.9<br>(197.9)    | 0.37              | 35.0<br>(31.9)   | 28.4<br>(26.4) | 17.1<br>(32.2)      | 0.16                 | 0.01   | 0.13      |
| Stiff <i>pap</i> and<br><i>imifino</i> (g) | 515.2<br>(93.2)      | 456.0<br>(76.3)  | 449.6<br>(81.6)     | 68.1<br>(214.5)                             | 135.5<br>(195.6) | 193.1<br>(192.2)    | 0.16              | 7.8<br>(40.9)  | 20.9<br>(32.9) | 32.2<br>(30.1)      | -0.17                | -0.26  | -0.04     |
| Porridge (g)                               | 449.0<br>(74.7)      | 438.9<br>(87.3)  | 403.9<br>(80.0)     | 168.4<br>(153.5)                            | 68.5<br>(117.7)  | 84.3<br>(113.8)     | 0.12              | 26.6<br>(28.8)   | 11.4<br>(26.5) | 15.8<br>(22.9)      | 0.01                 | 0.45   | 0.36      |
| Stiff <i>pap</i> (g)                       | 404.4<br>(84.8)      | 418.6<br>(91.4)  | 386.2<br>(103.7)    | -3.6<br>(148.3)                             | 70.4<br>(180.8)  | 70.9<br>(157.4)     | 0.31              | -7.7<br>(37.9)   | 5.5<br>(39.8)  | 10.9<br>(38.3)      | 0.14                 | 0.02   | 0.31      |
| <i>Umphokoqo</i> (g)                       | 343.8<br>(67.2)      | 346.7<br>(54.5)  | 322.7<br>(62.3)     | 164.0<br>(142.0)                            | 222.5<br>(117.4) | 230.7<br>(143.8)    | 0.29              | 34.7<br>(25.5)   | 45.7<br>(18.7) | 49.5<br>(28.0)      | -0.03                | -0.17  | -0.13     |
| Kernels (g)                                | 367.9<br>(140.8)     | 289.5<br>(90.1)  | 226.6<br>(83.3)     | 55.1<br>(116.8)                             | 139.0<br>(192.3) | 73.9<br>(133.8)     | 0.36              | 7.9<br>(41.4)  | 27.4<br>(42.6) | 20.5<br>(34.6)      | 0.68*                | -0.10  | 0.35      |

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Difference = test weight – reference weight

\* Spearman rank correlation coefficients significant at  $p < 0.05$

\*\* Spearman rank correlation coefficients significant at  $p < 0.01$

\*\*\* Spearman rank correlation coefficients significant at  $p < 0.001$

SD = standard deviation

Kruskal Wallis:  $p < 0.05$  = significant difference between two or more groups,  $p > 0.05$  = no significant difference between any groups

Kruskal Wallis: 1 - 3 = significant difference between education group 1&3, 1 - 2 = significant difference between education group 1 & 2, 2 - 3 = significant difference between education group 2 & 3

*Umngqusho* = Samp and beans (Dried kernels and sugar beans)

Soup = Whole kernels and sugar beans

Stiff *pap* and pumpkin = Stiff maize meal porridge and pumpkin

Stiff *pap* and *imifino* = Stiff maize meal porridge and spinach

Porridge = Thin maize meal porridge

Stiff *pap* = Stiff maize meal porridge

*Umphokoqo* = Crumbly *pap* (Dry maize meal porridge)

Kernels = Boiled whole maize kernels

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Table 5.16. Bland-Altman data for test and reference weights for each education group

| Education level                         | Agreement <sup>+</sup> |                        |                         | LOA            |                     |                      | Spearman correlation |                     |                      | Significance of LOA |                     |                      |
|---|------------------------|------------------------|-------------------------|----------------|---------------------|----------------------|----------------------|---------------------|----------------------|---------------------|---------------------|----------------------|
|   | None<br>n = 7          | Gr1 -<br>Gr7<br>n = 21 | Gr8 -<br>Gr12<br>n = 19 | None<br>n = 7  | Gr1 - Gr7<br>n = 21 | Gr8 - Gr12<br>n = 19 | None<br>n = 7        | Gr1 - Gr7<br>n = 21 | Gr8 - Gr12<br>n = 19 | None<br>n = 7       | Gr1 - Gr7<br>n = 21 | Gr8 - Gr12<br>n = 19 |
| Dish                                    | Mean                   | Mean                   | Mean                    | Lower - Upper  | Lower -<br>Upper    | Lower -<br>Upper     | r <sub>BA</sub>      | r <sub>BA</sub>     | r <sub>BA</sub>      |                     |                     |                      |
| <i>Umngqusho</i> (g)                    | 86.7                   | 100.0                  | 100.0                   | -304.2 – 656.2 | -252.6 – 664.2      | -194.1 – 639.9       | -0.38                | -0.04               | -0.24                | < 1 x Small portion | < 1 x Small portion | < 1 x Small portion  |
| Soup (g)                                | 85.7                   | 100.0                  | 88.5                    | -467.9 – 519.7 | -76.4 – 427.2       | -319.7 – 580.3       | -0.67**              | 0.19                | -0.30                | > 1 x Small portion | < 1 x Small portion | < 1 x Small portion  |
| Stiff <i>pap</i> and pumpkin (g)        | 92.3                   | 100.0                  | 92.3                    | -202.8 – 608.8 | -208.9 – 574.7      | -277.9 – 513.7       | -0.48                | 0.03                | -0.20                | < 1 x Small portion | < 1 x Small portion | < 1 x Small portion  |
| Stiff <i>pap</i> and <i>imifino</i> (g) | 93.3                   | 100.0                  | 95.8                    | -360.9 – 497.1 | -255.7 – 526.7      | -191.3 – 577.5       | -0.33                | 0.02                | -0.32                | ≈ 1 x Small portion | < 1 x Small portion | < 1 x Small portion  |
| Porridge (g)                            | 100.0                  | 90.9                   | 95.5                    | -138.6 – 475.4 | -166.9 – 303.9      | -143.3 – 311.9       | -0.33                | 0.25                | 0.17                 | < 1 x Small portion | < 1 x Small portion | < 1 x Small portion  |
| Stiff <i>pap</i> (g)                    | 100.0                  | 100.0                  | 100.0                   | -300.2 – 293.0 | -291.2 – 432.0      | -243.9 – 385.7       | 0.24                 | 0.57*               | 0.24                 | < 1 x Small portion | > 1 x Small portion | ≈ 1 x small portion  |
| <i>Umphokoqo</i> (g)                    | 100.0                  | 100.0                  | 100.0                   | -120.0 – 448.0 | -12.3 – 457.3       | -56.9 – 518.3        | 0.35                 | 0.49                | 0.24                 | < 1 x Small portion | < 1 x Small portion | < 1 x Small portion  |
| Kernels (g)                             | 100.0                  |                        |                         | -178.5 – 288.7 | -245.6 – 523.6      | -193.7 – 341.5       | 0.45                 | 0.50                | -0.09                | > 1 x Small portion | > 1 x Small portion | > 1 x Small portion  |

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‡ Percentage between the limits of agreement

g = gram

SD = standard deviation

LOA = Limits of agreement

Lower limit = mean difference between photograph weight and dished up weight – 2 standard deviations of the mean difference between the photograph weight and dished-up weight

Upper limit = mean difference between photograph weight and dished up weight + 2 standard deviations of the mean difference between the photograph weight and dished-up weight

$r_{BA}$  = Spearman correlation between the mean of photographic weight plus dished-up weight and the mean difference between the photograph weight and dished-up weight

\* Spearman rank correlation coefficients significant at  $p < 0.05$  level,

\*\* Spearman rank correlation coefficients significant at  $p < 0.01$  level,

\*\*\* Spearman rank correlation coefficients significant at  $p < 0.001$  level

Significance of LOA = mean difference between the test weight and reference weight  $\pm 1$  X small portion size

*Umngqusho* = Samp and beans (Dried kernels and sugar beans)

Soup = Whole kernels and sugar beans

Stiff *pap* and pumpkin = Stiff maize meal porridge and pumpkin

Stiff *pap* and *imifino* = Stiff maize meal porridge and spinach

Porridge = Thin maize meal porridge

Stiff *pap* = Stiff maize meal porridge

*Umphokoqo* = Crumbly *pap* (Dry maize meal porridge)

Kernels = Boiled whole maize kernels

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**Table 5.17. Tertile distribution and weighted Kappa statistics for the test and reference weights, for each education level**

| Education level                     | Tertiles      |                  |                     |                  |                      |                  | Kappa          |                |                |
|-------------------------------------|---------------|------------------|---------------------|------------------|----------------------|------------------|----------------|----------------|----------------|
|                                     | None<br>n = 7 |                  | Gr1 - Gr7<br>n = 21 |                  | Gr8 - Gr12<br>n = 19 |                  | None           | Gr1 - Gr7      | Gr8 - Gr12     |
| Dish                                | Same tertile  | Opposite tertile | Same tertile        | Opposite tertile | Same tertile         | Opposite tertile | K <sub>w</sub> | K <sub>w</sub> | K <sub>w</sub> |
| <i>Umngqusho</i>                    | 31.3          | 43.8             | 31.3                | 37.5             | 53.6                 | 17.9             | -0.02          | 0.00           | 0.06           |
| Soup                                | 53.3          | 20.0             | 43.8                | 25.0             | 34.6                 | 19.2             | 0.23           | 0.22*          | -0.08          |
| Stiff <i>pap</i> and pumpkin        | 35.7          | 14.3             | 56.3                | 25.0             | 50.0                 | 11.5             | 0.04           | 0.18           | 0.16           |
| Stiff <i>pap</i> and <i>imifino</i> | 12.5          | 25.0             | 37.5                | 25.0             | 50.0                 | 29.2             | -0.3           | -0.11          | 0.26*          |
| Porridge                            | 25.0          | 58.3             | 36.4                | 0.0              | 63.6                 | 9.1              | -0.09          | -0.17          | 0.19           |
| Stiff <i>pap</i>                    | 43.8          | 6.3              | 37.5                | 18.8             | 48.0                 | 12.0             | 0.09           | 0.12           | 0.09           |
| <i>Umphokoqo</i>                    | 56.3          | 18.8             | 33.3                | 26.7             | 40.7                 | 22.2             | 0.05           | 0.00           | -0.06          |
| Kernels                             | 86.7          | 90.9             | 46.7                | 13.3             | 81.8                 | 9.1              | 0.52**         | 0.05           | 0.00           |

K<sub>w</sub> weighted Kappa (Mason *et al.*, 2003:315)

Gr = grade

*Umngqusho* = Samp and beans (Dried kernels and sugar beans)

Soup = Whole kernels and sugar beans

Stiff *pap* and pumpkin = Stiff maize meal porridge and pumpkin

Stiff *pap* and *imifino* = Stiff maize meal porridge and spinach

Porridge = Thin maize meal porridge

Stiff *pap* = Stiff maize meal porridge

*Umphokoqo* = Crumbly *pap* (Dry maize meal porridge)

Kernels = Boiled whole maize kernels

Addendum 24 provides detailed portion size estimation validation results for each participant characteristic investigated, while Table 5.18 presents a summary of the final portion size validation results for each characteristic investigated. Validity of portion size photographs for *umngqusho* can generally be considered as being poor for all participants except males and those with education levels between Grade 8 and Grade 12. Soup portion size photographs on the other hand were considered to be valid amongst all participants except males, females and those with a BMI < 25, 26 - 30 kg/m<sup>2</sup> and those with a secondary education (Gr 8 – 12). Results of the portion size photographs of stiff *pap* and pumpkin indicated to poor validity in females, those between 45 and 65 years of age, those older than 65 years, with a BMI of < 25 and 26-30 kg/m<sup>2</sup> and those with no education and a primary education (Gr 1 – 7). Stiff *pap* and *imifino* were considered to have poor validity in all participants except those between the ages of 45 - 65 years, a BMI > 30 kg/m<sup>2</sup>, and those with a secondary education (Gr 8 – 12). Porridge photographs were considered to have poor validity in males, those between 45 and 65 years of age, those older than 65 years and those with no education, while stiff *pap* showed poor validity in males, participants between 18 – 44 years of age, those between 45 and 65 years, those with a BMI < 25, those with no education, an education levels between Gr 1 – 7. Generally *umphokoqo* photographs had poor validity, with only an acceptable validity for males. Kernel photographs were generally poor for obese participants (> 30 kg/m<sup>2</sup>), those with an education level between Gr 1 – 7 and those with a secondary education (Gr 8 – 12).

**Table 5.18. Summary of portion size validation results for each participant characteristic investigated**

| Dish                                | Gender     |            | Age (years) |            |            | Weight (kg/m <sup>2</sup> ) |            |            | Education  |            |            |
|-------------------------------------|------------|------------|-------------|------------|------------|-----------------------------|------------|------------|------------|------------|------------|
|                                     | Male       | Female     | 18 - 44     | 45 – 65    | > 65       | < 25                        | 25 – 29.9  | ≥ 30       | None       | Gr1 - Gr7  | Gr8 - Gr12 |
| <i>Umngqusho</i>                    | Acceptable | Poor       | Poor        | Poor       | Poor       | Poor                        | Poor       | Poor       | Poor       | Poor       | Acceptable |
| Soup                                | Poor       | Poor       | Acceptable  | Acceptable | Acceptable | Poor                        | Poor       | Acceptable | Acceptable | Acceptable | Poor       |
| Stiff <i>pap</i> and pumpkin        | Acceptable | Poor       | Acceptable  | Poor       | Poor       | Poor                        | Poor       | Acceptable | Poor       | Poor       | Acceptable |
| Stiff <i>pap</i> and <i>imifino</i> | Poor       | Poor       | Poor        | Acceptable | Poor       | Poor                        | Poor       | Acceptable | Poor       | Poor       | Acceptable |
| Porridge                            | Poor       | Acceptable | Acceptable  | Poor       | Poor       | Acceptable                  | Acceptable | Acceptable | Poor       | Acceptable | Acceptable |
| Stiff <i>pap</i>                    | Poor       | Acceptable | Poor        | Poor       | Acceptable | Poor                        | Acceptable | Acceptable | Poor       | Poor       | Acceptable |
| <i>Umphokoqo</i>                    | Acceptable | Poor       | Poor        | Poor       | Poor       | Poor                        | Poor       | Poor       | Poor       | Poor       | Poor       |
| Kernels                             | Acceptable | Acceptable | Acceptable  | Acceptable | Acceptable | Acceptable                  | Acceptable | Poor       | Acceptable | Poor       | Poor       |

Wilcoxon signed rank test agreement at group level  $p < 0.05$  = Good,  $p > 0.05$  Poor

Percentage difference:  $< 10\%$  = Good, 11 - 20% = Acceptable,  $> 20\%$  = Poor

Strength of association and correlation = Results from correlation coefficient (individual level)

*Umngqusho* = Samp and beans (Dried kernels and sugar beans)

Soup = Whole kernels and sugar beans

Stiff *pap* and pumpkin = Stiff maize meal porridge and pumpkin

Stiff *pap* and *imifino* = Stiff maize meal porridge and spinach

Porridge = Thin maize meal porridge

Stiff *pap* = Stiff maize meal porridge

*Umphokoqo* = Crumbly *pap* (Dry maize meal porridge)

Kernels = Boiled whole maize kernels

Good =  $> 0.50$ , Acceptable 0.21 – 0.50 and Poor  $< 0.20$

Agreement = Results from tertile classification (include chance) and Kappa statistics (exclude chance)

Tertile classification:  $> 50\%$  is same group = Good

Kappa statistics:  $< 0.20$  = Poor, 0.21 – 0.60 = Acceptable, 0.61 – 0.80 = Good

Bias = Bland-Altman data indication of bias on individual level (if  $r_{BA}$  is significant),

$r_{BA}$  = Spearman correlation coefficient for Bland-Altman data (correlation between mean and mean difference of intake)

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Agreement Bland-Altman:  $< 1 \times \text{RDA} = \text{Narrow LOA}$ ,  $\approx 1 \times \text{RDA} = \text{Acceptable}$ ,  $> 1 \times \text{RDA} = \text{Wide LOA}$   
Final validity\* = Four or more of statistical methods indicate agreement

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## 4. DISCUSSION

Visual aids are frequently used to assist in food portion size estimation (Lee & Nieman, 2007:104). While it is known that portion size photographs increase accuracy (Nelson *et al.*, 1994:649), little may be known about the size or direction of the errors involved. It is however expected that some error will always be present during portion size estimation, and this will influence the accuracy of classifying participants according to intake groups (Nelson *et al.*, 1994:650).

Results generally showed poor validity of the portion size photographs for all dishes (*umngqusho*, soup, stiff *pap* and pumpkin, stiff *pap* and *imifino*, porridge, stiff *pap* and *umphokoqo*) and valid portion size photographs for only one dish (kernels).

Agreement was poor at both individual (except kernels) and group level (especially for stiff *pap* and pumpkin and stiff *pap* and *imifino*), with poor associations found between the two weights (for all dishes except porridge and kernels). Bias was present for all dishes except for whole kernels. However, the small sample size for kernels influenced the results and a larger sample size would have given a more representative result.

The poor validity outcomes are very concerning. One possible explanation for this may be that the photo series that only includes three portion sizes (S, M, & L), do not necessarily reflect the portion usually consumed which may result in estimation errors. More portion size options (especially the inclusion of another portion between the medium and large size portions) may need to be considered to improve the validity of the tool in portion size estimation.

The fact that the food dishes tested in the validation study were all amorphous could have contributed to the poor portion size estimation using the photographs. Previous studies found that it is easier to estimate portion sizes of solid compared to amorphous foods. Since amorphous foods tend to take on the shape of the container it becomes more difficult to use visual cues (i.e., size and number of different pieces) to determine the portion size thereof (Venter *et al.*, 2000:213). The best estimates of PD in the present study were for stiff *pap* (4.2%), which is more solid and less amorphous compared with other dishes, such as porridge (17.6%), soup (16.5%), samp & beans (18.8%), kernels (19.6%) and *umphokoqo* (44.4%). Although the error in estimation for *umphokoqo* may seem to have serious implications, this dish is the least consumed maize dish based on information gathered from focus groups. A study conducted among Tswanas in the North

West Province found similar results for *umngqusho* with PDs ranging from -15.6% to 42%, while the difference for porridge ranged between 14.2% and 23% and that of stiff *pap* between -11.4 and 0% (Venter *et al.*, 2000:210).

Another factor that could have had an influence on portion size estimation was the fact that participants usually ate dishes from plates and bowls (at their own homes) that were not identical to the ones shown in the photographs; even though the researcher attempted to match eating utensils and crockery used in the photographs with that used in participants' homes as far as possible. However, for practical reasons only one type of container or plate could be used in the photographs. If containers other than these were used in participants' houses, they would most probably have had more difficulty in determining depth, which may have increased validity even more.

Some studies indicated that females reported more accurately than males, while other studies indicated no differences between the two genders (Yuhas *et al.*, 1980:1475, Robinson *et al.*, 1997:122, Venter *et al.*, 2000:214). Results from the present study indicated that the strength of association for the different dishes and agreement (with and without chance) was stronger in the male group than in the female group. Also, in the present study females tended to overestimate portion sizes more than males, although these differences were not significant. The latter could have been due to the small sample size of males. Similar results were found in black adults in the Northern Province (Venter *et al.*, 2000:214). An earlier study by Nelson *et al.*, (1994:661) showed that males underestimated portion size more than females.

In the current study no significant differences were found between the age groups, although participants older than 65 years tended to overestimate more than the other age groups. The difference between the age groups reached borderline significance, especially between the oldest and the youngest group. This corresponds with results found in an earlier study by Nelson *et al.*, (1996:36) who reported that persons over 65 years overestimated portions more frequently. However, results also indicated that the strength of association at individual level were stronger for the older group. Also, the individual agreement were better for the older group when chance agreement were included and excluded. The first two groups were distributed to provide near equal age ranges with near sample sizes per group, while the last age group was included because previous studies indicated perception differences between those younger than 65 years and those older than 65 years (Nelson *et al.*, 1994:661, Robinson *et al.*, 1994:122, Young & Nestle, 1995:154, Nelson *et al.*, 1998:22). However, in the current study the sample

size for those older than 65 years was much smaller when compared to the other groups and no real conclusions could be made regarding this. In the North West study (Venter *et al.*, 2000:214) no significant differences were found among the different age groups. However, the present study also indicated that the strength of association at individual level appeared to be stronger for the older group than for the other age groups.

According to the literature participants with a BMI  $\geq 30$  kg/m<sup>2</sup> under-estimate portion sizes (Nelson *et al.*, 1994:661, Nelson & Haraldsdóttir, 1998a:219). Although PDs increased as BMI of participants increased, differences between the weight groups were only significant for three dishes (*umngqusho*, stiff *pap*, and *imifino* and kernels). This is in accordance with a study by Nelson *et al.*, (1994:661) who indicated that participants with a BMI greater than 30 kg/m<sup>2</sup> more frequently underestimate portion size than those with a BMI less than 30 kg/m<sup>2</sup>. Also, results from the current study showed that agreement between the test and reference weights were poorer as BMI increased, that strength of association were poor for all weight groups. However, the results indicated that the overweight and obese group had less or no reporting bias and narrower LOA compared to the normal weight group and that the agreement (with or without chance) is better for the obese group. Once again, the small sample size of these groups made it difficult to make conclusions regarding the association between BMI and accuracy of the portion size estimation using the photographs.

Participants were subdivided according to their level of education to determine the differences between those with no schooling, those with primary schooling and those with secondary schooling. A similar study conducted in South Africa indicated no differences between the level of schooling and portion size estimation (Venter *et al.*, 2000:215). Results from the present study showed no significant differences between different education groups similar to that of the North West Province participants (Venter *et al.*, 2000:215). However, because of the small sample size of those with no education (n = 7) no formal conclusions could be drawn.

Finally, it needs to be mentioned that the sample size of the current photograph validation study was small, and a larger sample size may have resulted in smaller PDs, less perception bias, and increased agreement. This was also noted in the study undertaken by Nelson *et al.*, (1996:45).

## **5. CONCLUSION**

Although all efforts were made to decrease error in portion size estimation, it will always be present to some degree. Knowing the direction and the extent of errors provides an opportunity to interpret results with better understanding. Portion size photographs of the newly developed assessment method generally showed poor validation results, poor association between the test and reference weight and poor agreement at both individual and group level. Results indicated that error was mostly present when using the large portion sizes of dishes and not necessarily because of any specific characteristics of the participants. It must however be kept in mind that these validation results are only applicable to those living in Centane and not those in Bizana or any other areas.

University of Cape Town

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## Chapter 6

### RELIABILITY OF THE RAPP METHOD

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A village in Centane, a rural area in the Eastern Cape

## 1. INTRODUCTION

The RAPP method was developed firstly to assess the maize and thus fumonisin exposure and secondly, to assess the adequacy of nutrient intake of people living in a rural area in the Eastern Cape (EC). The method comprises a quantitative food frequency questionnaire (QFFQ) and food photograph series (FPS). These photographs were tested for validity to determine the size of perception error (Chapter 4).

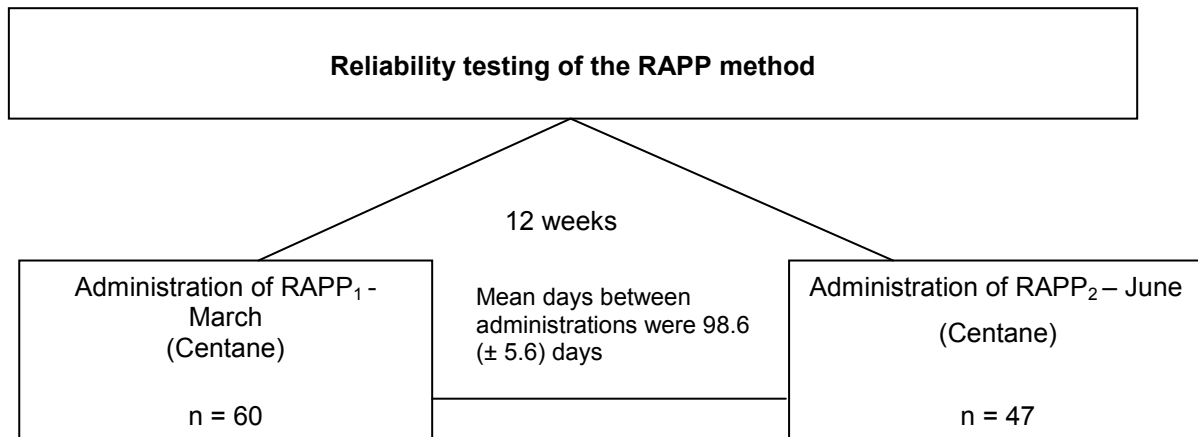
Before applying any newly developed dietary assessment method in a particular study, it is essential to determine the reliability of the method. Reliability refers to the amount of variation obtained in data when a dietary assessment method is repeated (Bates *et al.*, 1997:172). Generally the reliability of a dietary assessment method is determined using a test-retest design (Gibson, 2005:129). Most reproducibility studies repeated their measurements twice (Willett *et al.*, 1985:53; Rimm *et al.*, 1992b:1115, Boucher *et al.*, 2006:85, Shu *et al.*, 2004:20), although Pietinen *et al.*, (1988a:666) repeated their measurements three or more times.

## 2. MATERIALS AND METHODS

### 2.1. STUDY DESIGN AND POPULATION USED FOR RELIABILITY TESTING

The reliability study was done by means of a test-retest design (Gibson, 2005:129). The newly developed RAPP method was administered twice among the same participants twelve weeks apart. To reduce participant burden, these participants were from the same villages as those used in the photograph validation study (Chapter 5), but were new recruits. Sixty volunteers (males and females) between the ages 18 and 65 years were recruited on a door to door basis. Only one person per household was recruited. People were excluded if they had problems with their eyesight, or were younger than 18 years.

Results were compared to determine the extent of variation between the two measures. Figure 6.1 depicts the study design.



**Figure 6.1. Study design for the reliability testing of the newly developed RAPP method in a sample of sixty participants in rural areas of the Eastern Cape**

## 2.2. PROCEDURES FOLLOWED

Participants were interviewed twice at their homes, 12 weeks apart in an attempt to avoid learning bias and because of logistics (March and June 2004). To avoid inter-interviewer bias, the same interviewer interviewed the same participant each time. The full RAPP method was administered during both visits. Participants completed the RAPP method and other questionnaires (in March and June) with the assistance of a trained interviewer. Various other questionnaires (24-hour recalls, physical activity questionnaires, etc.) were administered as part of the validity testing (Chapter 7 & 8), however, the RAPP method was always administered first as this was the test method. Interviews were conducted in the participants' first language (isiXhosa) by trained fieldworkers (see Chapter 3, Section 4).

## 2.3. STATISTICAL ANALYSES USED FOR RELIABILITY TESTING

Data was first analysed in terms of food group intake. Food group classification was determined during the development of the questionnaire and was based on food groups classified by a previously developed urban questionnaire (Chapter 4). Food items and dishes were grouped according to the following groups: bread; cereals (maize dishes); combined dishes (combined maize and vegetable dishes); meat; condiments and beverages.

Average daily nutrient intakes were calculated with Foodfinder 3 (Grant *et al.*, 1992, FoodFinder 3, 2010) based on the South African Food Composition Tables. Nutrient analyses were done with FoodFinder 3 (FoodFinder dietary Software, 1992, FoodFinder 3, 2010). The nutrient contribution of each food item included in the QFFQ was calculated as the product frequency of intake indicated on the QFFQ and the portion size of the food item. The total monthly intake was calculated from the frequencies on the QFFQ and then divided

by 28 to provide the total intake per nutrient per day. The following nutrients were analysed: total energy, protein, fat, carbohydrates (CHO), folate, niacin, riboflavin, thiamine, vitamin A, vitamin B<sub>12</sub>, vitamin C, vitamin D, vitamin E, potassium (K), magnesium (Mg), sodium (Na), selenium (Se) and zinc (Zn).

Statistical analyses were conducted with Stata 6. Means, standard deviations (SD), medians and inter-quartile ranges (IQs) were calculated for descriptive reasons. The statistical tests conducted to assess reliability of the RAPP method (QFFQ + FPS) include Shapiro Wilk test (for normality), Wilcoxon signed rank test, mean percentage difference between the two measures, Spearman correlation coefficients, quantile classification, weighted Kappa statistics and Bland Altman plots. See further detail for interpretation of each individual test and final interpretation of all tests in Chapter 3, Section 5.

### **3. RESULTS**

#### **3.1. PARTICIPANTS**

Sixty participants initially agreed to participate in the study, with 13 dropping out for various reasons, including migration, participant burden and peer pressure. Eleven males and 36 females participated in the study. The average age (years) for both males and females were 43.8, the average height was 160 m, weight was 69.9 kg and the BMI was 26.8 kg/m<sup>2</sup>. Forty five percent of the participants had secondary schooling, 38% had a primary education, and 17% had no formal schooling. Forty seven percent were unemployed and 14% were mine workers. The rest had odd jobs. Most participants lived in mud houses (88.5%) and 11.5% in brick houses. Of the participants, 55.8% obtained their water from a river in the area, while 32.7% have a communal tap. Only 2% had a tap in the house. Seventy-three percent of the participants did not have a toilet, and made use of the bush. The majority of the participants (79%) had one person contributing financially, 9% had two contributors and 10% had no-one making a financial contribution to the household (see Chapter 7, Section 3,2 for more detail on the demographics of this group).

Overall the group are similar to the one used in the photo validation study in terms of location, sample size, language, culture and tradition.

### 3.2. RELIABILITY OF THE REPORTED INTAKE OF FOOD GROUPS

Intake from the food groups was significantly higher ( $p < 0.05$ ) for cereals, combined dishes and condiments for RAPP<sub>1</sub> compared to RAPP<sub>2</sub> (Table 6.1), indicating poor agreement. The percentage difference (PD) between the two test measures was acceptable (11 - 20% weight difference) for bread, meat, condiments and beverages and poor ( $> 20\%$  weight difference) for the cereals and combined dishes. Agreement at group level was therefore acceptable for most food groups, but poor for those consumed most frequently (cereal and combined dishes).

The Spearman rank correlation coefficients between the two measures were good for all food groups except for condiments ( $r < 0.2$ ) (Table 6.2), indicating acceptable association between the two RAPP measurements. Only two food groups had tertile classifications that were good ( $> 50\%$  classified in the same tertile) (cereals and beverages). It can therefore be deduced that agreement at individual level was poor. Kappa test results (Table 6.3) were acceptable (0.21 – 0.60) for beverages, cereals, combined dishes and meat, and poor ( $< 0.20$ ) for condiments and bread. Results indicated acceptable to good agreement at individual level for all food groups except for condiments and bread.

**Table 6.1. Mean, standard deviation, median, inter-quartile range, difference and percentage difference for food groups obtained from RAPP<sub>1</sub> and RAPP<sub>2</sub>**

| Food group             | RAPP <sub>1</sub>  |                         | RAPP <sub>2</sub>  |                          | Difference<br>(RAPP <sub>1</sub> – RAPP <sub>2</sub> ) |                      |                         | Percentage difference (%)<br>[(RAPP <sub>1</sub> – RAPP <sub>2</sub> ) / RAPP <sub>2</sub> * 100] |                      |                           |
|------------------------|--------------------|-------------------------|--------------------|--------------------------|--|----------------------|-------------------------|---|----------------------|---------------------------|
|                        | Mean<br>(SD)       | Median<br>(IQ Range)    | Mean<br>(SD)       | Median<br>(IQ Range)     | Mean<br>(SD)   | Median<br>(IQ Range) | 95% CI of<br>difference | Mean<br>(SD)  | Median<br>(IQ Range) | 95% CI of %<br>difference |
| n = 47                 |                    |                         |                    |                          |  |                      |                         |   |                      |                           |
| Bread (g)              | 415.1<br>(407.7)   | 266.0<br>(115-561)      | 338.2<br>(265.9)   | 244.0<br>(123.0-498.0)   | 76.9<br>(462.8)  | 49.9<br>(-221-245)   | -647.0-803.0            | 13.7<br>(129.8)   | 54.9<br>(-23.9-76.6) | -120.1-89.6               |
| Cereals (g)            | 700.6<br>(433.3)   | 651.0*<br>(381-955)     | 590.0<br>(661.7)   | 417.0.0<br>(178.0-720.0) | 110.6<br>(586.5)                                       | 153.8<br>(-47-476)   | -429.0-955.0            | 28.1<br>(59.0)  | 22.9<br>(-9.1-76.1)  | -66.8-99.3                |
| Combined dishes<br>(g) | 1150.3<br>(537.1)  | 1085.0***<br>(869-1430) | 827.0<br>(447.5)   | 782.0<br>(539.0-1051.0)  | 323.3<br>(571.9)                                       | 355.2<br>(-51-793)   | -511.0-1402.0           | 44.7<br>(60.4)  | 22.7<br>(-8.6-62.3)  | -54.9-86.7                |
| Meat (g)               | 91.6<br>(82.2)     | 72.0<br>(25-128)        | 39.7<br>(48.7)     | 19.0<br>(11.0-37.0)      | 51.9<br>(106.7)  | 9.9<br>(-37-69)      | -163.0-154.0            | 12.1<br>(97.5)  | 46.8<br>(-52.9-84.6) | -197.1-100.0              |
| Condiments (g)         | 131.6<br>(117.6)   | 97.0***<br>(59-156)     | 85.9<br>(159.5)    | 33.0<br>(21.0-72.0)      | 45.7<br>(310.3)  | 15.2<br>(-15-109)    | -218-421.0              | 13.0<br>(120.2)   | 54.8<br>(-4.8-82.1)  | -164.3-93.2               |
| Beverages (ml)         | 1385.9<br>(1005.2) | 1115.0<br>(549-2006)    | 1278.3<br>(1056.9) | 971.0<br>(651.0-1488.0)  | 107.6<br>(1129.9)                                      | 118.9<br>(-320-734)  | -1410.0-2060.0          | 9.4<br>(61.4)   | 12.4<br>(-10.6-57.7) | -100.2-87.4               |

RAPP<sub>1</sub> = Reported food group intake from RAPP method conducted in March

RAPP<sub>2</sub> = Reported food group intake from RAPP method conducted in June

\*Wilcoxon signed rank test, significance at p < 0.05

\*\*Wilcoxon signed rank test, significance at p < 0.01

\*\*\* Wilcoxon signed rank test, significance at p < 0.001

SD = Standard deviation

CI = Confidence intervals

IQ range = inter-quartile range (25<sup>th</sup> percentile - 75<sup>th</sup> percentile)

**Table 6.2. Spearman correlation coefficients, tertile distribution and weighted Kappa statistics for food groups obtained from RAPP<sub>1</sub> and RAPP<sub>2</sub>**

| Food group      | Spearman correlation | Percentage classified into tertiles |                 |                 | Weighted Kappa       |
|-----------------|----------------------|-------------------------------------|-----------------|-----------------|----------------------|
|                 |                      | Same                                | Adjacent        | Opposite        |                      |
| <b>n = 47</b>   | <b>r</b>             | <b>Same</b>                         | <b>Adjacent</b> | <b>Opposite</b> | <b>K<sub>w</sub></b> |
| Bread           | 0.23                 | 36.2                                | 40.4            | 23.4            | 0.06                 |
| Cereals         | 0.56***              | 53.2                                | 38.3            | 8.5             | 0.40                 |
| Combined dishes | 0.39**               | 42.5                                | 44.7            | 12.8            | 0.21                 |
| Meat            | 0.35                 | 46.8                                | 40.4            | 12.8            | 0.28                 |
| Condiments      | 0.11                 | 38.3                                | 36.2            | 25.5            | 0.06                 |
| Beverages       | 0.59***              | 57.5                                | 34.0            | 8.5             | 0.47                 |

RAPP<sub>1</sub> = Reported food group intake of the RAPP method conducted in March

RAPP<sub>2</sub> = Reported food group intake of the RAPP method conducted in June

CI = Confidence intervals

r = Spearman correlation coefficient

\* Significance at  $p < 0.05$

\*\* Significance at  $p < 0.01$

\*\*\* Significance at  $p < 0.001$

K<sub>w</sub> = Weighted Kappa statistics

Bland-Altman data (Table 6.3) for food group intakes indicate good agreement between group mean weights for the two applications of the RAPP method (> 95%) for all groups except for combined dishes (93.6%). (The average small portion size of all the dishes in that food group was used as a guide to determine the clinical acceptance of the width of the LOA). The limits of agreement (LOA) for most food groups were larger than 1 x average small portion. Spearman correlations between the mean intake and mean difference were not significant ( $p < 0.05$ ) for any food group, illustrating an absence of reporting bias for all food groups.

Figures 6.2 and 6.3 show the Bland-Altman plots for cereals (that include the main maize dishes) and combined dishes, respectively. Both plots show wide LOA, no significant bias and good agreement. The mean difference for cereal consumption was closer to zero (153.8 g), while that of the combined dishes was greater (323.3 g).

Table 6.3. Bland-Altman calculations for food groups obtained from RAPP<sub>1</sub> and RAPP<sub>2</sub>

| Food groups         | Percentage Agreement <sup>‡</sup> | Limits of agreement |             | Spearman correlation | LOA vs portion size                  |
|---------------------|-----------------------------------|---------------------|-------------|----------------------|--------------------------------------|
|                     |                                   | Lower limit         | Upper limit |                      |                                      |
| <b>n = 47</b>       |                                   |                     |             | $r_{BA}$             | <b>(Average small portion size)</b>  |
| Bread (g)           | 95.7                              | -848.7              | 1002.5      | 0.08                 | > 1 x Average small portion (178 g)  |
| Cereals (g)         | 95.7                              | -1062.4             | 1283.6      | -0.02                | > 1 x Average small portion (224 g)  |
| Combined dishes (g) | 93.6                              | -820.5              | 1467.1      | 0.07                 | > 1 x Average small portion (415 g)  |
| Meat (g)            | 95.7                              | -161.5              | 265.3       | 0.07                 | > 1 x Average small portion (156 g)  |
| Condiments (g)      | 97.9                              | -574.9              | 666.3       | 0.10                 | > 1 x Average small portion (2 g)    |
| Beverages (mℓ)      | 95.7                              | -2152.2             | 2267.4      | 0.17                 | > 1 x Average small portion (364 mℓ) |

<sup>‡</sup> Percentage of data points between the limits of agreement

LOA = Limits of agreement

Lower limit = Mean difference of RAPP<sub>1</sub> and RAPP<sub>2</sub> – 2 standard deviations of the mean difference

Upper limit = Mean difference of RAPP<sub>1</sub> and RAPP<sub>2</sub> + 2 standard deviations of the mean difference

$r_{BA}$  = Spearman correlation between the mean of RAPP<sub>1</sub> and RAPP<sub>2</sub> and the mean difference between RAPP<sub>1</sub> and RAPP<sub>2</sub>

\* Significance at  $p < 0.05$

\*\* Significance at  $p < 0.01$

\*\*\* Significance at  $p < 0.001$

LOA vs portion size = mean difference  $\pm$  1 X average small portion

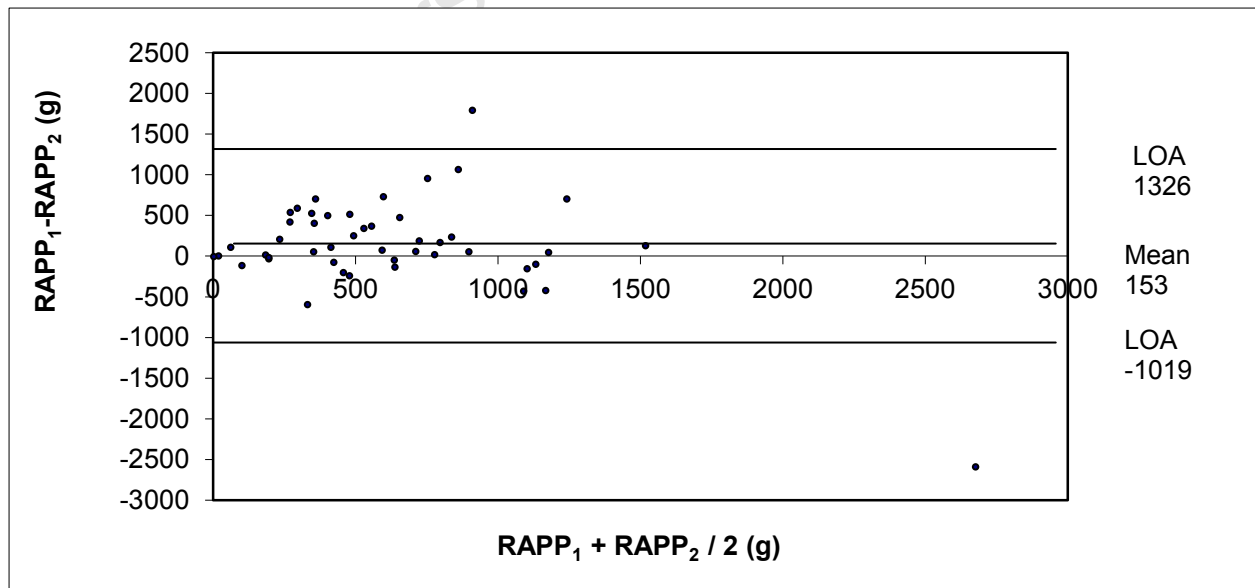
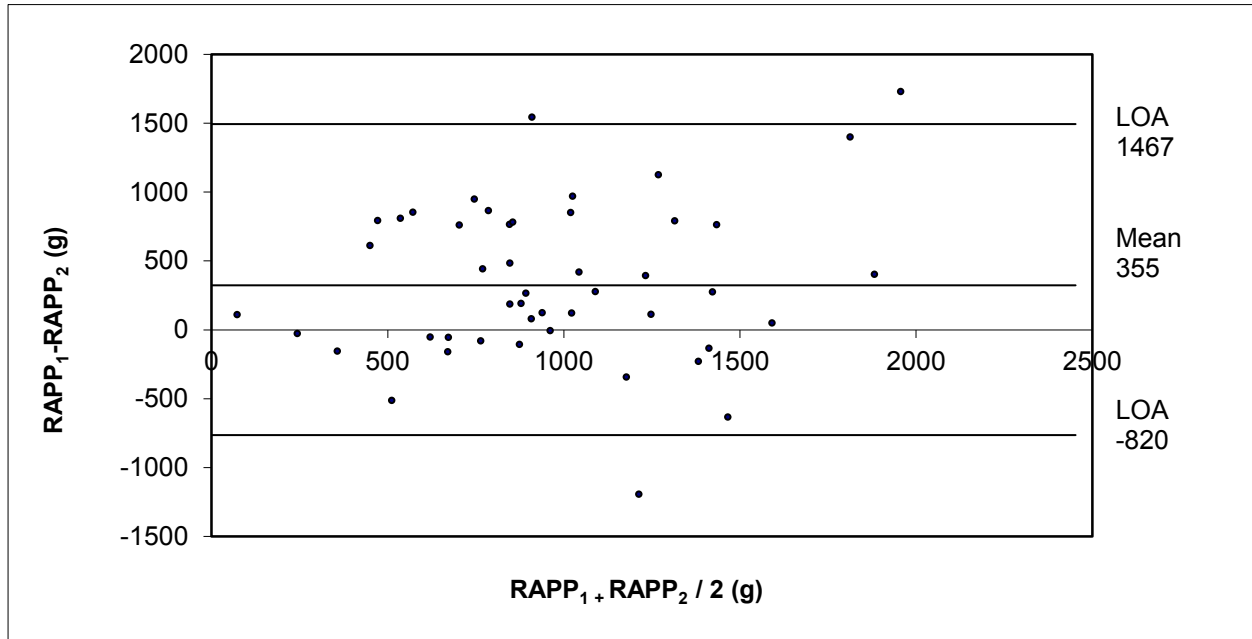


Figure 6.2. Bland-Altman plot comparing mean intake of cereal reported for RAPP<sub>1</sub> and RAPP<sub>2</sub> against the difference of the two measures (n = 47)



**Figure 6.3. Bland-Altman plot comparing mean intake of combined dishes reported for  $RAPP_1$  and  $RAPP_2$  against the difference of the two measures ( $n = 47$ )**

Table 6.4 provides a summary of the outcomes of the different statistical procedures applied to assess the reliability of data when reporting by means of food groups.

**Table 6.4. Summary of statistical results for the food group intake during reliability testing of the RAPP method**

| Food group                 | Agreement                 | Agreement             | Strength of association | Agreement (Including chance) | Agreement (Excluding chance) | Presence of bias                    | Limits of Agreement         | Final Reliability                  |
|----------------------------|---------------------------|-----------------------|-------------------------|------------------------------|------------------------------|-------------------------------------|-----------------------------|------------------------------------|
| n = 47                     | Wilcoxon Signed rank test | Percentage difference | Spearman correlations   | Tertile classification       | Kappa statistics             | Bland – Altman Spearman correlation | Bland-Altman (Width of LOA) | > 3 acceptable reliability results |
| <b>Level of validation</b> | <b>Group</b>              | <b>Group</b>          | <b>Individual</b>       | <b>Individual</b>            | <b>Individual</b>            | <b>Individual</b>                   | <b>Individual</b>           |                                    |
| Bread                      | Good                      | Acceptable            | Acceptable              | Poor                         | Poor                         | Absent                              | Wide                        | Acceptable                         |
| Cereals                    | Poor                      | Poor                  | Good                    | Acceptable                   | Acceptable                   | Absent                              | Wide                        | Acceptable                         |
| Combined dishes            | Poor                      | Poor                  | Good                    | Poor                         | Acceptable                   | Absent                              | Wide                        | Poor                               |
| Meat                       | Good                      | Acceptable            | Good                    | Poor                         | Acceptable                   | Absent                              | Wide                        | Acceptable                         |
| Condiments                 | Poor                      | Acceptable            | Poor                    | Poor                         | Poor                         | Absent                              | Wide                        | Poor                               |
| Beverages                  | Good                      | Good                  | Good                    | Acceptable                   | Acceptable                   | Absent                              | Wide                        | Acceptable                         |

Strength of association and correlation = Results from correlation coefficient (individual level)

Agreement = Results from tertile classification (include chance) and Kappa statistics (exclude chance)

Bias = Bland-Altman data indication of bias on individual level (if  $r_{BA}$  is significant),

$r_{BA}$  = Spearman correlation coefficient for Bland-Altman data (correlation between mean intake and mean difference of intake)

Wilcoxon signed rank test agreement at group level

### 3.3. RELIABILITY OF REPORTED NUTRIENT INTAKE

Table 6.5 shows the results for the Wilcoxon signed rank test for nutrients. Intakes were significantly higher ( $p < 0.05$ ) for all nutrients with RAPP<sub>1</sub> compared to RAPP<sub>2</sub>, with the exception of Na and Se.

The PD was poor for most of the nutrients when comparing RAPP<sub>1</sub> with RAPP<sub>2</sub> (> 20% weight difference) (Table 6.5). The PD was good (< 10% weight difference) only for vitamin B<sub>12</sub> and Se and acceptable (11 – 20%) for total energy, fat, niacin, vitamin E and Zn. This illustrates an overall poor agreement at group level.

Spearman rank correlation coefficients for agreement between RAPP<sub>1</sub> and RAPP<sub>2</sub> nutrients ranged between 0.02 (vitamin C) and 0.46 (energy) (Table 6.6). Correlation coefficients for vitamin B<sub>12</sub>, vitamin C, and vitamin E were poor ( $r < 0.2$ ), while the rest of the nutrients had acceptable ( $r$  0.20 - 0.50) correlation coefficients. These correlations therefore illustrated acceptable strength of association between the two measures for most of the nutrients.

Five nutrients were correctly classified (> 50%) in the same tertile (fat, thiamine, Mg, Na and Zn). More than 75% of participants fell in the same or adjacent tertiles (Table 6.6). Regardless of this, all nutrients were misclassified with more than 10% of the respondents classified in the opposite tertile.

Kappa values were mostly acceptable (0.20 – 0.60) for energy, fat, niacin, thiamine, vitamin A, iron, magnesium, sodium, selenium and zinc and poor (< 0.20) for protein, carbohydrates, folate, riboflavin, vitamin B<sub>12</sub>, vitamin C, vitamin D, vitamin E, calcium, and potassium (Table 6.6). Kappa values of 13 nutrients (energy, protein, fat, carbohydrates, folate, niacin, thiamine, vitamin A, calcium, iron, magnesium, sodium, selenium and zinc) were significant, indicating good agreement between RAPP<sub>1</sub> and RAPP<sub>2</sub> measures.

**Table 6.5. Mean, standard deviation, median, inter-quartile range, difference and percentage difference for nutrient intakes obtained from RAPP<sub>1</sub> and RAPP<sub>2</sub>**

| Nutrient             | RAPP <sub>1</sub> |                             | RAPP <sub>2</sub> |                        | Difference<br>(RAPP <sub>1</sub> – RAPP <sub>2</sub> ) |                           |                         | Percentage difference (%)<br>[(RAPP <sub>1</sub> – RAPP <sub>2</sub> ) / RAPP <sub>2</sub> * 100] |                          |                           |
|----------------------|-------------------|-----------------------------|-------------------|------------------------|--|---------------------------|-------------------------|---|--------------------------|---------------------------|
|                      | Mean<br>(SD)      | Median<br>(IQ Range)        | Mean<br>(SD)      | Median<br>(IQ Range)   | Mean<br>(SD)   | Median<br>(IQ Range)      | 95% CI of<br>difference | Mean<br>(SD)  | Median<br>(IQ Range)     | 95% CI of %<br>difference |
| Energy (kJ)          | 18033<br>(8089)   | 17338*<br>(12211 - 21910)   | 12463<br>(5854)   | 12475<br>(7686-17091)  | 5570<br>(9584)   | 2923<br>(-2824 – 8235)    | -13437 - 13293          | 20.3<br>(64.3)  | 13.8<br>(-34.3 – 50.6)   | -77.7 – 122.9             |
| Protein (g)          | 95.6<br>(36.4)    | 92.8**<br>(81.8 – 116.5)    | 67.3<br>(34.0)    | 66.0<br>(44.7-91.3)    | 28.3<br>(46.9)   | 29.6<br>(-6.1 - 44.0)     | -61.8 – 84.9            | 25.6<br>(70.1)  | 28.9<br>(-36.3 – 58.8)   | -75.3 – 154.6             |
| Fat (g)              | 97.3<br>(56.6)    | 83.8*<br>(61.7 – 131.3)     | 69.5<br>(46.9)    | 54.8<br>(38.3 – 86.5)  | 27.8<br>(106.1)  | 23.3<br>(-25.8 – 50.5)    | -84.3 – 129.6           | 13.7<br>(83.5)  | -10.1<br>(-50.0 – 68.3)  | -84.4 – 165.5             |
| Carbohydrates<br>(g) | 703.4<br>(334.5)  | 673.2*<br>(443.2 – 834.8)   | 475.7<br>(222.3)  | 475.7<br>(289.1-618.3) | 227.7<br>(401.3)                                       | 127.2<br>(-39.9 – 344.8)  | -370.4 – 770.1          | 22.3<br>(63.5)  | 11.8<br>(-24.9 – 58.3)   | -76.4 – 131.1             |
| Folate (mcg)         | 774.4<br>(427.5)  | 656.0*<br>(481.0 – 947.0)   | 558.0<br>(355.2)  | 454.0<br>(331.0-788.0) | 216.4<br>(474.0)                                       | 183.0<br>(-109.0 – 430.0) | -109 – 430              | 27.2<br>(129.6)   | 4.4<br>(-45.3 – 68.3)    | -78.6 – 160.3             |
| Niacin (mg)          | 14.0<br>(6.5)     | 13.4*<br>(9.8 – 16.4)       | 10.8<br>(6.5)     | 9.8<br>(6.6-14.2)      | 3.2<br>(8.9)   | 3.3<br>(-2.4 – 6.6)       | -11.9 – 12.8            | 12.0<br>(67.4)  | 12.7<br>(-47.1 – 51.7)   | -74.1 – 129.5             |
| Riboflavin (mg)      | 1.1<br>(0.5)      | 1.2***<br>(0.8 – 1.4)       | 0.7<br>(0.5)      | 0.6<br>(0.4-0.9)       | 0.4<br>(0.6)   | 0.4<br>(-0.04 – 0.8)      | -1.0 – 1.1              | 37.5<br>(88.9)  | 21.7<br>(-34.8 – 129.5)  | -77.3 – 180.9             |
| Thiamine (mg)        | 2.0<br>(1.0)      | 2.1**<br>(1.4 – 2.1)        | 1.4<br>(0.8)      | 1.4<br>(.9-1.7)        | 0.6<br>(1.1)   | 0.6<br>(0.02 – 1.0)       | -2.0 – 1.9              | 25.0<br>(66.1)  | 17.3<br>(-47.1 – 64.0)   | -73.9 – 124.4             |
| Vitamin A (mcg)      | 532.6<br>(519.6)  | 343.0***<br>(151.0 – 807.0) | 192.6<br>(231.1)  | 92.0<br>(51.0-242.0)   | 340.0<br>(482.0)                                       | 186.0<br>(13.0 – 563.0)   | -350.0 – 1048           | 162.5<br>(270.5)  | 110.4<br>(-68.2 – 330.0) | -96.8 – 650.0             |

| Nutrient                         | RAPP <sub>1</sub> |                             | RAPP <sub>2</sub>  |                          | Difference<br>(RAPP <sub>1</sub> – RAPP <sub>2</sub> ) |                         |                         | Percentage difference (%)<br>[(RAPP <sub>1</sub> – RAPP <sub>2</sub> ) / RAPP <sub>2</sub> * 100] |                          |                           |
|----------------------------------|-------------------|-----------------------------|--------------------|--------------------------|--|-------------------------|-------------------------|---|--------------------------|---------------------------|
|                                  | Mean<br>(SD)      | Median<br>(IQ Range)        | Mean<br>(SD)       | Median<br>(IQ Range)     | Mean<br>(SD)   | Median<br>(IQ Range)    | 95% CI of<br>difference | Mean<br>(SD)  | Median<br>(IQ Range)     | 95% CI of %<br>difference |
| <b>n = 47</b>                    |                   |                             |                    |                          |  |                         |                         |   |                          |                           |
| Vitamin B <sub>12</sub><br>(mcg) | 1.1<br>(0.9)      | 0.9**<br>(0.4 – 1.7)        | 0.5<br>(0.7)       | 0.3<br>(0.0-1.0)         | 0.6<br>(1.2)   | 0.8<br>(-0.2 – 1.3)     | -1.2 – 2.6              | 8.3<br>(208.1)  | 100.0<br>(-70.0 – 100.0) | -96.2 – 500.0             |
| Vitamin C (mg)                   | 92.6<br>(68.7)    | 81.0***<br>(43.0 – 150.0)   | 45.0<br>(40.7)     | 28.0<br>(15.0-62.0)      | 47.6<br>(80.5)   | 37.0<br>(-18.0 – 106.0) | -69.0 – 169.0           | 97.6<br>(217.8)   | 14.0<br>(-73.3 – 246.7)  | -96.6 – 536.4             |
| Vitamin D (mcg)                  | 1.9<br>(2.3)      | 1.35*<br>(0.06 – 2.8)       | 1.0<br>(2.0)       | 0.1<br>(0.0-4.8)         | 0.9<br>(3.0)   | 0.3<br>(0.0 – 2.4)      | -4.6 – 6.1              | 72.7<br>(174.1)   | 100.0<br>(-85.9 – 100.0) | -100.0 – 472.0            |
| Vitamin E (mg)                   | 48.7<br>(31.6)    | 40.6*<br>(29.2 – 65.9)      | 35.4<br>(25.5)     | 28.1<br>(17.7-47.8)      | 13.3<br>(59.0)   | 11.9<br>(-14.3 – 27.4)  | -51.6 – 63.1            | 11.1<br>(84.0)  | -9.6<br>(-51.0 – 64.7)   | -89.9 – 134.8             |
| Calcium (mg)                     | 733.1<br>(390.1)  | 768.0**<br>(409.0 – 1023.0) | 416.1<br>(275.9)   | 330.0<br>(204.0 – 650.5) | 317.0<br>(387.8)                                       | 273.0<br>(64.0 – 495.0) | -310.0 – 1046.0         | 76.2<br>(120.8)   | 34.3<br>(-30.7 – 149.1)  | -91.1 – 339.3             |
| Iron (mg)                        | 17.4<br>(8.6)     | 16.5***<br>(11.2 – 20.5)    | 12.1<br>(7.1)      | 12.3<br>(6.5-15.7)       | 5.3<br>(8.9)   | 3.5<br>(1.4 – 9.5)      | -8.6 – 22.7             | 43.8<br>(85.8)  | 22.0<br>(0.0 – 103.7)    | -77.2 – 220.0             |
| Potassium (mg)                   | 3986<br>(1788)    | 3913**<br>(2725 – 4774)     | 2701<br>(1527)     | 2503<br>(1660-3441)      | 1285<br>(1841)   | 1043.0<br>(-181 – 2749) | -2256 – 3572            | 35.6<br>(76.4)  | 21.7<br>(-20.9 – 84.9)   | -81.0 – 177.4             |
| Magnesium<br>(mg)                | 621.4<br>(315.3)  | 609.0**<br>(384.0 – 746.0)  | 447.5<br>(282.8)   | 406.0<br>(261.0-564.0)   | 173.9<br>(372.1)                                       | 135.0<br>(19.0 – 289.0) | -690.0 – 554.0          | 21.8<br>(65.8)  | 17.2<br>(-29.4 – 64.2)   | -79.4 – 128.8             |
| Sodium (mg)                      | 5883<br>(13964)   | 2922<br>(2579 – 4588)       | 4207.9<br>(4761.6) | 3018<br>(2424-4159)      | 1675.1<br>(1487.5)                                     | -151<br>(-1044 – 4792)  | -6256 – 4792            | 31.5<br>(60.7)  | 13.8<br>(-22.4 – 49.2)   | -68.4 – 1333.3            |
| Selenium (mcg)                   | 37.8<br>(23.1)    | 34.8<br>(21.0 – 54.6)       | 32.1<br>(36.6)     | 16.9<br>(8.5-34.6)       | 5.7<br>(49.9)  | 10.0<br>(-5.7 – 28.7)   | -97.1 – 52.4            | -2.1<br>(88.9)  | -31.9<br>(-78.1 – 53.1)  | -92.7 – 184.2             |

| Nutrient  | RAPP <sub>1</sub> |                        | RAPP <sub>2</sub> |                      | Difference<br>(RAPP <sub>1</sub> – RAPP <sub>2</sub> ) |                      |                         | Percentage difference (%)<br>[(RAPP <sub>1</sub> – RAPP <sub>2</sub> ) / RAPP <sub>2</sub> * 100] |                        |                           |
|-----------|-------------------|------------------------|-------------------|----------------------|--|----------------------|-------------------------|---|------------------------|---------------------------|
|           | Mean<br>(SD)      | Median<br>(IQ Range)   | Mean<br>(SD)      | Median<br>(IQ Range) | Mean<br>(SD)   | Median<br>(IQ Range) | 95% CI of<br>difference | Mean<br>(SD)  | Median<br>(IQ Range)   | 95% CI of %<br>difference |
| n = 47    |                   |                        |                   |                      |  |                      |                         |   |                        |                           |
| Zinc (mg) | 10.6<br>(4.7)     | 10.5**<br>(7.3 – 13.2) | 7.7<br>(4.4)      | 7.0<br>(4.7-9.6)     | 2.9<br>(6.2)   | 3.11<br>(-0.2 – 5.4) | -12.7 – 9.1             | 19.3<br>(68.4)  | 28.4<br>(-41.1 – 80.8) | -77.5 – 132.7             |

\* Wilcoxon signed rank test, significance levels set at  $p < 0.05$ ,

\*\* Wilcoxon signed rank test, significance levels set at  $p < 0.01$ ,

\*\*\* Wilcoxon signed rank test, significance levels set at  $p < 0.001$

SD = standard deviation

IQ Range = Inter-quartile range (25<sup>th</sup> percentile - 75<sup>th</sup> percentile)

CI = Confidence intervals for differences and percentage differences set at 95%

RAPP<sub>1</sub> = Reported nutrient intake from RAPP method conducted in March

RAPP<sub>2</sub> = Reported nutrient intake from RAPP method conducted in June

**Table 6.6. Spearman correlation coefficients, tertile distribution and weighted Kappa statistics for nutrients obtained from RAPP<sub>1</sub> and RAPP<sub>2</sub>**

| Nutrient                      | Spearman correlations | Percentage classified in tertiles |                   |                   | Weighted Kappa       |
|-------------------------------|-----------------------|-----------------------------------|-------------------|-------------------|----------------------|
|                               |                       | Same tertiles                     | Adjacent tertiles | Opposite tertiles |                      |
| <b>n = 47</b>                 | <b>r</b>              |                                   |                   |                   | <b>K<sub>w</sub></b> |
| Energy (kJ)                   | 0.46***               | 46.8                              | 42.6              | 10.6              | 0.20*                |
| Protein (g)                   | 0.35**                | 46.8                              | 34.0              | 19.2              | 0.19*                |
| Fat (g)                       | 0.23                  | 55.3                              | 23.4              | 21.3              | 0.32**               |
| Carbohydrates (g)             | 0.43***               | 44.7                              | 38.3              | 17.0              | 0.17*                |
| Folate (mcg)                  | 0.36*                 | 46.8                              | 40.4              | 12.8              | 0.19*                |
| Niacin (mg)                   | 0.33*                 | 46.8                              | 34.0              | 19.2              | 0.20*                |
| Riboflavin (mg)               | 0.42**                | 38.3                              | 48.9              | 12.8              | 0.07                 |
| Thiamine (mg)                 | 0.41**                | 55.3                              | 31.9              | 12.8              | 0.33***              |
| Vitamin A (mcg)               | 0.43**                | 46.8                              | 34.0              | 19.2              | 0.20*                |
| Vitamin B <sub>12</sub> (mcg) | 0.10                  | 29.8                              | 55.3              | 14.9              | -0.06                |
| Vitamin C (mg)                | 0.02                  | 31.9                              | 48.9              | 19.2              | -0.02                |
| Vitamin D (mcg)               | 0.21                  | 48.9                              | 29.8              | 21.3              | 0.15                 |
| Vitamin E (mg)                | 0.18                  | 42.6                              | 38.3              | 19.2              | 0.13                 |
| Calcium (mg)                  | 0.40***               | 44.7                              | 44.7              | 10.6              | 0.17*                |
| Iron (mg)                     | 0.29*                 | 46.8                              | 36.2              | 17.0              | 0.21*                |
| Potassium (mg)                | 0.32*                 | 44.7                              | 31.9              | 23.4              | 0.17                 |
| Magnesium (mg)                | 0.44**                | 51.1                              | 36.2              | 12.8              | 0.26**               |
| Sodium (mg)                   | 0.43***               | 51.1                              | 37.8              | 11.1              | 0.27**               |
| Selenium (mcg)                | 0.26                  | 46.8                              | 29.8              | 23.4              | 0.20*                |
| Zinc (mg)                     | 0.38**                | 53.2                              | 27.7              | 19.2              | 0.30**               |

r = Spearman correlation coefficient

CI = Confidence intervals

\* Significant level at p &lt; 0.05

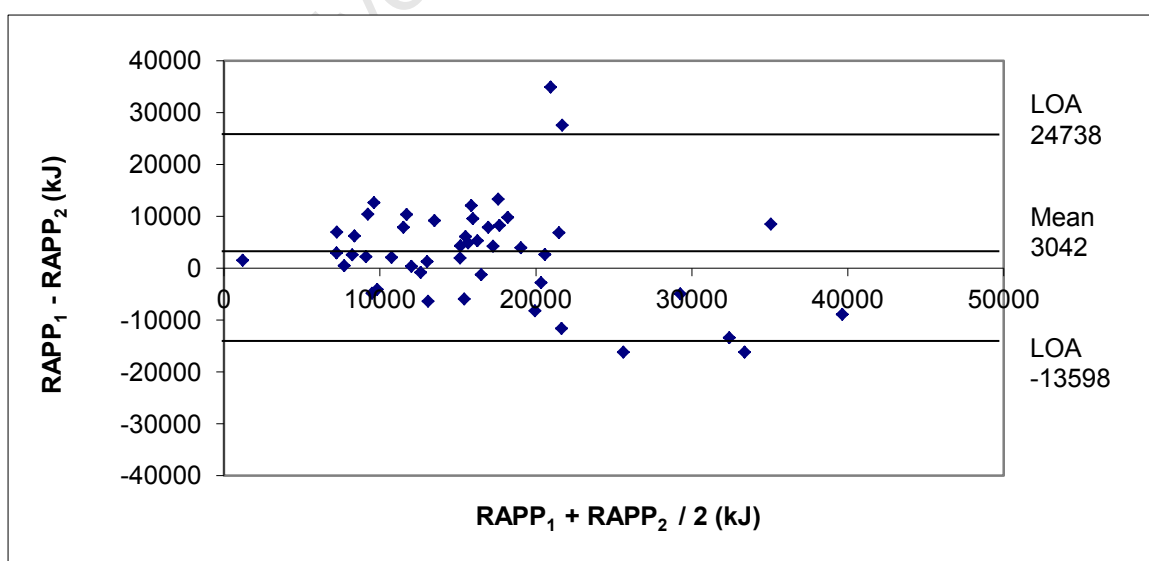
\*\*Significant level at p &lt; 0.01

\*\*\* Significant level at p &lt; 0.001

K<sub>w</sub> weighted Kappa

According to Bland-Altman data, nutrient intakes showed good agreement (> 95% within LOA) for six of the nutrients (riboflavin, vitamin A, vitamin C, Ca, Se and Zn) while the rest showed agreement between 90% and 95% (Table 6.7). Agreement was poor for Na with only 80% of the participants falling within the LOA. The LOA for most nutrients (except riboflavin, vitamin B<sub>12</sub> and vitamin C) were larger than one time the recommended daily allowance (RDA) (Standing Committee on the Scientific Evaluation of Dietary Intake References, 1997, 1998, 2000, 2001, 2004), making the LOA too wide to be clinically acceptable. Limits of agreement were especially wide for protein, CHO, folate, thiamine, vitamin E, Fe and Mg. For these nutrients repeated measures differed between two to seven times the RDA, indicating poor reliability. Spearman correlations were significant ( $p < 0.05$ ) for vitamin A, vitamin B<sub>12</sub>, vitamin C, vitamin D and Ca, indicating the presence of proportional bias.

The Bland-Altman plot for energy (Figure 6.4) showed good agreement with only two participants not lying within the LOA. However, the LOA were wide, ranging mostly between -16126 kJ and 22210 kJ (> 1 x RDA), indicating that the LOA were not clinically acceptable. The plot shows no sign of proportional bias, which is substantiated by the Spearman correlation coefficients that did not reach statistical significance. However, even though the mean differences were close to zero, over-estimating by 3042 kJ. Although the mean difference is close to zero, no bias is present and agreement is good, the reliability of the RAPP method based on Bland-Altman data for energy can be questioned because of the wide LOA.



**Figure 6.4.** Bland-Altman plot comparing mean energy consumption reported from RAPP<sub>1</sub> plus RAPP<sub>2</sub> against the difference of the two measures (n = 47)

**Table 6.7. Bland-Altman calculations for nutrients obtained from RAPP<sub>1</sub> and RAPP<sub>2</sub>**

| Nutrient                      | Percentage Agreement <sup>‡</sup> | Limits of agreement |             | Spearman correlations | LOA vs RDA <sup>#</sup>     |
|-------------------------------|-----------------------------------|---------------------|-------------|-----------------------|-----------------------------|
|                               |                                   | Lower limit         | Upper limit | r                     | (mean difference ± 1 X RDA) |
| <b>n = 47</b>                 |                                   |                     |             |                       |                             |
| Energy (kJ)                   | 90.5                              | -13598              | 24738       | -0.14                 | > 1 X RDA<br>(10093)        |
| Protein (g)                   | 93.6                              | -64.6               | 122.1       | -0.22                 | > 1 X RDA<br>(46)           |
| Fat (g)                       | 93.6                              | -184.4              | 240.0       | -0.05                 | RDA ne                      |
| Carbohydrates (g)             | 93.6                              | -574.9              | 1030.3      | -0.02                 | > 1 X RDA<br>(130)          |
| Folate (mcg)                  | 91.5                              | -731.6              | 1164.4      | 0.02                  | > 1 X RDA<br>(400)          |
| Niacin (mg)                   | 93.6                              | -14.6               | 21.0        | -0.14                 | > 1 X RDA<br>(14)           |
| Riboflavin (mg)               | 95.7                              | -0.8                | 1.6         | -0.05                 | ≈ 1 X RDA<br>(1.1)          |
| Thiamine (mg)                 | 93.7                              | -1.6                | 2.8         | -0.05                 | > 1 X RDA<br>(1.1)          |
| Vitamin A (mcg)               | 95.7                              | -624                | 1304        | 0.68***               | > 1 X RDA<br>(700)          |
| Vitamin B <sub>12</sub> (mcg) | 93.6                              | -1.8                | 3.0         | 0.32*                 | ≈ 1 X RDA<br>(2.4)          |
| Vitamin C (mg)                | 97.9                              | -113.4              | 208.6       | 0.38**                | < 1 X RDA<br>(75)           |
| Vitamin D (mcg)               | 91.5                              | -5.1                | 6.9         | 0.32*                 | > 1 X RDA<br>(5.0)          |
| Vitamin E (mg)                | 93.6                              | -104.7              | 131.3       | -0.03                 | > 1 X RDA<br>(15)           |
| Calcium (mg)                  | 95.7                              | -458.6              | 1092.6      | 0.35*                 | RDA ne                      |
| Iron (mg)                     | 91.5                              | -11.6               | 23.1        | 0.15                  | > 1 X RDA<br>(8)            |
| Potassium (mg)                | 93.6                              | -2394               | 3310        | -0.06                 | RDA ne                      |
| Magnesium (mg)                | 91.5                              | -570.3              | 918.1       | -0.02                 | > 1 X RDA<br>(310)          |
| Sodium (mg)                   | 80.0                              | -1299.9             | 4650.1      | -0.14                 | RDA ne                      |
| Selenium (mcg)                | 95.7                              | -94.1               | 105.5       | -0.17                 | > 1 X RDA<br>(55)           |

| Nutrient  | Percentage Agreement <sup>‡</sup> | Limits of agreement |             | Spearman correlations | LOA vs RDA <sup>#</sup>     |
|-----------|-----------------------------------|---------------------|-------------|-----------------------|-----------------------------|
|           |                                   | Lower limit         | Upper limit |                       |                             |
| n = 47    |                                   |                     |             | r                     | (mean difference ± 1 X RDA) |
| Zinc (mg) | 100.0                             | -9.5                | 15.3        | -0.10                 | > 1 X RDA<br>(8)            |

<sup>‡</sup> Percentage of data points between the limits of agreement

LOA = Limits of agreement

RDA = Recommended dietary allowances (Standing Committee on the Scientific Evaluation of Dietary Intake References, 1997, 1998, 2000, 2001, 2004)

ne = not established

Lower limit = Mean difference of RAPP<sub>1</sub> and RAPP<sub>2</sub> -2 standard deviations of the mean difference

Upper limit = Mean difference of RAPP<sub>1</sub> and RAPP<sub>2</sub> +2 standard deviations of the mean difference

r<sub>BA</sub> = Spearman correlation between the mean intake of RAPP<sub>1</sub> and RAPP<sub>2</sub> and the mean difference of RAPP<sub>1</sub> and RAPP<sub>2</sub>

\*Significance at p < 0.05

\*\*Significance at p < 0.01

\*\*\*Significance at p < 0.001

# LOA is considered clinical acceptable if it is smaller than mean difference ± 1 X RDA

Table 6.8 presents an overall summary on the results of different aspects of reliability results for the nutrient intake of the two measures using the RAPP method.

**Table 6.8. Summary of statistical results for the nutrient intake during reliability testing of the RAPP method**

| <b>Nutrient</b>               | <b>Agreement</b>                 | <b>Agreement</b>             | <b>Strength of association</b> | <b>Agreement (Including chance)</b> | <b>Agreement (Excluding chance)</b> | <b>Presence of bias</b>                      | <b>Limits of agreement</b>         | <b>Final Reliability</b>                     |
|-------------------------------|----------------------------------|------------------------------|--------------------------------|-------------------------------------|-------------------------------------|--|------------------------------------|--|
| <b>N = 47</b>                 | <b>Wilcoxon Signed rank test</b> | <b>Percentage difference</b> | <b>Spearman correlations</b>   | <b>Tertile classification</b>       | <b>Weighted Kappa statistics</b>    | <b>Bland – Altman (Spearman correlation)</b> | <b>Bland-Altman (Width of LOA)</b> | <b>&gt; 3 acceptable reliability results</b> |
| <b>Level of validation</b>    | <b>Group</b>                     | <b>Group</b>                 | <b>Individual</b>              | <b>Individual</b>                   | <b>Individual</b>                   | <b>Individual</b>                            | <b>Individual</b>                  |  |
| Energy (KJ)                   | Poor                             | Poor                         | Good                           | Poor                                | Acceptable                          | Absent                                       | Wide                               | Poor   |
| Protein (g)                   | Poor                             | Poor                         | Good                           | Poor                                | Poor                                | Absent                                       | Wide                               | Poor   |
| Fat (g)                       | Poor                             | Acceptable                   | Good                           | Acceptable                          | Acceptable                          | Absent                                       | ne                                 | Acceptable                                   |
| Carbohydrates (g)             | Poor                             | Poor                         | Good                           | Poor                                | Poor                                | Absent                                       | Wide                               | Acceptable                                   |
| Folate (mcg)                  | Poor                             | Poor                         | Good                           | Poor                                | Poor                                | Absent                                       | Wide                               | Poor   |
| Niacin (mg)                   | Poor                             | Acceptable                   | Good                           | Poor                                | Acceptable                          | Absent                                       | Wide                               | Acceptable                                   |
| Riboflavin (mg)               | Poor                             | Poor                         | Good                           | Poor                                | Poor                                | Absent                                       | Acceptable                         | Poor   |
| Thiamine (mg)                 | Poor                             | Poor                         | Good                           | Acceptable                          | Acceptable                          | Absent                                       | Wide                               | Acceptable                                   |
| Vitamin A (mcg)               | Poor                             | Poor                         | Good                           | Poor                                | Acceptable                          | Present                                      | Wide                               | Poor   |
| Vitamin B <sub>12</sub> (mcg) | Poor                             | Good                         | Poor                           | Poor                                | Poor                                | Present                                      | Acceptable                         | Poor   |
| Vitamin C (mg)                | Poor                             | Poor                         | Poor                           | Poor                                | Poor                                | Present                                      | Acceptable                         | Poor   |
| Vitamin D (mcg)               | Poor                             | Poor                         | Poor                           | Poor                                | Poor                                | Present                                      | Wide                               | Poor   |
| Vitamin E (mg)                | Poor                             | Acceptable                   | Poor                           | Poor                                | Poor                                | Absent                                       | Wide                               | Poor   |
| Calcium (mg)                  | Poor                             | Poor                         | Good                           | Poor                                | Poor                                | Present                                      | ne                                 | Poor   |
| Iron (mg)                     | Poor                             | Poor                         | Good                           | Poor                                | Acceptable                          | Absent                                       | Wide                               | Acceptable                                   |

| Nutrient            | Agreement                 | Agreement             | Strength of association | Agreement (Including chance) | Agreement (Excluding chance) | Presence of bias                      | Limits of agreement         | Final Reliability                  |
|---------------------|---------------------------|-----------------------|-------------------------|------------------------------|------------------------------|---------------------------------------|-----------------------------|------------------------------------|
| N = 47              | Wilcoxon Signed rank test | Percentage difference | Spearman correlations   | Tertile classification       | Weighted Kappa statistics    | Bland – Altman (Spearman correlation) | Bland-Altman (Width of LOA) | > 3 acceptable reliability results |
| Level of validation | Group                     | Group                 | Individual              | Individual                   | Individual                   | Individual                            | Individual                  |                                    |
| Potassium (mg)      | Poor                      | Poor                  | Good                    | Poor                         | Poor                         | Absent                                | ne                          | Poor                               |
| Magnesium (mg)      | Poor                      | Poor                  | Good                    | Acceptable                   | Acceptable                   | Absent                                | Wide                        | Acceptable                         |
| Sodium (mg)         | Good                      | Poor                  | Good                    | Acceptable                   | Acceptable                   | Absent                                | ne                          | Acceptable                         |
| Selenium (mcg)      | Good                      | Good                  | Good                    | Poor                         | Acceptable                   | Absent                                | Wide                        | Acceptable                         |
| Zinc (mg)           | Poor                      | Acceptable            | Good                    | Acceptable                   | Acceptable                   | Absent                                | Wide                        | Acceptable                         |

Wilcoxon signed rank test agreement at group level  $p < 0.05$  = Good,  $p > 0.05$  Poor

Percentage difference:  $< 10\%$  = Good,  $10 - 20\%$  = Acceptable,  $> 20\%$  = Poor

Strength of association and correlation = Results from correlation coefficient (individual level)

Good =  $> 0.50$ , Acceptable  $> 0.20 - 0.50$  and Poor  $< 0.20$

Agreement = Results from tertile classification (include chance) and Kappa statistics (exclude chance)

Bias = Bland-Altman data indication of bias on individual level (if  $r_{BA}$  is significant),

$r_{BA}$  = Spearman correlation coefficient for Bland-Altman data (correlation between mean intake and mean difference of intake)

Agreement Bland-Altman:  $< 1 \times RDA$  = Narrow,  $\approx 1 \times RDA$  = Acceptable,  $> 1 \times RDA$  = Wide

ne = not established

## 4. DISCUSSION

The aim of this chapter was to determine the reliability of the RAPP method in reporting on food group and nutrient intake. For these purposes the RAPP method was repeated twice,  $\pm$  12 weeks apart.

Overall, strength of association (correlations) between the two RAPP tests was acceptable for all food groups (Masson *et al.*, 2003:313), with the exception of condiments. The strength of association (based on Spearman correlation coefficients) was good for the majority of nutrient intakes (except for vitamin B<sub>12</sub>, vitamin C, vitamin D, and vitamin E). A strong indication of association shows a linear association between the two test measurements. Correlations in the present study for food groups compare well with a study conducted in a similar population in North West Province of South Africa (MacIntyre *et al.*, 2001a:55). Correlations ranged between 0.11 (condiments) and 0.59 (beverages) in the current study, while correlations for bread and meat were lower than those reported by black adults tested in North-West Province.

MacIntyre *et al.*, (2001a:64) reported correlations for nutrients ranging from 0.14 (Ca) to 0.75 (alcohol), while correlations in the present study ranged from 0.02 (vitamin C) to 0.46 (energy). Spearman rank correlations for energy in the present study were better than those in the North-West Province (0.46 compared to 0.28). Vitamin C correlation coefficient was poor (0.02) compared to that of the North West Province (0.38). According to Gibson (2005:135), the ideal correlation coefficients for QFFQs' should range between 0.5 and 0.8 (depending on population and sample size). Neither the present study nor the North-West study achieved such high correlation coefficients.

Regardless of its strength of association, the RAPP method has a limited ability to repeatedly rank participants according to intake (tertile classification) for both food groups and nutrients. Only cereals, beverages, fat, thiamine, Mg, Na and Zn were repeatedly ranked into the same category of intake. The level of misclassification is important because QFFQs, such as the RAPP method, are designed to rank participants according to intake (Masson *et al.*, 2003:313). Although the percentage of participants being misclassified in the present study was high for the majority of nutrients and food groups, it was never higher than 25% and a larger sample size would probably have lowered this to acceptable values.

Because chance can also influence level of classification (Gibson, 2005:143), weighted Kappa statistics were used to shed more light on the level of classification (Masson *et al.*,

2003:314, Gibson, 2005:143). Cohen's weighted Kappa statistics ranged between 0.06 (bread and condiments) and 0.47 (beverages) for food groups and between -0.06 (vitamin B<sub>12</sub>) and 0.33 (thiamine) for nutrients. Kappa statistics reached significance ( $p < 0.05$ ) for none of the food groups and for most nutrients (including energy, protein, fat, carbohydrates, folate, niacin, thiamine, vitamin A, calcium, iron, magnesium, sodium, selenium and zinc), showing that generally levels of agreement were acceptable for nutrients. Although agreement is lower compared to other studies (Masson *et al.*, 2003:313), low agreement in the current study was expected because of the population's low level of literacy and lack of sophistication. Previously, participants had no reason to think of food in a scientific, descriptive way and might not have understood the importance of the research study. Furthermore, the effect of seasonal differences also played a role in the expected results as food availability and therefore diversity (especially regarding mixed dishes such as stiff *pap* and pumpkin) is even less in winter. This could unfortunately not be prevented because of logistic limitations.

The LOA for the food groups were generally too wide to be clinically acceptable. Reliability for nutrients was poor overall because of the wide LOA, indicating that intakes could vary up to 7 x the RDA between different applications of the RAPP method. The LOA for nine of the twenty nutrients were not acceptable or they were wider than 2 x the RDA. This indicates that participants may be classified as having a low intake of a nutrient at one test measure, while at another test using the same method, can be ranked as having a high intake of that nutrient. This is of clinical relevance when determining the risk for OC for certain nutrients. In the present study, therefore, the following nutrient data: CHO, protein, folate, thiamine, vitamin C, vitamin E, iron and Mg; cannot be regarded as being reliable.

The percentage of participants that fell within the LOA were generally between 90 - 100% for both the food groups and nutrients. Ideally these should be between 95% and 100%, therefore agreement at individual level for the majority of food groups and nutrients were acceptable but not good. Bland-Altman data indicated no reporting bias for any of the food groups although the presence of reporting bias was detected for vitamin A, vitamin B<sub>12</sub>, vitamin C, vitamin D and Ca.

The most frequently consumed food items (bread) and some of the most infrequently consumed food items (meat) showed the best reproducibility in the current study. However, Pietinen *et al.*, (1988a:664) concluded from their study that this may be the result of over-reporting of desirable food items and under-reporting of undesirable food items, biasing the

outcomes. An earlier study showed that only the most frequently consumed items had good reproducibility (MacIntyre *et al.*, 2001a:60).

Various factors can influence reliability results and the difficulty lies in determining whether the measured differences are as a result of measurement error or because of other changes (Goldbohm *et al.*, 1995:421; Gibson, 2005:141). The period between the two measures, sample size, the variability of the method, inter-interviewer bias, with-in person variability and seasonal variability are all factors that influence reliability.

Block and Hartman (1989:1134) suggested that two measures should be at least four to eight weeks apart in order to reduce a learning effect. This was complied with in the present study and the time between repeated measurements was approximately 12 weeks.

In general, it can be said that the mean reported nutrient intakes of the first RAPP measurement were higher than that of the second measurement. This compares well with other studies (Pietinen *et al.*, 1988a:660, Munger *et al.*, 1992:196, Shu *et al.*, 2004:21), although some have also found higher mean intakes in the second measurement (MacIntyre *et al.*, 2001a:55, Erkkola *et al.*, 2001:469, Fornés *et al.*, 2003:823). It is possible that participants had a more realistic idea of their dietary intake at the second administration, and this may have decreased overestimation (Pietinen *et al.*, 1988:665), although it still signifies poor reliability.

Another factor that may have influenced reliability results is the sample size, which was relatively small compared to sample sizes of other reliability studies (44 – 196) (Munger *et al.*, 1992:196, MacIntyre *et al.*, 2001a:57, Fornés *et al.*, 2003:824, Shu, *et al.*, 2004:20, Boucher *et al.*, 2006:85). The current study included only 47 participants because of the time limitations, limited funding and other limitations set by the study design. A larger sample size would have decreased the between-person variation, and thereby improving results. Of the 47 who completed the study, 36 were females and 11 males. It is expected that the small sample of males may have introduced selection bias; however it was very difficult to obtain more male participants.

Another aspect that influences reliability is the variability of the method (Block & Hartman, 1989:1134). The RAPP method allows quite a range of reporting variety, since it includes three portion sizes and three ratio pictures for some of the most frequently consumed dishes. The number of response frequencies allowed may also affect its reliability. The RAPP method includes a variety of responses varying from “less than a month” to “daily”

intake and it is hence expected that its reproducibility will be lower. Also, results from the photo validation study (Chapter 5) indicated poor validity of the FPS. This will definitely impact on the reliability of the QFFQ. Although the RAPP method was developed in two areas (Centane and Bizana) while validation only included participants from Centane this should have had an impact on the reliability results as all food items consumed in Centane were included in the QFFQ and FPS.

In the present study the same interviewer interviewed the same participant both times to avoid inter-interviewer bias. It is not clear how this affected the reliability results of the present study if the comments by Teufel (1997:1177) are considered. The latter suggested that different interviewers interview participants so that the participants do not feel obliged to remember previous responses, however, if a particular interviewer makes errors these will remain constant if the same interviewer repeats the interview.

Lastly, it needs to be borne in mind that seasonal variation could also have influenced the results since the participants were mostly rural farmers. The produce available in the areas studied varied according to season and hence the ratios of vegetables and maize may have changed to some extent, resulting in poor correlations for vitamins and minerals, especially vitamin A (maize meal and pumpkin) and Fe (maize meal and spinach). Other studies in low-income countries have also reported that seasonal differences play a role in the intake of specific nutrients, namely vitamin A, vitamin C, Fe and fat (Kigutha, 1997:1172S).

## **5. CONCLUSION**

Although reliability seems poor compared to many international studies, it compared favourably with the only similar study conducted in black adults in a rural area of South Africa (MacIntyre *et al.*, 2001a:60).

Based on the previously mentioned factors it can be concluded that the RAPP method was considered to be acceptable for intake of bread, cereals, meat and beverages of the people living in high oesophageal cancer (OC) areas in the Eastern Cape (EC) Province. However, reliability of the RAPP method was poor for mixed dishes and condiment intake (salt and added sugar) of people living in these areas. Reliability was acceptable for CHO, fat, niacin, thiamine, Fe, Mg, Na, Se and Zn intakes but poor for total energy, protein, riboflavin, vitamin A, vitamin B<sub>12</sub>, vitamin C, vitamin D, vitamin E, folate, Ca and K intakes.

Reliability outcomes thus indicate that the RAPP method is acceptable when assessing food group intake, especially those containing maize, which are the primary determinants of

fumonisin intake and the primary objective of the study. Regardless of the limitations set by the small sample size; gender and level of education of participants; and the impact of seasonal variability; it can be concluded that the RAPP method can be deemed reliable to determine maize intake of people living in high OC areas (primary aim) and that the reliability for assessment of nutrient deficiencies (secondary aim) is limited.

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## Chapter 7

# THE RAPP METHOD VALIDATED AGAINST 24- HOUR RECALL QUESTIONNAIRES



An interviewer, interviewing a participant during the validation of the RAPP method

## 1. INTRODUCTION

The RAPP method (test method) was developed (Chapter 4) with the use of focus groups and interviews. The method comprises a quantified food frequency questionnaire (QFFQ) and food photograph series (FPS) (portion sizes and ratio photographs). The validity of the photographs in portion size estimation was evaluated to determine how participants perceived the portion sizes (Chapter 5) and the reliability of the method was assessed using a test-retest design (Chapter 6). The next step in the development of the test measure was to assess the validity of the RAPP method against a “gold standard.”

Relative validity is the most frequently used validation method for dietary assessment methods. This method requires the test method to be measured against another, more accurate method (also known as the gold standard) (Katzenellenbogen *et al.*, 2001:92). Because no dietary assessment method is perfect, validation cannot compare the test method against the truth, but only against another dietary assessment method (reference method). In this regard food frequency questionnaires can either be validated against multiple food records or against repeated 24-hour recalls (Cade *et al.*, 2002:575).

Clearly a variety of factors need to be taken into consideration when testing the validity of a dietary assessment method. For this study it was decided to validate the test method (RAPP method) against four 24-hour dietary recalls as the reference method. This decision was based on the fact that the weighed dietary record method would be difficult to implement since many participants were semi-literate. The choice of using four 24-hour recalls was based on studies conducted by Rosner and Willett (1988:385) and Stram *et al.*, (1995:353) who recommended two to five 24-hour recalls as reference method in validation studies.

Different factors influence the accuracy of validation studies, including age, gender, body mass index (BMI), medical conditions, day of the week, season and the use of supplements (Gibson, 2005:152, Marks *et al.*, 2006:399). Hence, the study must be conducted bearing these factors in mind. The following procedures were therefore considered in the design of this validation study: 1) the population tested should be part of the target population (Cade *et al.*, 2002:574); 2) the test and reference methods should cover the same time frame (Nelson, 1997:250); and 3) the test method must be conducted before the reference method to prevent a learning bias (Gibson, 2005:150).

## 2. MATERIALS AND METHODS

### 2.1. STUDY POPULATION

The study population used for the reliability study was used for the validation study. The study population is described in Chapter 6, section 2.1.

### 2.2. PROCEDURES

Participants were visited four separate times, twice in March and twice in June 2004 to complete 24 hour recalls covering one weekend and three week days. Ideally the 24-hour recalls should have reflected the same time frame as the QFFQ (one month). This was not possible due to logistical limitations and the fact that this study was part of a larger study. However, the residents in these areas are mostly subsistence farmers and have very monotonous dietary patterns, potentially mitigating the effect on the outcomes. During each of the two visits anthropometric measurements were taken and questionnaires, including the 24-hour recalls were completed. Table 7.1 and Figure 7.1 describe the procedures followed during the validation study and include the various questionnaires completed.

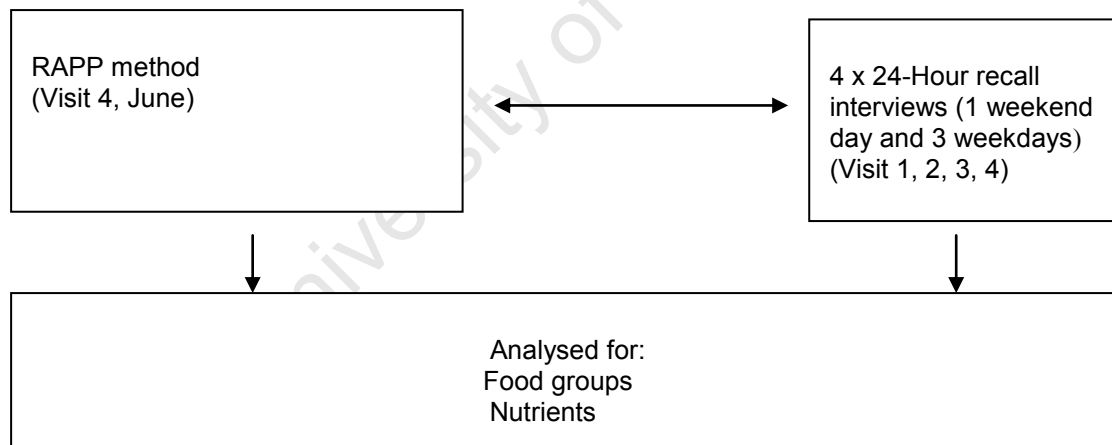


Figure 7.1. Steps followed during the validation testing of the RAPP method

**Table 7.1. Procedures followed during the validation testing of the RAPP method**

| Visit 1 (March)           | Visit 2 (March)                 | Visit 3 (June)       | Visit 4 (June)                               |
|---------------------------|---------------------------------|----------------------|--|
| Information               |                                 |                      |  |
| Consent                   |                                 |                      |  |
| Demographic questionnaire |                                 |                      |  |
|                           | RAPP <sub>1</sub> (Reliability) |                      | RAPP <sub>2</sub> (Reliability and validity) |
| 24-Hour recall            | 24-Hour recall                  | 24-Hour recall       | 24-Hour recall                               |
|                           | Hunger questionnaire            | Hunger questionnaire |  |
| Anthropometry             |                                 | Anthropometry        |  |

### 2.2.1. Interviewing procedures

Participants were visited at home, where they were most comfortable and privacy was ensured. The exact location of each participant's home was noted, and recorded with a global positioning system (GPS) monitor (GPS tracker satellite navigator Magellan). To exclude inter-interviewer bias, the same interviewer interviewed the same participants each time.

### 2.3. ASSESSMENTS

Anthropometric assessments (weight and height) were taken. Chapter 3, Section 3.2 provides detail on methodology of anthropometry.

A socio-demographic questionnaire was complete with each participant (Chapter 3, Section 3.3), as well as four 24 hour recalls. One recall was based on a weekend day intake (Saturday or Sunday) and three on week day intakes (Monday to Friday) (Chapter 3, Section 3.3). The RAPP method was completed during the final visit. All questionnaires were interviewer administered and conducted in the participants' first language (isiXhosa).

### 2.4. STATISTICAL ANALYSES USED FOR VALIDATION

Food items and dishes were divided into food groups based on their main ingredients, namely, bread, cereals, combined dishes, meat, condiments (sugar and salt) and beverages.

Nutrient analyses were done with FoodFinder 3 (FoodFinder dietary Software, 1992, FoodFinder 3, 2010). The nutrient content of the **test method (RAPP<sub>2</sub>)** for each food item was calculated as the product of each frequency of intake identified on the QFFQ and the

portion size of the food item. The total monthly intake was calculated from the frequencies on the QFFQ and then divided by 28 to provide the total intake per nutrient per day.

The total nutrient intake from the reported intake of the reference method (24-hour recalls) was calculated as the sum of the total intake per nutrient per portion size (Flegal *et al.*, 1990:1048). This was then divided by four to give an average nutrient intake of the four recalls.

The dietary intake reported on both dietary assessment methods was analysed for total energy, protein, fat, carbohydrates (CHO), folate, niacin, riboflavin, thiamine, vitamin A, vitamin B<sub>12</sub>, vitamin C, vitamin D, vitamin E, calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), sodium (Na), selenium (Se) and Zinc (Zn). Food group and nutrient intakes according to the test method were statistically compared with food group and nutrient intakes according to the reference method.

The detail of the statistical tests applied for these purposes is described in Chapter 3, Section 5. In summary data was firstly tested for normality with the Shapiro Wilk test, after which the Wilcoxon signed rank test and Spearman correlation coefficients were computed. Participants were classified into tertiles, weighted Kappa statistics were conducted and Bland-Altman data was calculated.

Recent validation studies adjusted dietary data for within and between-person variation by de-attenuating correlations (Segovia-Siapco *et al.*, 2007:179, Wong *et al.*, 2008:541, Cheng *et al.*, 2008:168, Satia *et al.*, 2009:505, Carithers *et al.*, 2009:1186, Lora *et al.*, 2010:553). Segovia-Siapco *et al.*, (2007:179) estimated the within-person variation in a study conducted among 87 adults in Southern California and found extremely high correction factors for some nutrients that could not be applied in analyses. These researchers concluded that the small sample size may explain the high correction factors. After consulting with an independent statistician it was decided not to implement adjustment for within and between individual variations in this study as only a small sample (47 participants) could be recruited. The fact that four 24-hour recalls were included may have contributed to reduced intra-individual variation, as Cole, 2007:70, Gibson, 2005:138, Kaaks *et al.*, 2002:971 indicated that the more repetitions included, the lower the intra-individual variation.

### 3. RESULTS

#### 3.1. SOCIO DEMOGRAPHIC AND ANTHROPOMETRIC PROFILE OF THE STUDY SAMPLE

Sixty participants initially agreed to participate in the study, 13 dropped out for various reasons, including urbanisation, participant burden and peer pressure. Of the 47 who completed the study, 36 were females and 11 males.

Table 7.2 presents demographic information of all the participants.

**Table 7.2. Mean ( $\pm$  SD) of the age and anthropometric measurements of participants**

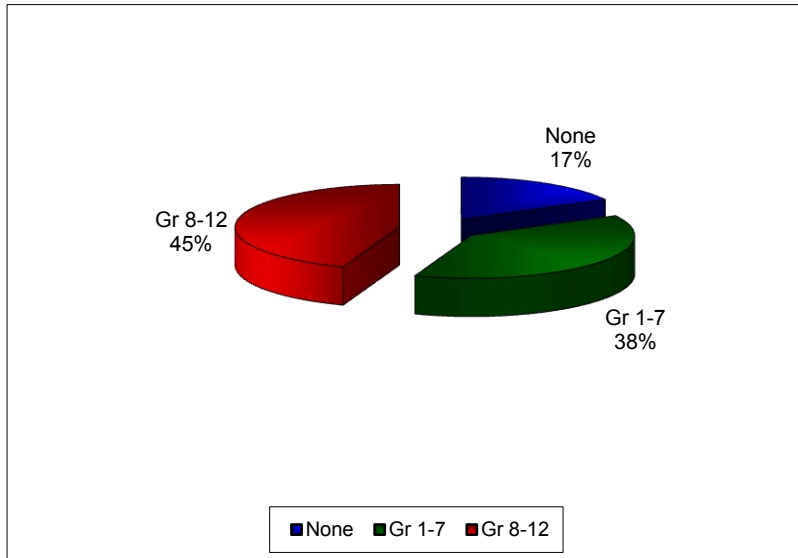
| Measurements             | Males<br>n = 11<br>Mean (SD) | Females<br>n = 36<br>Mean (SD) | Total<br>n = 47<br>Mean (SD) |
|--------------------------|------------------------------|--------------------------------|------------------------------|
| Age (years)              | 40.5 (16.4)                  | 45.0 (12.7)                    | 43.8 (13.8)                  |
| Height (cm)              | 170 (0.1)                    | 160 (0.1)                      | 160 (0.1)                    |
| Weight (kg)              | 62.3 (8.3)                   | 72.2 (17.7)                    | 69.9 (16.3)                  |
| BMI (kg/m <sup>2</sup> ) | 22.0 (3.0)                   | 28.2 (6.7)                     | 26.8 (6.6)                   |

BMI = Body mass index  
n = Sample size  
SD = Standard deviation  
kg = kilogram  
cm = centimetre

The majority of participants (45%) had secondary schooling, while 38% of them only had a primary education, and 17% had no formal education (Figure 7.2).

Employment status of the participants showed that 47% were unemployed, while 14% were mine workers. The rest had odd jobs including hotel work, fishing and assisting in the local shops. Most participants lived in mud houses (88.5%) and 11.5% in brick houses. Of the participants, 55.8% obtained their water from a river in the area, while 32.7% made use of a communal tap. Only 2% had a tap in the house. Seventy-three percent of the participants did not have a toilet, and made use of the bush. Most participants (87.5%) cooked their food outside the house, while 12.5% reported to cook inside. All the participants, however, used wood as an energy source for cooking.

The majority of the participants (79%) had a single person contributing to the household financially, with 9% reporting two contributors and 10% reported to have no-one making a financial contribution to the household.



**Figure 7.2. Different education levels of the participants**

The average income per family was R 695 ( $\pm$  R 540) per month. Fifty percent of the participants reported to have received a grant from the government (for old age, a child or disability).

Of the females, 75% had never smoked tobacco, 2.4% had smoked in the past but not at the time of questioning, while 22% were current smokers. Of the males, 13% had never smoked, 13% had smoked in the past and 73% were current smokers. Forty-five percent of the participants reported not to drink maize beer, while 54% did so.

### **3.2. REPORTED FOOD GROUP INTAKE OF THE RAPP METHOD COMPARED WITH 24-HOUR DIETARY RECALLS**

The Wilcoxon signed rank test (Table 7.3) indicated a significant difference ( $p < 0.05$ ) between the test method (RAPP) and reference method (24-hour recalls) for meat only, indicating that agreement at group level was acceptable for all other food groups. The percentage difference (PD) ranged between -19.4% (condiments) and 39.6% (bread). Percentage differences between methods were good ( $< 10\%$ ) for combined dishes, acceptable (11 – 20%) for cereals, meat and condiments and poor ( $> 20\%$ ) for bread and beverages. Results illustrate that overall there was acceptable agreement at group level except for bread and beverages.

Spearman correlation coefficients were poor ( $< 0.20$ ) between the two dietary methods for bread (-0.03) and meat (0.12), while the correlations for cereal, condiments, and beverages were acceptable (0.21 – 0.50) (Table 7.4). Correlation coefficient for bread was negative (-

0.03) indicating a negative association (when one measure is high, the other is low). The correlation coefficient for combined dishes was good (0.59).

Classification of participants into the same tertile ranged between 39.5% (bread) and 62.2% (combined dishes) (Table 7.4). Classification into the same tertile was poor (< 50%) for bread, cereal and beverages. When chance agreement was removed by using Kappa statistics, results showed poor agreement for bread and cereal (< 0.20).

Agreement at individual level assessed by using Bland-Altman data (percentage of reported intakes within the limits of agreement [LOA]) was good (> 95%) for all food groups except for bread and meat (93.0% and 93.3% respectively) (Table 7.5). However, regardless of the high level of individual agreement, the LOA were wide for all food groups (except meat), with a difference larger than 1 X the mean small portion size of a food group. Spearman correlation values ( $r_{BA}$ ) illustrated the presence of reporting bias, only for beverages ( $r$  is high and significant).

Table 7.6 provides a summary of the results of the statistical methods used for the analyses of the food groups for validation purposes.

**Table 7.3. Means, standard deviation, medians, inter-quartile ranges, differences and percentage differences of daily food group intake between the test and reference methods**

| Food Group          | Test method (g)    |                          | Reference method (g) |                         | Difference (g)                     |                             |                         | Percentage difference (%)                                 |                         |                           |
|---------------------|--------------------|--------------------------|----------------------|-------------------------|------------------------------------|-----------------------------|-------------------------|---|-------------------------|---------------------------|
|                     | RAPP <sub>2</sub>  |                          | (Mean recalls)       |                         | (RAPP <sub>2</sub> - Mean Recalls) |                             |                         | [(RAPP <sub>2</sub> - Mean Recalls) / Mean Recalls * 100] |                         |                           |
| N = 47              | Mean<br>(SD)       | Median<br>(IQ Range)     | Mean<br>(SD)         | Median<br>(IQ Range)    | Mean<br>(SD)                       | Median<br>(IQ Range)        | 95% CI of<br>difference | Mean<br>(SD)  | Median<br>(IQ Range)    | 95% CI of %<br>difference |
| Bread (g)           | 338.2<br>(265.9)   | 244.0<br>(123.0-498.0)   | 242.3<br>(251.1)     | 165.5<br>(100.0-253.5)  | 95.9<br>(394.9)                    | 48.0<br>(-61.5-283.0)       | -683.0-718.5            | 39.6<br>(237.2)   | -13.6<br>(-81.0-62.0)   | -92.4-196.2               |
| Cereal (g)          | 590.0<br>(661.7)   | 417.0.0<br>(178.0-720.0) | 515.0<br>(325.8)     | 483.3<br>(277.0-698.0)  | 48.8<br>(660.8)                    | -86.0<br>(-278.8-221.8)     | -656.8-966.8            | 14.6<br>(95.3)  | -16.8<br>(-65.3-68.2)   | -97.4-153.8               |
| Combined dishes (g) | 827.0<br>(447.5)   | 782.0<br>(539.0-1051.0)  | 901.9<br>(422.7)     | 831.0<br>(637.5-1217.5) | -74.9<br>(414.6)                   | -45.5<br>(-377.8-151.3)     | -72.1-116.4             | -8.2<br>(44.9)  | -9.2<br>(-38.8-16.6)    | -72.1-71.5                |
| Meat (g)            | 39.7<br>(48.7)     | 19.0*<br>(11.0-37.0)     | 44.2<br>(53.5)       | 26.0<br>(1.0-65.0)      | -8.1<br>(59.8)                     | 2.0<br>(-39.5-18.0)         | -112.0-93.0             | -10.2<br>(160.9)  | -84.5<br>(-95.2-23.1)   | -100.0-245.6              |
| Condiments (g)      | 85.9<br>(159.5)    | 33.0<br>(21.0-72.0)      | 106.6<br>(100.6)     | 74.3<br>(50.3-124.3)    | -20.7<br>(164.3)                   | -31.5<br>(-54.8 - -4.5)     | -141.0 – 80.8           | -19.4<br>(96.8)   | -37.6<br>(-69.1 - -4.0) | -86.6 – 76.5              |
| Beverages (ml)      | 1278.3<br>(1056.9) | 971.0<br>(651.0-1488.0)  | 1026.9<br>(395.1)    | 900.0<br>(800.0-1175.0) | 251.3<br>(988.6)                   | 12.5<br>(-314.0–<br>1330.0) | -756.5-1745.0           | 24.6<br>(88.7)  | 2.0<br>(-32.5-60.3)     | -63.5 – 195.0             |

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\* Wilcoxon signed rank t-test significant at  $p < 0.05$

\*\*Wilcoxon signed rank t-test significant at  $p < 0.01$

\*\*\* Wilcoxon signed rank t-test significant at  $p < 0.001$

SD = Standard deviation

IQ Range = Inter Quartile range (25<sup>th</sup> and 75<sup>th</sup> percentile)

CI = 95% Confidence interval

RAPP<sub>2</sub> with Recall<sub>Mean</sub>  $n = 47$

Test method = Reported nutrient intake from RAPP method conducted in June

Reference method = Mean reported nutrient intake from 4 x 24-hour recall questionnaires conducted in March and June

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**Table 7.4. Spearman correlation coefficients, tertile distribution and weighted Kappa statistics for food groups obtained from the test and reference methods**

| Food group      | Spearman correlation | Percentage classified in tertiles |                         |                         | Weighted Kappa       |
|-----------------|----------------------|-----------------------------------|-------------------------|-------------------------|----------------------|
|                 |                      | Same tertile                      | Adjacent tertile        | Opposite tertile        |                      |
| <b>N = 47</b>   | <b>r</b>             | <b>Same tertile</b>               | <b>Adjacent tertile</b> | <b>Opposite tertile</b> | <b>K<sub>w</sub></b> |
| Bread           | -0.03                | 39.5                              | 27.9                    | 32.6                    | 0.06                 |
| Cereal          | 0.39**               | 47.7                              | 38.6                    | 13.6                    | 0.15                 |
| Combined dishes | 0.59***              | 62.2                              | 28.9                    | 8.9                     | 0.43***              |
| Meat            | 0.12                 | 55.6                              | 35.6                    | 8.9                     | 0.33***              |
| Condiments      | 0.38**               | 53.3                              | 35.6                    | 11.1                    | 0.30**               |
| Beverages       | 0.38**               | 48.9                              | 31.1                    | 20.0                    | 0.21**               |

RAPP<sub>2</sub> with 24-H recall<sub>Mean</sub> n = 47

r = Spearman correlation coefficient

\* Significance set at p &lt; 0.05

\*\*Significance set at p &lt; 0.01

\*\*\* Significance set at p &lt; 0.001

K<sub>w</sub> weighted Kappa**Table 7.5. Bland-Altman calculations for food groups as obtained from the test and reference methods**

| Food groups         | Percentage Agreement <sup>‡</sup> | Limits of agreement |                    | Spearman correlation  | LOA vs. mean portion size |
|---------------------|-----------------------------------|---------------------|--------------------|-----------------------|---------------------------|
|                     |                                   | Lower limit         | Upper limit        |                       |                           |
| <b>N = 47</b>       |                                   | <b>Lower limit</b>  | <b>Upper limit</b> | <b>r<sub>BA</sub></b> |                           |
| Bread (g)           | 93.0                              | -693.9              | 885.7              | 0.22                  | > Small portion           |
| Cereal (g)          | 97.7                              | -1272.8             | 1370.4             | 0.24                  | > Small portion           |
| Combined dishes (g) | 95.6                              | -904.1              | 754.3              | 0.10                  | > Small portion           |
| Meat (g)            | 93.3                              | -127.7              | 111.5              | -0.23                 | > Small portion           |
| Condiments (g)      | 95.6                              | -349.3              | 307.9              | 0.01                  | > Small portion           |
| Beverages (ml)      | 95.6                              | -1725.9             | 2228.5             | 0.59***               | > Small portion           |

RAPP<sub>2</sub> with 24-hour recall<sub>Mean</sub> n = 47<sup>‡</sup> Percentage of data points between the limits of agreement

Lower limit = mean difference of RAPP and 24-hour recalls – 2 standard deviations

Upper limit = mean difference of RAPP and 24-hour recalls + 2 standard deviations

r<sub>BA</sub> = Spearman correlation between the mean intake of the RAPP and 24-hour recalls and the mean difference between the RAPP<sub>2</sub> and 24-hour recalls

Significance of the Spearman correlation between mean intake and mean difference

\* Significance set at p &lt; 0.05,

\*\* Significance set at p &lt; 0.01,

\*\*\* Significance set at p &lt; 0.001

Mean portion size = the mean of the small portion sizes for all the different dishes and food items in that food group

<sup>#</sup> LOA is considered clinical acceptable if it is smaller than mean difference ± 1 X mean small portion

**Table 7.6. Summary of statistical results for the different food groups from the validation of the test method with a reference methods**

| Food group          | Agreement                 | Agreement             | Strength of association | Agreement (including chance) | Agreement (excluding chance) | Bias                                | Agreement                   | Final validity                    |
|---------------------|---------------------------|-----------------------|-------------------------|------------------------------|------------------------------|-------------------------------------|-----------------------------|-----------------------------------|
|                     | Wilcoxon Signed rank test | Percentage difference | Spearman correlation    | Tertile classification       | Kappa statistic              | Bland-Altman (Spearman correlation) | Bland-Altman (Width of LOA) | > 3 Acceptable validation results |
| Level of validation | Group                     | Group                 | Individual              | Individual                   | Individual                   | Individual                          | Individual                  |                                   |
| Bread (g)           | Good                      | Poor                  | Poor                    | Poor                         | Poor                         | Absent                              | Wide                        | Poor                              |
| Cereal (g)          | Good                      | Acceptable            | Acceptable              | Poor                         | Poor                         | Absent                              | Wide                        | Acceptable                        |
| Combined dishes (g) | Good                      | Good                  | Good                    | Good                         | Acceptable                   | Absent                              | Wide                        | Acceptable                        |
| Meat (g)            | Poor                      | Acceptable            | Poor                    | Good                         | Acceptable                   | Absent                              | Wide                        | Acceptable                        |
| Condiments (g)      | Good                      | Acceptable            | Acceptable              | Good                         | Acceptable                   | Absent                              | Wide                        | Acceptable                        |
| Beverages (mℓ)      | Good                      | Poor                  | Acceptable              | Poor                         | Acceptable                   | Present                             | Wide                        | Poor                              |

Wilcoxon signed rank test agreement at group level  $p < 0.05$  = Good,  $p > 0.05$  Poor

Percentage difference:  $< 10\%$  = Good, 11 - 20% = Acceptable,  $> 20\%$  = Poor

Strength of association and correlation = Results from correlation coefficient (individual level)

Good =  $> 0.50$ , Acceptable 0.21 – 0.50 and Poor  $< 0.20$

Agreement = Results from tertile classification (include chance) and Kappa statistics (exclude chance)

Tertile classification:  $> 50\%$  is same group = Good

Kappa statistics:  $< 0.20$  = Poor, 0.21 – 0.60 = Acceptable, 0.61 – 0.80 = Good

Bias = Bland-Altman data indication of bias on individual level (if  $r_{BA}$  is significant),

$r_{BA}$  = Spearman correlation coefficient for Bland-Altman data (correlation between mean and mean difference of intake)

Agreement Bland-Altman:  $< 1 \times$  mean small portion = Narrow LOA,  $\approx 1 \times$  mean small portion = Acceptable,  $> 1 \times$  mean small portion = Wide LOA

Final validity\* = Four or more of statistical methods indicate agreement

### 3.3. REPORTED NUTRIENT INTAKE OF THE RAPP METHOD COMPARED WITH 24-HOUR DIETARY RECALLS

Nutrient intakes derived from the reference method (24-hour recalls) and the test method (RAPP method) are reported in Table 7.7. The Wilcoxon signed rank test showed significant differences ( $p < 0.05$ ) for most nutrients, indicating poor agreement at group level between the test and reference method. Percentage differences between the two methods were good ( $< 10\%$ ) for energy, fat, CHO and vitamin E, and acceptable (11 - 20%) for protein and niacin. The PD was poor ( $> 20\%$  difference) for other nutrients (folate, riboflavin, thiamine, vitamin A, vitamin B<sub>12</sub>, vitamin C, vitamin D, Ca, Fe, K, Mg, Na, Se and Zn). Agreement was hence, best for macronutrients and only for a few micronutrients (vitamin E, niacin, riboflavin).

Spearman correlation coefficients between the reference and test methods, were generally low, ranging between 0.01 and 0.45 (Table 7.8). Correlation coefficients for fat, riboflavin, vitamin A, vitamin B<sub>12</sub>, vitamin C, vitamin E and Ca were poor ( $< 0.20$ ), while coefficients for the rest of the nutrients (energy, protein, CHO, folate, niacin, thiamine, vitamin D, Fe, K, Mg, Na, Se and Zn) were acceptable (0.21 – 0.50). None of the correlation coefficients indicated a negative association.

Tertile classification into the same tertiles ranged between 34% (fat and vitamin A) and 63.8% (Mg) (Table 7.8). Many nutrients ( $> 20\%$ ) were classified into opposite tertiles including protein, fat, riboflavin, vitamin B<sub>12</sub>, vitamin C, vitamin E, K and Mg. This is indicative of poor agreement between methods for these nutrients. Kappa statistics were poor ( $< 0.20$ ) for protein, fat, vitamin A, vitamin B<sub>12</sub>, vitamin C, vitamin E, Ca and Se and acceptable (0.21 – 0.60) for energy, CHO, folate, niacin, riboflavin, thiamine, vitamin D, Fe, K, Mg, Na and Zn. Once chance agreement was removed, it appears that agreement at individual level between the test and reference method was acceptable for two thirds of the nutrients.

According to Bland-Altman data, agreement was good for eleven of the nutrients ( $> 95\%$  in LOA) with the exception of energy, niacin, riboflavin, thiamine, vitamin A, vitamin B<sub>12</sub> vitamin D, calcium and selenium (Table 7.9). The LOAs were wider than 1 X RDA for all nutrients except vitamin D. Spearman correlation coefficients ( $r_{BA}$ ) between the mean total intake and the mean difference between the two assessment methods illustrated the presence of proportional bias for energy, protein, CHO, folate, Fe, Mg, Na, Se and Zn.

**Table 7.7. Means, standard deviation, medians, inter-quartile ranges, differences and percentage differences of daily nutrient intake between the test and reference methods**

| Nutrient             | RDA   |         | Test method (g)<br>(RAPP <sub>2</sub> ) |                         | Reference method (g)<br>(Mean Recalls) |                        | Difference (g)<br>(RAPP <sub>2</sub> – Mean Recalls) |                         |                         | Percentage difference (%)<br>[(RAPP <sub>2</sub> – Mean Recalls) / Mean Recall * 100] |                           |                         |
|----------------------|-------|---------|---|-------------------------|--|------------------------|--|-------------------------|-------------------------|---|---------------------------|-------------------------|
|                      | Males | Females | Mean<br>(SD)                            | Median<br>(IQ Range)    | Mean<br>(SD)                           | Median<br>(IQ Range)   | Mean<br>(SD)   | 95% CI of<br>difference | Median<br>(IQ Range)    | Mean<br>(SD)  | 95% CI of %<br>difference | Median<br>(IQ Range)    |
| Energy (kJ)          | 12881 | 10093   | 12463<br>(5854)                         | 12475<br>(7686-17091)   | 13819<br>(3677)                        | 13661<br>(11003-16225) | -1356<br>(6025)                                      | -9192 - 8602            | -1642<br>(-6439 - 2830) | -9.8<br>(47.7)  | -72.5-68.6                | -10.4<br>(-37.1-20.4)   |
| Protein (g)          | 56    | 46      | 67.3<br>(34.0)                          | 66.0**<br>(44.7-91.3)   | 84.9<br>(26.2)                         | 81.6<br>(68.8-99.5)    | -17.6<br>(38.5)                                      | -75.5-48.0              | -19.3<br>(-42.4 - 4.2)  | -19.1<br>(45.2)   | -80.4-73.7                | -18.5<br>(-48.5-4.0)    |
| Fat (g)              | 118   | 93      | 69.5<br>(46.9)                          | 54.8<br>(38.3 – 86.5)   | 83.9<br>(31.5)                         | 82.8<br>(62.7-102.5)   | -14.4<br>(55.5)                                      | -83.3-62.1              | -12.8<br>(-54.0-16.9)   | -6.9<br>(81.3)  | -82.7-113.4               | -23.4<br>(-55.3-31.4)   |
| Carbohydrates<br>(g) | 130   | 130     | 475.7<br>(222.3)                        | 475.7<br>(289.1-618.3)  | 494.1<br>(148.6)                       | 487.7<br>(397.3-572.0) | -18.5<br>(215.4)                                     | -326.2-325.5            | -16.3<br>(-153.1-140.3) | -1.4<br>(46.4)  | -70.4-61.2                | -2.1<br>(-31.1-22.6)    |
| Folate (mcg)         | 400   | 400     | 558.0<br>(355.2)                        | 454.0*<br>(331.0-788.0) | 419.6<br>(235.7)                       | 405.8<br>(247.5-552.7) | 138.4<br>(393.7)                                     | -401.6-842.9            | 108.6<br>(0.9-305.0)    | 33.0<br>(164.5)   | -74.1-215.4               | 36.2<br>(0.2-84.1)      |
| Niacin (mg)          | 16    | 14      | 10.8<br>(6.5)                           | 9.8*<br>(6.6-14.2)      | 14.0<br>(6.0)                          | 12.8<br>(10.0-17.2)    | -3.2<br>(8.3)  | -13.9-8.8               | -3.0<br>(-6.9-2.0)      | -16.3<br>(51.0)   | -83.8-87.4                | -22.4<br>(-55.4-11.8)   |
| Riboflavin (mg)      | 1.3   | 1.1     | 0.7<br>(0.5)                            | 0.6**<br>(0.4-0.9)      | 1.0<br>(0.4)                           | 0.9<br>(0.7-1.2)       | -0.3<br>(0.6)  | -1.1-0.5                | -0.3<br>(-0.5-1.0)      | -20.3<br>(55.4)   | -84.5-59.7                | -38.0<br>(-56.8-6.9)    |
| Thiamine (mg)        | 1.2   | 1.1     | 1.4<br>(0.8)                            | 1.4***<br>(.9-1.7)      | 1.9<br>(0.6)                           | 1.8<br>(1.5-2.4)       | -0.6<br>(0.8)  | -1.9-0.7                | -0.6<br>(-1.1- -0.2)    | -26.6<br>(39.3)   | -79.7-45.3                | -31.1<br>(-59.2- -10.0) |
| Vitamin A (mcg)      | 900   | 700     | 192.6<br>(231.1)                        | 92.0**<br>(51.0-242.0)  | 346.7<br>(276.9)                       | 277.0<br>(171.1-402.4) | -154.2<br>(360.9)                                    | -895.2-373.9            | -105.2<br>(-278.8-5.1)  | -22.9<br>(88.7)   | -98.7-195.0               | -50.1<br>(-82.4-6.4)    |

| Nutrient                         | RDA   |         | Test method (g)<br>(RAPP <sub>2</sub> ) |                          | Reference method (g)<br>(Mean Recalls) |                          | Difference (g)<br>(RAPP <sub>2</sub> – Mean Recalls) |                         |                            | Percentage difference (%)<br>[(RAPP <sub>2</sub> – Mean Recalls) / Mean Recall * 100] |                           |                          |
|----------------------------------|-------|---------|---|--------------------------|--|--------------------------|--|-------------------------|----------------------------|---|---------------------------|--------------------------|
|                                  | Males | Females | Mean<br>(SD)                            | Median<br>(IQ Range)     | Mean<br>(SD)                           | Median<br>(IQ Range)     | Mean<br>(SD)   | 95% CI of<br>difference | Median<br>(IQ Range)       | Mean<br>(SD)  | 95% CI of %<br>difference | Median<br>(IQ Range)     |
| Vitamin B <sub>12</sub><br>(mcg) | 2.4   | 2.4     | 0.5<br>(0.7)                            | 0.3**<br>(0.0-1.0)       | 1.3<br>(1.2)                           | 0.8<br>(0.5-1.6)         | -0.7<br>(1.4)  | -3.9-1.1                | -0.3<br>(0.12 - -0.1)      | -35.8<br>(82.8)   | -100.0-163.6              | -65.2<br>(-100.0- -14.5) |
| Vitamin C (mg)                   | 90    | 75      | 45.0<br>(40.7)                          | 28.0**<br>(15.0-62.0)    | 71.8<br>(54.1)                         | 52.3<br>(34.5-100.1)     | -26.8<br>(61.4)                                      | -101.1-70.1             | -27.3<br>(-63.7-10.4)      | -37.7<br>(147.6)  | -92.1-254.9               | -47.8<br>(-76.0-75.9)    |
| Vitamin D (mcg)                  | 5.0   | 5.0     | 1.0<br>(2.0)                            | 0.1<br>(0.0-4.8)         | 0.8<br>(1.1)                           | 0.33<br>(0.1-1.3)        | 0.2<br>(1.9)   | -2.8-4.0                | -0.1<br>(-0.4-2.0)         | 25.0<br>(2079.2)  | -100.0-282.0              | -58.6<br>(-100.0-56.5)   |
| Vitamin E (mg)                   | 15    | 15      | 35.4<br>(25.5)                          | 28.1<br>(17.7-47.8)      | 36.7<br>(15.7)                         | 36.8<br>(25.7-46.0)      | -1.3<br>(28.9)                                       | -39.9-39.1              | -4.7<br>(-23.7-10.8)       | -3.5<br>(99.2)  | -80.5-218.5               | -23.8<br>(-56.0-32.8)    |
| Calcium (mg)                     | ne    | Ne      | 416.1<br>(275.9)                        | 330.0<br>(204.0 – 650.5) | 603.0<br>(338.9)                       | 500.9<br>(369.3 – 752.9) | -253.8<br>(402.9)                                    | -930.0 – 331.4          | -234.5<br>(-430.6- - 47.2) | -30.9<br>(62.0)   | -100.0 –<br>71.9          | -47.3<br>(-83.1 - -13.3) |
| Iron (mg)                        | 8     | 18      | 12.1<br>(7.1)                           | 12.3<br>(6.5-15.7)       | 16.1<br>(5.5)                          | 15.4<br>(11.4-0.2)       | -6.0<br>(8.2)  | -18.9 – 10.5            | -6.2<br>(-11.1- -1.7)      | -24.8<br>(49.9)   | -100.0 –<br>65.8          | -35.3<br>(-73.6 - -17.1) |
| Potassium (mg)                   | 4000  | 5000    | 2701<br>(1527)                          | 2503***<br>(1660-3441)   | 3925<br>(1076)                         | 3750<br>(3253-4811)      | -1224<br>(1517)                                      | -3417-1697              | -1191<br>(-2500 - -504)    | -30.3<br>(37.1)   | -77.5-35.9                | -32.4<br>(-58.9- -18.1)  |
| Magnesium<br>(mg)                | 420   | 320     | 447.5<br>(282.8)                        | 406.0**<br>(261.0-564.0) | 587.9<br>(178.8)                       | 560.9<br>(457.1-708.1)   | -140.4<br>(273.3)                                    | -484.9-395.9            | -162.7<br>(-302.1 - -3.1)  | -23.3<br>(43.0)   | -79.8-72.1                | -31.1<br>(-58.5 - -3.0)  |
| Sodium (mg)                      | 500   | 500     | 4207.9<br>(4761.6)                      | 3018*<br>(2424-4159)     | 2528<br>(1132)                         | 2370<br>(1768-2964)      | 1679<br>(4733)                                       | -1484-5597              | 758.7<br>(-11.5-1788)      | 66.0<br>(227.8)   | -45.7-264.6               | 36.3<br>(-0.8-75.5)      |
| Selenium (mcg)                   | 55.0  | 55      | 32.1<br>(36.6)                          | 16.9<br>(8.5-34.6)       | 25.9<br>(22.2)                         | 19.1<br>(12.7-35.6)      | 6.2<br>(29.5)  | -28.8-73.6              | 1.5<br>(-13.1-14.4)        | 23.7<br>(508.6)   | -88.6-478.1               | 2.1<br>(-56.2-107.8)     |
| Zinc (mg)                        | 11    | 8.0     | 7.7<br>(4.4)                            | 7.0***<br>(4.7-9.6)      | 10.7<br>(3.1)                          | 10.7<br>(8.3-12.7)       | -3.1<br>(4.6)  | -9.1-5.1                | -3.3<br>(-5.8- -1.0)       | -27.3<br>(40.7)   | -81.7-52.6                | -29.5<br>(-55.7- -10.5)  |

| Nutrient | RDA   |         | Test method (g)<br>(RAPP <sub>2</sub> ) |                      | Reference method (g)<br>(Mean Recalls) |                      | Difference (g)<br>(RAPP <sub>2</sub> – Mean Recalls) |                         |                      | Percentage difference (%)<br>[(RAPP <sub>2</sub> – Mean Recalls) / Mean Recall *<br>100] |                           |                      |
|----------|-------|---------|---|----------------------|--|----------------------|--|-------------------------|----------------------|--|---------------------------|----------------------|
|          | Males | Females | Mean<br>(SD)                            | Median<br>(IQ Range) | Mean<br>(SD)                           | Median<br>(IQ Range) | Mean<br>(SD)   | 95% CI of<br>difference | Median<br>(IQ Range) | Mean<br>(SD)   | 95% CI of %<br>difference | Median<br>(IQ Range) |
|          |       |         |   |                      |  |                      |  |                         |                      |  |                           |                      |

RDA Recommended dietary allowance (Standing committee on the scientific evaluation of dietary reference intakes, 1997, 1998, 2000, 2001, 2004, 2005)  
n = 47

Recall<sub>Mean</sub> = Mean reported nutrient intake from 4 x 24-hour recall questionnaires conducted in March and June

\* Wilcoxon signed rank t-test significant at p < 0.05

\*\* Wilcoxon signed rank t-test significant at p < 0.01

\*\*\* Wilcoxon signed rank t-test significant at p < 0.001

SD = standard deviation

IQ Range = inter quartile range (25<sup>th</sup> percentile – 75<sup>th</sup> percentile)

CI = 95% Confidence interval

SD = Standard deviation

IQ Range = Inter quartile range (25<sup>th</sup> and 75<sup>th</sup> percentage)

**Table 7.8. Spearman correlation coefficients, tertile distribution and weighted Kappa statistics for nutrients obtained from the test and reference methods**

| Nutrient                      | Spearman correlation | Percentage classified in tertiles |                         |                         | Weighted Kappa       |
|-------------------------------|----------------------|-----------------------------------|-------------------------|-------------------------|----------------------|
|                               |                      | Same tertile                      | Adjacent tertile        | Opposite tertile        |                      |
| <b>N = 47</b>                 | <b>r</b>             | <b>Same tertile</b>               | <b>Adjacent tertile</b> | <b>Opposite tertile</b> | <b>K<sub>w</sub></b> |
| Energy (kJ)                   | 0.26                 | 46.8                              | 34.0                    | 19.2                    | 0.20*                |
| Protein (g)                   | 0.23                 | 42.6                              | 34.0                    | 23.4                    | 0.12                 |
| Fat (g)                       | 0.01                 | 34.0                              | 34.0                    | 31.9                    | -0.01                |
| Carbohydrates (g)             | 0.40***              | 50.0                              | 32.6                    | 17.4                    | 0.25**               |
| Folate (mcg)                  | 0.40**               | 53.2                              | 38.3                    | 8.5                     | 0.30**               |
| Niacin (mcg)                  | 0.21                 | 46.8                              | 34.0                    | 19.2                    | 0.20*                |
| Riboflavin (mg)               | 0.03                 | 46.8                              | 27.7                    | 25.5                    | 0.21*                |
| Thiamine (mg)                 | 0.36*                | 53.2                              | 31.2                    | 14.9                    | 0.30**               |
| Vitamin A (mcg)               | 0.15                 | 34.0                              | 51.1                    | 14.9                    | 0.03                 |
| Vitamin B <sub>12</sub> (mcg) | 0.11                 | 42.6                              | 42.6                    | 23.4                    | 0.00                 |
| Vitamin C (mg)                | 0.14                 | 40.4                              | 34.0                    | 25.5                    | 0.10                 |
| Vitamin D (mcg)               | 0.37**               | 51.1                              | 34.0                    | 14.9                    | 0.26**               |
| Vitamin E (mg)                | 0.03                 | 40.4                              | 27.7                    | 31.9                    | 0.08                 |
| Calcium (mg)                  | 0.11                 | 40.4                              | 38.3                    | 21.3                    | 0.11                 |
| Iron (mg)                     | 0.38**               | 51.1                              | 25.5                    | 23.4                    | 0.29**               |
| Potassium (mg)                | 0.35*                | 48.9                              | 29.8                    | 21.3                    | 0.23**               |
| Magnesium (mg)                | 0.45***              | 63.8                              | 14.9                    | 21.3                    | 0.45***              |
| Sodium (mg)                   | 0.42***              | 47.8                              | 39.1                    | 13.0                    | 0.20*                |
| Selenium (mcg)                | 0.27                 | 36.2                              | 44.7                    | 19.2                    | 0.04                 |
| Zinc (mg)                     | 0.34*                | 48.9                              | 34.0                    | 17.0                    | 0.23*                |

RAPP<sub>2</sub> with 24-hour recall<sub>Mean</sub> n = 47

r Spearman correlation coefficient

\* Spearman rank correlation coefficient significant at p &lt; 0.05

\*\*Spearman rank correlation coefficient significant at p &lt; 0.01

\*\*\* Spearman rank correlation coefficient significant at p &lt; 0.001

CI = Confidence intervals

K<sub>w</sub> weighted Kappa

**Table 7.9. Bland-Altman calculations for nutrients as obtained from the test and reference methods**

| Nutrient                      | Percentage Agreement <sup>‡</sup> | Limits of agreement |             | Spearman correlations | LOA vs. RDA (RDA)       |
|-------------------------------|-----------------------------------|---------------------|-------------|-----------------------|-------------------------|
|                               |                                   | Lower limit         | Upper limit |                       |                         |
| <b>N = 47</b>                 |                                   |                     |             | <b>r<sub>BA</sub></b> |                         |
| Energy (kJ)                   | 93.6                              | -13406              | 10694       | 0.49***               | > 1 X RDA<br>(10093 kJ) |
| Protein (g)                   | 95.7                              | -94.6               | 59.4        | 0.34*                 | > 1 X RDA<br>(46 g)     |
| Fat (g)                       | 97.9                              | -125.4              | 96.6        | 0.27                  | RDA ne                  |
| Carbohydrates (g)             | 95.7                              | -449.3              | 412.3       | 0.46**                | > 1 X RDA<br>(130 g)    |
| Folate (mcg)                  | 95.7                              | -649.0              | 925.8       | 0.42**                | > 1 X RDA<br>(400 mcg)  |
| Niacin (mg)                   | 91.5                              | -19.8               | 13.4        | 0.21                  | > 1 X RDA<br>(14 mg)    |
| Riboflavin (mg)               | 93.6                              | -1.5                | 0.9         | 0.13                  | > 1 X RDA<br>(1.1 mg)   |
| Thiamine (mg)                 | 93.6                              | -2.2                | 1.0         | 0.23                  | > 1 X RDA<br>(1.1 mg)   |
| Vitamin A (mcg)               | 89.4                              | -876.0              | 567.6       | -0.15                 | > 1 X RDA<br>(700 mcg)  |
| Vitamin B <sub>12</sub> (mcg) | 93.6                              | -3.5                | 2.1         | -0.23                 | > 1 X RDA<br>(2.4 mcg)  |
| Vitamin C (mg)                | 100.0                             | -149.6              | 96.0        | -0.26                 | > 1 X RDA<br>(75 mg)    |
| Vitamin D (mcg)               | 93.6                              | -3.8                | 4.0         | 0.08                  | < 1 X RDA<br>(5.0 mcg)  |
| Vitamin E (mg)                | 97.9                              | -59.1               | 56.5        | 0.25                  | > 1 X RDA<br>(15 mg)    |
| Calcium (mg)                  | 94.6                              | -1059.6             | 552.0       | -0.16                 | RDA ne                  |
| Iron (mg)                     | 98.2                              | -22.4               | 10.4        | -0.41**               | > 1 X RDA<br>(8 mg)     |
| Potassium (mg)                | 97.9                              | -4258.0             | 1810.0      | 0.16                  | RDA ne                  |
| Magnesium (mg)                | 95.7                              | -687.0              | 406.2       | 0.32*                 | > 1 X RDA<br>(310 mg)   |
| Sodium (mg)                   | 97.9                              | -7787.0             | 11145       | 0.56***               | RDA ne                  |

| Nutrient       | Percentage Agreement <sup>‡</sup> | Limits of agreement |             | Spearman correlations | LOA vs. RDA (RDA)     |
|----------------|-----------------------------------|---------------------|-------------|-----------------------|-----------------------|
|                |                                   | Lower limit         | Upper limit |                       |                       |
| <b>N = 47</b>  |                                   |                     |             | $r_{BA}$              |                       |
| Selenium (mcg) | 93.6                              | -52.8               | 65.2        | 0.30*                 | > 1 X RDA<br>(55 mcg) |
| Zinc (mg)      | 95.7                              | -12.3               | 6.1         | 0.33*                 | > 1 X RDA<br>(8 mg)   |

RAPP<sub>2</sub> with 24-hour recall<sub>Mean</sub> n = 47

<sup>‡</sup> Percentage of data points between the limits of agreement

Lower limit = mean difference of RAPP and 24-hour recalls – 2 standard deviations

Upper limit = mean difference of RAPP and 24-hour recalls + 2 standard deviations

$r_{BA}$  Spearman correlation between the mean intake of the RAPP and 24-hour recalls and the mean difference between the RAPP and 24-hour recalls

\*Spearman rank correlation coefficients significant at  $p < 0.05$ ,

\*\*Spearman rank correlation coefficients significant at  $p < 0.01$ ,

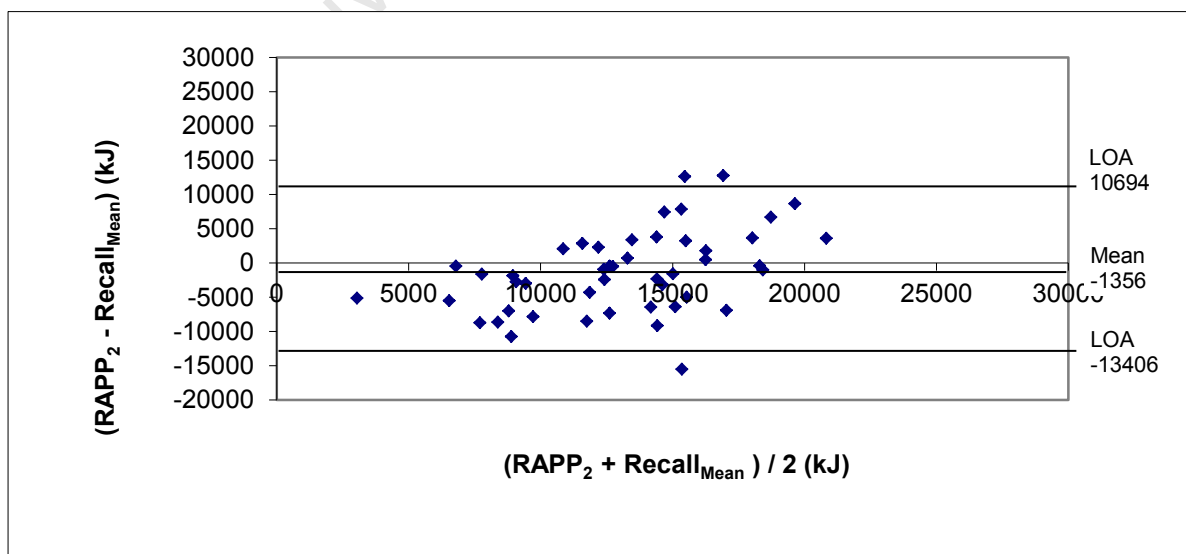
\*\*\*Spearman rank correlation coefficients significant at  $p < 0.001$

RDA = Recommended dietary allowance for adult females (Standing committee on scientific evaluation of dietary reference intakes, 1997, 1998, 2000, 2001, 2004, 2005)

ne = not established

# LOA is considered clinical acceptable if it is smaller than mean difference  $\pm 1$  X RDA

Figure 7.3 shows the Bland-Altman plot for reported energy intake between the means of the two methods and the mean difference of the two methods. The LOA were wide ( $> 1$  x RDA for energy), with poor agreement (93.6%). The mean difference was however, relatively close to zero (-1356 kJ). The plot shows an energy intake of between approximately 7 000 kJ and 21 000 kJ. The Spearman correlation coefficient between the mean intake and mean difference was 0.49 (and significant) indicating clear visual bias.



**Figure 7.3. Bland-Altman plot comparing the mean of the test and reference method against the difference between the test and reference method for energy intake**

Table 7.10 presents a summary of all the statistical outcomes for each nutrient.

**Table 7.10. Summary of statistical results for the different nutrients from the validation of the test method against a reference method**

| <b>Nutrient</b>               | <b>Agreement</b>                 | <b>Agreement</b>             | <b>Strength of association</b> | <b>Agreement (including chance)</b> | <b>Agreement (excluding chance)</b> | <b>Presence of bias</b>                    | <b>Limits of agreement</b>         | <b>Final validity</b>                       |
|-------------------------------|----------------------------------|------------------------------|--------------------------------|-------------------------------------|-------------------------------------|--|------------------------------------|---|
|                               | <b>Wilcoxon Signed rank test</b> | <b>Percentage difference</b> | <b>Spearman correlation</b>    | <b>Tertile classification</b>       | <b>Kappa statistics</b>             | <b>Bland-Altman (Spearman correlation)</b> | <b>Bland-Altman (Width of LOA)</b> | <b>&gt; 3 Acceptable validation results</b> |
| <b>Level of validation</b>    | <b>Group</b>                     | <b>Group</b>                 | <b>Individual</b>              | <b>Individual</b>                   | <b>Individual</b>                   | <b>Individual</b>                          | <b>Individual</b>                  |   |
| Energy (KJ)                   | Good                             | Good                         | Acceptable                     | Poor                                | Acceptable                          | Present                                    | Wide                               | Acceptable                                  |
| Protein (g)                   | Poor                             | Acceptable                   | Acceptable                     | Poor                                | Poor                                | Present                                    | Wide                               | Poor  |
| Fat (g)                       | Good                             | Good                         | Poor                           | Poor                                | Poor                                | Absent                                     | Ne                                 | Poor  |
| Carbohydrates (g)             | Good                             | Good                         | Good                           | Good                                | Acceptable                          | Present                                    | Wide                               | Acceptable                                  |
| Folate (mcg)                  | Poor                             | Poor                         | Good                           | Good                                | Acceptable                          | Present                                    | Wide                               | Poor  |
| Niacin (mg)                   | Poor                             | Acceptable                   | Acceptable                     | Poor                                | Acceptable                          | Absent                                     | Wide                               | Acceptable                                  |
| Riboflavin (mg)               | Poor                             | Acceptable                   | Poor                           | Poor                                | Acceptable                          | Absent                                     | Wide                               | Poor  |
| Thiamine (mg)                 | Poor                             | Poor                         | Acceptable                     | Good                                | Acceptable                          | Absent                                     | Wide                               | Acceptable                                  |
| Vitamin A (mcg)               | Poor                             | Poor                         | Poor                           | Poor                                | Poor                                | Absent                                     | Wide                               | Poor  |
| Vitamin B <sub>12</sub> (mcg) | Poor                             | Poor                         | Poor                           | Poor                                | Poor                                | Absent                                     | Wide                               | Poor  |
| Vitamin C (mg)                | Poor                             | Poor                         | Poor                           | Poor                                | Poor                                | Absent                                     | Wide                               | Poor  |
| Vitamin D (mcg)               | Good                             | Poor                         | Acceptable                     | Good                                | Acceptable                          | Absent                                     | Narrow                             | Acceptable                                  |
| Vitamin E (mg)                | Good                             | Good                         | Poor                           | Poor                                | Poor                                | Absent                                     | Wide                               | Poor  |
| Calcium (mg)                  | Good                             | Poor                         | Acceptable                     | Good                                | Poor                                | Absent                                     | Ne                                 | Acceptable                                  |
| Fe (mg)                       | Good                             | Poor                         | Acceptable                     | Good                                | Acceptable                          | Present                                    | Wide                               | Acceptable                                  |

| Nutrient            | Agreement                 | Agreement             | Strength of association | Agreement (including chance) | Agreement (excluding chance) | Presence of bias                    | Limits of agreement         | Final validity                    |
|---------------------|---------------------------|-----------------------|-------------------------|------------------------------|------------------------------|-------------------------------------|-----------------------------|-----------------------------------|
|                     | Wilcoxon Signed rank test | Percentage difference | Spearman correlation    | Tertile classification       | Kappa statistics             | Bland-Altman (Spearman correlation) | Bland-Altman (Width of LOA) | > 3 Acceptable validation results |
| Level of validation | Group                     | Group                 | Individual              | Individual                   | Individual                   | Individual                          | Individual                  |                                   |
| Potassium (mg)      | Poor                      | Poor                  | Acceptable              | Poor                         | Acceptable                   | Absent                              | Ne                          | Poor                              |
| Magnesium (mg)      | Poor                      | Poor                  | Good                    | Poor                         | Acceptable                   | Present                             | Wide                        | Poor                              |
| Sodium (mg)         | Poor                      | Poor                  | Good                    | Poor                         | Acceptable                   | Present                             | Ne                          | Poor                              |
| Selenium (mcg)      | Good                      | Poor                  | Acceptable              | Poor                         | Poor                         | Present                             | Wide                        | Poor                              |
| Zinc (mg)           | Poor                      | Poor                  | Acceptable              | Poor                         | Acceptable                   | Present                             | Wide                        | Poor                              |

Wilcoxon signed rank test agreement at group level  $p < 0.05$  = Good,  $p > 0.05$  Poor

Percentage difference:  $< 10\%$  = Good,  $11 - 20\%$  = Acceptable,  $> 20\%$  = Poor

Strength of association and correlation = Results from correlation coefficient (individual level)

Good =  $> 0.50$ , Acceptable  $0.21 - 0.50$  and Poor  $< 0.20$

Agreement = Results from tertile classification (include chance) and Kappa statistics (exclude chance)

Tertile classification:  $> 50\%$  is same group = Good

Kappa statistics:  $< 0.20$  = Poor,  $0.21 - 0.60$  = Acceptable,  $0.61 - 0.80$  = Good

Bias = Bland-Altman data indication of bias on individual level (if  $r_{BA}$  is significant),

$r_{BA}$  = Spearman correlation coefficient for Bland-Altman data (correlation between mean and mean difference of intake)

Agreement Bland-Altman:  $< 1 \times RDA$  = Narrow LOA,  $\approx 1 \times RDA$  = Acceptable,  $> 1 \times RDA$  = Wide LOA

RDA Recommended dietary allowance (Standing committee on the scientific evaluation of dietary reference intakes, 1997, 1998, 2000, 2001, 2004, 2005)

Final validity\* = Four or more of statistical methods indicate agreement

ne = not established

## 4. DISCUSSION

The primary aim of the current study was for the RAPP method to measure fumonisin exposure accurately. Fumonisin grows on maize and therefore it was imperative that maize dishes and CHO intake as measured by the RAPP method should be valid. Secondly, it was important to determine the validity of the RAPP method in assessing micronutrient intake, particularly those associated with oesophageal cancer (OC) risk.

Results showed acceptable validity (four or more of the indicated tests indicating acceptable validity as summarized in Table 7.10) of the test method for measuring dietary intake for the following food groups: cereals, combined dishes, meat and condiments (sugar and salt), but not for estimation of bread and beverage intake. Acceptable validity (four or more of the indicated tests indicating acceptable validity) was also found for a limited range of nutrient estimations, including energy, CHO, niacin, thiamine, vitamin D, Ca and Fe but not for the remaining 12 nutrients.

Agreement at group level was generally good for all food groups and poor for most micronutrients (folate, thiamine, vitamin A, vitamin B<sub>12</sub>, vitamin C, K, Mg, Na and Zn). The PD of total energy intake between the test and reference methods was minimal. However, certain micronutrients, including folate, niacin, riboflavin, thiamine, vitamin A, vitamin B<sub>12</sub>, vitamin C, Ca, Fe, K, Mg and Zn were under reported on the test method. Of these micronutrients, vitamin A, beta-carotene, vitamin E, riboflavin, Mg, Zn, and Se have been specifically associated with cancer (Table 2.2). Under-reporting of micronutrients may be due to the exclusion of fruit and vegetables as a group on its own in the RAPP method. However, fruit and vegetables were not consumed frequently enough to warrant inclusion on the QFFQ and vegetables were generally consumed combined with maize.

The large difference between the two dietary assessments for vitamin A could possibly be ascribed to the exclusion of potatoes and margarine in the test method. These items were excluded because of their extreme low intake frequency. The large mean differences found between the two methods for folate and sodium could be because the test method over-reports on bread and salt respectively.

A study undertaken in South Africa among a similar population, found that the FFQ under-reported in comparison with weighed dietary records (MacIntyre *et al.*, 2001b:65), while Erkkola *et al.*, (2001:471) reported higher intakes when using the FFQ than when using weighed records. However, in the present study Wilcoxon signed rank test only illustrated a significant difference between methods for meat intake and micronutrients.

Strength of association between the two dietary methods was acceptable for all food groups except for bread and meat. Strength of association was also regarded as acceptable for micronutrients

studied with the exception of fat, riboflavin, vitamin A, vitamin B<sub>12</sub>, vitamin C and vitamin E. Spearman correlation coefficients showed a relationship between the two methods, although this relationship was not always strong (good). It is possible that weaker (poor) relationships could be the result of with-in person variation resulting from the use of the reference method (Gibson, 2005:183). According to Willett (1994:174S), correlation coefficients for FFQ's compared with 24-hour recall questionnaires should be in the range of  $r = 0.6$  or  $r = 0.7$ . Although the correlation coefficients found in this study were much lower ( $r = -0.03 - 0.59$  for food groups and  $r = 0.01 - 0.45$  for nutrients), they were still similar to those found in other studies (Bohlscheid-Thomas *et al.*, 1997:S74, MacIntyre *et al.*, 2001b:68).

Furthermore, a validation study by Pietinen *et al.*, (1988:664) indicated that participants over-reported on food items they considered to be healthy. Correlation coefficients for the different food groups among Finish males ranged between 0.20 (berries) and 0.82 (alcohol). Another study in rural Japan (Ogawa *et al.*, 2003:153) reported Spearman correlation coefficients ranging between -0.06 (meat) and 0.67 (dairy) for males and between 0.21 (pulses) and 0.63 (dairy) for females. Correlations in the present study for food groups and food items were much lower, and ranged between -0.03 (bread) and 0.59 (combined dishes). Contrary to the Finish study, it seems as if participants in the present study reported infrequently consumed, luxury food items (meat) erroneously, while reporting more accurately on items consumed daily (i.e. cereal and combined maize dishes). This is a positive outcome, since the test method was designed mainly to report on maize intake, which makes up the bulk of the diet and is the major source of fumonisins.

Validity assessment of the RAPP method indicated acceptable agreement at group level for all food groups. However, agreement at individual level tested poorly for bread and cereals. Combined dishes, meat and condiments showed good agreement at individual level between the methods. Bias was found to be absent in most of the food groups tested, except for beverages. Other researchers who have reported poor correlations for micronutrients were Tjønneland *et al.*, (1991:909), Katsouyanni *et al.*, (1997:S122), Jain *et al.*, (2002:82), Rodrigues *et al.*, (2002:694) and Fornés *et al.*, (2003:823).

When chance agreement was included, combined dishes, meat and condiments were repeatedly ranked in the same tertiles, while bread, cereal and beverages were poorly ranked. Only six nutrients were repeatedly ranked in the same tertile (CHO, folate, thiamine, vitamin D, Ca and Fe). However, more than 75% of participants were classified as falling within two adjacent tertiles, for the majority of nutrients, except for fat and vitamin E. Although the ideal is that more than 50% of participants be classified in the same tertile, Masson *et al.*, (2003:319) reported prevalences of 22% - 61% for males and 35% - 78% for females lying in the same tertile. These results are similar to those found in the current study. The level of correct classification as well as the level of misclassification was good for CHO in the current study. According to the National Food

Consumption Survey (NFCS) 65% – 70% of the energy consumption of South African children (0 - 9 years) is from CHO and therefore accuracy when measuring CHO consumption is very important (Nel & Steyn, 2002).

Bland-Altman data showed the absence of reporting bias for all food groups except for beverages. Additionally, Bland-Altman data illustrated the absence of reporting bias for 11 nutrients while the other nine nutrients (energy, protein, CHO, folate, Fe, Mg, Na, Se and Zn) showed reporting bias. Limits of agreement were wide for all food groups and the majority of the nutrients. Very few published studies to date have used Bland-Altman plots to determine validity of their dietary assessment methods and therefore it is difficult to make comparisons. Agreement between methods was not always acceptable (> 95% of the participants within the LOA) and the LOA were frequently clinically too wide (> 1 x RDA) to be considered acceptable.

The agreement between methods for vitamin C in the current study was considered as being very good (100% agreement, narrow LOA, mean difference close to zero) compared to that found by Seele, (2007:91) who reported LOA > 1 x RDA for male athletes, a large mean difference and poor agreement. This may possibly be due to the fact that adults in the current study had a low vitamin C intake.

However, various factors influenced the above mentioned results. The type of information the test method is expected to provide (group data vs. individual data) is crucial when validating a dietary assessment method. Because of the characteristics of OC, it is important that the test method provides accurate data both at individual and group level. Consequently, in the development of the test method, all efforts were made to simplify the frequency of reporting. Additionally, it should be remembered that FFQs are analysed according to single nutrients per dish, while recalls are analysed according to different recipes (Flegal *et al.*, 1990:1055), making analyses of recalls more precise than those of FFQs. A certain level of error can therefore be expected.

Another source of error relates to the participants themselves. Black *et al.*, (1991:591) refer to an observer effect occurring when participants are aware that they are being studied and consequently may under-report intake in order to appear in a good light. This effect may have been present during the current study, but rather than under-reporting, participants may have over-reported. This may be because of their severe level of poverty.

The poor validation results for micronutrients can possibly be attributed to seasonal changes and the inclusion of ratio photographs. The ratio photographs comprised different quantities of maize mixed with either pumpkin or a green leafy vegetable when available. For logistic and practical reasons data was collected over a period of 12 weeks. During this period seasonal changes may have caused changes in dietary consumption patterns, particularly between the first and last test measurements. These changes would have been mainly due to the low intake of fruits that were

consumed very intermittently (when available). Seasonal differences could also have influenced the availability of vegetables commonly used in the combined dishes such as pumpkin and *imifino*.

A further factor that may have contributed to the validity outcomes is the fact that the validity of the food photograph series in estimation of portion size was found to be poor (Chapter 4). As mentioned before, the small sample size is a major limitation.

## **5. CONCLUSION**

The results of the validity testing of the RAPP method for specified food groups show acceptable validity for cereals (including maize), combined dishes (including maize), meat and condiments, but not for assessing bread or beverage intake. As maize intake reflects fumonisin exposure of people living in rural areas in the EC it can be concluded that the test method (RAPP method) is a valid measure of intake of cereals and combined dishes reflecting maize and fumonisins intake (primary aim of the study).

The results of the validity testing of the RAPP method for nutrients show acceptable validity for total energy, CHO, niacin, thiamine, vitamin D, Ca and Fe, but not for protein, fat, folate, riboflavin, vitamin A, vitamin B<sub>12</sub>, vitamin C, vitamin E, K, Mg, Na, Se and Zn.

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## Chapter 8

# THE DIETARY ASSESSMENT METHOD VALIDATED WITH BIOMARKERS

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A rural house with a typical “*kraal*” and field where maize and vegetables are grown  
(photograph by H.J. Lombard)

## 1. INTRODUCTION

A RAPP method [Quantitative Food Frequency Questionnaire (QFFQ) + Food Photograph Series (FPS)] was developed with the use of focus group discussions and interviews in the rural areas of the Eastern Cape (EC). The photographs were tested to determine respondents' perception about the portion sizes (Chapter 5) and the reliability of the RAPP method was assessed using the test-retest design (Chapter 6). In chapter 7 the validity of the RAPP method was assessed against four 24-hour dietary recalls. In this chapter the validity of the RAPP method is assessed against biomarkers.

Ideally, a newly developed dietary assessment method should be validated against a "gold standard" (Bingham & Day, 1997:1130S). Unfortunately, the weighed record, which is frequently used as the dietary "gold standard" could not be used in the current study because of illiteracy amongst many participants. Four 24-hour recalls were hence used as a reference standard; however, validity may be compromised by errors associated with the newly developed method, as well as the reference method (Cade *et al.*, 2002:575). To account for this, additional criterion methods, independent of the dietary methods, were used for further validation of the RAPP method.

These methods includes 1) doubly-labelled water for energy expenditure (EE), 2) accelerometers for measuring EE, 3) Schofield equations for calculating EE, 4) physical activity questionnaires for measuring EE, 5) urinary biochemical markers and 6) blood biomarkers. Each of the above mentioned methods have different advantages and disadvantages (Addendum 5) and the methods used for validation should be selected with care.

The aim of this part of the research study (Chapter 8) was to assess the validity of the RAPP method using an internationally used physical activity questionnaire [the global physical activity questionnaire (GPAQ)], energy expenditure (EE) from Schofield equations, urinary biomarkers [sodium (Na), potassium (K) and nitrogen (N)] (four 24-hour urine samples per participant) and single blood biomarkers [red blood cell folate, selenium (Se) and vitamin B<sub>12</sub>] (Figure 8.1).



**Figure 8.1. A makeshift “clinic”, where blood was taken to determine the validation of the RAPP method with blood markers**

## **2. MATERIALS AND METHODS**

### **2.1. STUDY POPULATION**

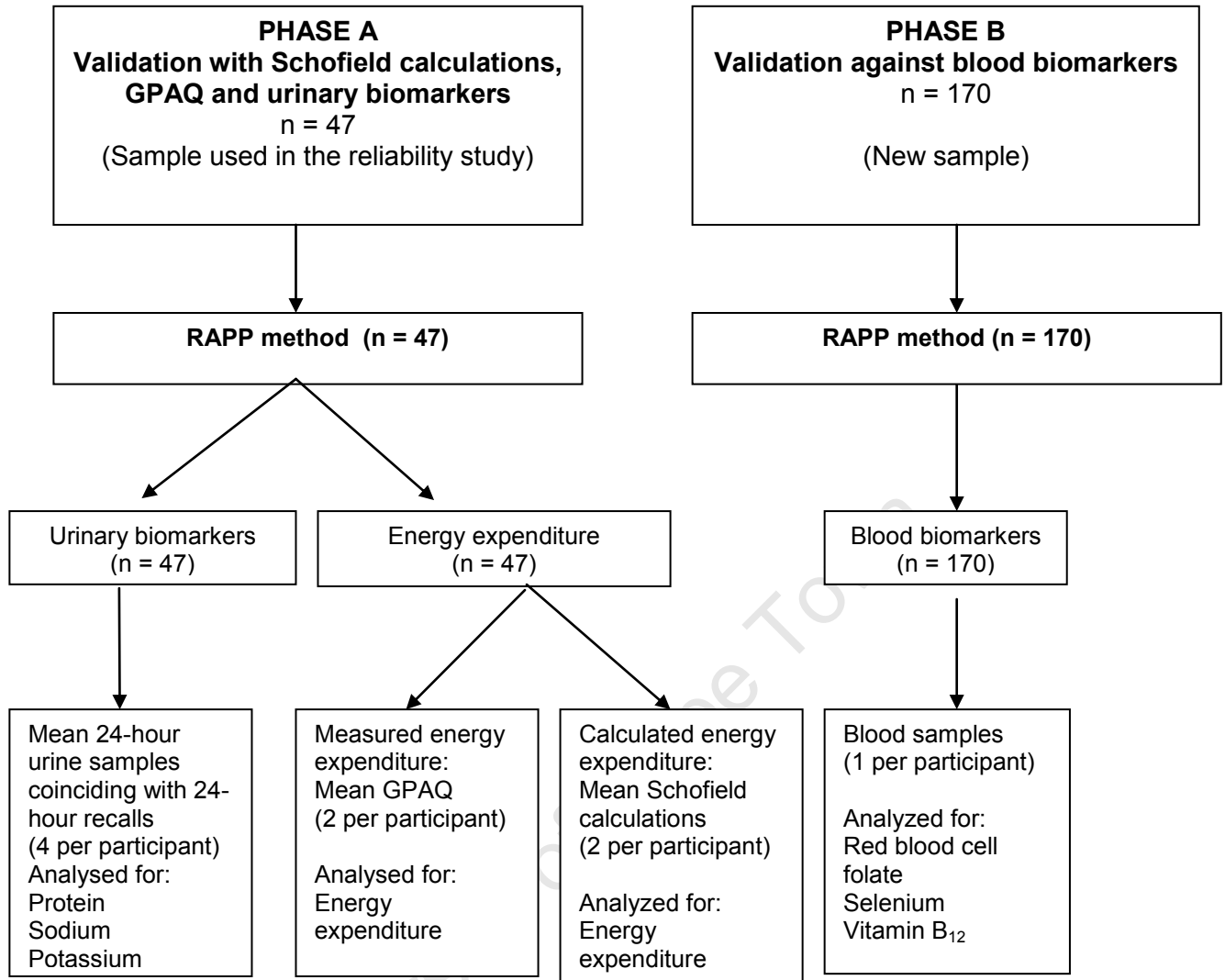
Two different, independent samples were used to further assess the validity of the RAPP method. Figure 8.2 describes the study design used in both phase A and phase B of this part of the study.

#### **2.1.1 Phase A: Validation against physical activity questionnaires, Schofield’s equation and urinary biomarkers**

The study population used in phase A of the validation study is described in Chapter 5, Section 2.1.

#### **2.1.2 Phase B: Validation against blood biomarkers**

A total of 170 new volunteers (males and females between the ages 18 and 65 years) who had not participated in any other phases of the dietary research, were recruited from five rural villages in Centane. These participants were volunteers recruited using snowball sampling. They also participated in the parent project run by the Medical Research Council (MRC) Programme for Mycotoxins and Experimental Carcinogens Unit (PROMEC). People with a diagnosed chronic disease and those who did not fit the age profile (18 – 65 years) were excluded from the study. Information about the study was provided to participants’ in their first language (isiXhosa) and consent was obtained from each participant before inclusion into the study (Chapter 1).



**Figure 8.2 Steps taken during the validation of the RAPP method with biomarkers**

## 2.2. PROCEDURES

### 2.2.1 Phase A

Participants were visited at four separate times, twice in March and twice in June 2004 (Table 8.1). During these visits anthropometric measures were taken, questionnaires completed and urine samples collected. The aim was to collect four sets of 24-hour urine samples per participant, one day prior to the interview for each 24-hour recall assessment.

**Table 8.1. Procedures followed during phase A of the validation testing of the RAPP method**

| Visit 1 (March)           | Visit 2 (March)              | Visit 3 (June)          | Visit 4 (June)                               |
|---------------------------|------------------------------|-------------------------|--|
| Information               |                              |                         |  |
| Consent                   |                              |                         |  |
| Demographic questionnaire |                              |                         |  |
|                           | RAPP method<br>(Reliability) |                         | RAPP method<br>(Reliability and<br>validity) |
| GPAQ                      |                              | GPAQ                    |  |
| 24-hour urine             | 24-hour urine                | 24-hour urine           | 24-hour urine                                |
|                           | Hunger<br>questionnaire      | Hunger<br>questionnaire |  |
| Anthropometry             |                              | Anthropometry           |  |

Each participant received a urine collection kit, to keep for the duration of the study (Figure 8.3) for urine collection purposes. The kit included a urine collection tag; a pictorial pamphlet indicating when to collect the urine; a pin reminding them to collect urine during the whole day; and a container for the urine. Participants were instructed to collect urine from 6:00 in the morning until 6:00 the following morning on the specific day of collection. The total 24-hour volume was measured and recorded (Figure 8.4). Three five ml aliquots were stored at  $-20^{\circ}\text{C}$  for analysis (MacIntyre *et al.*, 2001b:64). Aliquots were clearly labelled and stored in a sealed plastic bag, which was marked with the participant's number. In order to encourage complete 24-hour samples, participants each received a gift (food parcel) at the end of the four 24-hour urine collections. This was regarded as a donation for the many hours which the participants had spent participating in the study.

Anthropometric measurements were taken in order to calculate EE with the Schofield equations. Techniques were described in Chapter 3 (Section 4.3) (Figure 8.5).

Five questionnaires were administered during phase A, including the newly developed RAPP method, four 24-hour recalls (reference method), the socio-demographic questionnaire and the GPAQ.



Figure 8.3. The urine collection kit participants received during validation of the RAPP method



Figure 8.4. Urine sample volumes were recorded before being stored and transported to Cape Town



**Figure 8.5. Anthropometric measurements taken during phase A and phase B to calculate energy expenditure using the Schofield equations**

### 2.2.2 Phase B

Participants were recruited at their homes and asked to meet the research team at a central point (local shop) the following day. Different research stations were established and participants moved from one to the other (Figure 8.6). These stations included an information and consent location, a station for questionnaire completion (socio-demographic questionnaire and RAPP method), and a station where blood was drawn (Figure 8.7). At completion of all tests participants received a snack and beverage.

Two questionnaires were administered during Phase B, including the newly developed RAPP method and the socio-demographic questionnaire.



Figure 8.6. The different stations set up to collect information during the cross sectional study



Figure 8.7. A trained nurse drawing blood from a participant

### 2.3. URINARY AND BLOOD ANALYSES

Urine was analysed by National Health Laboratories Services (NHLS) in Cape Town. Potassium and Na urinary results are not influenced by other factors (faecal losses etc.) (Bates *et al.*, 1997:189). Twenty four hour urinary K and Na were analysed using the direct ion selective electrode (ISE) method. Nitrogen was analysed using urease enzymatic conductivity rates<sup>7.1</sup> and the Kjeldahl method<sup>7.2</sup>. The equivalent protein intake

<sup>7.1</sup> Urease enzymatic conductivity rates are a process where changes in the electrical conductivity of a specific solution containing the reaction products of a urea-urease reaction can be monitored.

and nitrogen content of the urine sample ( $N_u$ ) was adjusted for skin and faecal losses using the formula:

$$\text{Nitrogen intake} = \frac{\text{Protein intake (g/24h)}}{6.25 \text{g Protein / g nitrogen}}$$

Nitrogen output = Urinary Urea Nitrogen (g/24-hour) + 4g Nitrogen (Czajka-Narins, 1992:303).

$$\text{Protein intake} = 6.25(N_u + 2) \text{ (Isaksson, 1980:4)}$$

Urinary urea ( $N_u$ ) was determined to calculate protein intake (Table 8.2) and it was assumed the participants were in nitrogen balance (no gain due to growth *etc.* and no loss to starvation or dieting) (Czajka-Narins, 1992:303, Bingham, 2003:922).

Average daily creatinine values are 0.6 – 1.2 mg/dl (53 – 106  $\mu\text{mol/L}$ ) ideal body weight for males and 0.5 – 1.1 mg/dl (44 – 97  $\mu\text{mol/L}$ ) for females. Urine samples with creatinine values lower than these values were excluded (Litchford, 2008:415), as well as urine volumes less than 500 ml.

Although para-amino benzoic acid (PABA) is a more sensitive way of measuring urine to verify completeness, this method was deemed as not being appropriate for the current study because traditionally people in this region would be suspicious of taking “medicine” from strangers. The research team was well aware of the fact that creatinine excretion is less accurate, but it was considered to be the only practical method to use in this population.

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This is monitored without interference from the enzyme as it passes a urea solution through a column containing immobilized urea. The conductivity of the solution is measured after passage through the column, and compared with either the conductivity of the urea solution before passage through the column or against the conductivity of a standard urea solution (United States Patent 3915804, 2010)..

<sup>7.2</sup> Samples are digested in  $\text{H}_2\text{SO}_4$  to produce ammonia. The ammonia is then distilled into a saturated boric acid solution (an alkaline) and titrated with a standardized hydrochloric acid solution (Konstantinides *et al.*, 1988:2519).

**Table 8.2. Calculations and cut-offs used during the validation of the RAPP method with energy expenditure and biomarkers**

| Calculations   | Reference   |
|--|---|
| $El_{(rep)} = TEE_{(calc)}$<br>Total calculated energy expenditure   | Black 2000:1119   |
| <b>Total energy expenditure</b>  | Livingstone & Black, 2003:897S  |
| $TEE_{(calc)} = BMR_{(calc)} \times PAL_{(calc)}$<br><b>Basal metabolic rate</b><br>Adults: 18 to 30 years:<br>Male = $0.063 (\text{weight}) - 0.042 (\text{height}) + 2,953$<br>Female = $0.057 (\text{weight}) + 1.184 (\text{height}) + 0.411$<br>Adults: 30 to 60 years:<br>Male = $0.048 (\text{weight}) - 0.011 (\text{height}) + 3.670$<br>Female = $0.034 (\text{weight}) + 0.006 (\text{height}) + 3.530$<br>BMR is given in MJ / 24 hour, weight in kilogram and height in meter | Schofield, 1985:19  |
| <b>Physical activity level</b>   | FAO/WHO/UNU 1985:78   |
| The physical activity level was determined as 1.55, according to WHO guidelines for 'light' activity.  |   |
| <b>Goldberg cut-off points</b>   |   |
| PAL is expressed as $El_{(rep)} : TEE_{(calc)}$  |   |
| Goldberg cut-off points < 1.35: under reporters  | Goldberg <i>et al.</i> , 1991:573   |
| Goldberg cut-off points 1.35-2.39: normal reporters  |   |
| Goldberg cut-off points 2.4: over-reporters  | Goldberg <i>et al.</i> , 1991:575   |
| <b>Global physical activity questionnaire (TEE<sub>meas</sub>)</b>   | Ainsworth <i>et al.</i> , 2000  |
| The following MET scores were used:  | Department of Chronic Diseases and Health Promotion Surveillance and Population-Based Prevention, WHO |
| Moderate PA = 4.0 METs   |   |
| Vigorous PA = 8.0 METs   |   |
| Transport related walking / cycling = 4 METs   |   |
| MET-minutes were determined by the following formula:  |   |
| MET-minute = Frequency in days X (time in minutes X MET value).  | Bull, 2003.   |
| <b>Urinary nitrogen</b>  |   |
| Protein intake = $6.25 (N_u + 2)$  | Isaksson, 1980:4  |
| Where $N_u$ is the 24-hour $N_u$ output (g) and 2 is the average daily extra renal nitrogen losses: (1 g for faecal losses and 1 g for dermal losses)  |   |
| $El_{rep}$ = Energy intake (reported)<br>$TEE_{calc}$ = Total Energy Expenditure (Calculated)<br>$BMR_{calc}$ = Basal Metabolic Rate (Calculated)<br>$PAL_{calc}$ = Physical Activity Level (Calculated)<br>WHO = World Health Organisation<br>$TEE_{meas}$ = Total Energy Expenditure (measured)<br>MET = Metabolic Equivalent score<br>$N_u$ = Nitrogen (urine)  |   |

Venous blood (20 ml) was drawn into aliquots (with the vacutainer system<sup>7.3</sup>) by a registered nursing sister. Special containers for the safe disposal of contaminated needles and alcohol swabs were used. The participants' study numbers were indicated on the aliquots to ensure anonymity. For the preparation of serum, 15 ml of blood was allowed to clot at room temperature in glass tubes, followed by centrifugation at 3000 revolutions per minute (rpm) for 15 minutes. For the preparation of plasma, 5 ml blood was mixed with ethylenediaminetetraacetic acid (EDTA<sup>7.4</sup>) (purple top tube) and was then centrifuged at 3000 rpm for 10 minutes. Serum and plasma aliquots were stored at -20<sup>0</sup> in a field freezer. Blood was analysed by the NHLS in Cape Town. Selenium, vitamin B<sub>12</sub> and red blood cell folate were analysed with high performance liquid chromatography (HPLC<sup>7.5</sup>) method.

## 2.4. CALCULATIONS FOR ENERGY EXPENDITURE

### 2.4.1. Calculation of energy expenditure (Phase A) and identification of under- and over-reporters

Total calculated energy expenditure [EE<sub>(calc)</sub>] was calculated for each participant by multiplying the calculated basal metabolic rate [BMR<sub>(calc)</sub>] with the calculated physical activity level [PAL<sub>(calc)</sub>]. Although participants were classified as farmers, their work periods are sporadic during the year according to season. During the time of the study participants were observed to be inactive since no farming activities were taking place. Low physical activities amongst rural people (in a similar study) were also reported by Kruger *et al.*, (2003:16). It was therefore decided to use the PAL for light activity (PAL = 1.55). Table 8.2 provides all the calculations used to determine TEE<sub>calc</sub>.

Goldberg cut-off points were used to determine the percentage of participants who under- and over-reported (Goldberg *et al.*, 1991:573, Black, 2000:395) (Table 8.2). The PAL was expressed as a ratio EI<sub>(rep)</sub>: TEE<sub>(calc)</sub>. Goldberg cut-off points less than 1.35 are indicative of under-reporters, Goldberg cut-off points between 1.35 – 2.39 indicate normal reporters and Goldberg cut-off points larger than 2.4 are indicative of over-reporters. It should be kept in mind that the Goldberg cut-off points have limitations, including 1) the cut-off points assume that everybody in the population has a sedentary lifestyle; 2) the equations assume everybody in the population weighs a maximum of 84 kg (which is not the case in

<sup>7.3</sup> Vacutained tubes are evacuated collection tubes that are designed to fill with a predetermined volume of blood by vacuum (Ahmed, 2010).

<sup>7.4</sup> EDTA is a powerful anticoagulant (Ahmed, 2010).

<sup>7.5</sup> HPLC is a mechanism that separate molecules according to their solubility in water, solubility in organic solvents, net positive charge, net negative charge, or size (Bird, 1989:787).

the current study); 3) it assumes that everybody in the population is in energy balance ( $EE = EI$ ) (Gibson, 2005:169).

#### 2.4.2. Measured energy expenditure (Phase A)

Measured energy expenditure ( $TEE_{meas}$ ) was determined using the GPAQ (Armstrong & Bull, 2006:66). The GPAQ was administered twice and the mean  $TEE_{meas}$  was used. The GPAQ was developed by the World Health Organisation (WHO) to improve physical activity surveillance in different developing countries. The questionnaire includes 16 questions on physical activity, including sedentary behaviour, activities at work, travel activities and recreational activities (Armstrong & Bull, 2006:66). Metabolic equivalent (MET) scores are generally used to describe the intensity of physical activity and consequently the GPAQ data is analysed using MET scores. One MET equivalent is the “ratio of the working metabolic rate in relation to resting metabolic rate,” with one MET equivalent being “the energy needed to sit quietly and being equivalent to 1 kcal/kg/hour.” It can therefore be deduced that compared to sitting still, energy consumption is four times as high with moderate activity and eight times as high with vigorous activity (Armstrong & Bull, 2006:66). In other words, the GPAQ assigns 4 METs for moderate activities and 8 METs for vigorous activities. In the current population at the time of the study the physical activities reported most commonly were carrying water from the river (Figure 8.8) and working in the field (Figure 8.9).



Figure 8.8. A rural woman in the Eastern Cape carrying river water home.



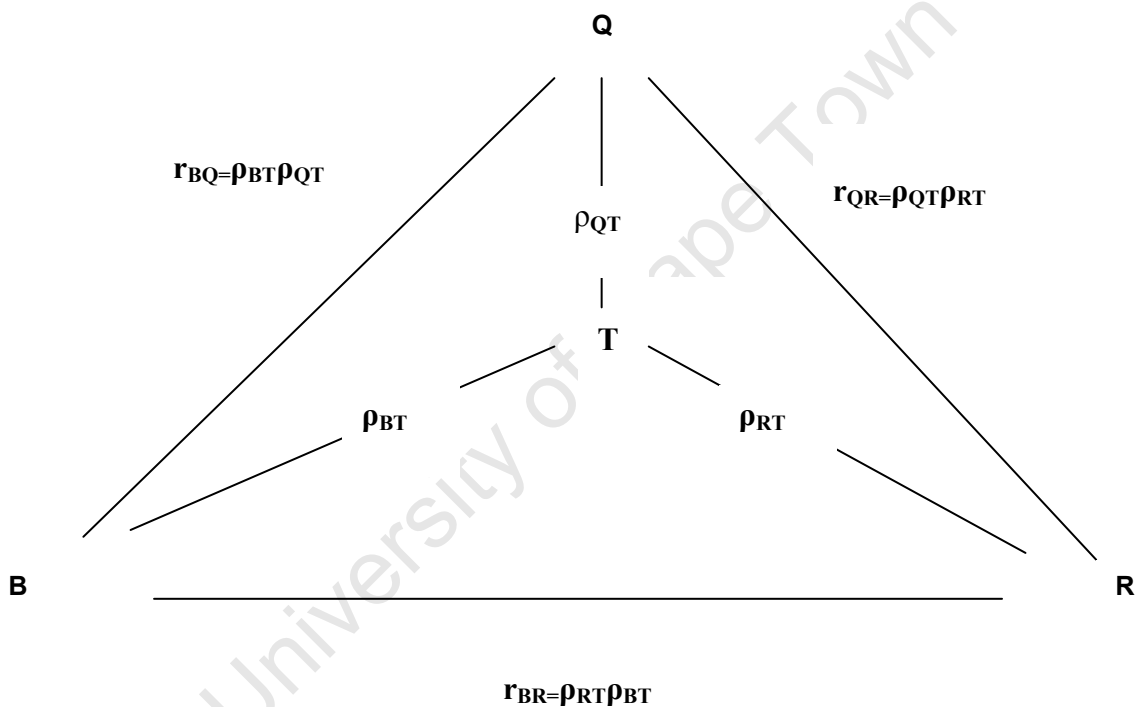
**Figure 8.9. A rural Eastern Cape family working in the field next to their home**

## **2.5. STATISTICAL ANALYSES**

Energy, K, Na and protein intake derived from the RAPP method (Phase A) were statistically compared with  $EE_{\text{calc}}$ ,  $EE_{\text{meas}}$ ,  $N_u$ , K and Na biomarkers (urinary levels) and Se, folate and vitamin B<sub>12</sub> intake reported on the RAPP method were compared with the relevant blood (red blood cell levels) biomarkers (Phase B) (Figure 8.2).

Statistical methods used were discussed in detail in Chapter 3, Section 5. In summary data was tested for normality with the Shapiro Wilk test, after which the Wilcoxon signed rank test and Spearman correlation coefficients were computed. Participants were then classified into tertiles, weighted Kappa statistics were conducted and Bland-Altman data was calculated.

Finally, the method of triads was also used (Figure 8.10) to determine the validation coefficient (VC). The method of triads included the following calculations: Spearman correlation coefficients between all three methods were determined. The VCs between the true intake, and the estimated intake using different methods, were determined by generating correlation coefficients from the formulas indicated in Figure 8.10. The VCs obtained for each assessment method (RAPP method, recalls and biomarkers) illustrate agreement between that method and the actual true intake. However, in some cases the VC might be greater than one (Heywood cases). In such a case the VC can be set at one. Heywood cases usually occur when the product of two or three samples is larger than the third (Ocke & Kaaks, 1997:1241S).



$\rho$  = validation coefficient

R = Reference method (Recall<sub>Mean</sub>)

B = Urinary biomarker (Na, K and protein)

T = True validation

Q = RAPP method (RAPP<sub>2</sub>)

The correlations were estimated as follows: (Kaaks, 1997:1236S)

$$\rho_{QR} = \sqrt{r_{BQ}r_{QR}/r_{RB}}$$

$$\rho_{RT} = \sqrt{r_{QR}r_{RB}/r_{BQ}}$$

$$\rho_{BT} = \sqrt{r_{RB}r_{BQ}/r_{QR}}$$

$\rho$  is the validation coefficient (Kaaks, 1997:1236S).

**Figure 8.10. An example of the method of triads for correlation (Kaaks, 1997:1236S)**

A triangular comparison was made between the RAPP method (RAPP method) (Q), the reference method (recalls) (R) and the biochemical marker (urinary or blood) (B). The VC of Na, K and protein from the RAPP method was compared with four 24-hour urinary biomarkers and four coinciding 24-hour recalls. Similarly, folate, Se and vitamin B<sub>12</sub> reported from the RAPP method were compared with single 24-hour recalls and single blood markers (Figure 8.2). The model assumes that measurements Q, R and B are linearly related to the true intake (T) and that their random errors are uncorrelated. The correlations between the measurements can therefore be expressed as products of the measurements' correlation coefficients with the latent true intake factor (Kaaks, 1997:1236S).

Because each statistical method describes a different facet of the validity of the dietary assessment method, and because there are no set criteria for determining the validity of the dietary assessment method, a nutrient is considered to be valid if four or more of the different statistical methods show validity, and poor if three or less show validity.

### 3. RESULTS

#### 3.1. RESULTS FROM PHASE A

Demographic results of the 47 who completed most assessments are reported in Chapter 7 (Section 3).

Of these 47 participants 25 participants did not meet the criteria for complete 24-hour samples (either because of a volume less than 500 ml was collected or due to low creatinine values) (Table 8.3).

**Table 8.3 Summary of complete 24-hour urine results**

| Urine collection                             | Visit 1 | Visit 1-2 | Visit 1-3 | Visit 1-4 |
|--|---------|-----------|-----------|-----------|
| Number of participants with complete samples | 47      | 35        | 26        | 22        |

Means, medians and percentage differences of reported energy intake ( $EE_{rep}$ ) on RAPP method (Phase A) compared with either  $EE_{calc}$  (Schofield's equation) or  $EE_{meas}$  (GPAQ) are presented in Table 8.4. Results of the Wilcoxon signed rank test indicate good agreement, (no significant differences) for energy between the RAPP method with  $EE_{calc}$  (Schofield's equation). However, there appears to be poor agreement at group level for

the RAPP method when compared to  $EE_{\text{meas}}$  (GPAQ) (Table 8.4). The RAPP method over-reported energy intake (12463 kJ) compared to the GPAQ (8889 kJ) at group level.

Mean PD between  $EE_{\text{calc}}$  (Schofield's equation) and reported intake from the RAPP method was acceptable (-12.9%) (Table 8.4). Results however, indicated poor agreement at group level when compared against  $EE_{\text{meas}}$  (GPAQ) (PD 40.2%).

Table 8.5 presents the Goldberg cut-off points for under- and over-reporters identified when using the RAPP method. Overall, the RAPP method resulted in a large number of under-reporters (53.2%). The mean EI:BMR for the RAPP method was 1.34 ( $\pm$  0.7) indicating overall under-reporting (results not included in a table).

University of Cape Town

**Table 8.4. The means, standard deviations, medians, inter-quartile ranges, differences and percentage differences of energy calculated from the RAPP method and energy biomarkers during Phase A**

| Biomarker                                    | EI from RAPP method |                          | EE calculated / measured |                        | Difference<br>(RAPP <sub>2</sub> - Mean calculated energy expenditure) |                         |                      | Percentage difference (%)<br>[(RAPP <sub>2</sub> - Mean calculated energy expenditure) / Mean calculated energy expenditure * 100] |                         |                        |
|--|---------------------|--------------------------|--------------------------|------------------------|--|-------------------------|----------------------|--|-------------------------|------------------------|
|  | Mean (SD)           | Median (IQ Range)        | Mean (SD)                | Median (IQ Range)      | Mean (SD)  | Median (IQ Range)       | 95% CI of difference | Mean (SD)  | Median (IQ Range)       | 95% CI of % difference |
| n = 47                                       |                     |                          |                          |                        |  |                         |                      |  |                         |                        |
| Energy (kJ) n = 47                           |                     |                          |                          |                        |  |                         |                      |  |                         |                        |
| RAPP <sub>2</sub> :Schofield <sub>Mean</sub> | 12463<br>(5854)     | 12475<br>(7686-17091)    | 14315<br>(1561)          | 14262<br>(12948-15321) | -1851<br>(6527)  | -1997<br>(-6861-1902)   | -11313 - 9665        | -12.9<br>(47.1)  | -13.3<br>(-46.3-12.5)   | -76.3-79.9             |
| RAPP <sub>2</sub> :GPAQ <sub>Mean</sub>      | 12463<br>(5854)     | 12475***<br>(7686-17091) | 8889<br>(7429)           | 6650<br>(3201-10737)   | 3574<br>(10288)  | 4994<br>(-2021 - 11657) | -17224-16942         | 40.2<br>(132.3)  | -18.0<br>(-73.4 – 90.6) | -91.6 – 287.3          |

RAPP<sub>2</sub> = Reported nutrient intake from RAPP method conducted in June

EI = Energy intake

EE= Energy expenditure

Schofield<sub>Mean</sub> = Mean energy expenditure calculated from mean height and weight taken in March and June

\* Wilcoxon signed rank t-test significant at p < 0.05

\*\*Wilcoxon signed rank t-test significant at p < 0.01

\*\*\* Wilcoxon signed rank t-test significant at p < 0.001

SD = standard deviation

IQ Range = inter quartile range (25<sup>th</sup> percentile – 75<sup>th</sup> percentile)

CI = 95% Confidence interval

**Table 8.5. Percentage over- and under-reporters with use of the RAPP method**

| Questionnaire | Goldberg cut-off points |                      |                |
|---------------|-------------------------|----------------------|----------------|
|               | Under-reporting         | Acceptable reporting | Over-reporting |
| RAPP method   | 53.2                    | 40.4                 | 6.4            |

Under-reporting : Goldberg cut-off points < 1.35: under-reporters

Acceptable reporting : Goldberg cut-off points 1.35-2.39: normal reporters

Over-reporting : Goldberg cut-off points 2.4: over-reporters

Spearman correlation coefficients between the RAPP method and  $EE_{calc}$  (Schofield's equations) were poor (Table 8.6), indicating poor or no association between the RAPP method and  $EE_{calc}$  (Schofield's equations). Correlation coefficients between the RAPP method and GPAQ for EE were equally poor (-0.23), indicating almost no linear association. However, the correlation coefficients showed a trend towards an inverse linear association, indicating that when one measure was large, the other was small.

Tertile classification was similarly poor for the RAPP method when classifying participants regarding EI compared with  $EE_{calc}$  (Schofield's equation) or  $EE_{meas}$  (GPAQ) (Table 8.6). The percentage of participants correctly classified in the same tertile, was low (23.4 and 25.5%, respectively), however more than 40% fell in the adjacent tertiles for both biomarkers. Results from the tertile classification and the Kappa statistics for  $EE_{calc}$  (Schofield's equation) indicated a poor ability of the RAPP method to classify participants accurately, regardless of chance. Kappa statistics were however acceptable for the RAPP method when compared against the GPAQ ( $EE_{meas}$ ).

Bland-Altman plots for the RAPP method compared with the calculated and measured EE are presented in Figures 8.11 and 8.12; and the actual values are presented in Table 8.6. Spearman's correlation coefficient for the mean energy intake compared with the mean difference was good (close to zero) for the RAPP method compared against  $EE_{meas}$  (GPAQ) (0.00), indicating a total lack of reporting bias (Figure 8.12 and Table 8.6) as well as for  $EE_{calc}$  (Schofield's equation) (0.17). Agreement, in terms of participants lying within the LOA, was acceptable (> 95%) for the RAPP method compared with  $EE_{calc}$  (Schofield's equation), but poor for the RAPP method compared with  $EE_{meas}$  (GPAQ). However, the LOA were extremely wide for the RAPP method compared with  $EE_{meas}$  (GPAQ) as well as for  $EE_{calc}$  (Schofield's equation), and may therefore be considered to be clinically unacceptable.

Final validation results indicated that the RAPP method was not valid in reflecting usual energy intake when compared against  $EE_{calc}$  (Table 8.7) nor when compared against  $EE_{meas}$ .

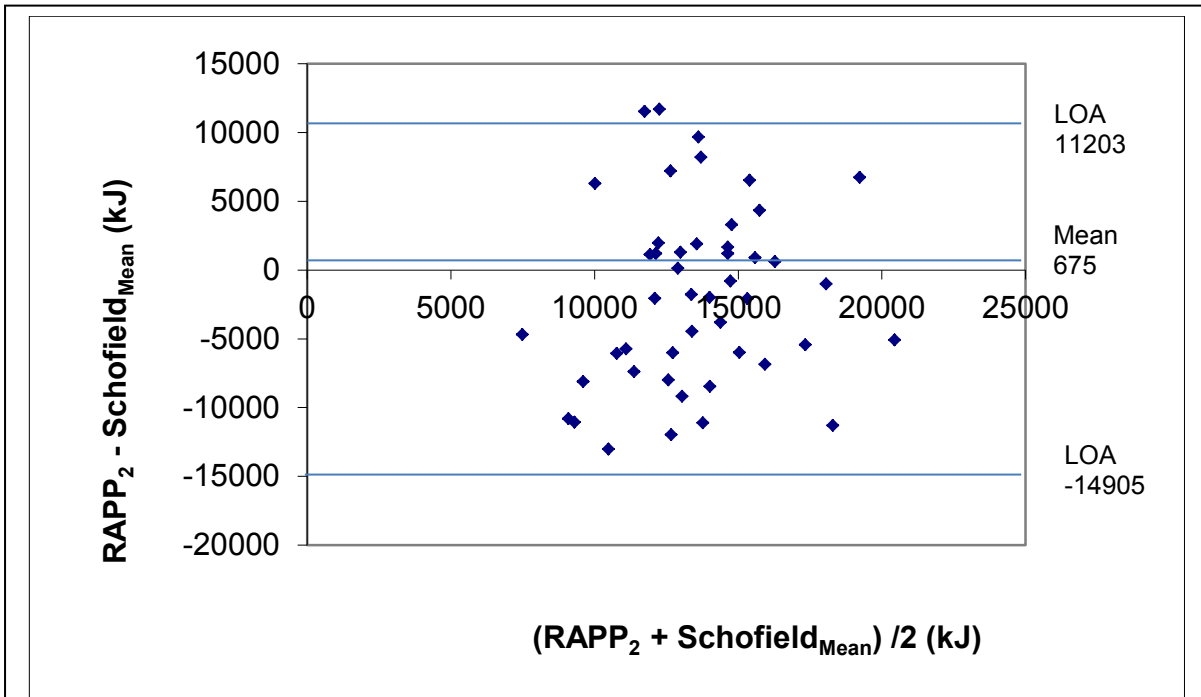


Figure 8.11. Bland-Altman plot comparing reported energy intake and calculated energy expenditure

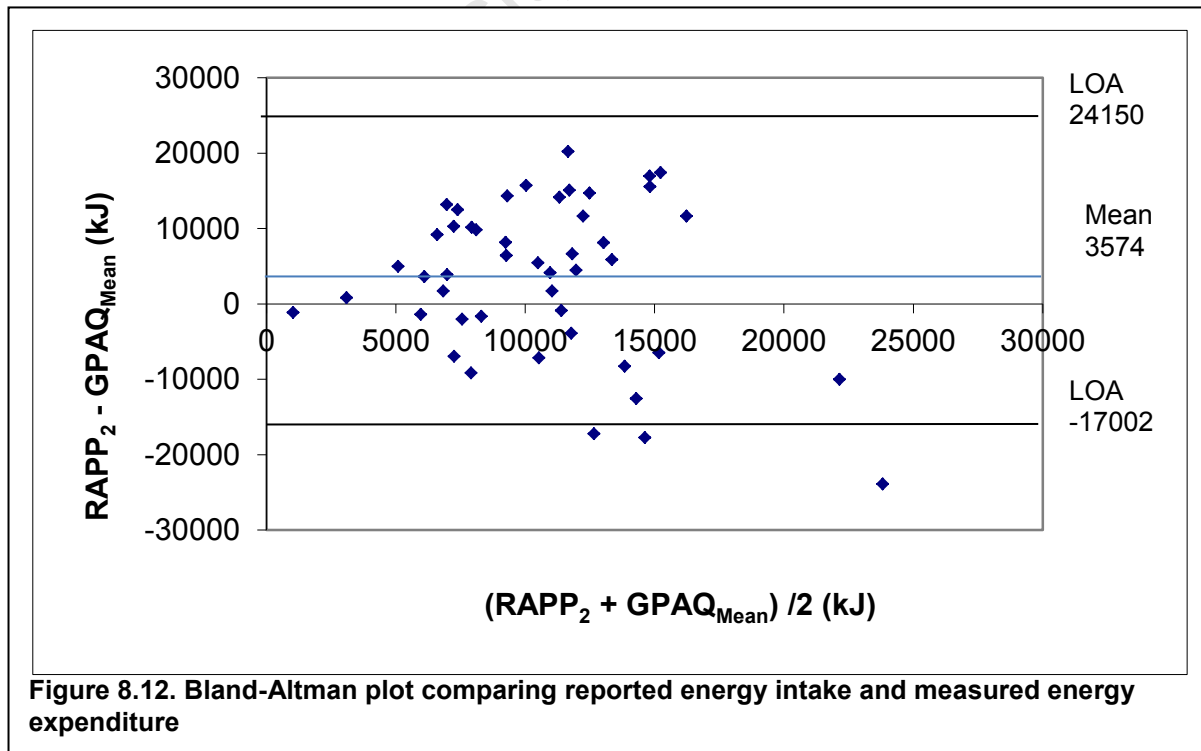


Figure 8.12. Bland-Altman plot comparing reported energy intake and measured energy expenditure

**Table 8.6. Spearman correlation coefficients, tertile distribution, weighted Kappa statistics and Bland-Altman data for biomarkers and RAPP method**

| Biomarker                                    | Spearman correlations | Percentage classified in tertiles |                  |                  | Kw Group | Percentage Agreement | Limits of agreement |             | Spearman correlations | LOA vs RDA        |
|--|-----------------------|-----------------------------------|------------------|------------------|----------|----------------------|---------------------|-------------|-----------------------|-------------------|
|  |                       | Same tertile                      | Adjacent tertile | Opposite tertile |          |                      | Lower limit         | Upper limit |                       |                   |
| n = 47                                       | r                     |                                   |                  |                  |          |                      |                     |             |                       |                   |
| Energy n = 47                                |                       |                                   |                  |                  |          |                      |                     |             |                       |                   |
| RAPP <sub>2</sub> :Schofield <sub>Mean</sub> | -0.35                 | 23.4                              | 42.6             | 34.0             | -0.15    | 95.7                 | -14905.0            | 11233       | 0.17                  | > 1 X RDA (10093) |
| RAPP <sub>2</sub> :GPAQ <sub>Mean</sub>      | -0.23                 | 25.5                              | 47.7             | 29.8             | 0.22*    | 91.5                 | -17002.0            | 24150       | 0.00                  | >1 X RDA (10093)  |

r = Spearman correlation coefficient

\* Spearman rank correlation coefficient significant at  $p < 0.05$

\*\* Spearman rank correlation significant at  $p < 0.01$

\*\*\* Spearman rank correlation significant at  $p < 0.001$

$K_w$  weighted Kappa statistics

Lower limit = mean difference of RAPP and energy expenditure – 2 standard deviations

Upper limit = mean difference of RAPP and energy expenditure + 2 standard deviations

$r_{BA}$  = Spearman correlation between the biomarkers and RAPP method

Significance of the Spearman correlation between mean intake and mean difference

\* Spearman rank correlation coefficients significant at  $p < 0.05$  level,

\*\* Spearman rank correlation coefficients significant at  $p < 0.01$  level,

\*\*\* Spearman rank correlation coefficients significant at  $p < 0.001$  level

# LOA is considered clinical acceptable if it is smaller than mean difference  $\pm 1$  X RDA

RDA Recommended dietary allowance (Standing committee on the scientific evaluation of dietary reference intakes, 1997, 1998, 2000, 2001, 2004, 2005)

**Table 8.7. Summary of statistical results for validation of the RAPP method with energy biomarkers**

| Biomarker   | Agreement                 | Agreement             | Strength of association | Agreement (including chance) | Agreement (excluding chance) | Presence of bias                    | Limits of Agreement         | Final Validity                  |
|---|---------------------------|-----------------------|-------------------------|------------------------------|------------------------------|-------------------------------------|-----------------------------|---------------------------------|
|   | Wilcoxon Signed rank test | Percentage difference | Spearman correlation    | Tertile classification       | Kappa statistics             | Bland-Altman (Spearman correlation) | Bland-Altman (Width of LOA) | > 3 Acceptable validity results |
| Level of validation                               | Group                     | Group                 | Individual              | Individual                   | Individual                   | Individual                          | Individual                  | Individual                      |
| RAPP <sub>2</sub> :Schofield <sub>Mean</sub> (kJ) | Good                      | Acceptable            | Poor                    | Poor                         | Poor                         | Absent                              | Wide                        | Poor                            |
| RAPP <sub>2</sub> :GPAQ <sub>Mean</sub> (kJ)      | Poor                      | Poor                  | Poor                    | Poor                         | Acceptable                   | Absent                              | Wide                        | Poor                            |

Wilcoxon signed rank test agreement at group level  $p < 0.05$  = Good,  $p > 0.05$  Poor

Percentage difference:  $< 10\%$  = Good,  $11 - 20\%$  = Acceptable,  $> 20\%$  = Poor

Strength of association and correlation = Results from correlation coefficient (individual level)

Good =  $> 0.50$ , Acceptable  $0.21 - 0.50$  and Poor  $< 0.20$

Agreement = Results from tertile classification (include chance) and Kappa statistics (exclude chance)

Tertile classification:  $> 50\%$  is same group = Good

Kappa statistics:  $< 0.20$  = Poor,  $0.21 - 0.60$  = Acceptable,  $0.61 - 0.80$  = Good

Bias = Bland-Altman data indication of bias on individual level (if  $r_{BA}$  is significant),

$r_{BA}$  = Spearman correlation coefficient for Bland-Altman data (correlation between mean and mean difference of intake)

Agreement Bland-Altman:  $< 1 \times RDA$  = Narrow LOA,  $\approx 1 \times RDA$  = Acceptable,  $> 1 \times RDA$  = Wide LOA

RDA Recommended dietary allowance (Standing committee on the scientific evaluation of dietary reference intakes, 1997, 1998, 2000, 2001, 2004, 2005)

Final validity\* = Four or more of statistical methods indicate agreement

The Wilcoxon signed rank test results showed no significant differences between the urinary biomarkers (Na, K and protein) and the RAPP method ( $p < 0.05$ ) (Table 8.8). However, PDs were high, ranging between 112.7% and 150.0%. Spearman's correlation coefficients between the reported Na, K and protein intake from the RAPP method compared to the urinary excretion were poor, ranging from -0.31 – 0.10 (Table 8.9). Once again, Spearman correlation coefficients indicated an inverse linear association for K and protein, indicating that when one measure was large the other was small.

Tertile classification was poor when compared against the urinary biomarkers (Table 8.9). Results indicated a poor ability of the RAPP method to accurately classify participants in high or low intake groups, especially for K and protein.

Kappa statistics were also poor (Table 8.9). Results from the tertile classification and the Kappa statistics showed a poor ability of the RAPP method to accurately classify participants, regardless of chance.

Bland-Altman plots for the RAPP method compared with the different biomarkers are presented in Figures 8.13 – 8.15 and Table 8.9. Most participants' observations were within the LOA for each Bland-Altman plot, with the exception of the RAPP method compared with urinary Na. The width of the LOA for Na and K could not be measured as the RDA for these two nutrients has not been established. The LOA were however, wide for protein. Reporting bias was present for all three nutrients.

The VCs between the RAPP method and urinary biomarkers ranged from 0.00 to 1.00 with a large number of Heywood cases, especially for potassium and protein (Table 8.10). Although the VC results indicated that repeated 24-hour recalls may be the best method to measure sodium intake, the RAPP method can also be regarded as being acceptable to measure Na.

**Table 8.8. The means, standard deviations, medians, inter-quartile ranges, differences and percentage differences of selected nutrients calculated from the RAPP method and urinary biomarkers**

| Biomarker                                | Questionnaire  |                      | Biomarker     |                      | Difference<br>(RAPP <sub>2</sub> - Mean urinary results) |                      |                         | Percentage difference (%)<br>[(RAPP <sub>2</sub> - Mean urinary results) / Mean urinary results * 100] |                       |                           |
|--|----------------|----------------------|---------------|----------------------|--|----------------------|-------------------------|--|-----------------------|---------------------------|
|  | Mean<br>(SD)   | Median<br>(IQ Range) | Mean<br>(SD)  | Median<br>(IQ Range) | Mean<br>(SD)   | Median<br>(IQ Range) | 95% CI of<br>difference | Mean<br>(SD)   | Median<br>(IQ Range)  | 95% CI of %<br>difference |
| <b>n = 22</b>                            |                |                      |               |                      |  |                      |                         |  |                       |                           |
| Sodium (mg)                              |                |                      |               |                      |  |                      |                         |  |                       |                           |
| RAPP <sub>2</sub> :Urine <sub>Mean</sub> | 3595<br>(2270) | 3001<br>(2424-3983)  | 1690<br>(791) | 1471<br>(1147-2212)  | 1905<br>(2475)   | 1510<br>(547 - 2750) | -799-7158               | 112.7<br>(149.4)   | 94.1<br>(23.1–153.2)  | -69.7-439.8               |
| Potassium (mg)                           |                |                      |               |                      |  |                      |                         |  |                       |                           |
| RAPP <sub>2</sub> :Urine <sub>Mean</sub> | 2701<br>(1526) | 2503<br>(1660-3441)  | 1044<br>(643) | 846<br>(635-1350)    | 1656<br>(1806)   | 1507<br>(310-2778)   | -820-5085               | 144.9<br>(209.9)   | 55.1<br>(-18.5–357.4) | -89.9-540.9               |
| Protein (g)                              |                |                      |               |                      |  |                      |                         |  |                       |                           |
| RAPP <sub>2</sub> :Urine <sub>Mean</sub> | 67.3<br>(34.0) | 66.0<br>(47.7-91.3)  | 26.8<br>(8.3) | 22.9<br>(20.5-34.1)  | 40.5<br>(36.3)   | 46.3<br>(16.4-60.1)  | -13.9-106.5             | 150.0<br>(176.4)   | 124.1<br>(44.9-318.8) | -51.2 – 483.4             |

RAPP<sub>2</sub> = Reported nutrient intake from RAPP method conducted in June

Mean intake = (RAPP<sub>2</sub> + Mean urinary results) / 2

SD = standard deviation

IQ Range = inter quartile range (25<sup>th</sup> percentile – 75<sup>th</sup> percentile)

CI = 95% Confidence interval

**Table 8.9. Spearman correlation coefficients, tertile distribution, weighted Kappa statistics and Bland-Altman data for urinary biomarkers and RAPP method**

| Biomarker                                    | Spearman correlations | Percentage classified in tertiles |                  |                  | Kw Group | Percentage Agreement | Limits of agreement |             | Spearman correlations | LOA vs RDA        |
|--|-----------------------|-----------------------------------|------------------|------------------|----------|----------------------|---------------------|-------------|-----------------------|-------------------|
|  |                       | Same tertile                      | Adjacent tertile | Opposite tertile |          |                      | Lower limit         | Upper limit |                       |                   |
| <b>n = 22</b>                                | <b>r</b>              |                                   |                  |                  | $K_w$    |                      |                     |             | $r_{BA}$              | (RDA)             |
| Sodium                                       |                       |                                   |                  |                  |          |                      |                     |             |                       |                   |
| RAPP <sub>2</sub> :NaUrine <sub>Mean</sub>   | 0.10                  | 43.5                              | 34.8             | 21.7             | 0.14     | 91.3                 | -3045.0             | 6855        | 0.61***               | RDA ne            |
| Potassium                                    |                       |                                   |                  |                  |          |                      |                     |             |                       |                   |
| RAPP <sub>2</sub> :KUrine <sub>Mean</sub>    | -0.31                 | 19.2                              | 38.3             | 42.6             | -0.23    | 97.9                 | -1956.0             | 5268        | 0.61***               | RDA ne            |
| Protein                                      |                       |                                   |                  |                  |          |                      |                     |             |                       |                   |
| RAPP <sub>2</sub> :ProtUrine <sub>Mean</sub> | -0.23                 | 23.4                              | 46.8             | 29.8             | -0.15    | 97.9                 | -32.1               | 113.1       | 0.87***               | > 1 X RDA<br>(46) |

RAPP<sub>2</sub> with urinary biomarkers n = 22

r = Spearman correlation coefficient

\* Spearman rank correlation coefficient significant at p < 0.05

\*\* Spearman rank correlation significant at p < 0.01

\*\*\* Spearman rank correlation significant at p < 0.001

$K_w$  weighted Kappa

Lower limit = mean difference of RAPP and urinary biomarker results – 2 standard deviations

Upper limit = mean difference of RAPP and urinary biomarker results + 2 standard deviations

RDA Recommended dietary allowance (Standing committee on the scientific evaluation of dietary reference intakes, 1997, 1998, 2000, 2001, 2004, 2005)

$r_{BA}$  Spearman correlation between the mean intake of the RAPP and urinary biomarker results and the mean difference between the RAPP and urinary biomarker results

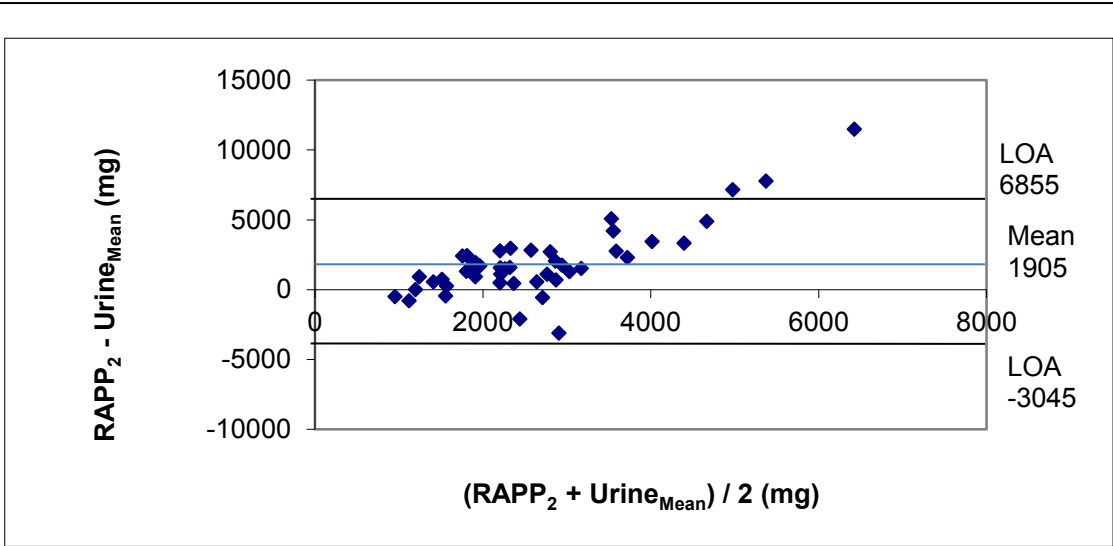


Figure 8.13. Bland-Altman plot comparing reported sodium intake and urinary excreted sodium

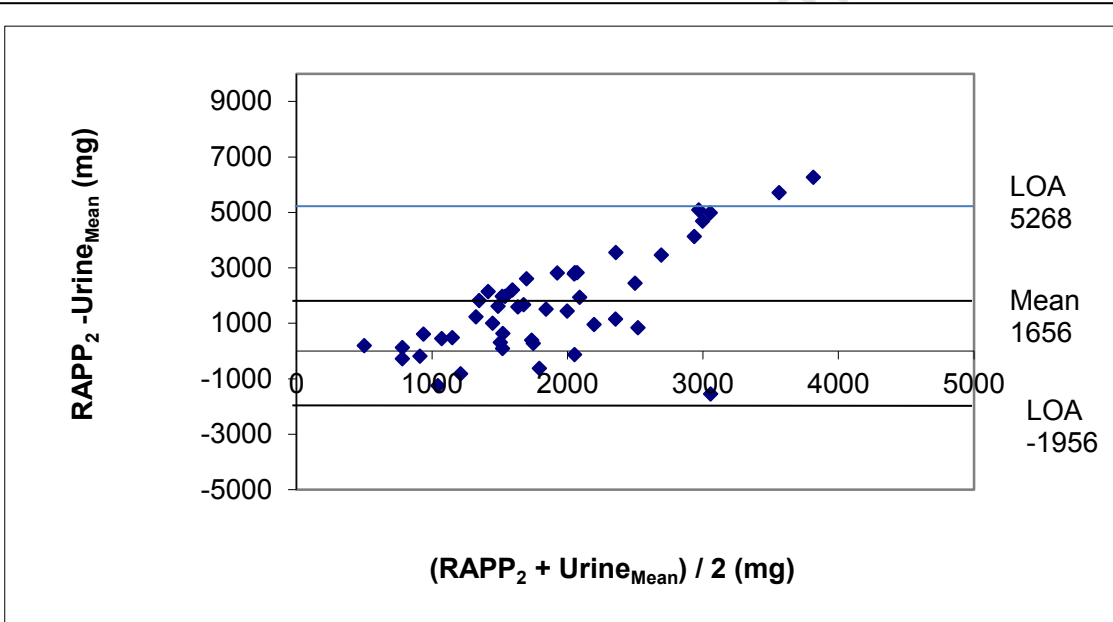


Figure 8.14. Bland-Altman plot comparing reported potassium intake and urinary excreted potassium

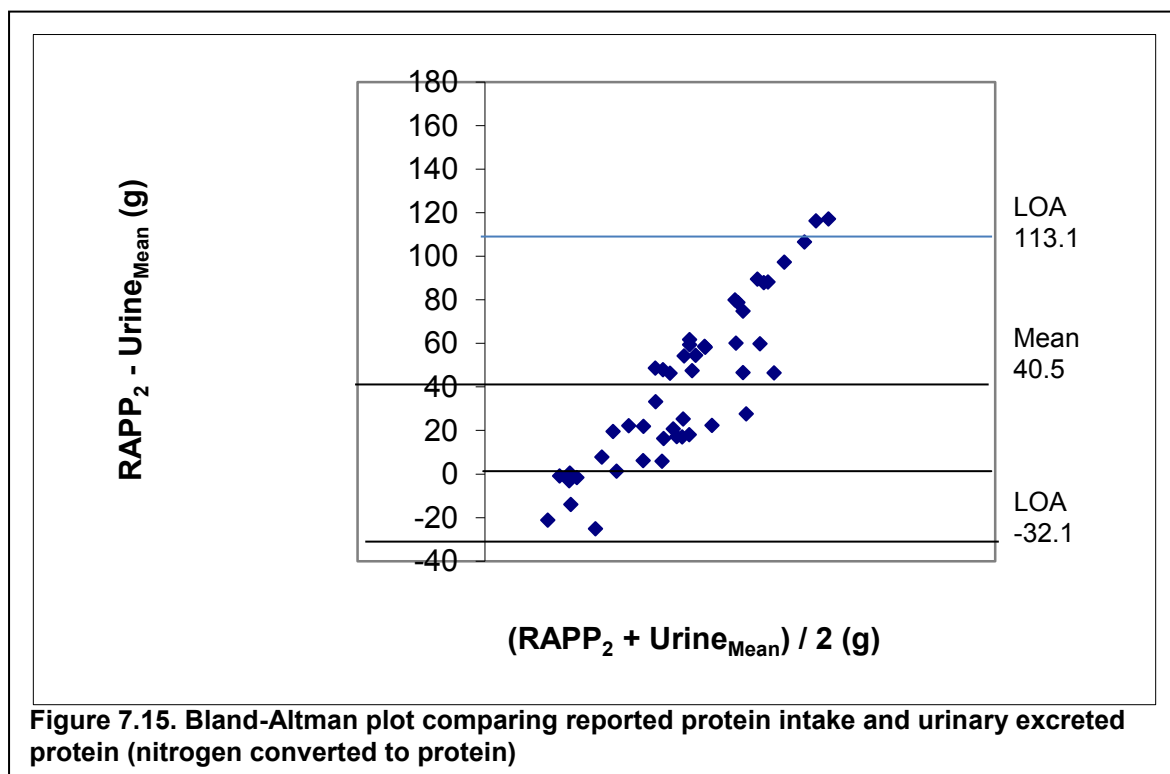


Table 8.10. Validity coefficients for selected nutrients derived from the RAPP method, recalls and urinary biomarkers

| Nutrient  | Biomarker                   | VC                |
|-----------|-----------------------------|-------------------|
| Sodium    | Recall sodium vs. Truth     | 1.00*             |
|           | RAPP sodium vs. Truth       | 0.82              |
|           | Urinary sodium vs. Truth    | 0.82              |
| Potassium | Recall potassium vs. Truth  | 0.00 <sup>^</sup> |
|           | RAPP potassium vs. Truth    | 0.00 <sup>^</sup> |
|           | Urinary potassium vs. Truth | 0.00 <sup>^</sup> |
| Protein   | Recall potassium vs. Truth  | 0.00 <sup>^</sup> |
|           | RAPP potassium vs. Truth    | 0.00 <sup>^</sup> |
|           | Urinary potassium vs. Truth | 0.00 <sup>^</sup> |

Validity coefficients > 1 (Heywood cases) were set to 1.

<sup>^</sup> Validity coefficients < 0.0 (Heywood cases) were set to 0.

**Table 8.11. Summary of statistical results for validation of the RAPP method with urinary biomarkers**

| <b>Biomarker</b>                             | <b>Agreement</b>                 | <b>Agreement</b>             | <b>Strength of association</b> | <b>Agreement (Including chance)</b> | <b>Agreement (Excluding chance)</b> | <b>Presence of Bias</b>                    | <b>Limits of Agreement</b>         | <b>Final validity</b>                     |
|--|----------------------------------|------------------------------|--------------------------------|-------------------------------------|-------------------------------------|--|------------------------------------|---|
|  | <b>Wilcoxon Signed rank test</b> | <b>Percentage difference</b> | <b>Spearman correlation</b>    | <b>Tertile classification</b>       | <b>Kappa statistics</b>             | <b>Bland-Altman (Spearman correlation)</b> | <b>Bland-Altman (Width of LOA)</b> | <b>&gt; 3 acceptable validity results</b> |
| <b>Level of validation</b>                   | <b>Group</b>                     | <b>Group</b>                 | <b>Individual</b>              | <b>Individual</b>                   | <b>Individual</b>                   | <b>Individual</b>                          | <b>Individual</b>                  | <b>Individual</b>                         |
| RAPP <sub>2</sub> :NaUrine <sub>Mean</sub>   | Good                             | Poor                         | Poor                           | Poor                                | Poor                                | Present                                    | Ne                                 | Poor                                      |
| RAPP <sub>2</sub> :KUrine <sub>Mean</sub>    | Good                             | Poor                         | Poor                           | Poor                                | Poor                                | Present                                    | Ne                                 | Poor                                      |
| RAPP <sub>2</sub> :ProtUrine <sub>Mean</sub> | Good                             | Poor                         | Poor                           | Poor                                | Poor                                | Present                                    | Poor                               | Poor                                      |

Wilcoxon signed rank test agreement at group level  $p < 0.05$  = Good,  $p > 0.05$  Poor

Percentage difference:  $< 10\%$  = Good,  $11 - 20\%$  = Acceptable,  $> 20\%$  = Poor

Strength of association and correlation = Results from correlation coefficient (individual level)

Good =  $> 0.50$ , Acceptable  $0.21 - 0.50$  and Poor  $< 0.20$

Agreement = Results from tertile classification (include chance) and Kappa statistics (exclude chance)

Tertile classification:  $> 50\%$  is same group = Good

Kappa statistics:  $< 0.20$  = Poor,  $0.21 - 0.60$  = Acceptable,  $0.61 - 0.80$  = Good

Bias = Bland-Altman data indication of bias on individual level (if  $r_{BA}$  is significant),

$r_{BA}$  = Spearman correlation coefficient for Bland-Altman data (correlation between mean and mean difference of intake)

Agreement Bland-Altman:  $< 1 \times RDA$  = Narrow LOA,  $\approx 1 \times RDA$  = Acceptable,  $> 1 \times RDA$  = Wide LOA

RDA Recommended dietary allowance (Standing committee on the scientific evaluation of dietary reference intakes, 1997, 1998, 2000, 2001, 2004, 2005)

Final validity\* = Four or more of statistical methods indicate agreement

### 3.2 RESULTS FROM PHASE B

Results showed poor agreement at group level for the RAPP method when compared with the blood biomarkers (Se, Folate and vitamin B<sub>12</sub>) (n = 170 single samples) since all three biomarkers had highly significant Wilcoxon signed rank results (Table 8.12). Folate intake was significantly over-reported compared with the blood results, having a PD of 75.2%. On the other hand, the RAPP method under-reported Se and vitamin B<sub>12</sub> compared with the blood results (PD of -38.9% and 72.2%, respectively).

Spearman correlation coefficients between the RAPP method and blood markers were poor (< 0.20) for folate (-0.05) and Se (0.17), indicating little agreement between the blood markers and the RAPP method (Table 8.13). The Spearman correlation coefficient for vitamin B<sub>12</sub> was acceptable (0.26) and indicated agreement between the RAPP method and the blood markers although not a high level of agreement.

Classification of participants into tertiles was poor (< 50%) for all three blood markers compared with the RAPP method; with percentages ranging between 27.5% (Se) and 46.1% (vitamin B<sub>12</sub>) in the same tertiles (Table 8.13). These results indicate a poor ability of the RAPP method to accurately classify participants into high or low intake groups. Kappa statistics were also considered poor (< 0.20) for all blood markers compared with the RAPP method, indicating that the poor tertile classification was not merely because of chance.

Bland-Altman data is presented in Table 8.13. Percentage agreement for all three blood markers was good, with more than 95% of the responses within the LOA. However, the LOA were wide (> 1 x RDA) and not considered clinically acceptable. Regardless of this, no reporting bias was present as the Spearman correlations for all three blood markers were low and not significant.

Finally, the VCs (Table 8.14) for the RAPP method and reference method (4 single 24-hour recalls) and for each blood biomarker were determined according to the method of triads shown in Figure 8.10. Heywood cases occurred for all three variables (RAPP method, reference method and the blood marker) for red blood cell folate and Se. Unfortunately the RAPP method presented with a Heywood case that cannot be interpreted. The VC for Se indicated that the RAPP method is able to accurately measure Se, as results indicated a perfect VC of 1.0. The VCs for vitamin B<sub>12</sub> indicate that the RAPP method was the best method available to determine the unknown true measurement. Both the blood biomarker and the reference method had poor VCs compared with the RAPP method for vitamin B<sub>12</sub>.

**Table 8.12. The means, standard deviations, medians, inter-quartile ranges, differences and percentage differences of selected nutrients calculated from the RAPP method and blood biomarkers during Phase B**

| Biomarker  | RAPP method      |                           | Blood biomarker  |                        | Difference<br>(RAPP – Mean blood values) |                        |                         | Percentage difference (%)<br>[(RAPP - Mean blood values) / Mean<br>blood values * 100] |                         |                           |
|--|------------------|---------------------------|------------------|------------------------|--|------------------------|-------------------------|--|-------------------------|---------------------------|
|  | Mean<br>(SD)     | Median<br>(IQ Range)      | Mean<br>(SD)     | Median<br>(IQ Range)   | Mean<br>(SD)                             | Median<br>(IQ Range)   | 95% CI of<br>difference | Mean<br>(SD)   | Median<br>(IQ Range)    | 95% CI of %<br>difference |
| n = 170  |                  |                           |                  |                        |  |                        |                         |  |                         |                           |
| RAPP:Folate (mcg)<br>n = 170                     | 716.2<br>(463.4) | 597.5***<br>(389.5-896.0) | 445.9<br>(133.3) | 425.5<br>(355.4-524.7) | 380.3<br>(496.0)                         | 137.6<br>(-65.2-447.3) | -344.9 - 1208.6         | 75.2<br>(134.3)  | 36.6<br>(-13.6-123.8)   | -54.5 – 359.6             |
| RAPP:Se (mcg)<br>n = 170                         | 42.9<br>(68.6)   | 28.0***<br>(15.7-51.3)    | 69.7<br>(17.7)   | 70.0<br>(57.0-82.0)    | -26.8<br>(75.1)                          | -13.0<br>(-47.2- 16.7) | -71.4-89.2              | -38.9<br>(106.3)   | -54.9<br>(-77.8- -17.9) | -90.6-36.4                |
| RAPP:Vitamin B <sub>12</sub><br>(mcg)<br>n = 170 | 1.0<br>(2.5)     | 0.4***<br>(0.1-1.3)       | 3.6<br>(1.4)     | 3.4<br>(2.7-4.2)       | -2.6<br>(2.7)                            | -2.6<br>(-3.5- -1.9)   | -5.1 – 0.03             | -72.2<br>(62.5)  | -86.5<br>(-96.4- -64.1) | -100.0- - 1.6             |

RAPP<sub>2</sub> = Reported nutrient intake from RAPP method conducted in June

Biomarker = Mean blood values for each biomarker

\* Wilcoxon signed rank t-test significant at p < 0.05

\*\*Wilcoxon signed rank t-test significant at p < 0.01

\*\*\* Wilcoxon signed rank t-test significant at p < 0.001

**Table 8.13. Spearman correlation coefficients, tertile distribution, weighted Kappa statistics and Bland-Altman data for blood biomarkers and the RAPP method**

| Biomarker                    | Spearman correlations | Percentage classified in tertiles |                  |                  | Kw Group             | Percentage Agreement | Limits of agreement |        | Spearman correlations <sub>BA</sub> | LOA vs RDA         |
|------------------------------|-----------------------|-----------------------------------|------------------|------------------|----------------------|----------------------|---------------------|--------|-------------------------------------|--------------------|
|                              |                       | Same tertile                      | Adjacent tertile | Opposite tertile |                      |                      | Lower               | Upper  |                                     |                    |
| <b>n = 170</b>               | <b>r</b>              |                                   |                  |                  | <b>K<sub>w</sub></b> |                      |                     |        | <b>r<sub>BA</sub></b>               |                    |
| RAPP:Folate                  | -0.05                 | 33.8                              | 37.6             | 28.7             | 0.00                 | 99.4                 | -1626.6             | 2298.2 | 0.05                                | > 1 X RDA<br>(400) |
| RAPP:Se                      | 0.17                  | 27.5                              | 60.6             | 11.9             | -0.04                | 98.1                 | -177.0              | 123.4  | 0.15                                | > 1 X RDA<br>(55)  |
| RAPP:Vitamin B <sub>12</sub> | 0.26**                | 46.1                              | 33.8             | 20.1             | 0.17**               | 98.1                 | -8.0                | 2.8    | 0.01                                | > 1 X RDA<br>(2.4) |

Spearman \* Significance set at  $p < 0.05$

Spearman \*\*Significance set at  $p < 0.01$

Spearman \*\*\* Significance set at  $p < 0.001$

K<sub>w</sub> weighted Kappa

Lower limit = mean difference of RAPP and mean biomarker value – 2 standard deviations

Upper limit = mean difference of RAPP and mean biomarker value + 2 standard deviations

r<sub>BA</sub> Spearman correlation between the mean intake of the RAPP and mean biomarker value and the mean difference between the RAPP and mean biomarker value

**Table 8.14. Validity coefficients for selected nutrients derived from the RAPP method, recalls and blood biomarkers**

| <b>Nutrient</b>   | <b>Biomarker</b>                         | <b>VC</b>         |
|---|--|-------------------|
| Folate  |  |                   |
|   | Recall folate vs. Truth                  | 0.00 <sup>^</sup> |
|   | RAPP folate vs. Truth                    | 0.00 <sup>^</sup> |
|   | Blood folate vs. Truth                   | 0.00 <sup>^</sup> |
| Selenium  |  |                   |
|   | Recall selenium vs. Truth                | 0.22              |
|   | RAPP selenium vs. Truth                  | 1.00 <sup>*</sup> |
|   | Blood selenium vs. Truth                 | 0.14              |
| Vitamin B <sub>12</sub>   |  |                   |
|   | Recall vitamin B <sub>12</sub> vs. Truth | 0.15              |
|   | RAPP vitamin B <sub>12</sub> vs. Truth   | 0.77              |
|   | Blood vitamin B <sub>12</sub> vs. Truth  | 0.34              |
| *Validity coefficients > 1.0 (Heywood cases) = 0.00             |  |                   |
| <sup>^</sup> Validity coefficients < 0.0 (Heywood cases) = 0.00 |  |                   |

A summary of validation results of the RAPP method with blood biomarkers (except for the method of triads) is presented in Table 8.15.

**Table 8.15. Summary of statistical results for validation of the RAPP method with different blood biomarkers**

| <b>Biomarker</b>             | <b>Agreement</b>                 | <b>Agreement</b>             | <b>Strength of association</b> | <b>Agree (including chance)</b> | <b>Agreement (excluding chance)</b> | <b>Bias</b>                                | <b>Agreement</b>                   | <b>Final validity</b>                     |
|------------------------------|----------------------------------|------------------------------|--------------------------------|---------------------------------|-------------------------------------|--|------------------------------------|---|
|                              | <b>Wilcoxon Signed rank test</b> | <b>Percentage difference</b> | <b>Spearman correlation</b>    | <b>Tertile classification</b>   | <b>Kappa statistics</b>             | <b>Bland-Altman (Spearman correlation)</b> | <b>Bland-Altman (Width of LOA)</b> | <b>&gt; 3 Acceptable validity results</b> |
| <b>Level of validation</b>   | <b>Group</b>                     | <b>Group</b>                 | <b>Individual</b>              | <b>Individual</b>               | <b>Individual</b>                   | <b>Individual</b>                          | <b>Individual</b>                  | <b>Individual</b>                         |
| RAPP:Folate                  | Poor                             | Poor                         | Poor                           | Poor                            | Poor                                | Absent                                     | Wide                               | Poor                                      |
| RAPP:Se                      | Poor                             | Poor                         | Poor                           | Poor                            | Poor                                | Absent                                     | Wide                               | Poor                                      |
| RAPP:Vitamin B <sub>12</sub> | Poor                             | Poor                         | Acceptable                     | Poor                            | Poor                                | Absent                                     | Wide                               | Poor                                      |

‡ Percentage of data points between the limits of agreement

Lower limit = mean difference between biomarker and RAPP<sub>2</sub> – 2 standard deviations of the mean difference between the biomarker and RAPP<sub>2</sub>

Upper limit = mean difference between biomarker and RAPP<sub>2</sub> + 2 standard deviations of the mean difference between the biomarker and RAPP<sub>2</sub>

r<sub>BA</sub> = Spearman correlation between the mean of photographic weight and dished-up weight and the mean difference between the photograph weight and dished-up weight

Significance of the Spearman correlation between mean intake and mean difference

\* Spearman rank correlation coefficients significant at p < 0.05 level,

\*\* Spearman rank correlation coefficients significant at p < 0.01 level,

\*\*\* Spearman rank correlation coefficients significant at p < 0.001 level

# LOA is considered clinical acceptable if it is smaller than mean difference ± 1 X RDA

## 4. DISCUSSION

The validity of RAPP method was assessed against four 24-hour recall questionnaires (Chapter 7) to determine the size and the direction of reporting errors. The recalls were chosen as being the most appropriate reference method available for this specific population group. Validity against the recalls was good for measuring food group intake but less so for measuring nutrient intake. However, as both dietary methods rely on the memory of participants, which may result in related reporting bias, it was important to compare the RAPP method with further reference values that did not involve memory. Hence, it was decided to compare the RAPP method with biomarkers, including EE; urinary and blood biomarkers. The main objective of this part of the study was therefore to validate the RAPP method against biomarkers other than dietary assessment methods.

The RAPP method showed poor validity when compared against both  $EE_{\text{calc}}$  (Schofield's equation) and  $EE_{\text{meas}}$  (GPAQ). Spearman correlation coefficient results showed no agreement with coefficient values being negative, reflecting an inverse association (although insignificant).

The  $EE_{\text{meas}}$  (GPAQ), also performed poorly on all the validation tests when compared with the RAPP method (RAPP method). It is possible that the error lies with  $EE_{\text{meas}}$  (the GPAQ) rather than the dietary assessment method. From a researcher's perspective, it appeared throughout the study that participants did not fully understand the GPAQ questions even though they were repeatedly explained. In particular participants had difficulty in separating leisure and work time and in understanding the concept of "usually." This might explain the inverse linear association found with the Spearman correlation coefficients.

The mean EI:BMR for the RAPP method was 1.34 ( $\pm$  0.71), while the four recalls showed a mean of 1.48 ( $\pm$  0.2). The latter is similar to a study on black adults in North West Province (MacIntyre *et al.*, 2000:68) which reported EI:BMR ratios of 1.42 ( $\pm$  0.43) for the FFQ and 1.47 ( $\pm$  0.43) for weighed records that were used as reference method. Overall, in all these studies the ratio tended to be low and closer to that of under-reporting (<1.35) than the normal range (1.35 - 2.39), as was found for the RAPP method in this study.

A review conducted by Black (2000:395) concluded that adults mostly under-report food consumption in all ethnic groups. Black *et al.*, (1991:585) compared EI of participants in 37 different studies. In the present study 53% of participants were classified as being under-reporters. In a study done by Black (2000:401) among black adults in the United States of

America (USA), it was found that 3% of participants over-reported intake, 67% reported acceptable intake, while 30% under-reported intake. In one of the few studies in South Africa in this regard, in black adults, Steyn *et al.*, (2001:25) conducted four recalls per adult in a rural area of Limpopo Province (Dikgale) and found that 54% of participants under-reported. They concluded that this was mostly because of the high prevalence of underweight in this population, and suggested that the cut-off points be adjusted for rural non-Western areas. Mennen *et al.*, (2000:286) also investigated differences in energy intake reporting among African participants in Cameroon in different settings. Their results indicated that rural participants were the least likely to under-report (6%). However, the cut off values for under-reporters used by them were very low (1.15) compared to the 1.35 cut-off value suggested by Goldberg (1991:573).

Although participants in the present study included a similar number of under-reporters to those in the Dikgale area, the average BMI was not as low as that found in Dikgale (Steyn *et al.*, 2001:25). Results from the OPEN study (Millen *et al.*, 2009:1201) found that low energy reporters mostly under-reported the frequency of consumption of food groups. According to them, results did not differ by gender or by BMI. Bahareh *et al.*, (2008:119) on the other hand found a strong association between BMI and the level of under-reporting.

When interpreting under-reporting results the limitations of using Goldberg's cut-off points also need to be interrogated. The 1.55 x BMR is based on the assumption of a sedentary lifestyle. This is used in an attempt to avoid over-estimating the under-reporters (Black, 2000:396).

Urinary biomarkers were used as a further step in the validation of the RAPP method because unlike recalls, that also involve recall bias, the biomarkers include independent errors (Kaaks, 1997:232S) and serve as the least subjective measurement (Marshall, 2003:883S). Biomarkers (urinary, etc.) are not gold standards and have their own set of limitations, therefore they should be used in conjunction with other validation methods (Potischman, 2003:875S).

Expected correlations between reported intake (RAPP method) and urinary excretion should be around 0.5 (Bingham, 2003:924S), while the results in this study were much lower. MacIntrye *et al.*, (2000:68) and Day *et al.*, (2001:312) reported similar low correlations in rural and urban participants. One explanation for the poor correlations could relate to

incompleteness of urine samples. Williams and Bingham (1986:21) concluded that the inclusion of incomplete urine samples could lead to large underestimations of Na and K.

Verification of completeness and the number of repeats of urinary samples was very problematic in this study. Using creatinine excretion as an indication of completeness was not effective. Creatinine excretion is correlated with creatinine intake (mostly meat), and is therefore not always an accurate measure of completeness (Bingham, 2003:924). Williams and Bingham (1986:16) reported creatinine levels of 16 mmol/l (males) and 11.2 mmol/l (females) for complete urine samples. In the current study creatinine excretion was much lower than normal values for most participants, which could be because of low animal protein consumption as is reflected in the low reported portion sizes from the recalls.

*Para*-amino benzoic acid (PABA) is a more accurate measure of completeness of 24 hour urine samples. Using the PABA was, however, not possible in this particular population because of cultural and educational limitations. MacIntyre *et al.*, (2001:69) and Charlton *et al.*, (2008:86) used PABA tablets in similar populations and also reported high levels of incomplete samples (64%).

As a compromise, total urine volume was also used as an indicator of completeness. Samples less than 500 ml were considered as too little to realistically represent a 24-hour urine sample. Based on this premise it was evident that a large portion of urine samples were incomplete. As also explained by MacIntyre *et al.*, (2001:69) the reasons for these incomplete samples might have been as a result of participants not understanding the collection methods or the importance of a 24-hour collection, as well as difficult circumstances for collecting the urine, such as working and a lack of privacy.

The use of 24-hour urinary nitrogen could be effective as part of the validation of dietary assessment instruments. This is the most frequently used and best known biomarker (Bingham, 2003:921S). However, urinary nitrogen is based on the assumption that participants are in a nitrogen balance (Bingham, 2003:922S). In this study it was difficult to determine whether participants were in nitrogen balance. Although they were not prone to dieting and were grown adults, there was a risk of hunger. Regardless of this, there was no real change in the weight during the two visits ( $68.1 \pm 15.6$  kg, versus  $69.9 \pm 16.3$  kg).

The RAPP method is based on a habitual intake of one month, whereas the urinary biomarkers are unable to measure more than daily excretion. This could also have impacted

negatively on the correlation between the RAPP method and the urinary biomarkers. Also, four urinary excretions may be too few to overcome within-person differences.

It was concluded that urinary biomarkers were not successfully used in this population. This was mostly because of the fact that eight or more repeats are needed and the effort of collecting urine, and transporting the samples makes collecting so many samples impossible. MacIntyre *et al.*, (2000:70) also concluded that biomarkers were not a suitable validation method for their population, which was similar to the population in the current study.

Correlations between the RAPP method and the three blood markers were generally poor; with the exception of vitamin B<sub>12</sub> ( $r = 0.26$ ) which was regarded as acceptable. Selenium and folate correlation coefficients were lower than vitamin B<sub>12</sub> and were not significant. Vitamin B<sub>12</sub> content of food composition tables is complete, however, disadvantage of Vitamin B<sub>12</sub> is the fact that it may have a high day to day variation depending on whether meat (high in Vitamin B<sub>12</sub>) was eaten. Furthermore, plasma vitamin B<sub>12</sub> levels and recalls reflect short term intake while the RAPP method indicates longer term consumption.

Correlation coefficients for vitamin B<sub>12</sub> blood markers compared against a FFQ in a study by Green *et al.* were lower than the weighed food records used as a reference method (Green *et al.*, 1998:1668). They reported vitamin B<sub>12</sub> classifications of 42% (food records) and 32% (QFFQ) in comparison with the current study results of 35.7% (recalls) and 46.1% (RAPP). Green *et al.*, (1998:1669) also reported low levels (< 50%) of participants being correctly classified (the same or adjacent quartile).

Van't Veer *et al.*, (1993:S60) reported a Pearson correlation coefficient of 0.15 between plasma Se and dietary history as the reference method, while the current study reported  $r = 0.17$  between plasma Se and the RAPP method. The results for Se were not unexpected since the South African food composition tables are not complete for selenium with many items having missing values. Furthermore, Se intake is difficult to measure accurately, as its content varies geographically (Gibson, 2005:770). Selenium deficiencies have been reported in past studies done in the high oesophageal cancer areas in the EC (Jaskiewicz *et al.*, 1988:2638).

Finally, structural equation modelling (method of triads) is a more recent method to assess the validity of dietary assessment methods. The method of triads is an example of such a method and can be used when dietary information is available from a test method (the RAPP

method), the reference method (4 repeated 24-hour recalls) and a biomarker (blood levels) (Kaaks, 1997:1237S). Usefulness of the method of triads was limited because of the high occurrence of Heywood cases in the folate data. The VCs generated for Vitamin B<sub>12</sub> indicated that the RAPP method (VC = 0.77) measures the truth better than the recalls (VC = 0.15).

Kabagambe *et al.* (2001:1134) suggested that Heywood cases could occur as a result of random sampling error, which may have been the case in the present study. To prevent this it is recommended that samples should be larger than 120 (Kabagambe *et al.*, 2001:1134). Due to cost and other logistic reasons it was not possible to include 120 in each sample in the present study. Another possible explanation for the Heywood cases may be that there were violations of the underlying model assumptions (Ocke & Kaaks, 1997:1241S). The model assumes linear relations between the different measurements and the unknown true intake; as well as a lack of random errors between the different measurements (Ocke & Kaaks, 1997:1241S). However, it is possible that errors between the RAPP method and the recalls agree because of the use of the same food composition tables. It should also be remembered that the food composition tables do not include the fortified nutrient values for maize and bread since they were developed before fortification. However, this would only be applicable to folate.

Various cultural and logistical limitations influenced the results of this phase of the validation of the RAPP assessment method. These include the following:

- 1) The poor validity of the portion sizes represented on the portion size photographs in portion size estimation (Chapter 5).
- 2) Collecting complete urine samples was difficult because of cultural beliefs and a lack of privacy. The same was true for determining the completeness of the urine samples;
- 3) Because of the extreme lack of infrastructure in this area, random sampling was not possible as very few households could be accessed by road. There was no formal postal system (residents use the local trading store), and no formal enumeration areas. Participants were therefore recruited using snowball sampling. This could have introduced a bias as those that were willing to participate were more interested in food. Also, participants who were approached were those living near access roads,

which may also have introduced a bias as these residents may have more access to the trading store and therefore to commercially available foods.

- 4) The sample of Phase A of this study was small with a large drop-out rate (22%). This was mostly attributed to the large participant burden and the duration of the study (12 weeks). This was especially true for the collection of urine which resulted in a poor recovery of 24-hour urine samples. A larger sample size would possibly have improved the results and also lowered within and between person variations. Unfortunately, funding and other logistic limitations prevented the use of a larger sample size.

## 5. CONCLUSION

Validity of the energy intake of the RAPP method was considered to be poor when validated against  $EE_{calc}$  (Schofield's equation). In terms of Goldberg's cut-off points, there were many participants who under-reported their intake. Validity of the RAPP method compared with the GPAQ was considered to be poor, and this was believed to be due to errors related to  $EE_{meas}$  (the GPAQ) rather than the RAPP method. The RAPP method also performed poorly when compared against urinary biomarkers. As a very small percentage of participants provided four complete 24-hour urinary biomarkers, indicates that urinary biomarkers are most probably not feasible validation options in this population. Finally, the RAPP method performed poorly against two of the three blood biomarkers. This may have been due to incomplete values (for Se) in the food composition database and lack of inclusion of fortification values (for folate) in the food composition database. Various cultural and logistic limitations introduced bias to the study and hence influenced the study results. The RAPP method did however perform better against Vitamin B<sub>12</sub> as blood biomarker based on the VC results.

In summary, validity of the RAPP method for energy, nitrogen and micronutrient intake could not be confirmed using biomarkers.

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## Chapter 9

# HABITUAL DIETARY INTAKE OF PEOPLE LIVING IN HIGH OESOPHAGEAL CANCER AREAS

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Home grown maize is ground at the local shop

## 1. INTRODUCTION

There is a high prevalence of oesophageal cancer (OC) in certain rural areas of the Eastern Cape (EC) Province of South Africa (Somdyala *et al.*, 2003:10). It is a cancer with an irregular geographic distribution and is mostly prevalent amongst rural, semi or illiterate people, and is more common in males (Somdyala *et al.*, 2003:5). Various risk factors, including alcohol consumption, tobacco smoking, tannin intake, *N*-nitrosamines intake, *injonga* use, mycotoxin exposure, specific dietary habits, and nutrient deficiencies have been associated with the development of OC.

The consumption of mouldy maize, pickled vegetables (Franceschi, 1993:616), high meat consumption and low consumption of fruit and vegetables (Stefani *et al.*, 1999:35) are possible dietary habits that increase the risk of developing OC. Nutrient deficiencies associated with OC include vitamin C (Guo *et al.*, 1990:124), nicotinamide, magnesium (Mg) (Craddock, 1992:93), selenium (Se) (Mark *et al.*, 2000:1753), zinc (Zn) (Fong *et al.*, 1998:1595) and riboflavin (Guo *et al.*, 1990:125).

Another risk factor associated with OC is mycotoxin exposure. Gelderblom *et al.* (1988) first described Fumonisin mycotoxins in 1988. This is a fungus produced on maize which flourishes in a cool environment. The strain most often associated with OC is *F. moniliforme* (Craddock, 1992:95). These fungi produce toxins that are associated with carcinogenicity (Craddock, 1992:95) and research has shown that these mycotoxins are not only hepatocarcinogenic, but also nephrocarcinogenic (Gelderblom *et al.*, 1991, Howard *et al.*, 2001).

Marasas *et al.*, (1988) reported that maize from high OC rate households in rural areas in the EC had significantly higher levels of the fungi than households in low OC rate areas (Marasas *et al.*, 1988:112). Xhosa people in the EC rural areas are subsistence maize farmers (Figure 9.1) and contaminated maize is stored (Figure 9.2) and used for human consumption. Contaminated maize (Figure 9.3) is also preferred for home brewed beer (Craddock, 1992:95).

In order to conduct a risk assessment of fumonisin exposure comprehensive information on the food consumption of the target population is needed. Exposure assessments have been conducted in developed countries (De Nijs *et al.*, 1998, Kuiper-Goodman *et al.*, 1998, Petersen and Thorup, 2001, Humphreys *et al.*, 2001, Leblanc *et al.*, 2005) but assessments in developing countries have been few and problematic because of a lack of

adequate and accurate food consumption data (Gelderblom *et al.*, 1996:291, Solovey *et al.*, 1999:327, Machinski & Soares, 2000:876, Yasdanpanah *et al.*, 2006:399).

The primary aim of this study was therefore to determine and describe habitual dietary intake for all food groups (especially regarding maize consumption) and to calculate fumonisin exposure. The secondary aim of the study was to determine the nutrient intake of the people living in high OC rate areas in the EC, with specific reference to nutrients associated with OC development.



**Figure 9.1.** A typical smallholding in Centane where participants grow their maize supply



**Figure 9.2.** Maize is dried and stored in the house.



Figure 9.3. Mouldy maize grown and consumed in the rural areas of the Eastern Cape

## 2. MATERIALS AND METHODS

### 2.1. STUDY POPULATION

One hundred and seventy ( $n = 170$ ) Xhosa participants including both males and females between the ages of 18 and 65 years were part of this cross-sectional study conducted between November 2005 and February 2006. Participants were recruited on a voluntary basis from five different rural villages in the Centane area. Fieldworkers went from door to door in order to select participants. Each participant completed the RAPP method with the help of a trained fieldworker and provided a blood sample for the analyses of vitamin B<sub>12</sub>, Se, and red blood cell folate (used in Chapter 8 for validation purposes). Recruitment, inclusion and exclusion criteria as well as the exact procedures followed for the collection of the blood samples were described and discussed in Chapter 8.

### 2.2. STATISTICAL ANALYSES CONDUCTED FOR THE CROSS-SECTIONAL STUDY

Quantitative Food Frequency Questionnaire data were analysed in terms of food groups and nutrients. Food items and dishes were divided into six food groups based on their main nutrient content and on the food groups used in the original adapted urban Xhosa questionnaire, namely: bread, cereal, combined dishes, meat, condiments (sugar & salt) and beverages. The average portion size of each food item consumed during the previous month was multiplied with the reported consumption frequency, to provide a

monthly consumption per dish. The total for each dish was divided by 28 in order to obtain a daily intake in grams. The daily intake of all food items in a given food group was summed to provide a daily consumption per food group. Home grown maize and commercially obtained maize were analysed separately because of their different fumonisin levels. The daily cooked maize portions of each maize source were converted to raw maize consumption (ratio information obtained during cooking session – see Chapter 4). Fumonisin exposure was then calculated by multiplying the daily raw maize consumption by the fumonisin level for each maize source and dividing the outcome by the participant's body weight in order to provide fumonisin consumption per kg body mass. The fumonisin exposure for home grown and commercially obtained maize was summed to provide a final fumonisin exposure level for each participant.

It is known from previous studies (Shephard *et al.*, 2007:625) that the total fumonisin content of home-grown maize is much higher than that of commercial maize. After the mean daily dry maize intake was calculated, the total fumonisin exposure was calculated.

Nutrient analysis was done with FoodFinder 3 (FoodFinder dietary Software, 1992, FoodFinder 3, 2010) based on the MRC food composition tables developed for South African foods (Langenhoven *et al.*, 1991). The nutrient content for each food item or dish was calculated as the product of each frequency identified on the quantitative food frequency questionnaire (QFFQ), by its portion size. The total monthly intake was calculated from the frequencies on the QFFQ and then divided by 28 to provide the total nutrient intake per day. Nutrient intakes were compared to the recommended daily allowance (RDA's) for each nutrient.

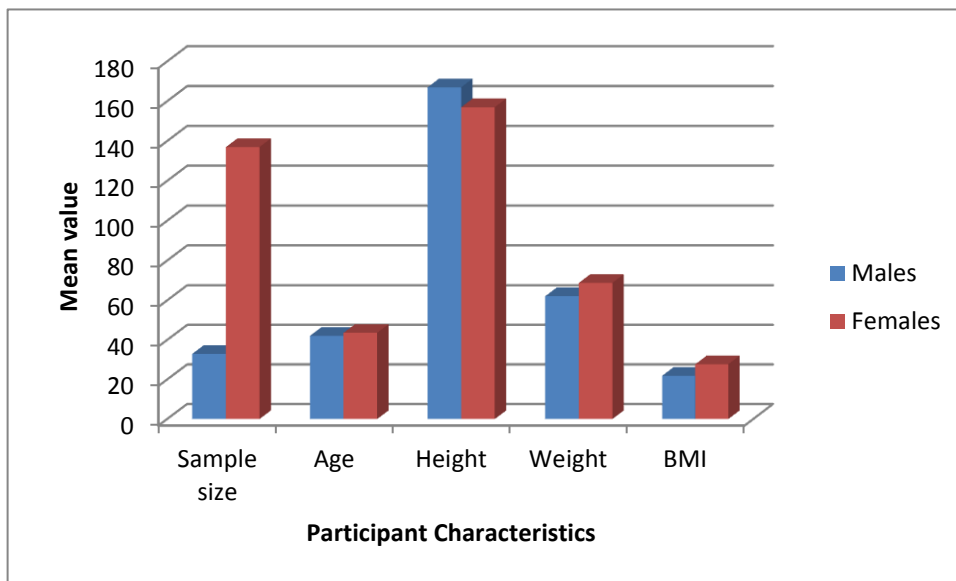
### **3. RESULTS FROM THE CROSS-SECTIONAL STUDY**

Hundred and seventy participants volunteered to participate in the study, including 137 females and 33 males. Data from all the participants were used. The average age of the participants was 43.3 years, average weight was 67.5 kilogram (kg) and average height was 1.6 meter (m). Body mass index (BMI) for this group of people was on average 26.6 kg/m<sup>2</sup>. Figure 9.5 give the frequency distribution of the different BMI groups as classified by the WHO (2006). This was similar to information reported in earlier chapters (Table 9.1) (Figure 9.4).

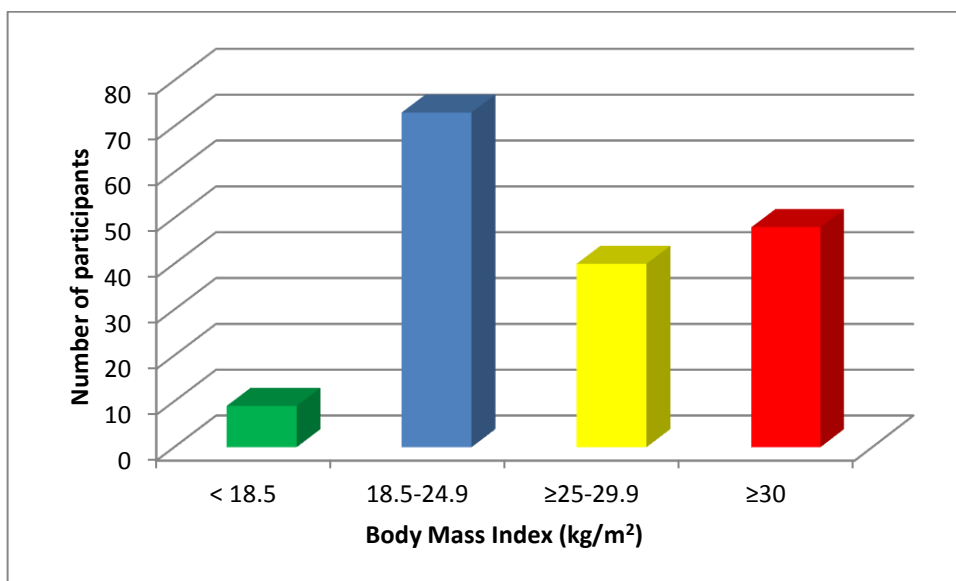
**Table 9.1. Mean ( $\pm$  SD) of the age and anthropometric measurements of participants**

| Measurements             | Males<br>n = 33<br>Mean ( $\pm$ SD) | Females<br>n = 137<br>Mean ( $\pm$ SD) | Total<br>n = 170<br>Mean ( $\pm$ SD) |
|--------------------------|-------------------------------------|--|--------------------------------------|
| Age (years)              | 42.1 (14.3)                         | 43.6 (13.8)                            | 43.3 (13.9)                          |
| Height (centimetres)     | 167.0 (0.01)                        | 157.0 (0.06)                           | 160.0 (0.08)                         |
| Weight (kilogram)        | 62.1 (11.9)                         | 68.8 (15.4)                            | 67.5 (15.0)                          |
| BMI (kg/m <sup>2</sup> ) | 21.9 (3.3)                          | 27.7 (5.7)                             | 26.6 (5.8)                           |

BMI = Body mass index  
n = sample size  
SD = standard deviation

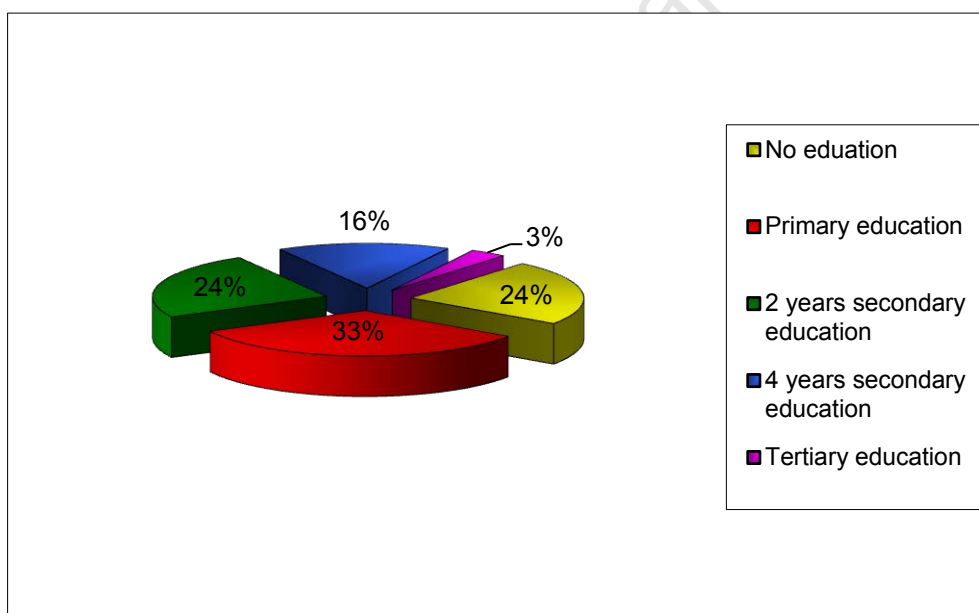


**Figure 9.4 Means of male and female participants regarding sample size, age, height, weight and BMI**



**Figure 9.5** Frequency distribution of the body mass index of participants

Figure 9.6 provides information regarding the different education levels of the participants that participated in the cross sectional study.



**Figure 9.6.** Education levels of participants

Forty-seven percent of the participants were unemployed while 14% were mine workers. The rest of the participants had different jobs including hotel work, fishing, assisting in the local shops and producing crafts. The majority of participants lived in mud houses (63.1%) while 32.2% lived in brick houses while another 4.7% lived in informal housing. The average household included five to seven people. Almost three quarters of the participants (73.8%) used water from a river as their main source of water, while 12.1%

made use of a communal tap, and 14.1% had their own taps. Seventy-three percent of participants did not have a toilet in the home or yard. The majority of participants (69.2%) cooked their food both inside and outside (fire) the house. Almost 13% of the participants only cooked inside while 17.9% only cooked outside. Only 0.9% used electricity as a source of energy while 5.1% used gas, 6.8% used paraffin and the majority (87.2%) used wood.

Generally participants (79%) claimed to have a single person contributing financially to the household; another 9% reported two contributors while 10% of the participants had nobody contributing. The average income per household was R 699.60 (87 USD) ( $\pm$  R 512.20) per month. Of these participants, 84.7% claimed to receive either a child grant or an old age grant from the government.

Twenty-four percent of participants confirmed that one of their parents had died of cancer. Of those, 63% had OC, 8% breast cancer, 6.8% bladder cancer and 4.1% lung cancer. The remainder of the participants claimed other types of cancer. Sixty-eight percent of the participants had never smoked, 10.3% had smoked in the past and quit, while 21.4% were currently smoking. Thirty-three percent claimed to consume home-made beer (made from mouldy maize) and the average age to start drinking this beer was 23.8 years ( $\pm$  8.7 years).

People living in rural areas and peri-urban areas usually have a small piece of land where maize and vegetables are grown (even if residents have an alternative income). Therefore, a certain amount of maize consumed is home grown. Maize is dried inside the house (Figure 9.3), on the roof or stored in special containers (Figure 9.7). After drying the maize, women and children remove the kernels from the cob (Figure 9.8). These kernels are either stamped or ground at home, or ground at the local shop (Figure 9.9).



**Figure 9.7. Special containers in which maize is stored in the Eastern Cape rural areas.**



**Figure 9.8. After the maize kernels are dried, women and children remove it from the cob.**



**Figure 9.9.** Home-grown maize is ground at the local shops.

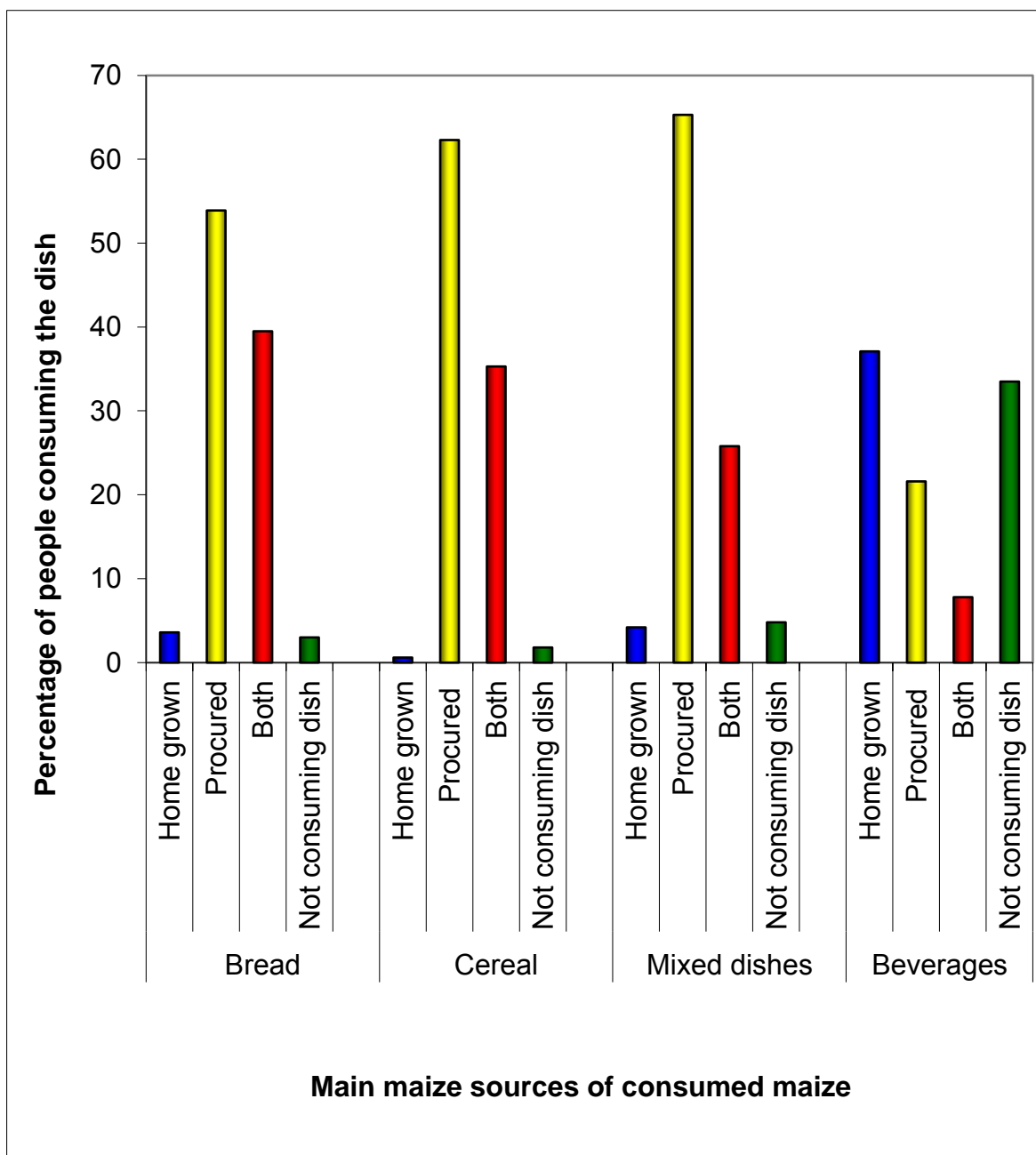
Table 9.2 and Figure 9.10 show a breakdown of maize sources of different maize-based dishes and beverages. The standard daily intake of home-grown maize was less than that of commercially bought maize (Table 9.2). Very little home-grown maize is currently used for consumption, and participants' preferred using commercial maize (Figure 9.10) for specific dishes (mostly for baking bread and the main maize dishes and combined dishes). On the other hand, home-grown maize seemed to be used mainly for maize-based beverages, including home-made beer.

**Table 9.2. Mean ( $\pm$  SD) daily intake and source of maize of cooked maize-based food items, dishes and beverages consumed by participants**

| Maize dish             | Maize source | RAPP method      |                  |       |                  |         |                  |
|------------------------|--------------|------------------|------------------|-------|------------------|---------|------------------|
|                        |              | All participants |                  | Males |                  | Females |                  |
|                        |              | n                | Mean (SD)        | n     | Mean (SD)        | n       | Mean (SD)        |
| Maize bread (g)        | Home grown   | 72               | 231.8<br>(274.6) | 15    | 297.6<br>(298.8) | 57      | 214.5<br>(268.0) |
|                        | Procured     | 156              | 497.5<br>(484.0) | 29    | 671.6<br>(556.2) | 127     | 457.8<br>(459.2) |
| Maize meal (g)         | Home grown   | 62               | 168.9<br>(455.4) | 6     | 178.5<br>(209.3) | 56      | 167.9<br>(475.4) |
|                        | Procured     | 170              | 543.5<br>(415.4) | 30    | 592.9<br>(343.6) | 130     | 532.4<br>(430.3) |
| Maize & vegetables (g) | Home grown   | 50               | 258.7<br>(380.4) | 7     | 87.3<br>(65.5)   | 43      | 286.6<br>(403.1) |
|                        | Procured     | 152              | 738.3<br>(617.8) | 28    | 853.1<br>(847.4) | 124     | 712.4<br>(554.3) |
| Maize beverages (g)    | Home grown   | 75               | 395.5<br>(478.7) | 17    | 403.6<br>(477.5) | 37      | 637.3<br>(610.9) |
|                        | Procured     | 49               | 565.2<br>(558.4) | 12    | 342.8<br>(259.6) | 58      | 393.1<br>(483.2) |

n = sample size

SD = Standard deviation



**Figure 9.10. Percentage of participants using different maize sources for the main maize dishes**

The RAPP method reported a total dry maize consumption of 539.5 g/day (115 g + 424 g) (Table 9.3). The probable daily intake (PDI) for fumonisin is based on the amount of dry maize consumed and was determined as  $8.4 \mu\text{g}/\text{kg}/\text{day}^{-1}$ . Whether the maize consumed was home-grown or commercially bought, was of great importance because of the different fumonisin levels. The levels in commercial maize were much lower. To improve the accuracy of fumonisin exposure data, the intakes and percentages of participants using either home-grown maize or commercial maize were assessed. The PDI for home-grown maize was compared to the consumption of commercial maize.

**Table 9.3. Total fumonisin intakes and exposure calculated for home-grown and commercial maize in study participants**

| RAPP method   |                     |                     |   |
|---|---------------------|---------------------|---|
|   | Home-grown<br>maize | Commercial<br>maize | Total maize<br>intake and maize<br>exposure |
| N   | 138                 | 170                 | 170   |
| Mean body weight (kg)   | 68.9                | 68.9                | 68.9  |
| Mean daily dry maize<br>consumption<br>(g/person/day)             | 115                 | 424                 | 539   |
| Total fumonisin<br>( $\mu\text{g}/\text{kg}^{-1}$ )*              | 1142                | 222                 | 1364  |
| Fumonisin exposure<br>( $\mu\text{g}/\text{kg}/\text{day}^{-1}$ ) | 1.9<br>(2.4)        | 1.3<br>(0.9)        | 3.2   |

\* Shephard *et al.* 2007:625

Table 9.4 presents the mean nutrient intake of people living in the Centane area, using the RAPP method. More females (n = 137) than males (n = 33) participated in this cross-sectional study. Total energy intake was higher for both males and females when compared with the RDA, which helps to explain the high prevalence of overweight. Total protein intake was higher than the RDA, although most of the protein was from plant and not animal origin (data not shown). The findings show low or deficient intakes for niacin, riboflavin, vitamin A, vitamin B<sub>12</sub>, vitamin C, vitamin D and Se.

Table 9.5 shows the mean and median intake of folate, Se and vitamin B<sub>12</sub> compared to the blood test results. Red blood cell folate levels were adequate (> 372 nmol/l) while reported dietary intake levels were high (150% RDA). Both reported Se and vitamin B<sub>12</sub> levels were lower than the blood results.

**Table 9.4. Mean ( $\pm$  SD) daily nutrient intake from the RAPP method compared with the recommended dietary allowance in study participants**

| Nutrient                      | RAPP method         |                     |                    | RDA   |         | % RDA intake (Males) | % RDA intake (Females) |
|-------------------------------|---------------------|---------------------|--------------------|-------|---------|----------------------|------------------------|
|                               | Mean ( $\pm$ SD)    | Mean ( $\pm$ SD)    | Mean ( $\pm$ SD)   |       |         | Mean ( $\pm$ SD)     | Mean ( $\pm$ SD)       |
|                               | Males               | Females             | Total              | Males | Females |                      |                        |
| Energy (kJ)                   | 15069.9<br>(7459.9) | 14132.7<br>(4792.7) | 14307.8<br>(537.5) | 12881 | 10093   | 139.7<br>(94.1)      | 170.4<br>(85.5)        |
| Protein (g)                   | 85.8<br>(49.8)      | 81.0<br>(28.2)      | 81.9<br>(33.2)     | 56    | 46      | 182.1<br>(134.4)     | 207.9<br>(94.8)        |
| Fat (g)                       | 82.5<br>(57.1)      | 80.1<br>(38.5)      | 80.6<br>(42.4)     | ne    | ne      | ne                   | ne                     |
| Carbohydrates (g)             | 581.9<br>(264.2)    | 537.2<br>(192.8)    | 545.6<br>(207.8)   | 130   | 130     | 447.6<br>(342.5)     | 413.2<br>(251.1)       |
| Folate (mcg)                  | 606.4<br>(463.4)    | 600.3<br>(310.0)    | 601.5<br>(342.2)   | 400   | 400     | 176.6<br>(141.9)     | 176.6<br>(106.7)       |
| Niacin (mg)                   | 14.0<br>(8.3)       | 13.2<br>(5.2)       | 13.4<br>(5.9)      | 16    | 14      | 104.3<br>(79.1)      | 112.9<br>(57.4)        |
| Riboflavin (mg)               | 1.0<br>(0.7)        | 0.9<br>(0.4)        | 0.9<br>(0.5)       | 1.3   | 1.1     | 93.7<br>(76.4)       | 93.8<br>(49.4)         |
| Thiamine (mg)                 | 1.8<br>(1.1)        | 1.6<br>(0.7)        | 1.7<br>(0.8)       | 1.2   | 1.1     | 184.3<br>(154.3)     | 173.3<br>(81.7)        |
| Vitamin A (mcg)               | 530.8<br>(664.8)    | 393.2<br>(411.6)    | 418.9<br>(469.9)   | 900   | 700     | 61.4<br>(77.3)       | 63.5<br>(86.5)         |
| Vitamin B <sub>12</sub> (mcg) | 0.9<br>(1.6)        | 0.7<br>(0.8)        | 0.7<br>(1.0)       | 2.4   | 2.4     | 43.9<br>(73.9)       | 33.3<br>(44.0)         |
| Vitamin C (mg)                | 80.3<br>(82.4)      | 69.8<br>(56.3)      | 71.8<br>(61.9)     | 90    | 75      | 97.3<br>(100.4)      | 103.9<br>(91.5)        |
| Vitamin D (mcg)               | 1.2<br>(1.7)        | 1.1<br>(2.2)        | 1.1<br>(2.1)       | 50    | 50      | 2.5<br>(3.3)         | 2.1<br>(4.3)           |
| Vitamin E (mg)                | 41.1<br>(28.6)      | 40.4<br>(22.0)      | 40.5<br>(23.3)     | 15    | 15      | 310.2<br>(243.4)     | 343.2<br>(285.8)       |
| Potassium (mg)                | 3389.3<br>(2187.4)  | 3131.4<br>(1231.0)  | 3179.6<br>(1452.9) | ne    | ne      | ne                   | ne                     |
| Magnesium (mg)                | 583.3<br>(373.6)    | 532.2<br>(200.0)    | 541.8<br>(241.3)   | 400   | 310     | 174.1<br>(138.8)     | 193.0<br>(86.1)        |
| Sodium (mg)                   | 8154.6<br>(4531.8)  | 7413.8<br>(5734.4)  | 7552.1<br>(5524.8) | ne    | ne      | ne                   | ne                     |
| Selenium (mcg)                | 34.3<br>(26.8)      | 31.5<br>(25.7)      | 32.0<br>(25.8)     | 55    | 55      | 73.3<br>(64.8)       | 67.9<br>(55.7)         |
| Zinc (mg)                     | 9.9<br>(6.8)        | 8.9<br>(3.4)        | 9.1<br>(4.2)       | 11    | 8       | 110.5<br>(94.9)      | 129.9<br>(59.8)        |

RDA = Recommended dietary allowance

RAPP = Ratio and Portion size Photographic dietary assessment method

ne = not established

**Table 9.5. Means ( $\pm$  SD) and medians (IQ range) of nutrients reported from the RAPP method and analysed in blood samples**

| n = 170                       | RAPP method      |                        | Blood results    |                        |                                  |
|-------------------------------|------------------|------------------------|------------------|------------------------|----------------------------------|
|                               | Mean (SD)        | Median (IQ Range)      | Mean (SD)        | Median (IQ Range)      | Normal reference values          |
| Folate (mcg)                  | 601.5<br>(342.2) | 597.5<br>(389.5-896.0) | 445.9<br>(133.3) | 425.5<br>(355.4-524.7) | 280.0 – 791.0 $\mu\text{g}/\ell$ |
| Se (mcg)                      | 32.0<br>(25.8)   | 28.0<br>(15.7-51.3)    | 69.7<br>(17.7)   | 70.0<br>(57.0-82.0)    | 46.0 – 143.0 $\mu\text{g}/\ell$  |
| Vitamin B <sub>12</sub> (mcg) | 1.0<br>(2.5)     | 0.4<br>(0.1-1.3)       | 3.6<br>(1.4)     | 3.4<br>(2.7-4.2)       | 211.0 – 911.0 ng/l               |

SD = Standard deviation

IQ range = Inter quartile range

#### 4. DISCUSSION

Participants included in this research, living in high OC areas in the EC province have the same socio-demographic profile as described in the literature for people living in high OC risk areas (Day, 1975:3306, Rose, 1979:30, Gabriel *et al.*, 1982:788, Li *et al.*, 1989:758, Negri *et al.*, 1992:1170, Guar *et al.*, 1997:2129, White *et al.*, 2002:462, Somdyala *et al.*, 2003:5). In the present sample 57% had no education or only a primary education; an average monthly income per family of R 699.60 (85 USD). The mean BMI of participants fell in the overweight category ( $\geq 25 - 29.9 \text{ kg}/\text{m}^2$ ).

Rose (1972:1353) reported that in good agricultural production years, Xhosa people living in rural areas have a diet low in animal protein, fat and minerals but sufficient in energy, total protein, carbohydrate (CHO) and fibre. The current study confirmed this. The planting, storage habits and cooking methods were still similar to those practiced in the 1970s (Rose, 1972:1353).

According to dietary results from using the RAPP method, the common risk factors for OC were prevalent in the diet of the Xhosa people, namely alcohol consumption (maize-brewed beer); and mycotoxin exposure (from home-grown maize). Tobacco use was not evaluated. With regard to micronutrient deficiencies associated with OC the following was found: mean folate, Mg and Zn intakes were above their respective RDA, (176.6%, 193.0% and 129.9% of RDA, respectively); mean niacin, riboflavin and vitamin C intakes were close to the respective RDAs (112.9%, 93.8% and 103.9% of RDAs respectively), while Se intake was poor (67.9% of RDA). Blood results for folate and Se were within the normal range, substantiating the dietary folate intake, but indicating underestimation of dietary intake of Se (see discussion below for further comments on this finding).

Frequent alcohol consumption is another possible risk factor for OC in this population. Only 33% claimed to consume home-made beer and the average age to start drinking this beer was reported to be 23.8 years ( $\pm$  8.7 years). Consumption of this beer mostly occurred over weekends and at month end. The RAPP method was not validated and tested for reliability of alcohol intake as the study mainly focused on the maize content of the traditional beer.

People living in rural areas of the EC frequently consume large amounts of non-alcoholic beverages made from mouldy maize (*amagewu*). Fungi that grow on certain food items, produce mycotoxins that increase the risk for a variety of diseases (Van Rensburg, 1985:30). It is known that mouldy maize contains high levels of *Fusarium* mycotoxins, which have been identified as being a risk factor for OC (Van Rensburg 1985:30 and Craddock 1992:94). According to Shephard *et al.*, (2007:265), there is a large difference in fumonisin content between home-grown maize and commercial maize because of various reasons (storage, exposure etc). According to information obtained in this cross-sectional study, Xhosa people consume large amounts of commercially obtained and home-grown maize daily, which amounted to 539 g dry maize per day. Furthermore, the study showed that most of the maize consumed in maize-based dishes was from commercially acquired maize, although specific dishes (especially the whole kernels) and beverages (*amagewu* and traditional beer) were made from home-grown maize. The RAPP method provided information on dry maize consumed, translating to an average fumonisin consumption of 3.2  $\mu\text{g}/\text{kg}/\text{day}$ . This is higher than FAO/WHO recommended PMTDI of 2  $\mu\text{g}/\text{kg}/\text{day}$  (Bolger *et al.*, 2001:270).

The current global economic crisis has caused a dramatic increase in the price of maize between 2005 and 2008 as the domestic price of maize increased from < R 600 / ton (2005) to > R 2000 / ton (2008) (First National Bank, AgriWeekly). It is speculated that further increases in the price of commercial maize may force people living in the EC areas to increase their consumption of home-grown maize. Therefore, it can be expected that the total daily fumonisin exposure will consequently increase in the near future. This increase may subsequently increase the risk of OC, and therefore, it is imperative that levels of fumonisin exposure be evaluated regularly to monitor exposure levels.

When considering the results of the food group (specifically maize intake) energy and nutrient intakes in the cross-sectional study, the outcomes of the reliability study (Chapter 6) and validity (Chapters 7 and 8) testing of the RAPP method need to be considered. Validation outcomes showed that the RAPP was found to be an acceptable method for

obtaining accurate data on maize dishes (cereals & combined dishes) at both group and individual level. The same can be said for energy and carbohydrate intakes, both of which reflect maize intake. In terms of reliability, all food groups (except condiments) performed acceptable in terms of reliability and validity testing. Hence the data on maize obtained from this study can be considered to have acceptable validity and reliability.

Validation results of the RAPP method showed were “poor” validity for vitamin C, Mg, Se and Zn, while those for niacin and riboflavin were “acceptable.” Reliability of niacin, vitamin C, Mg and Zn was “poor” and was “acceptable” for riboflavin and Se. Bearing this in mind, it can not be conclusively stated that low selenium intake is a clear risk for the development of OC in the investigated population. Conversely, it can also not be stated that the adequate intakes of the other risk nutrients (niacin, riboflavin, vitamin C, Mg, Zn) reflects the truth thus low risk in this regard, as only the validity of niacin and riboflavin was found to be acceptable. However, when interpreting these results it must also be taken into account that the food items and dishes on the QFFQ were analysed by means of raw ingredients and therefore a certain amount of loss would be expected to occur. This was not accounted for in the above mentioned tables.

Specific dietary patterns have also been identified as risk factors for OC. Amongst the risk dietary patterns are high meat consumption (De Stefani *et al.*, 1999:35). Results from the cross-sectional study found that the average intake of meat was very low (43.3 g). In the current study chicken was mainly consumed, although not daily. Red meat was not consumed frequently enough to justify inclusion in the food frequency questionnaire. This is also supported by the very low intake of vitamin B<sub>12</sub> (33.3% RDA) which is generally low when meat intake is low. Traditionally red meat is consumed at special social occasions such as weddings and funerals during which large amounts are consumed at a time. It can therefore be concluded that high and frequent consumption of meat is not a risk factor among the people in the current population. Results from the validation results indicated that both validity and reliability of the RAPP method were “acceptable” when measuring meat consumption as a food group.

Infrequent and low consumption of fruit and vegetables (Stefani *et al.*, 1999:35) are also risk factors for the development of OC. The RAPP method was found to be valid and reliable when measuring the consumption of mixed dishes, which were the main form of vegetable intake in this population. This is supported by the adequate intake of folate (from *imfino*). However, vegetable intake itself may not have been underestimated to an extent since vegetables were not included in the QFFQ as single items. Fruit

consumption was so infrequent and sporadic that it was not included on the QFFQ of the RAPP method. The overall pattern and intake of vegetable consumption therefore appeared to be far below that recommended. This is supported by the low intake of vitamin A (63.5% RDA).

Srivastava (1997:97) found in a case-control study of the nutritional intake of people with OC in India that intake of green leafy vegetables and fresh fruit less than three times per week increased the risk of OC (1.98 - 2.37 times). This may be as a result of a lower intake of vitamin C and  $\beta$ -carotene. These are both anti-oxidants and therefore free radical scavengers. Vitamin C also blocks the formation of *N*-nitrosamines (Srivastava 1997:97).

In the present study vitamin A dietary intake levels were found to be very low. This data is supported by the National Food Consumption Survey – Fortification Baseline (NFCS-FBI) conducted in South Africa in 2005 which found that in the EC one in four women had a marginal vitamin A deficiency as measured by a vitamin A concentration of < 120ug/dL (Labadarios *et al.*, 2008:261). Some researchers believe vitamin A deficiency plays a role in the process of atrophy that occurs in the epithelium of the oesophagus (Van Rensburg, 1987:10), while others believe that the deficiency increases carcinogenesis (Groenewalt *et al.*, 1981:967). Regardless of the pathophysiology, it seems that vitamin A deficiency does play a role in the development of OC, and that it may be present in the population studied.

## 5. CONCLUSION

Results from the cross-sectional survey showed that mouldy maize, containing high levels of fumonisins (3.2  $\mu$ g/kg/day) is consumed, especially in the form of snacks (whole kernels), combined maize dishes and as maize beverages in the study population and may be contributing to OC risk in the study area. Other possible dietary OC risk factors that were identified include low consumption of fruit and vegetables; vitamin A, vitamin C and Se, but this result needs to be confirmed using dietary methodology with proven validity for the assessment of these particular nutrients.

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## Chapter 10

### OVERARCHING CONCLUSIONS

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A woman removing kernels from the cob in preparation for storage.

The RAPP dietary assessment method that was developed and tested for reliability and validity in this research comprises a quantified food frequency questionnaire (QFFQ) that includes 33 food items, as well as a food photographic series (FPS) comprising commonly eaten foods and portion sizes and ratios. The food items included in the QFFQ and depicted in the FPS were categorized according to six food groups, namely: bread, cereal, combined dishes, meat, condiments and beverages. These food items represent the food items and dishes consumed most frequently by people living in a high oesophageal cancer (OC) risk area in the Eastern Cape. These food items, more specifically the cereal and combined dishes groups, provide information on the daily amount of maize consumed and therefore fumonisins exposure of the target population.

In order to assist with portion size estimation, the RAPP method includes portion size and ratio photographs. Validation results (Chapter 5), however, show that the validity of this tool is poor, with participants not being able to accurately relate the portion sizes depicted on the photographs to their actual intake. This might impact negatively on the reliability and validity of food intake estimations obtained through application of the RAPP method.

Reliability testing of the RAPP method indicated that the method is reliable when measuring habitual intake of the food groups represented in the QFFQ, especially for bread, cereals, meat and beverages. However, results also indicated that the reliability of the RAPP method was poor for nutrients, especially energy, protein, riboflavin, vitamin A, vitamin B<sub>12</sub>, vitamin C, vitamin D, vitamin E, folate, Ca and potassium (K).

Validity results indicate that the RAPP method is valid for estimation of the intake of cereals (includes maize), combined dishes (includes maize), meat and condiments, but not for estimation of bread and beverage intake when using four repeated 24 hour recalls as reference method. The RAPP method was also found to be valid for estimation of total energy intake when using four repeated 24 hour recalls as reference method. However, this outcome was not supported by the results of validation of the RAPP method against calculated energy expenditure (Schofield's equations), with a large percentage of the participants under-reported energy intake.

As far as individual nutrients are concerned, the RAPP method was found to be valid for estimation of CHO, niacin, riboflavin, thiamine, vitamin D, Ca and Fe intake, but not for estimation of protein, fat, folate, vitamin A, vitamin B<sub>12</sub>, vitamin C, vitamin E, K, magnesium, sodium, selenium and zinc intake when using four repeated 24 hour recalls as reference method. The poor validity of the RAPP method in estimation of folate, vitamin B<sub>12</sub> and selenium intake was confirmed by the results of the validation of the

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RAPP method against blood biomarkers (red blood cell folate, vitamin B12 and selenium levels).

These conclusions need to be considered within the context of the following limitations:

- 1) The study was part of a larger (parent) study conducted by the Medical Research Council (MRC) Programme for Mycotoxins and Experimental Carcinogens (PROMEC) Unit, limiting the study design to what was feasible within the context of the larger study.
- 2) Transport and accommodation costs were extremely high because of limited accommodation choices and the large distance between Cape Town and the study area. Because of limited funding available, the number of days in the study area was restricted.
- 3) An extreme lack of infrastructure (no / poor roads to villages, no postal system, no enumeration areas nor any arial photographs) in this area prevented the use of random sampling and participants were therefore recruited using the snowball sampling technique, resulting in possible selection bias. Those who were willing to participate might have been more interested in their health and eating habits than those who did not want to participate. Furthermore, people living in villages close to access roads were approached for practical reasons. The eating habits of these people may differ from the eating habits of people living further from access roads as the former (participants in this research) may have had access to a greater variety of foods.
- 4) Because of large participant burden different participants were used for different sections of this study. Ideally the same participants should have been used for all sections of the study.
- 5) The sample size for the reliability assessment and validation phases (Phase A) of this study was small ( $n = 47$ ) with a large drop-out rate (22%). This was mostly attributed to the large participant burden and the duration of the study (12 weeks). A larger sample size may have improved results and also decreased between person variation. Because of limited funding and resources, it was decided to rather increase the number of repeats per participant for the 24 hour recalls and the urinary biomarkers (thereby decreasing the with-in person variation) than to increase the number of participants.

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- 6) The reference method used in the current study may have impacted on the validation results obtained. For logistical reasons weighed records could not be considered and four repeated 24-hour recalls were thus used as reference method. Participants might have forgotten what they ate the previous day as this method relied on memory. Furthermore, a 24-hour recall does not necessarily reflect usual intake. This limitation was addressed by conducting four non-consecutive 24-hour recalls (including one weekend and three week days) per participant to better reflect usual intake.
  - 7) Within- and between-person variation was not calculated because of the small sample size. It is possible that adjustment for these factors may have resulted in better validation outcomes. It must however, be borne in mind that the fact that four 24-hour recalls were taken per participant may have contributed to a reduction in the within-person variation.
  - 7) The validity (Phase A) and reliability testing of the RAPP method was conducted over a period of 12 weeks for logistic reasons. During the twelve weeks seasonal changes may have occurred which may have influenced results, especially regarding availability of vegetables in the mixed dishes.
  - 8) It was aimed to collect four 24-hour recalls and four 24-hour urine samples during the 12 weeks of the validation study. Ideally, these samples should have been taken during the same time frame as the RAPP method (4 weeks). Once again, because of logistic reasons this was not possible.
  - 9) Collecting complete urine samples was very challenging, mainly because of a lack of privacy experienced by participants as well as cultural beliefs in this regard (see point 11). The number of complete urine samples collected was so small that no meaningful validation tests could be conducted using this data.
  - 10) The poor validity of food portion photographs may have contributed to the poor reliability and validity of the RAPP method for estimation of nutrient and energy intake.
  - 11) Cultural perspectives played a large role throughout the study. It was important for the research team to preserve and respect these. This resulted in some of the standard procedures being adapted, which may have influenced the results. Firstly, many participants believed that weighing and measuring was for burial

purposes and initially refused to participate because of that. The method was adapted in order for the participants to feel comfortable about this. As indicated, these adaptations had a negligible effect on  $EE_{calc}$  from Schofield calculations. Secondly, participants believed that diseases can be transferred if their urine is taken from them. This limited the number of willing participants for the validation study. The same was true for determining completeness of urine samples with para-amino benzoic acid (PABA) (a tablet to be taken).

Bearing in mind the above mentioned limitations, it is concluded that the RAPP method is valid for the estimation of maize intake and thus fumonisin exposure in the target population, which was the primary aim of developing the RAPP method. The method was subsequently successfully applied in a cross-sectional study to assess fumonisin exposure (Chapter 9). It can therefore be recommended that the RAPP method is a suitable tool to assess fumonisin exposure in the target population, especially as no other dietary method to assess fumonisin exposure is available. If the RAPP method is specifically used for these purposes, it can be considered to shorten the QFFQ by excluding food items and dishes that do not include maize. However, the validity of such a shortened questionnaire would need to be confirmed.

Because of the poor reliability and validity of the RAPP method in the estimation of energy and nutrient intake in the target population, it cannot be recommended as a method for the estimation of energy and nutrient intake in this population.

Finally, the statistical approach applied in the assessment of the reliability and validity of the different components of the RAPP method is novel. As there is no consensus on the most appropriate statistical method(s) that should be applied to assess reliability and validity of dietary assessment methods, this study used a range of seven different statistical tests that provide insights in different aspects of validity and reliability. Published and novel criteria for the interpretation of individual statistical tests to reflect poor, acceptable or good validity/reliability were applied to provide a comprehensive perspective on the reliability and validity of the different components of the RAPP method. As validation of dietary assessment methods is an ongoing challenge faced by every researcher attempting to assess the dietary intake of individuals/groups, the statistical approach developed for and implemented in this validation study makes a methodological contribution to a very challenging field of research.

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