

**The feasibility of implementing a point-of-use micronutrient fortification among
African pre-school children: A pilot study**

Ogunlade, Adebayo Olakunle
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Supervisor: Prof. HS Kruger
Co-supervisor: Prof. JC Jerling
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NORTH-WEST UNIVERSITY
YUNIBESITHI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

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ABSTRACT

Background

The high prevalence of micronutrient deficiencies among South African preschool children reinforces the need for an intensified micronutrient malnutrition control strategy targeting children at home or in school. The use of recently developed micronutrient powders also known as “in-home fortification” or “point-of-use fortification” can be an effective long-term, sustainable approach in improving early childhood nutrition and cognitive developmental potential. However, before embarking on large-scale, long-term, point-of-use fortification trials, it is imperative to conduct external pilot trials in which the feasibility of implementing full-scale studies can be effectively assessed.

Objective

The objective of this study was to assess the feasibility of implementing a point-of-use micronutrient fortification trial among African preschool children, which would aid in pilot-testing the study design, logistics and implementation process as well as reveal limitations which can be addressed before the implementation of full-scale trials.

Methods

Preschool children ($n = 151$), aged 36 – 79 months with haemoglobin concentration (Hb) ≤ 12.5 g/dL, from eight schools in a low socio-economic community were randomly assigned to an intervention ($n = 76$) and a control ($n = 75$) groups, both receiving breakfast maize-meal porridge, either with added micronutrient or placebo powder for 52 school days. Several process evaluation indicators (fidelity, dose delivered, dose received, reach, recruitment and context) were used to assess trial feasibility. Selected indicators of early childhood development (Hb concentration, anthropometric indices and cognitive function) were used to evaluate the outcome of the intervention within the context of a pilot study.

Results

The process evaluation results indicate that the various implementation components were delivered with high fidelity. Capacity development and strengthening of the front-line staff (cognitive assessors and study assistants) was executed as planned. The point-of-use fortificant was well accepted among the children and the mean percentage of days the point-of-use fortificant or placebo was consumed (~85%) did not differ between the groups. There were significant increases in Hb concentration ($p < 0.05$) from baseline to follow-up in both the intervention [mean change: 0.38 g/dL (95% CI: 0.14, 0.61 g/dL)] and control [mean change:

0.57 g/dL (95% CI: 0.35, 0.80 g/dL)] groups, however, mean change did not differ significantly between the groups ($p = 0.250$). The intervention did not improve any of the anthropometric indices measured in the intervention group compared to control. However, there was a medium likelihood for practical significance for the two global cognitive scores assessed, nonverbal index [intervention effects: 7.20 (95% CI: 2.60, 11.81); $p = 0.002$, effect size: 0.55] and mental processing index [intervention effects: 2.73 (95% CI: 0.25, 5.70); $p = 0.072$, effect size: 0.36] on the Kaufman Assessment Battery for Children, second edition.

Conclusion

The feasibility of implementing a point-of-use micronutrient fortification trial was demonstrated among African preschool children with potential benefits of improving their cognitive function. The most important lessons learned from this trial that could help improve similar future large-scale trials included the recruitment and training of the most eligible front-line staff as well as identifying that the use of a simple field-friendly finger prick method to measure Hb concentration may not be sufficiently sensitive to show differences in iron status after the intervention.

Keywords: Point-of-use, fortification, preschool, feasibility, pilot, South Africa, micronutrient powders, implementation

OPSOMMING

Agtergrond

Die hoë voorkoms van mikronutriënttekorte onder Suid-Afrikaanse voorskoolse kinders versterk die behoefte vir 'n beheerstrategie wat kinders se mikronutriëntwanvoeding by die huis of by skole kan aanspreek. Die gebruik van mikronutriëntpoeiers wat onlangs ontwikkel is en bekend staan as "tuisfortifisering" of "kitsfortifisering" of "plek-van-gebruik-fortifisering" kan 'n effektiewe langtermyn, volhoubare benadering wees om kinders se voeding en kognitiewe ontwikkelingspotensiaal te verbeter. Alvorens grootskaalse, langtermyn, kitsfortifiseringseksperimente aangepak word is dit belangrik om loodsstudies uit te voer sodat die uitvoerbaarheid van volskaalse studies effektief geassesseer kan word.

Doelwit

Die doelwit van die studie was om die uitvoerbaarheid van kitsmikronutriëntfortifisering onder swart voorskoolse kinders te assesser, wat hulp sal verleen tydens die loodsstudietoetsings van die studie-ontwerp, logistieke en implementeringsproses asook wat beperkings sal aanwys wat voor implementering op grootskaal aangespreek kan word.

Metodes

Voorskoolse kinders ($n = 151$), tussen 36 – 79 maande oud met hemoglobienkonsentrasies ($Hb \leq 12.5$ g/dL, van agt skole in 'n lae sosio-ekonomiese gemeenskap was ewekansig verdeel in 'n eksperimentele ($n = 76$) en 'n kontrole ($n = 75$) groep, beide groepe het mieliepap vir ontbyt ontvang. Die eksperimentele groep het bygevoegde mikronutriëntpoeier en die kontrole groep het plasebo-poeier by die pap ontvang vir 52 skooldae. Verskeie prosesevalueringsaanwysers (presisie, dosis ontvang, omvang, werwing en konteks) was gebruik om uitvoerbaarheid te bepaal. Geselekteerde aanwysers van vroeë kinderjaarontwikkeling (Hb -konsentrasies, antropometriele indikatore en kognitiewe funksie) was gebruik om die uitkoms van die intervensie te evalueer binne die konteks van die loodsstudie.

Resultate

Die prosesevaluering het aangedui dat verskeie implementeringskomponente uitgevoer was met hoë presisie. Kapasiteitontwikkeling van die veldwerkers (vir kognitiewe metings en skoolassistentie) is uitgevoer soos beplan. Die kitsfortifisering was goed aanvaar deur die kinders. Die persentasie van die gemiddelde aantal dae waarop kitsfortifisering of die plasebo ingeneem was was ~85% en dit het nie verskil tussen die twee groepe nie. Daar was 'n betekenisvolle verhoging in hemoglobienkonsentrasie ($p < 0.05$) van basislyn tot die opvolg in

beide die intervensie [gemiddelde verandering: 0.38 g/dL (95% VI: 0.14, 0.61 g/dL)] en die kontrolegroep [gemiddelde verandering: 0.57 g/dL (95% VI: 0.35, 0.80 g/dL)], maar die gemiddelde verandering het nie betekenisvol tussen die twee groepe verskil nie ($p = 0.250$). Die intervensie het nie tot enige veranderinge van die antropometriese indikatore in die intervensiegroep in vergelyking met die kontrole gelei nie. Praktiese betekenisvolheid vir die twee globale kognitiewe assesseringstegnieke, nie-verbale indeks [effek van intervensie: 7.20 (95% VI: 2.60, 11.81); $p = 0.002$, effekgrootte: 0.55] en verstandelike prosesseringsindeks [effek van intervensie: 2.73 (95% VI: 0.25, 5.70); $p = 0.072$, effekgrootte: 0.36] op die Kaufman Assesserings Battery vir kinders (tweede weergawe) is gevind.

Gevolgtrekking

Die uitvoerbaarheid van kitsmikronutriëntfortifisering is gedemonstreer onder swart voorskoolse kinders met potensiële voordele van verbetering van hulle kognitiewe funksie is gedemonstreer. Die belangrikste lesse wat in die studie geleer is, kan gebruik word in die beplanning en/of implementering van tuisfortifiseringsintervensies in Suid-Afrikaanse voorskoolse kinders. Geskikte en goed opgeleide veldwerkers van dieselfde agtergrond as die kinders help om kommunikasieprobleme uit te skakel en is uiters belangrik vir suksesvolle implementering. Gebruik van 'n sensitiewe kognitiewe toetsbattery vir die etniese en ouderdomsgroep van studie maak dit moontlik om die effek van die intervensie op kognitiewe funksie aan te toon. Gebruik van veldvriendelike vingerpriktoetse om hemoglobien te meet is waarskynlik nie sensitief genoeg om verskille in ysterstatus met 'n fortifiseringsintervensie aan te toon nie.

Kernwoorde: plek-van-gebruik-fortifisering, kitsfortifisering, voorskool, uitvoerbaarheid, loodsstudie, Suid-Afrika, mikronutriëntpoeiers, implementering

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ACRONYMS AND ABBREVIATIONS

BMI	Body mass index
DALY	Disability-adjusted life year
EAR / AI	Estimated average requirement / Adequate intake
ECD	early childhood development
FAO	Food and Agricultural Organization (of the United Nations)
GDP	Gross domestic product
Hb	Haemoglobin
HIV / AIDS	Human immune deficiency virus / Acquired immune deficiency syndrome
ICC	Intraclass correlation co-efficient
IDA	Iron deficiency anemia
IDD	Iodine deficiency disorders
IQ	Intelligence quotient
KABC-II	Kauffman Assessment Battery for Children, second edition
MPI	Mental processing index
MSE	Mean square error
MTHFR	Methyltetrahydrofolate reductase
NFCS	National food consumption survey
NVI	Non-verbal index
RDA	Recommended dietary allowance
RE	Retinol equivalent
SAVACG	South African vitamin A Consultative Group
SPSS	Statistical Package for the Social Sciences
VAD	Vitamin A deficiency
WHO	World Health Organization
YLL	Years of life lost

GLOSSARY

Anthropometry indicators	Anthropometric indicators provide useful summary measures of nutritional status based on measures of body size and composition, often relative to their distribution in a reference population.
Cognition	A broad range of high level physiological processes of brain functioning such as learning, memory, thinking, reasoning, movement, coordination, attention and language.
Cognitive development	The changes or progressive improvement in cognitive or brain processes observed in a child over long periods of time (months or years)
Cognitive function	An intellectual or mental process that involves symbolic operations such as learning, memory, thinking, movement, reasoning, attention and language. Cognitive function can be clustered into six main domains namely - executive, memory, attention, perception and psychomotor functions as well as language skills.
Context	Aspects of the larger social, political and economic environment that may influence intervention implementation.
Dose delivered	The number of intended units of each intervention or each component delivered or provided. Dose delivered is a function of efforts of the intervention provider.
Dose received	The extent to which participants actively engage with, interact with, are receptive to, and/or use materials recommended resources.

Fidelity	The extent to which the intervention was delivered as planned. It represents the quality and integrity of the intervention as conceived by the developers. Fidelity is a function of the intervention providers.
Infants	Children from birth to 24 months.
Index	An index is usually made up of two or more unrelated variables that are used together to measure an underlying characteristics.
Micronutrient powders	Vitamin and minerals added to traditional foods; used as “point-of-use fortificants” or “home fortificants.”
Point-of-use fortificants	Micronutrient powders [such as Sprinkles™ or MixmePlus®] that can be added to ready to eat food.
Point-of-use micronutrient fortification	Addition of a micronutrient powder to cooked meal just before consumption.
Preschool-age children	Children aged 2 to 6 years.
Psychomotor	Relating to movement or muscular activity associated with mental processes such as movement, coordination, manipulation or dexterity.
Psychomotor development	The progressive attainment (by a child) of skills that involve both mental and muscular activity.
Preschool-based	Nutrition intervention targeting preschool-age children and using existing crèche infrastructure in the implementation of the nutritional intervention.

Reach

The proportion of intended target audience that participates in an intervention. It is often measured by attendance.

Recruitment

Procedure used to approach and attract participants. Recruitment often occurs at individual and organisational / community levels.

School-age children

Children aged 6 to 15 years.

CHAPTER 1

INTRODUCTION

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND AND MOTIVATION

Globally, more than 2 billion people are adversely affected by micronutrient malnutrition (Sanghvi *et al.*, 2007). In developing countries, more than 10 million preventable child deaths occur yearly (Black *et al.*, 2003), and an additional 200 million children under five years are not reaching their full cognitive developmental potential mainly due to poor nutrition, especially micronutrient deficiencies (Grantham-McGregor *et al.*, 2007). In South Africa for instance, micronutrient deficiency is still a major public health challenge especially among preschool children (Labadarios *et al.*, 2005; Labadarios *et al.*, 2008).

Micronutrient deficiencies especially of iron and vitamin A and zinc are widespread among South African preschool children as reported in the South African Vitamin A Consultative Group study (SAVACG, 1995), which is in agreement with data from the National Food Consumption Survey in 1999 (Labadarios *et al.*, 2000; Labadarios *et al.*, 2005). Additionally, the most recent national survey in 2005 reported a rather worsening situation especially for iron and vitamin A deficiencies when compared to the previous national survey conducted in 1994 (Labadarios *et al.*, 2008).

Several studies consistently show that micronutrient deficiencies are associated with impaired cognitive development in young children (Bryan *et al.*, 2004). The preschool-age period, therefore, appears to be a missed opportunity in terms of the micronutrient intervention strategies which are targeted mainly at school-age children (Van Stuijvenberg *et al.*, 1999; Van Stuijvenberg *et al.*, 2001a; Van Stuijvenberg *et al.*, 2001b; Van Jaarsveld *et al.*, 2005). The preschool children are, therefore, in urgent need of attention, if they are to attain their full physical and mental potential. The use of multiple micronutrient formulations as part of meals for preschool children could improve their micronutrient status (Menon *et al.*, 2007; Zlotkin & Tondeur, 2007) and could also positively influence their cognitive function and development (Black, 2003; Faber *et al.*, 2005). The use of micronutrient powders also known as “in-home fortification” or “point-of-use fortification” can be an effective long-term, sustainable approach in addressing micronutrient deficiencies especially among infants, preschool children and women of child bearing age (Zlotkin & Tondeur, 2007; De Pee *et al.*, 2008; Troesch *et al.*, 2009).

However, experience with the use of point-of-use fortificants [such as “MixMe plus[®]” (DSM Nutritional Products Ltd, Basel, Switzerland) and “Sprinkles[™]” (Sprinkles Global Health Initiative, Toronto, Canada)] in larger-scale trials and in preschool settings for instance is limited (De Pee *et al.*, 2008).

Therefore, before implementing large-scale, long-term point-of-use fortification trials, it is important to conduct external or stand-alone pilot trials under normal field conditions and programmatic constraint in which the feasibility of conducting a full-scale study can be effectively assessed (Anderson & Prentice, 1999).

1.2 RATIONALE FOR CONDUCTING A PILOT TRIAL

According to Lancaster *et al.* (2004), pilot trials play an important role in health research, as they can provide useful information for the design, planning, implementation and justification for future full-scale randomised controlled trials (Anderson & Prentice, 1999). Pilot studies are relevant to best practice in research, but their potential and usefulness by researchers appears to be underutilised (Van Teijlingen *et al.*, 2001). Most of the pilot studies reported in the literature are focused mainly on reporting the results of the pilot trials in terms of outcomes (Colangelo *et al.*, 2005; Williams *et al.*, 2007; Kalman *et al.*, 2008; Kalman *et al.*, 2009) and not on the implementation process (Van Teijlingen *et al.*, 2001; Kong *et al.*, 2009). The implementation process may also provide valuable insights on the design and operational aspects, as well as the readiness for implementing full-scale randomised controlled trials (Gardner *et al.*, 2003).

A pilot or feasibility trial is a small study, designed to test logistics and gather information on the implementation processes prior to a larger study in order to improve the efficiency and quality of the full-scale trial. A pilot study can reveal deficiencies in the design of a proposed study and these can be addressed before time and resources are expended on large-scale studies (Gardner *et al.*, 2003; Lancaster *et al.*, 2004). However, with particular reference to the well designed preschool-based pilot trial conducted in this dissertation, a process evaluation approach was employed to help assess the feasibility of implementing the study under normal field conditions and programme constraints.

1.2.1 USE OF PROCESS EVALUATION WITHIN INTERVENTION TRIALS

Rossi *et al.* (2004) defined process evaluation as a form of program monitoring, designed to assess how well a program is operating and whether a program is delivered as intended to the target recipients. Program monitoring is the systematic documentation of aspects of program performance (program inputs, activities, outputs and outcomes) which give indications whether the program is functioning as intended (Saunders *et al.*, 2005). Furthermore, according to Habicht *et al.* (2008), use of process evaluation embedded within intervention studies can help identify breaks in the chain of the implementation pathway, and this can provide useful information for modification in future studies.

The use of process evaluation in assessing the feasibility, as well as the implementation processes of several recent intervention trials have been unequivocally demonstrated (Toroyan *et al.*, 2004; Robert *et al.*, 2006; Robert *et al.*, 2007; Kong *et al.*, 2009; Loechl *et al.*, 2009). Specifically for the pilot study in this dissertation, the development of the process evaluation methodology began by creating a model (**Figure 1.1**; see simplified version in Chapter 3) as described by Rossi *et al.* (2004), which gives a comprehensive detail of the expected implementation pathway leading to the expected intervention outcomes [assessed using selected indicators of early childhood development (Behrman *et al.*, 2007)]. Additionally, several process evaluation indicators (fidelity, dose delivered, dose received, reach, recruitment and context) were, however, linked to salient points of the model to help assess the feasibility and the implementation process of the pilot trial.

1.3 AIMS AND OBJECTIVES

This dissertation is based on a preschool-based randomised controlled pilot trial. The overall goal of the study was to pilot-test the design, methodology and implementation of a preschool-based micronutrient intervention designed to improve the micronutrient status, anthropometric indices and cognitive developmental potentials of preschool-age children. The findings of the pilot trial, both the process and outcome are, therefore, presented in this dissertation.

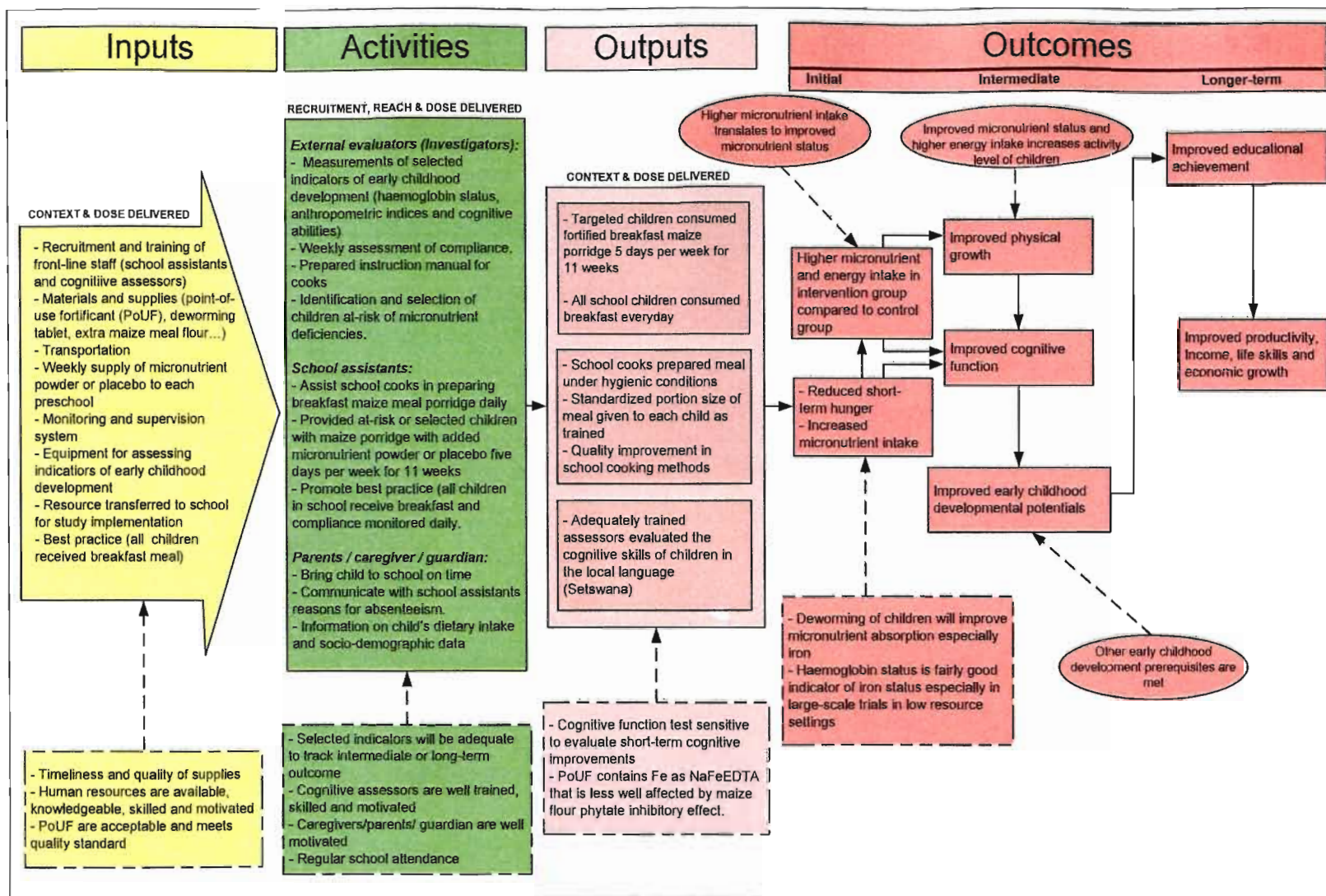


Figure 1.1 Logic model of the implementation pathway of the preschool-based pilot trial including indicators of process evaluation. The various components of the implementation model are categorised as inputs, activities, outputs and outcomes. Theoretical (circles) and trial (dashed boxes) assumptions are shown.

The aim of the **article** presented in **Chapter 3** was to assess the feasibility of implementing a point-of-use micronutrient fortification trial in a preschool setting.

The specific objectives were:

- (i) To evaluate the processes involved in implementing a preschool-based, point-of-use micronutrient fortification trial.
- (ii) To use selected indicators of early childhood development [haemoglobin status, anthropometric indices (weight, height, weight-for-age z-score, height-for-age z-score, body mass index-for-age z-score, mid-upper arm circumference and triceps skin-fold) and cognitive function (assessed by the Kaufman Assessment Battery for Children second edition, KABC- II)] to evaluate the outcome of the intervention within the context of a pilot study.

1.4 STRUCTURE OF DISSERTATION

This dissertation is in article format and consists of three chapters and one article manuscript which will be submitted for publication. The introductory chapter (Chapter 1) contains the background information of the dissertation, aims and objectives of the study, as well as the contributions of the authors to the study described in this dissertation. The subsequent chapter is a narrative literature review (Chapter 2), which provides additional background information for the interpretation of the results from the article presented in Chapter 3. Chapter 3, with the title **“Point-of-use micronutrient fortification: Lessons learned in implementing a preschool-based pilot trial in South Africa”**, conveys the research process of the pilot trial. This article describes the lessons learned (implementation process) in the pilot preschool-based micronutrient fortification trial. In the last chapter (Chapter 4) a general summary of the main findings are provided, conclusions are drawn and recommendations made.

The references of the chapters are provided at the end of each chapter. The technical style and references of Chapters 1, 2 and 4 are according to the guidelines stipulated by the North-West University (Van der Walt, 2006), but Chapter 3 is written according to the instruction for authors for the **International Journal of Food Sciences and Nutrition** where the article will be submitted for publication.

1.5 CONTRIBUTIONS OF THE AUTHORS

The experimental study reported in this dissertation was planned and executed by a team of researchers. The contribution of each of the researchers is given in **Table 1.1**.

Table 1.1 Qualifications and roles of the research team in the study

NAME	ROLE IN STUDY
Mr. A.O. Ogunlade (M. Sc Student)	Responsible for all aspects of the study. Literature review, data collection, compilation, statistical analysis, interpretation and writing up of the data.
Prof. H.S. Kruger Ph.D (Dietitian and Pharmacist)	Supervision of the entire project. Guidance in all aspects of the project considering the design, planning, execution and documentation of the study. Supervisor of Mr. A.O. Ogunlade.
Prof. J.C. Jerling Ph.D (Nutritionist)	Study leader of the entire project. Advisory role in all aspects of the project considering the design, planning, execution and documentation of the study. Co-supervisor of Mr. A.O. Ogunlade.
Prof. C.M. Smuts Ph.D (Biochemist)	Advisory role in all aspects of the study, especially in the aspect of project implementation and analysis of cognitive assessment data.
Dr. N. Govic Ph.D (Nutritionist)	Guidance in the recruitment, training and supervision of the front-line staff. (Cognitive assessors and school assistants).
Dr. S.M. Hanekom Ph.D (Nutritionist)	Guidance in all dietary aspects of the project.

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

REFERENCES

- ANDERSON, G.L. & PRENTICE, R.L. 1999. Individually randomized intervention trials for disease prevention and control. *Statistical methods in medical research*, 8(4):287-309.
- BEHRMAN, J.R., GLEWWE, P. & MIGUEL, E., eds. 2007. Methodologies to evaluate early childhood development programs. Washington D.C.: World Bank.
- BLACK, M.M. 2003. Micronutrient deficiencies and cognitive functioning. *Journal of nutrition*, 133(11 Suppl 2):3927S-3931S.
- BLACK, R.E., MORRIS, S.S. & BRYCE, J. 2003. Where and why are 10 million children dying every year? *Lancet*, 361(9376):2226-2234.
- BRYAN, J., OSENDARP, S., HUGHES, D., CALVARESI, E., BAGHURST, K. & VAN KLINKEN, J.W. 2004. Nutrients for cognitive development in school-aged children. *Nutrition reviews*, 62(8):295-306.
- COLANGELO, L.A., CHIU, B.C., LOPEZ, P., SCHOLTENS, D., WILLIS, L.C., HENDRICK, R.E. & GAPSTUR, S.M. 2006. A pilot study of vitamin D, calcium, and percent breast density in Hispanic women. 26(1):11-15.
- DE PEE, S., KRAEMER, K., VAN DEN BRIEL, T., BOY, E., GRASSET, C., MOENCH-PFANNER, R., ZLOTKIN, S., BLOEM, M.W., WORLD FOOD PROGRAMME & SPRINKLES GLOBAL HEALTH INITIATIVE. 2008. Quality criteria for micronutrient powder products: Report of a meeting organized by the world food programme and sprinkles global health initiative. *Food and nutrition bulletin*, 29(3):232-241.
- FABER, M., KVALSVIG, J.D., LOMBARD, C.J. & BENADE, A.J. 2005. Effect of a fortified maize-meal porridge on anemia, micronutrient status, and motor development of infants. *American journal of clinical nutrition*, 82(5):1032-1039.
- GARDNER, G., GARDNER, A., MACLELLAN, L. & OSBORNE, S. 2003. Reconceptualising the objectives of a pilot study for clinical research. *International journal of nursing studies*, 40(7):719-724.
- GRANTHAM-MCGREGOR, S., CHEUNG, Y.B., CUETO, S., GLEWWE, P., RICHTER, L., STRUPP, B. & INTERNATIONAL CHILD DEVELOPMENT STEERING GROUP. 2007. Developmental potential in the first 5 years for children in developing countries. *Lancet*, 369(9555):60-70.
- HABICHT, J-P., PELTO, G.H. & LAPPA, J. 2008. Methodologies to evaluate the impact of large-scale nutrition projects. Washington D.C.: World Bank.

JOOSTE, P.L., WEIGHT, M.J. & LOMBARD, C.J. 2000. Short-term effectiveness of mandatory iodization of table salt, at an elevated iodine concentration, on the iodine and goiter status of schoolchildren with endemic goiter. *American journal of clinical nutrition*, 71(1):75-80.

KALMAN, D.S., FELDMAN, S., FELDMAN, R., SCHWARTZ, H.I., KRIEGER, D.R. & GARRISON, R. 2008. Effect of a proprietary magnolia and phellodendron extract on stress levels in healthy women: A pilot, double-blind, placebo-controlled clinical trial. *Nutrition journal*, 7:11.

KALMAN, D.S., LOU, L., SCHWARTZ, H.I., FELDMAN, S. & KRIEGER, D.R. 2009. A pilot trial comparing the availability of vitamins C, B(6), and B(12) from a vitamin-fortified water and food source in humans. *International journal of food sciences and nutrition*, 1-11 [Epub ahead of print].

KONG, A.S., SUSSMAN, A.L., NEGRETE, S., PATTERSON, N., MITTLEMAN, R. & HOUGH, R. 2009. Implementation of a walking school bus: Lessons learned. *The journal of school health*, 79(7):319-325

LABADARIOS, D., STEYN, N.P., MAUNDER, E., MACINTYRE, U., SWART, R. and GERICKE, G., 2000. The national food consumption survey (NFCS): Children Aged 1-9 years, South Africa, 1999. Pretoria: Department of Health.

LABADARIOS, D., STEYN, N.P., MAUNDER, E., MACINTYRE, U., GERICKE, G., SWART, R., HUSKISSON, J., DANNHAUSER, A., VORSTER, H.H., NESMVUNI, A.E. & NEL, J.H. 2005. The national food consumption survey (NFCS): South Africa, 1999. *Public health nutrition*, 8(5):533-543.

LABADARIOS, D., SWART, R., MAUNDER, E., KRUGER, H.S., GERICKE, G., KUZWAYO, P.M.N., NTSIE, P.R., STEYN, N.P., SCHLOSS, I., DHANSAY, M.A., JOOSTE, P.L., DANNHAUSER, A., NELL, J.H., MOLEFE, D. & KOTZE, T.J.W. 2008. Executive summary of the national food consumption survey-fortification baseline (NFCS-FB-I): South Africa, 2005. *South African journal of clinical nutrition*, (supplement 2). 21(3):245-300.

LANCASTER, G.A., DODD, S. & WILLIAMSON, P.R. 2004. Design and analysis of pilot studies: Recommendations for good practice. *Journal of evaluation in clinical practice*, 10(2):307-312.

LOECHL, C.U., MENON, P., ARIMOND, M., RUEL, M.T., PELTO, G., HABICHT, J.P. & MICHAUD, L. 2009. Using programme theory to assess the feasibility of delivering micronutrient sprinkles through a food-assisted maternal and child health and nutrition programme in rural Haiti. *Maternal & child nutrition*, 5(1):33-48.

MENON, P., RUEL, M.T., LOECHL, C.U., ARIMOND, M., HABICHT, J.P., PELTO, G. & MICHAUD, L. 2007. Micronutrient sprinkles reduce anemia among 9- to 24-mo-old children when delivered through an integrated health and nutrition program in rural Haiti. *Journal of nutrition*, 137(4):1023-1030.

ROBERT, R.C., GITTELSON, J., CREED-KANASHIRO, H.M., PENNY, M.E., CAULFIELD, L.E., NARRO, M.R. & BLACK, R.E. 2006. Process evaluation determines the pathway of success for a health center-delivered, nutrition education intervention for infants in Trujillo, Peru. *Journal of nutrition*, 136(3):634-641.

ROBERT, R.C., GITTELSON, J., CREED-KANASHIRO, H.M., PENNY, M.E., CAULFIELD, L.E., NARRO, M.R., STECKLER, A. & BLACK, R.E. 2007. Implementation examined in a health center-delivered, educational intervention that improved infant growth in Trujillo, Peru: Successes and challenges. *Health education research*, 22(3):318-331.

ROSSI, P.H., LIPSEY, M.W. & FREEMAN, H.E. 2004. Evaluation: A systematic approach. 7th ed. Thousand Oaks, CA.: Sage.

SANGHVI, T., VAN AMERINGEN, M., BAKER, J., FIEDLER, J., BORWANKAR, R., PHILLIPS, M., HOUSTON, R., ROSS, J., HEYMANN, H. & DARY, O. 2007. Vitamin and mineral deficiencies technical situation analysis: A report for the ten year strategy for the reduction of vitamin and mineral deficiencies. *Food and nutrition bulletin*, 28(1 Suppl Vitamin):S160-219.

SAUNDERS, R.P., EVANS, M.H. & JOSHI, P. 2005. Developing a process-evaluation plan for assessing health promotion program implementation: A how-to guide. *Health promotion practice*, 6(2):134-147.

SAVACG see SOUTH AFRICAN VITAMIN A CONSULTATIVE GROUP.

SOUTH AFRICAN VITAMIN A CONSULTATIVE GROUP. 1996. Anthropometric, vitamin A, iron and immunisation coverage status in children aged 6-71 months in South Africa, 1994. *South African medical journal*, 86(4):354-357.

TOROYAN, T., OAKLEY, A., LAING, G., ROBERTS, I., MUGFORD, M. & TURNER, J. 2004. The impact of day care on socially disadvantaged families: An example of the use of process evaluation within a randomized controlled trial. *Child: Care, health and development*, 30(6):691-698.

TROESCH, B., EGLI, I., ZEDER, C., HURRELL, R.F., DE PEE, S. & ZIMMERMANN, M.B. 2009. Optimization of a phytase-containing micronutrient powder with low amounts of highly bioavailable iron for in-home fortification of complementary foods. *American journal of clinical nutrition*, 89(2):539-544.

VAN DER WALT, E.J. 2006. Quoting sources. Potchefstroom: Ferdinand postma library. 75p.

VAN JAARSVELD, P.J., FABER, M., TANUMIHARDJO, S.A., NESTEL, P., LOMBARD, C.J. & BENADE, A.J. 2005. Beta-carotene-rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. *American journal of clinical nutrition*, 81(5):1080-1087.

VAN STUIJVENBERG, M.E., KVALSVIG, J.D., FABER, M., KRUGER, M., KENOYER, D.G. & BENADE, A.J. 1999. Effect of iron-, iodine-, and beta-carotene-fortified biscuits on the micronutrient status of primary school children: A randomized controlled trial. *American journal of clinical nutrition*, 69(3):497-503.

VAN STUIJVENBERG, M.E., DHANSAY, M.A., LOMBARD, C.J., FABER, M. & BENADE, A.J. 2001a. The effect of a biscuit with red palm oil as a source of beta-carotene on the vitamin A status of primary school children: A comparison with beta-carotene from a synthetic source in a randomised controlled trial. *European journal of clinical nutrition*, 55(8):657-662.

VAN STUIJVENBERG, M.E., DHANSAY, M.A., SMUTS, C.M., LOMBARD, C.J., JOGESSAR, V.B. & BENADE, A.J. 2001b. Long-term evaluation of a micronutrient-fortified biscuit used for addressing micronutrient deficiencies in primary school children. *Public health nutrition*, 4(6):1201-1209.

VAN STUIJVENBERG, M.E., SMUTS, C.M., WOLMARANS, P., LOMBARD, C.J. & DHANSAY, M.A. 2006. The efficacy of ferrous bisglycinate and electrolytic iron as fortificants in bread in iron-deficient school children. *British journal of nutrition*, 95(3):532-538.

VAN STUIJVENBERG, M.E., SMUTS, C.M., LOMBARD, C.J. & DHANSAY, M.A. 2008. Fortifying brown bread with sodium iron EDTA, ferrous fumarate, or electrolytic iron does not affect iron status in South African schoolchildren. *Journal of nutrition*, 138(4):782-786.

VAN TEIJLINGEN, E.R., RENNIE, A.M., HUNDLEY, V. & GRAHAM, W. 2001. The importance of conducting and reporting pilot studies: The example of the Scottish births survey. *Journal of advanced nursing*, 34(3):289-295.

WILLIAMS, C.L., STROBINO, B.A. & BROTHANEK, J. 2007. Weight control among obese adolescents: A pilot study. *International journal of food sciences and nutrition*, 58(3):217-230.

ZLOTKIN, S.H. & TONDEUR, M. 2007. Successful approaches: Sprinkles. (In Kraemer, K. & Zimmermann, M.B., eds. Nutritional anemia. Switzerland: Sight and Life Press, p. 269-284.)

CHAPTER 2

MICRONUTRIENTS AND COGNITION IN CHILDREN: A REVIEW OF THE LITERATURE

CHAPTER 2: MICRONUTRIENTS AND COGNITION IN CHILDREN: A REVIEW OF THE LITERATURE

2.1 INTRODUCTION

Micronutrient malnutrition resulting mainly from the insufficient dietary intake of nutrients such as iron, zinc, vitamin A and iodine affects the health and survival of over two billion people worldwide (Allen, 2005; Sanghvi *et al.*, 2007). Women and children in developing countries are the most adversely affected (West, 2002; Stoltzfus, 2003; De Benoist *et al.*, 2008b; Fischer-Walker *et al.*, 2008; McLean *et al.*, 2009). Other micronutrient deficiencies of public health concern include folate and vitamin B12 (McLean *et al.*, 2008). The deficiencies of these micronutrients are closely linked with more than ten million preventable childhood mortality cases every year in developing countries (Black *et al.*, 2003).

The impacts of these deficiencies have long been studied on the physical health of women and young children. In recent years, research interests are seriously growing in examining the relationship of these nutrients on growth, behavioural and cognitive development of young children (Bhatnagar & Taneja, 2001; Sachdev *et al.*, 2005a; Beard, 2007; Grantham-McGregor *et al.*, 2007; McCann & Ames, 2007; Isaacs *et al.*, 2008). In spite of these growing interests, most studies have focused on controlling single micronutrient deficiencies and little is known about the impact of multiple micronutrient malnutrition on the cognition of undernourished children in developing countries (Black, 2003b; Bryan *et al.*, 2004).

A recent meta-analysis by Ramakrishnan *et al.* (2009) showed that multiple micronutrient interventions improved linear growth while single interventions including iron or vitamin A had no effect on growth. This suggests that several nutrient deficiencies might co-exist (Castejon *et al.*, 2004; Pathak *et al.*, 2007) and addressing nutrient deficiencies through the use of multiple micronutrient formulations might be more beneficial and effective (Zimmermann *et al.*, 2002; Ramakrishnan *et al.*, 2004; Ouédraogo *et al.*, 2008; Ramakrishnan *et al.*, 2009).

This chapter will, therefore, focus on the following: 1) the epidemiology and aetiology of micronutrient malnutrition in developing countries; 2) the burden of micronutrient malnutrition in developing countries; 3) the prevention and control of micronutrient malnutrition; 4) micronutrients in cognitive development and function; 5) micronutrient interactions in child nutrition; 6) the effect of multiple micronutrients on cognition of young children in developing countries and 7) the standardised methods of assessing cognition in preschool children.

2.2 EPIDEMIOLOGY AND AETIOLOGY OF MICRONUTRIENT MALNUTRITION IN DEVELOPING COUNTRIES

2.2.1 EPIDEMIOLOGY OF MICRONUTRIENT MALNUTRITION

Globally, deficiencies of micronutrients [such as iron, vitamin A, zinc, iodine, folate and co-existing multiple micronutrient deficiencies] affect over two billion people (Sanghvi *et al.*, 2007). Countries in Sub-Saharan Africa and South Asia have the largest prevalence and the largest absolute numbers of micronutrient-deficient individuals (De Benoist *et al.*, 2008b; WHO, 2008; UNICEF, 2008; WHO, 2009).

Although there has been significant global progress in reducing the prevalence of iodine, vitamin A and folate deficiencies especially in their severe forms, the prevalence still remains high in some countries (Ramakrishnan, 2002; Sanghvi *et al.*, 2007). Additionally, global prevalence data on iron deficiency are non-existent and the available information on a related indicator, anaemia, suggests little progress (WHO, 2008; McLean *et al.*, 2009). In general, emerging data on the prevalence of micronutrient deficiencies and undernutrition in children from developing countries continue to show a rather worsening situation of public health concern (Black *et al.*, 2008).

2.2.1.1 IRON DEFICIENCY

Iron deficiency anaemia still remains the most widespread nutritional deficiency with public health implications and pregnant women as well as young children are particularly vulnerable (Zimmermann & Hurrell, 2007; McLean *et al.*, 2009). The most recent World Health Organization (WHO) global database on anaemia indicated that globally, 293 million (47.4%) preschool-age children, 305 million (23.4%) school-age children, 56

million (41.8%) pregnant women and 468 million (30.2%) non-pregnant women are anaemic (WHO, 2008). However, the highest proportion of individuals affected is in Africa with 83.5 million (67.6%) preschool-age children being anaemic (WHO, 2008).

Severe iron deficiency results in nutrition anaemia, which is the most widely used indicator of nutritional iron deficiency anaemia (WHO *et al.*, 2001a; McLean *et al.*, 2009). However, using haemoglobin concentration alone as a proxy for the prevalence of nutritional iron deficiency anaemia may be misleading due to the fact that other factors [such as nutritional deficiencies (folate, vitamin B12 and vitamin A deficiencies); genetic abnormalities (thalassemia, haemoglobinopathies); infectious disease (human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS); tuberculosis, malaria) and chronic diseases] could also result in anaemia (WHO *et al.*, 2001a; Zimmermann & Hurrell, 2007). Nevertheless, nutritional iron deficiency is estimated to be responsible for about 50 percent of all anaemia cases (WHO *et al.*, 2001a; Stoltzfus, 2003; WHO, 2008). Such estimates indicate that South East Asia has the highest prevalence of nutritional iron deficiency followed by Africa, Eastern Mediterranean countries and the Western Pacific (**Figure 2.1**) (WHO, 2008).

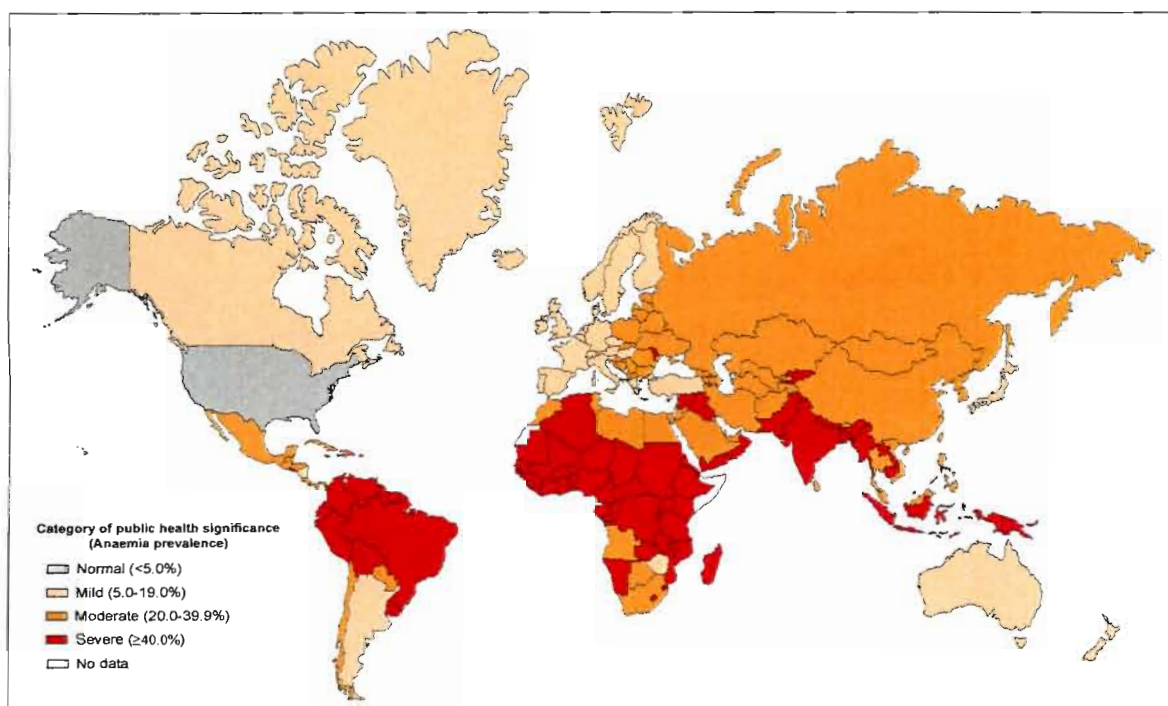


Figure 2.1 Prevalence of anaemia in preschool-age children (Source: WHO, 2008)

In South Africa, the first national survey of vitamin A and iron status in 1994 reported that 11% of children had haemoglobin concentration of less than 11 g/dL and 25% had low-iron stores (ferritin less than 12 µg/dL) (SAVACG, 1996). Additionally, the 1999 South African National Food Consumption Survey (NFCS) for children 1 to 9 years reported that about 41 to 63% of children had iron intakes of less than 50% of the recommended dietary allowance (RDA) (Labadarios *et al.*, 2000; Labadarios *et al.*, 2005). However, the most recent South African NFCS Fortification Baseline reported that the prevalence of poor iron status in children appeared to have increased when compared to previous national surveyed data, such that one out of every seven children have a poor iron status (Labadarios *et al.*, 2008).

2.2.1.2 VITAMIN A DEFICIENCY

According to the WHO global database on vitamin A deficiency (VAD), global estimation of approximately 190 million (33.3%) and 5.2 million (0.9%) preschool children are suffering from VAD with serum retinol less than 0.70 µmol/L and xerophthalmia (dry eyes), respectively (WHO, 2009). VAD is still a major problem of public health in many developing countries (**Figure 2.2**), where an estimated 250,000 to 500,000 vitamin A deficient children go blind every year (West & Darnton-Hill, 2001; West, 2003).

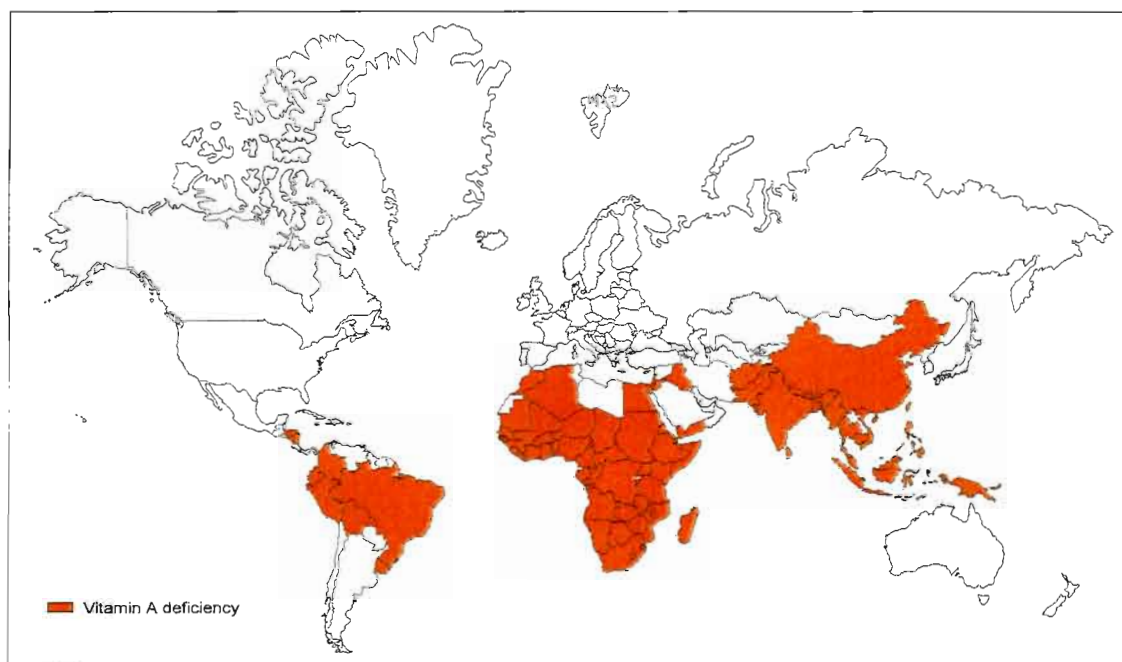


Figure 2.2 Prevalence of vitamin A deficiency in children under 5 years (Source: Black *et al.*, 2008)

Apart from acute eye symptoms, VAD also weakens the immune system, thus increasing the incidence and severity of infectious diseases (West & Darnton-Hill, 2001). Africa still accounts, however, for the highest proportion of preschool-age children at 56.4 million (44.4%) affected with serum retinol less than 0.70 $\mu\text{mol/L}$ when compared to other regions of the world (WHO, 2009). In South Africa, one in three preschool children has a serum retinol concentration less than 0.7 $\mu\text{mol/L}$, and 55 to 68% of children aged 1 to 9 years consume diets that supply less than 50% of the recommended dietary intake of vitamin A (700 μg retinol equivalents) (Labadarios *et al.*, 2000; Labadarios *et al.*, 2005). Despite several micronutrient control initiatives among South African children, a recent national survey revealed that 63.6% and 13.7% had inadequate vitamin A status (serum retinol < 0.70 $\mu\text{mol/L}$) and severe vitamin A deficiency (serum retinol < 0.35 $\mu\text{mol/L}$) respectively, and children living in rural areas were the most affected (Swart *et al.*, 2008).

2.2.1.3 IODINE DEFICIENCY

Iodine deprivation is the most common cause of preventable mental handicap worldwide (Zimmermann, 2009b). Prior to universal salt iodisation, the WHO micronutrient deficiency information system (MDIS) reported that worldwide, an estimated 655 million iodine deficient individuals suffered from goitre in many developing populations (WHO, 1993). Currently, because of widespread usage of iodised salt, significant improvement has been achieved in the control of this nutritional deficiency (De Benoist *et al.*, 2008b).

However, the risks of severe iodine deficiency are still prevalent especially among young preschool children and pregnant women living in remote places (Vanderpas, 2006). A recent study conducted by De Benoist *et al.* (2008b) on iodine nutrition reported that globally, an estimated 2 billion people have insufficient iodine intakes of which 31.5% (266 million) are school age children from low-income countries (**Figure 2.3**). Africa next to South-East Asia has the highest number of school-age children (57.7 million) suffering from low iodine intake (urinary iodine less than 100 $\mu\text{g/L}$) (De Benoist *et al.*, 2008b). Iodine deficiency occurs as a result of low iodine intake usually below the recommended levels (Zimmermann, 2009b). As reviewed by Jooste and Zimmerman (2008), substantial progress has been achieved in South Africa in eradicating iodine deficiency by the introduction of mandatory iodisation of table salt (40 to 50 ppm) since 1995.

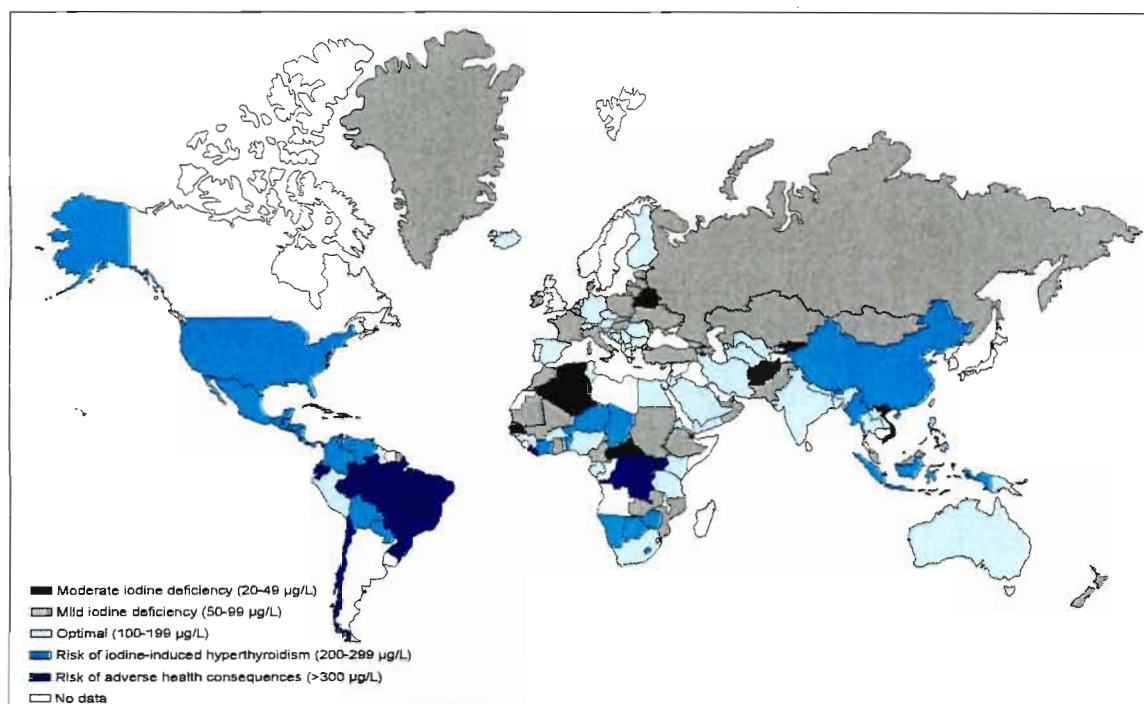


Figure 2.3 National iodine nutrition based on median urinary iodine (Source: De Benoist *et al.*, 2008b)

There seems to be a virtual elimination of iodine deficiency disorders with the possibility of excesses (urinary iodine greater than 300µg/L) in some areas evident by high urinary iodine concentration in some regions as assessed by the most recent NFCS in 2005 (Labadarios *et al.*, 2008). The incidence of high iodine intake in South Africa is a cause for serious concern as this might have detrimental effects on childhood intelligence (Liu *et al.*, 2009), however, the small risks of iodine excess are far outweighed by the substantial risks of iodine deficiency (Zimmermann, 2008a).

2.2.1.4 ZINC DEFICIENCY

There is an increasing global interest in the importance and role of zinc nutrition in public health (Hess *et al.*, 2009). Zinc deficiency resulting mainly from the inadequate dietary intake of absorbable zinc increases the risk and severity of a variety of infections such as malaria, diarrhea, pneumonia or other lower respiratory infection (Caulfield & Black, 2004), and is said to affect an estimated 25 to 33% of developing country populations (IZiNCG *et al.*, 2004). Additionally, zinc deprivation may also result in physical growth

and cognitive development delays in young children (Bhatnagar & Taneja, 2001; Black *et al.*, 2004a).

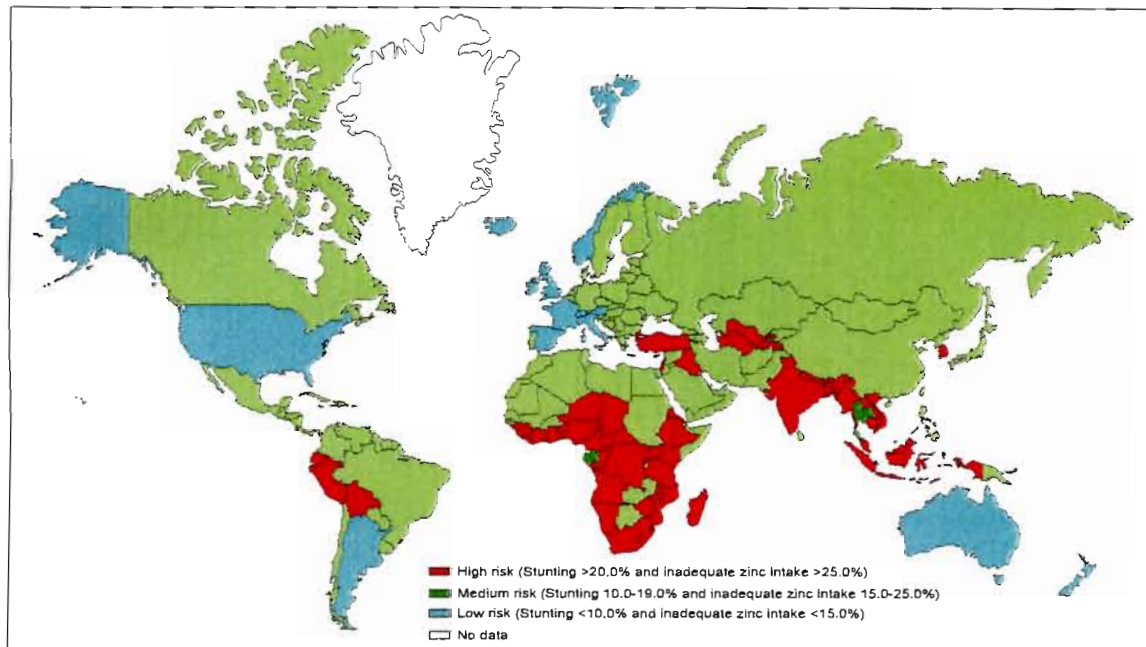


Figure 2.4 National risk of zinc deficiency in children under 5 years (Adapted from IZiNCG, 2004)

However, because no single indicator exists yet for assessing zinc status, different countries have been classified into three categories at risk of zinc deficiency (**Figure 2.4**), based on the combination of stunting (height-age-z score < -2SD) prevalence and inadequacy of zinc intake (zinc intake below the estimated average requirement (EAR)) (IZiNCG *et al.*, 2004). A recent study by Fischer-Walker *et al.* (2009) suggested that in developing countries, zinc deficiency in children under five years is responsible for approximately 453,000 deaths and 16 million disability-adjusted life years (DALYs) annually mainly from malaria, diarrhea and lower respiratory infection.

The highest prevalence rate of zinc deficiency in children under five years is in Africa where it causes about 260,000 deaths and 9.4 million disability adjusted life years (DALYs) yearly (Fischer-Walker *et al.*, 2009). South African children 1 to 9 years are at high risk of zinc deficiency and according to the NFCS (Labadarios *et al.*, 2000), 52-60% had zinc intake less than the RDA in which 21.6% of this at-risk children stunted. A recent review of previous and recent national surveys among South African children by

Swart *et al.* (2008) revealed that in 2005, 45.3% were zinc deficient (serum zinc < 65 µg/dL).

2.2.1.5 FOLATE DEFICIENCY

The public health concern with respect to folate deficiencies in developing countries is relatively new and there are indications that population groups consuming monotonous refined staple crops might be at risk (Cherian *et al.*, 2005; Allen, 2009). Deficiencies of folate during the pre-conceptional period may contribute to neural tube defects and negative brain development respectively (Black *et al.*, 2008); while during adulthood it is associated with greater risk of depression (Tiemeier *et al.*, 2002; Sachdev *et al.*, 2005b). Recent global prevalence data showed that folate deficiencies around the world appear to be a public health problem, but more representative larger prevalence data are still needed (MacLean *et al.*, 2008). Although most anaemia in developing countries is due to iron deficiency (Zimmermann & Hurrell, 2007), a proportion might also be due to deficiency of the vitamin B complex, principally folate (Allen, 2008).

Prior to folic acid fortification in South Africa, a large percentage of children and women of reproductive age had intakes less than 50% of the recommended daily allowance (Labadarios, 2000; Labadarios *et al.*, 2005). Inadequate folate intake during pregnancy is often associated with neural tube defects, low birth weight and impaired development of the foetus or child (Allen, 2005; Molloy *et al.*, 2008).

2.2.1.6 SUMMARY

In developing populations, the concurrent prevalence of several micronutrient deficiencies is common (Allen *et al.*, 2000; Dijkhuizen *et al.*, 2001; Adelekan, 2003; Ahmed *et al.*, 2008; Anderson *et al.*, 2008). Several observational studies from low-income countries consistently show that co-existence of two (Zimmermann *et al.*, 2000; Oelofse *et al.*, 2002; Palafox *et al.*, 2003), three (Seshadri, 2001; Pathak *et al.*, 2007; Duque *et al.*, 2007) or more (Thurlow *et al.*, 2006; Lander *et al.*, 2008) micronutrient deficiencies often occur. Therefore, addressing these deficiencies through the use of multiple micronutrient formulations might be more effective owing to the fact that several micronutrient deficiencies can be targeted at once (Gera *et al.*, 2009; Ramakrishnan *et al.*, 2009).

2.2.2 AETIOLOGY OF MICRONUTRIENT MALNUTRITION

The aetiology of micronutrient deficiencies is complex and multifactorial (Vorster *et al.*, 1997; Black *et al.*, 2008). The complexities and interrelated causes and consequences of undernutrition are shown in **Figure 2.5**, which clearly demonstrates that the consequences of undernutrition as well as micronutrient deficiencies are often exacerbated by the causes. For instance, undernutrition increases the risk of infectious diseases, while infectious diseases contribute to undernutrition. Although, poverty is the root cause of undernutrition, **Figure 2.5** further illustrates that to address undernutrition or micronutrient deficiencies, the interrelated contributory factors should be mitigated in community-based, intersectoral programs with focus on alleviating poverty and development of human capital (Vorster *et al.*, 1997).

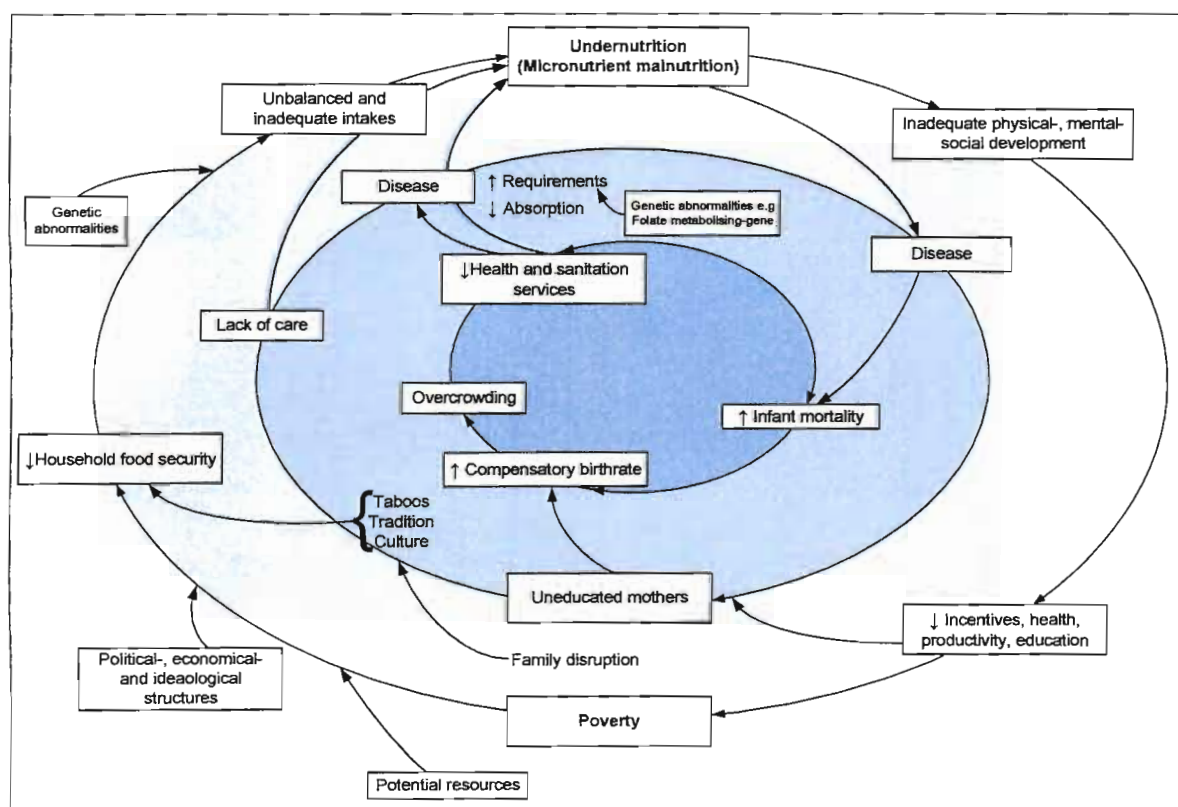


Figure 2.5 The vicious cycle of undernutrition causes and consequences (Adapted from Vorster *et al.*, 1997).

In view of these, the development of micronutrient deficiencies can thus be generally attributed to at least four contributory factors occurring either in isolation or in

combination (**Figure 2.5**). These include dietary factors, infectious diseases, genetic factors and environmental factors. However, one of the major preventable causes of micronutrient malnutrition is inadequate dietary intake of these essential nutrients. Other important causes of micronutrient deficiency of public health implication include malabsorption due to high infection rate such as HIV/AIDS, tuberculosis, malaria, parasites (Thurnham *et al.*, 2003; Thurnham & Northrop-Clewes, 2007); genetic abnormalities (haemoglobinopathy, genetic polymorphisms in folate metabolising enzyme); chronic diseases, poor sanitation (Berkman *et al.*, 2002) and poverty.

For the purpose of this review, the focus will be on the four major causes of micronutrient deficiencies [mainly of iron, zinc, vitamin A, iodine and folate] within the African and South African context.

2.2.2.1 DIETARY FACTORS

In most African countries, the most important cause of poor micronutrient status is low dietary intake of these essential nutrients. Some other causes of nutrient deficiencies of public health importance related to dietary issues include low bioavailability, presence of inhibitors, excessive intake of goitrogenic substances and losses during food processing.

Low consumption of micronutrient-rich foods

Inadequate intake of micronutrient-rich diet is one of the most important factors predisposing individuals in many developing populations to micronutrient deficiencies. In Sub-Saharan Africa for instance, consumption of micronutrient-rich foods, such as animal source foods (red meat, poultry, fish) and green leafy vegetables is often poor because of economic, cultural and religious constraints (Gibson *et al.*, 2006; Allen, 2008), while on the other hand, these populations subsist mainly on micronutrient-poor monotonous starchy (Zimmermann *et al.*, 2005a; Hotz & Gibson, 2007) or refined plant-based diets (Gibson & Hotz, 2001; Krittaphol *et al.*, 2006). Plant-based diets in developing populations consisting mainly of staple crops [such as rice, maize, yam, cassava, sorghum] are often associated with micronutrient deficits, exacerbated in part by poor micronutrient bioavailability of dietary iron, zinc, provitamin A carotenoids and folate (Gibson *et al.*, 2006).

Low bioavailability and presence of inhibitors

Micronutrient bioavailability is a function of micronutrients ingested in food that is absorbed and the ability to utilise the nutrients for normal biochemical, metabolic and physiological functions (Jackson, 1997). Low-income populations depend mainly on plant-based diets from staple cereals, starchy roots, tubers and legumes which are low in bioavailable micronutrients [iron, zinc, provitamin A carotenoids, folate] and high in inhibitors or anti-nutrients (Ferguson *et al.*, 1993; Gibson, 1994; Hotz *et al.*, 2001).

In iron and zinc nutrition, the high intake of inhibitors or anti-nutrients [such as phytic acid in cereal grains, polyphenols in beverages, oxalate in some vegetables] can form insoluble complexes with iron or zinc in the gastrointestinal tract, thereby inhibiting iron or zinc absorption (Hurrell *et al.*, 1999; Hotz *et al.*, 2001; Kim *et al.*, 2007). Additionally, evidence also exists that diets containing high dietary fibre content may also limit folate bioavailability in plant-based diets (Gregory, 1997). The negative effects of low micronutrient bioavailability on nutritional status and subsequent health are potentially quite substantial (Zimmermann *et al.*, 2005a; Thankachan *et al.*, 2007; Campbell *et al.*, 2009).

Excessive intake of goitrogenic foods

Excessive intake of goitrogenic foods may adversely affect iodine status, such that mild, moderate or even severe iodine deficiency may sometimes occur in the presence of adequate iodine intake (Vanderpas, 2006; Chandra *et al.*, 2008). Excessive intake of goitrogenic substances such as glucosinolates and cyanogenic glycosides which naturally occur in foods like cassava, sweet potato, lima seeds, sorghum, cruciferous vegetables, soy, and millet can interfere with iodine uptake in the thyroid gland (Lamberg, 1993; Vanderpas, 2006).

For instance, cassava or sorghum contains cyanogenic glycosides which are metabolised to thiocyanates that can block the uptake of iodine by the thyroid gland; additionally, cruciferous vegetables [such as cabbage or broccoli] contain glucosinolates which also compete with iodine for thyroidal uptake (Vanderpas, 2006). Developing populations living in remote areas that are subsisting mainly on staple crops containing goitrogens may be at risk (Akindahunsi *et al.*, 1998; Pineda-Lucatero *et al.*, 2008). It may

be important, however, to consider the possible impact of goitrogens in the aetiology of iodine deficiency when implementing table salt iodisation programs.

Losses during food processing

Provitamin A carotenoids and folates from vegetable sources are relatively unstable to oxidation or heat and large losses may occur during prolonged cooking and processing (Mosha *et al.*, 1997). For instance, boiling destroys 50 to 80% of folate in green leafy vegetables (McKillop *et al.*, 2002) and 50% of that of legumes (Dang *et al.*, 2000). Additionally, traditional processing practices involving sun drying and heat treatments [such as deep frying, prolonged cooking and baking], and a combination of multiple preparation and processing methods of vegetables common in many Sub-Saharan African countries can result in substantial losses of provitamin A carotenoids (Mosha *et al.*, 1997; Yadav & Sehgal, 1997; Rodriguez-Amaya, 2003; Muzhingi *et al.*, 2008).

2.2.2.2 INFECTION

Helminth infections

Over two billion people are estimated to be infected with one or more soil-transmitted helminths (Hall *et al.*, 2008). Helminth infection (intestinal parasites) [such as hookworm, roundworm and whipworm] in children may adversely affect nutrient uptake, absorption and micronutrient status. High helminthes infestation can interfere with the uptake of vitamin A or iron, such that the parasite can alter the structure of the intestinal mucosa leading to malabsorption of these micronutrients (Stoltzfus *et al.*, 1997; Hall *et al.*, 2008). For instance, intestinal parasitic infestation may contribute to negative iron balance through occult gastrointestinal blood loss (Haowdon & Hotez, 1996) and may interfere with iron absorption (Stoltzfus *et al.*, 1997). Several studies have demonstrated benefits of anthelmintic treatment in children such as improved growth, micronutrient status, appetite, physical activity and school performance (Simeon *et al.*, 1995; Stephenson *et al.*, 1993; Jinabhai *et al.*, 2001a; Stoltzfus *et al.*, 2004b).

Malaria infection

Global prevalence of micronutrient malnutrition in particular of iron deficiency anaemia still remains high, especially in areas of the world where malaria morbidity and mortality are high (Prentice *et al.*, 2007). Malaria is an important cause of anaemia in Sub-Saharan Africa (Snow *et al.*, 1999). Severe anaemia may be induced during malaria

infection mainly through the clearance and /or destruction of infected red blood cells by the malaria parasite *Plasmodium falciparum* (Lamikanra *et al.*, 2007). In malaria-endemic areas, the incidence and age pattern of severe anaemia were strongly dependent on the intensity of malaria transmission (Snow *et al.*, 1994). Malaria control trials have been associated with significant reductions in the prevalence of anaemia in children and pregnant women (Alonso *et al.*, 1991).

Human immune deficiency virus / Acquired immune deficiency syndrome

HIV infections negatively influence the nutritional status through its attacks on the immune system and its adverse effects on nutrient intake, absorption, metabolism and utilization are well known (ASSAF, 2007; Hendricks *et al.*, 2007). HIV infection may either induce excessive loss and/or impair the utilisation of micronutrients (Cunningham-Rundles *et al.*, 1996; Dreyfuss & Fawzi, 2002; Shet *et al.*, 2009). However, more than 95% of HIV infections in children are acquired through mother-to-child transmission (Newell, 1998). Furthermore, severe undernutrition and co-existing multiple micronutrient deficiencies are highly prevalent in HIV-infected children studied in several African countries (Kurawige *et al.*, 1993; Kessler *et al.*, 2000; Reddi *et al.*, 2007). Studies in South Africa for instance, demonstrated that more than 50% HIV infected children before antiretroviral therapy were stunted or underweight (Reddi *et al.*, 2007); additionally, other studies have also documented high prevalence rate of iron, zinc, vitamin A and co-existing multiple micronutrient deficiencies in these children (Eley *et al.*, 2002a; Eley *et al.*, 2002b).

Micronutrients are essential for immune functioning and deficiencies may, therefore, act as cofactor in HIV disease transmission and progression (Periquet *et al.*, 1995). On the other hand, according to a recent review by ASSAF (2007), there are some indications that micronutrient intervention with multivitamin (excluding vitamin A) supplementation in HIV-infected children and pregnant women may reduce the risk of the disease progression, adverse pregnancy outcomes and mortality (Fawzi *et al.*, 1998; Fawzi *et al.*, 2004; Villamor *et al.*, 2005).

2.2.2.3 GENETIC FACTORS

Although studies on the associations between nutrition and genetics are still at infancy in many developing populations, the pivotal role of genetics as it affects human well-being

is becoming an important concept in nutrition science (El-Soheemy, 2007). Available data suggests that genetics might be an important factor in the aetiology of some micronutrient deficiencies (Amouzou *et al.*, 2004; Tsaras *et al.*, 2009) which could have major intervention implications on micronutrient control measures in the affected individuals. However, for the purpose of the present review two major genetic polymorphisms that may predispose affected individuals to iron or folate deficiency will be briefly discussed.

Haemoglobinopathies

Haemoglobinopathies and other haemoglobin disorders are genetic haemoglobin disorders common in malaria endemic areas and have been associated with protection against the effects of severe malaria (Williams *et al.*, 2005; Wambua *et al.*, 2006a). The most common type of haemoglobinopathies are thalassemia and sickle cell disease. They are common in ethnic populations from Africa, where malaria infections are high. For instance, sickle cell trait occurs in approximately 300 million people worldwide, with the highest prevalence of approximately 30 to 40% in Sub-Saharan Africa (Tsaras *et al.*, 2009). Additionally, according to Diallo *et al.* (2008), about 150,000 to 300,000 sickle cell anaemia homozygous individuals are born every year. Most clinically significant haemoglobinopathies cause mild to acute anaemia, in sickle cell disease the red blood cells tend to assume a sickle shape under anaerobic conditions, leading to organ damage and circulatory problems, while in thalassemia there is ineffective production of red blood cells (erythropoietin) (Wambua *et al.*, 2006a; Wambua *et al.*, 2006b).

Genetic polymorphism in folate-metabolising gene

The polymorphism in the methyltetrahydrofolate reductase (MTHFR) gene [such as the 677TT and 1298C alleles] affects approximately 10 to 30% of Caucasians (Stevenson *et al.*, 1997). In a study among West African population by Amouzou *et al.*, (2004), moderate hyperhomocysteinemia (with folate < 6.75 nmol/L and MTHFRCT/TT genotype as major risk factors) was found in 62.3% and 29.4% of the subjects from the coastal and savanna regions, respectively. Furthermore, a recent study among a sample of 1,844 South African population revealed that the distribution of the mutant genotypes 677TT, heterozygous genotypes 677CT and homozygous wild-type 677CC were 0.81% (15), 15.24% (281) and 83.95% (1,548), respectively (Nienaber C, In preparation). The MTHFR polymorphism is associated with increased plasma homocysteine and a

tendency to lower serum and erythrocyte folate concentrations (Molloy *et al.*, 1997); this can increase the risks for neural tube defects (Van der Put *et al.*, 1998; Motulsky, 1996). However, the 677TT seems to be generally associated with much higher homocysteine levels in Africans than those described in Caucasians, Hispanics or Mexicans (Guéant-Rodriguez *et al.*, 2006).

2.2.2.4 ENVIRONMENTAL FACTORS

Poor hygiene

Poor hygiene and unhealthy sanitation lead to increased risks of infections and parasite infestations (Muoki *et al.*, 2008). Access to a clean and healthy environment reduces the risk of infectious diseases. Households need to practice good environmental and personal hygiene, drink safe water, and keep food safe and clean (Curtis & Cairncross, 2003; Muoki *et al.*, 2008).

Low micronutrient content in the soil

Soils deficient in their ability to supply micronutrients to crops are widespread across the globe (White & Zasoski, 1999). For instance, soils may become deficient of iodine or zinc due to leaching effects of glaciations, snow, high rainfall and floods. Micronutrient deficiencies in soils may not only limit crop production, but may also predispose animals and populations subsisting on plants grown in such regions to risk of micronutrient deficiencies (WHO *et al.*, 2001b; Yang *et al.*, 2006).

The association between soil-plant-animal and human related micronutrient deficiencies may have been suppressed in many developed countries due to regional and international movement of foods, food fortification and supplementation programs (Thornton & Alloway, 1974; White & Zasoski, 1999), the association still exists and is greatest in rural communities of many developing populations where animal and human diets are largely local in origin (Liu, 1994; Khin-Maung-Naing *et al.*, 1989; Gbadebo & Oyesanya, 2005; Assey *et al.*, 2006). Several initiatives are in place [for example biofortification, salt iodisation, use of micronutrient rich fertilizers] such that populations residing in these areas may not necessarily suffer from micronutrient deprivation (Yang *et al.*, 2006; WHO *et al.*, 2001b).

Taken together, the various causes of micronutrient deficiencies are broad and complex (**Figure 2.5**), so also the enormous burden they impose on human wellbeing and development globally. This however, has negative economic and developmental implications at national and international levels.

2.3 BURDEN OF MICRONUTRIENT MALNUTRITION

Historically, the severity and burden of undernutrition and micronutrient deficiencies in children have usually been assessed based on mortality statistics (Mosley & Chen, 2003; Black *et al.*, 2003; Jones *et al.*, 2003). However, mortality data alone may underestimate the adverse outcome of many nutritional deficiencies (Grantham-McGregor *et al.*, 2007), which may also have considerable long or short term health, social and economic consequences (Darnton-hill *et al.*, 2005; Victora *et al.*, 2008; Black *et al.*, 2008).

Recently, the use of DALYs as a single indicator of total loss of health gives a better indication of the negative impact of micronutrient deficiencies. The DALYs combine years of life lost (YLL) due to cause-specific premature death and years of life lived with disability (YLD) into a single index, such that one DALY can be considered as approximately one lost year of healthy life (WHO, 2002). Additionally, it provides an overall estimate of the magnitude of economic losses in a population due to disease (World Bank, 1993; Murray & Lopez, 1994).

Furthermore, several studies have adopted the use of DALYs in analysing either the burden of diseases attributed to micronutrient deficiencies (Nannan *et al.*, 2007; Nojilana *et al.*, 2007a; Nojilana *et al.*, 2007b; Black *et al.*, 2008) or the potential health and economic benefits of micronutrient intervention programs (Baltussen *et al.*, 2004; Robberstad *et al.*, 2004; Sharieff *et al.*, 2008; Stein *et al.*, 2005; Meenakshi *et al.*, 2007). For example, the recently published best practice papers commissioned by Copenhagen consensus centre as reported by Horton *et al.* (2009a) and Horton *et al.* (2009b), adopted the use of the DALY in their economic arguments in favour of several micronutrient control programs. In their analysis they assessed the potential cost benefits ratios of eliminating micronutrient deficiencies using the assumption that one DALY is valued at \$1000, however, this will be discussed in **section 2.4**. For the

purpose of this review section, the burden of diseases attributed to deficiencies of iron, zinc and vitamin A within the global and South African context will be discussed.

Burden of iron deficiency

Several adverse effects of iron deficiency or iron deficiency anaemia in developing populations have been related to certain physiological and clinical deficits such as poor pregnancy outcome, delayed development in infants and children (Grantham-McGregor, 2001; Stoltzfus *et al.*, 2004a), compromised immune function (Beard, 2001) and impaired work capacity or productivity in adults (Beard, 2001; Haas & Brownlie, 2001; Horton & Levin, 2001). Furthermore, iron deficient preschool children in Indonesia and India have been found to have poorer cognitive performance than those with normal iron status and lower cognitive performance was substantially improved after 12 weeks of iron supplementation (Soemantri *et al.*, 1985; Seshadri & Gopaldas, 1989).

In economic terms, Horton and Ross (2003) reported that in an analysis of ten developing countries, median total annual losses (due to the effect of iron deficiency on physical and cognitive skills combined) are estimated US \$16.78 per capital, or 4.05% of the GDP (gross domestic product). A recent analysis in children less than 5 years demonstrated that in Sub-Saharan Africa, iron deficiency anaemia is responsible for an estimated 21,000 deaths annually and over 14 million DALYs lost (Stoltzfus *et al.*, 2004a). In South Africa, Nojilana *et al.* (2007a) revealed that approximately 175,000 YLLs or 0.9% to 1.3% of all DALYs in South Africa in 2000 can be attributed to iron deficiency anaemia.

Burden of vitamin A deficiency

When vitamin A status deteriorates, serum retinol concentration remains unaffected until liver stores are depleted. Decline in serum retinol concentration before the appearance of clinical eye signs is associated with increased severity of infections or alteration in immune status (McLaren & Frigg, 2001). If vitamin A is not replenished, serum retinol will continue to fall until signs of xerophthalmia (dry eyes) appear (Sommer & West, 1996). Thus, the pathological changes due to VAD can contribute to the severity of respiratory, diarrhoeal, measles, malaria and HIV infections; subsequently decreasing the survival of affected children in developing populations (McLaren & Frigg, 2001).

According to Rice *et al.* (2004), VAD is responsible for 630,000 deaths annually in children less than 5 years. Sub-Saharan Africa seems to be hardest hit with an annual VAD attributed death rate of about 383,000, and approximately 13.6 million DALYs lost in children in this age group (Rice *et al.*, 2004). In South Africa, VAD account for approximately 111,000 healthy years lost and 0.5% to 8% of all DALYs in 2000. Additionally, VAD is also responsible for an estimated 3,000 annual deaths of children under 5 years (Nojilana *et al.*, 2007b) this situation may, however, have deteriorated due to worsening vitamin A status of children as reported by Labadarios *et al.* (2008).

Burden of zinc deficiency

Zinc is an essential micronutrient for human health and well known for its multiple structural and biochemical functions at cellular and sub-cellular levels, such as DNA and RNA metabolism, protein synthesis, gene expression, cell growth and differentiation, cell mediated immunity and neuro-behavioural development (Vallee & Falchuk, 1993; King *et al.*, 2000). Evidence from recent meta-analyses strongly implicates zinc deficiency with adverse functional outcomes that are strongly associated with diarrhea, pneumonia and stunting (growth retardation) in young children (Brown *et al.*, 2009; Haider & Bhutta, 2009). Caulfield and Black (2004) revealed that zinc deficiency is responsible for approximately 780,000 deaths each year in children under five years, out of which Sub-Saharan Africa accounts for about 400,000 deaths and 748,000 DALYs lost in children under five years (Caulfield & Black, 2004). Recently, Fischer-Walker *et al.* (2009) reported that zinc deficiency among African children less than five years results in 9.4 million DALYs yearly. With the current prevalence rate of zinc deficiency as high as 45.3% among South African children (1 to 9 years) (Labadarios *et al.*, 2008), the DALYs and mortality rate per year attributed to this deficiency will probably be quite substantial as well (Nannan *et al.*, 2007).

The social and economic costs (both nationally and internationally) of micronutrient deficiencies are enormous; however, strategies are in place to help break the intergenerational vicious cycle of undernutrition and its consequences in many developing populations.

2.4 PREVENTION AND CONTROL OF MICRONUTRIENT MALNUTRITION

According to the most recent Copenhagen consensus, controlling micronutrient deficiencies was considered the top-ranked strategies for improving human welfare globally particularly in developing countries, in terms of high economic benefits compared to costs as assessed by some of the world's most distinguished economists consisting of five Nobel laureates (Copenhagen consensus, 2008). The three topmost strategies that were unanimously selected for correcting micronutrient deficiencies in developing countries were: food fortification, biofortification and micronutrient supplementation.

Like many other African countries, the South African government designed a three-way food-based approach to combat micronutrient malnutrition; these include a micronutrient supplementation program for women and children, mandatory food fortification and nutritional educational program (Kloka, 2003). For the purpose of this review, the micronutrient malnutrition control strategies that were highly ranked in the 2008 Copenhagen consensus: food fortification, biofortification and micronutrient supplementation will be briefly and selectively discussed. While each of these can help reduce the burden of micronutrient deficiencies, none is capable of alleviating the problem on its own (Copenhagen consensus, 2008).

(i) Food fortification

Food fortification is perhaps the most cost effective, practical, medium and long-term solution capable of effectively controlling micronutrient deficiencies (WHO / FAO, 2006); it involves adding the required micronutrient to commonly eaten foods by large segments of the population. For food fortification program to be successful, it is of utmost importance to consider the nutrient requirement of the target population (**Table 2.1**), and periodically monitor the efficacy or effectiveness of interventions with the use of specific laboratory measures of micronutrient status (**Table 2.2**) in the target population where possible (Zimmermann & Hurrell, 2007). A food fortification approach usually involves either mass fortification of a food vehicle at industrial level (WHO / FAO, 2006) or targeted fortification at the point of use involving the use of micronutrient powders (Zlotkin & Tondeur, 2007; De Pee *et al.*, 2008).

Table 2.1 Recommended dietary allowance (RDA), mean intake and % RDA of some selected micronutrients in South African children aged 1 to 6 years (Adapted from Labadarios *et al.*, 2000)

Micronutrients	RDA		Mean Intake		% RDA	
	1 - 3y	4 - 6y	1 - 3y	4 - 6y	1 - 3y	4 - 6y
Iron (mg/day)	7	10	4.8	6.4	68.6	64.0
Zinc (mg/day)	7	10	4.2	5.3	60.0	53.0
Vitamin A (RE/day)	400	500	359	425	89.8	85.0
Folate (µg/day)	150	200	94	143	62.7	71.5

RDA, Recommended daily allowance; MI, Mean intake; % RDA, Percentage recommended daily allowance; RE, Retinol equivalent

Mass micronutrient fortification

The aim of mass fortification (usually at industrial level) is to add micronutrients to a vehicle (food or condiment) that is regularly consumed by the targeted population at a level that will correct an existing nutrient deficiency (Mannar & Gallego, 2002). This approach is relatively cost effective, for instance, the cost of iron fortification varies according to the iron compound and food vehicle used, but can be within the range of \$0.10 to \$0.12 per person per year with benefit to cost ratio of 7.8 to 1 (Horton & Ross, 2003; Horton *et al.*, 2009b).

Additionally, the benefit to cost ratio of salt iodization is approximately 30 to 1 at the cost of \$0.5 per person per year (Horton, 2006; Horton *et al.*, 2009b). In most African countries, the food vehicles most often used for mass fortification are the staple cereal flours. In South Africa, micronutrient fortification vehicles such as table salt, bread and maize flour are consistently consumed in most households and are thus currently fortified with iodine (for salt) and other essential micronutrients (for bread and maize flour) (Labadarios *et al.*, 2000; Kloka, 2003).

Table 2.2 Summary of cut-off values for blood levels of micronutrients (iron, zinc, vitamin A, iodine & folate) in preschool-age children (24 – 72 months) (Adapted from De Pee & Dary, 2002; IZNEG, 2004; WHO, 2008; Zimmermann *et al.*, 2005a; Zimmermann, 2008b)

Micronutrient Status	Biochemical Indices	Cut-off Value	
		≤60 Months	> 60 Months
Iron	Haemoglobin (g/dL)	< 7.0 Severe anemia 7.0 - 9.9 Moderate anemia 10.0 - 10.9 Mild anemia	< 7.0 Severe anemia 7.0 - 9.9 Moderate anemia 10.0 - 11.49 Mild anemia
	Plasma or serum ferritin (µg/L)	< 12.0	< 15.0
	Serum transferrin receptors (mg/L)		> 7.6
	Erythrocyte zinc protoporphyrin (µmol/mol haem)	> 70.0	> 80.0
Zinc	Plasma or serum zinc (µg/dL)	< 65.0	< 65.0
Vitamin A	Serum retinol (µmol/L)	< 0.35 Severe VAD < 0.70 Inadequate VAS	< 0.35 Severe VAD < 0.70 Inadequate VAS
Iodine	Median urinary iodine concentration (µg/L)	< 100.0	< 20.0 Severe ID 20.0 - 49.0 Moderate ID 50.0 – 99.0 Mild ID
	Serum or whole blood thyroglobulin (µg/L)		4.0 – 40.0 Iodine Sufficient
Folate	Serum folate (µg/L)	< 4.4	< 4.4

ID, Iodine deficiency; VAD, Vitamin A deficiency; VAS, Vitamin A status

Point-of-use micronutrient fortification

There has been rapid development of point-of-use fortificants [such as micronutrient powders, crushable tablets and lipid-based nutrient supplements] which are designed to address deficiencies in multiple micronutrient and many other nutrient deficiencies (Zlotkin & Tondeur, 2007). Untargeted micronutrient supplementation or fortification of young children in high infection areas [such as areas of high malaria endemicity] may increase morbidity and mortality (Sazawal *et al.*, 2006). In contrast to untargeted fortification, point-of-use fortification targets specific individuals at risk and can be an

effective, low-cost strategy for providing additional dietary iron and other micronutrients to vulnerable individuals (Christofides *et al.*, 2006).

Several micronutrient powders have been developed [such as “MixMe plus[®]” (DSM Nutritional Products Ltd, Basel, Switzerland) and “Sprinkles[™]” (Sprinkles Global Health Initiative, Toronto, Canada)]. These point-of-use micronutrient fortificants can be added directly to either already prepared complementary foods for infants or school meals after cooking (Zlotkin & Tondeur, 2007; Troesch *et al.*, 2009). A recent cost and impact analyses by Sharieff *et al.* (2008) concluded that benefit to cost ratio of “Sprinkles[™]” (containing iron and other micronutrients) can be as high as 37:1 at the cost of \$1.20 per person per year (for children 6 to 24 months).

Furthermore, in developing countries, complementary foods and school meals are predominantly plant-based and are often inadequate to meet the nutritional requirements of infants and young children. This may be due to low energy density and low bioavailability of essential nutrients [such as iron and zinc] due to presence inhibitors such as phytate in maize (Gibson *et al.*, 2006). The application of enzymes such as amylase or phytase containing micronutrient powder may help improve the nutrient density or micronutrient bioavailability of complementary foods or school meals that are predominantly plant-based (Owino *et al.*, 2007; Troesch *et al.*, 2009).

Several studies involving the use micronutrient powders conducted in Ghana, India, Bangladesh, and many other developing countries have consistently demonstrated that it positively influences the micronutrient status of infants when administered at home (Zlotkin & Tondeur, 2007). In South Africa as well as many developing countries, schools are being used as an effective delivery point for many health and nutrition programs (Van Stuijvenberg *et al.*, 1999; Steyn & Marais 2002; Van Stuijvenberg, 2005; Manger *et al.*, 2008). It can also be used for the implementation of point-of-use micronutrient fortification programs among children at-risk of micronutrient deficiencies.

(ii) Biofortification

Biofortification of staple crops is a relatively novel approach and represents an alternative as well as a sustainable strategy for controlling micronutrient deficiencies especially among the rural poor in developing countries (Bouis, 2000; Nestel *et al.*,

2006). It involves breeding new crop cultivars with increased micronutrient-density through conventional selective breeding or genetic engineering (Welch, 2002). Breeding programs are in place and several studies conducted in a range of staple crops normally consumed in many developing countries demonstrated that this micronutrient control approach is promising (Tako *et al.*, 2009; Howe *et al.*, 2009).

A randomised controlled trial conducted among South African primary school children showed that that consuming beta-carotene-rich sweet potato, which provided about 830 retinol activity equivalents/100 g cooked root, improved vitamin A liver stores as measured by the modified relative dose-response test (Van Jaarsveld *et al.*, 2005). Additionally, Haas *et al.* (2005) also revealed that in a double-blinded controlled intervention, iron-enhanced rice supplying 1.41 mg of iron per day was effective in improving serum ferritin concentrations and body iron levels in non-anemic women compared with the control locally consumed rice. The potential health benefits of biofortification, estimated in median cost per DALY saved is about \$10 per DALY saved (optimistic scenario) and \$120 per DALY (pessimistic scenario), at an estimated annual cost per crop per country from US\$500,000 to 1,000,000 (Meenakshi *et al.*, 2007). Further studies still need to be conducted to be able to determine the true public health potentials of this approach.

(iii) Micronutrient supplementation

Supplementation with pharmacological doses can effectively reduce micronutrient deficiencies within a short period of time, especially among vulnerable population groups, such as infants, school children and pregnant women (WHO *et al.*, 2001b). Meta-analyses of randomised controlled trials of the impact of zinc supplementation, suggest that therapeutic zinc for diarrhoea may be effective in reducing mortality risks, duration as well as severity of persistent diarrhoea among children ≤ 5 years (Bhutta *et al.*, 2000; Lukacik *et al.*, 2008). Furthermore, according to Copenhagen consensus best practices paper on micronutrient supplements (vitamin A and zinc) for child survival, estimated cost of vitamin A supplementation is \$1.20 per person per year with benefit to cost ratio of 17 to 1, while that of zinc supplementation is 13.7 to 1 at the cost of \$1.00 per person per year (Horton *et al.*, 2009a). In South Africa like many other African countries, high-dose vitamin A supplementation is being implemented nationally among children aged 6 to 60 months and in postpartum mothers within 6 to 8 weeks of delivery,

however, coverage rates are 72.8% for children 6-11 months old, and 13.9% for children 12-59 months old (Hendricks *et al.*, 2006).

In general, there is documented evidence that access to simple but vital micronutrients in at-risk individuals (infants, young children and pregnant women), either through food fortification and micronutrient supplementation as well as biofortification may improve child survival and mortality rate. However, these strategies may not only improve child growth and development (Lopriore *et al.*, 2004; Varma *et al.*, 2007; Rivera *et al.*, 2003; Ramakrishnan *et al.*, 2009), but also their cognitive skills (Stein *et al.*, 2008) as well as productivity (Hodinott *et al.*, 2008) in adulthood, which may also possibly result in substantial economic gains.

2.5 MICRONUTRIENTS IN COGNITIVE DEVELOPMENT AND FUNCTION IN CHILDREN

Micronutrient deprivation in developing countries is a global public health concern with long-term implications on early childhood neuropsychological or brain development (Black, 2003a; Black & Ackerman, 2008). The *Lancet Series* on child development in developing countries published in early 2007 reported that more than 200 million children under five years are not fulfilling their developmental potentials mainly due to poor nutrition (especially micronutrient deficiencies) among other risk factors (Grantham-McGregor *et al.*, 2007; Engle *et al.*, 2007; Walker *et al.*, 2007). The associations between micronutrients, structural brain development and neuropsychological function have attracted global attention as evidences continue to emerge documenting the negative consequences of nutrition deficiencies on infant cognitive and motor functioning (Black *et al.*, 2008; Benton & ILSI Europe, 2008). Findings from animal and human studies consistently show that nutrient deficiencies during early childhood can influence structural (both macrostructure and microstructure) and functional changes (in neurotransmitters) to the developing brain (Fernstrom, 2000; Grantham-McGregor *et al.*, 2000; Bryan *et al.*, 2004).

The first few years of life (gestation until two years) are particularly important because rapid growth and development occur in all brain domains (Dobbing & Sands, 1979; Pollitt, 1996), however, by this time the brain has reached approximately 80% of its adult

size (Dekaban & Sadowsky, 1978; Schaefer *et al.*, 1990; Jernigan & Tallal, 1990; Reiss *et al.*, 1996). During this critical period, the brain develops rapidly through neurogenesis, synaptogenesis, synaptic pruning, myelination and gliogenesis (Thompson & Nelson, 2001); any small perturbation in any of these processes can have long-term effects on the structural and functional capacity of the brain and may have lasting cognitive, behavioural and emotional effects (Bryan *et al.*, 2004; Benton & ILSI Europe, 2008).

Brain development is, however, not complete by the age of two years (**Figure 2.6**), as certain brain regions (especially the frontal, temporal and parietal lobes) are not yet fully developed, but continue to undergo less rapid anatomical changes throughout childhood (between 3 years to adolescence) (Thompson *et al.*, 2000; Thompson & Nelson, 2001; Lenroot & Giedd, 2006).

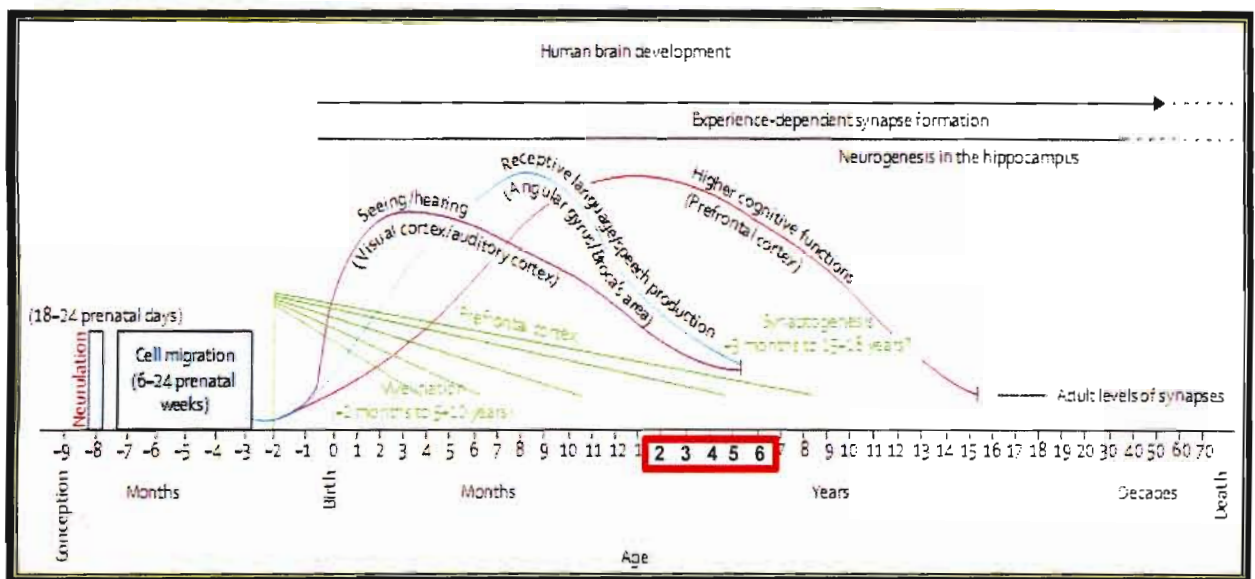


Figure 2.6 Human brain development (Adapted from Grantham-McGregor *et al.*, 2007).

Although, by age five years, the brain has reached approximately 90% of its adult size (Jernigan & Tallal, 1990; Reiss *et al.*, 1996), the most rapid, distinct, spurts of growth occurs in the frontal lobes (responsible for executive functions) from ages 3 to 6 years (Giedd *et al.*, 1999; Thompson *et al.*, 2000), and from 6 years to adolescence, the most substantial changes take place in the temporal and parietal lobes (especially those areas involving language and spatial relations) (Giedd *et al.*, 1999; Thompson *et al.*, 2000; Lenroot & Giedd, 2006). In general, increasing brain maturation combined with several

other important factors such as physical growth, interaction with the environment and the integration of stimuli provided by mother or caregiver in a broader social or economic context contributes to childhood cognitive development and function (Connolly & Grantham-McGregor, 1993; Grantham-McGregor *et al.*, 2007).

The mechanisms and evidence linking iron, zinc, iodine and vitamin A to brain development and function in children will now be selectively reviewed. These nutrients are chosen because their deficiencies are highly prevalent (Black *et al.*, 2008) and these nutrients are important in early childhood cognitive development (Bryan *et al.*, 2004).

2.5.1 IRON IN COGNITIVE DEVELOPMENT AND FUNCTION

Iron is the most abundant micronutrient in the human brain and it is well known that the human brain is responsive to dietary iron, such that insufficient dietary iron intake may alter the regulation of brain iron homeostasis (Beard, 2001; Lozoff & Georgieff, 2006; Beard, 2007). Altered brain iron homeostasis due to nutritional iron deficiency may result in delayed cognitive development or function during early childhood (Grantham-McGregor & Ani, 2001; Lozoff, 2007).

Several mechanisms have been proposed by which nutritional iron deficiency or iron deficiency anaemia may alter brain development and function. Neurodevelopmental delays or deficits may be a direct effect from anaemia causing a low oxygen delivery to the brain regions or cells (Stoltzfus *et al.*, 2001; Gordon, 2003). Additionally, iron deprivation can also alter some of the brain macrostructures [cortex, hippocampus and striatum] and/or microstructures (neurons, neurotransmitters and synapses) such that the proper myelination of the neurons or the syntheses of the neurotransmitters are adversely affected (Beard, 2001; Lozoff, 2007; Beard, 2007; Georgieff, 2008).

A causal link between iron deficiency anaemia and delays in child development may be mediated by a variety of direct or indirect pathways (Lozoff, 1998); the most obvious are associated decreases in haemoglobin concentration and oxygen delivery to tissues. Alternative theories relate to reductions in cerebral iron concentrations, including hypomyelination and impaired dopaminergic function (Connor & Menzies, 1996; Nelson *et al.*, 1997). Several observational as well as interventional studies in children have demonstrated that iron deficiency is associated with apathy, irritability, lethargy, lack of

concentration, and decreased cognitive and attention processes (Grantham-McGregor & Ani, 2001; Yager & Hartfield, 2002).

A systematic review of seventeen randomised controlled trials by Sachdev *et al.* (2005a) showed that iron supplementation improves mental development score modestly in initially anaemic or iron deficient children. However, documented evidence shows that cognitive or behavioural deficits in iron deficient anaemic children after the critical growth periods (gestation and early lactation) are often difficult to reverse (Beard, 2007).

2.5.2 ZINC IN COGNITIVE DEVELOPMENT AND FUNCTION

Zinc is required for a vast array of multiple physiologic and metabolic functions, such as physical growth, immuno-competence, reproductive function, and neurobehavioral development (Penland, 2000; Brown *et al.*, 2009). It is required for the activity of more than 300 different enzymes involved in various aspects of cellular metabolism (Falchuk, 1998). Zinc exerts its biological activity through its association with proteins, in which it may serve as a catalytic, structural, and regulatory element (McCall *et al.*, 2000). Additionally, zinc plays an important role in the process of gene replication, deoxyribonucleic acid transcription, ribonucleic acid translation, cellular division and protein synthesis (Vallee & Falchuk, 1993; Walsh *et al.*, 1994).

The human brain contains a significant quantity of zinc; in fact, it is second only to iron in terms of total concentration when compared to other micronutrients present in the human brain (Prasad, 1988). It is present in the brain bound to proteins and is important for its structure and function (Sandstead, 1985). Zinc is not uniformly distributed in the brain and this is evident in its concentration in specific areas of the brain such as the hippocampus and the cerebral cortex (Frederickson, 1989; Frederickson & Danscher, 1990), each of these regions has been associated with emotion, learning and memory (Baxter & Murray, 2002).

Zinc deficiency during embryonic and early infancy may impair brain development. Although the exact mechanism is not clear, it seems that zinc is required for neurogenesis, synaptogenesis, neuronal replication and migration (Bhatnagar & Taneja, 2001; Corniola *et al.*, 2008); compromised zinc status may lead to abnormal brain development. Although the exact mechanisms by which zinc modulates brain and

cognitive function is not yet clear, a high zinc concentration in the synaptic vesicles in the forebrain (Frederickson *et al.*, 2000) may indicate that it is important in some brain biochemical processes [such as myelination and functioning of the neurotransmitters] (Bhatnagar & Taneja, 2001).

Intervention trials gave supporting evidence that zinc supplementation improved developmental score (Friel *et al.*, 1993), activity levels (Sazawal *et al.*, 1996; Bentley *et al.*, 1997), neuropsychological test performance (Sandstead *et al.*, 1998), motor development and exploratory behaviours (Black *et al.*, 2004b) in both infants and preschool-age children. Other studies showed somewhat contrasting results as zinc supplementation did not significantly improve mental or psychomotor deficit in Brazilian (Ashworth *et al.*, 1998), Bangladeshi (Hamadani *et al.*, 2001) or Indian (Black *et al.*, 2004a) children.

Furthermore, a recent systematic review of nine randomised controlled trials of zinc supplementation with or without other additional micronutrients by Brown *et al.* (2009) revealed that neither the mental processing index nor the psychomotor development index as assessed by the Bayley scales were significantly improved. However, because of the few studies analysed more research is needed to ascertain the efficacy of zinc interventions on young children. Although the evidence for improved neurophysiological performance after zinc supplementation among originally zinc-deficient children exists (Friel *et al.*, 1993; Sandstead *et al.*, 1998; Black *et al.*, 2004b), more work is needed to replicate existing studies and clarify the effect of zinc on cognition in young children (Bhatnagar & Taneja, 2001; Black, 2003a).

2.5.3 IODINE IN COGNITIVE DEVELOPMENT AND FUNCTION

Iodine is an essential element required for normal growth and development in animals and humans. It is basically essential for the synthesis of thyroid hormones (triiodothyronine and thyroxine), which are essential for the growth and development of many body organs, especially the brain (Zoeller *et al.*, 2002; Zimmermann, 2008a). Specifically, during foetal and early postnatal development, the thyroid hormones are required for the myelination, normal neuronal movement, plasticity, synaptic transmission and maturation of the developing brain (Morreale *et al.*, 2004; Kester *et al.*, 2004; Dong *et al.*, 2005). Mental impairment and endemic neurological cretinism results

from insufficient supply of thyroid hormones to the developing brain due to lack of iodine (Delange, 2005). The resulting hypothyroidism, brain damage and loss of intellectual ability due to this deficiency can have long term negative economic implications (Delange, 2005).

Several observational studies have reported impaired cognitive and psychomotor development in children from iodine-deficient regions (Vermiglio *et al.*, 1990; Tiwari *et al.*, 1996; Santiago-Fernandez *et al.*, 2004). A report of two meta-analyses of observational and experimental trials, as reviewed by Zimmermann (2008a), reported that children with chronic or severe iodine deficiency had a mean reduction in IQ of 12 to 13.5 points when compared to other iodine sufficient children. Furthermore, randomized controlled trials conducted in several countries demonstrated that iodine treatment in initially iodine-deficient children either through supplementation (use of iodised oil) or fortification (use of iodised salt) improved the iodine status and mental performance of children in most (Bautista *et al.*, 1982; Van den Briel *et al.*, 2000; Zimmermann *et al.*, 2006; Gordon *et al.*, 2009), but not in all studies (Huda *et al.*, 2001).

2.5.4 VITAMIN A IN COGNITIVE DEVELOPMENT AND FUNCTION

Vitamin A is a generic term used for a group of structurally related chemical compounds known as retinoids that possess qualitatively the biological activity of retinol (Ahmed & Darnton-Hill, 2004). Although the retinoids are well known for the normal functioning of a large number of regulatory and physiological processes in the human body (Ross, 2003; Marill *et al.*, 2003), their specific roles and exact biochemical mechanism in human brain development is not clear. However, there are indications that the retinoids may be involved in numerous physiological functioning of the hippocampus (the brain region important for memory) (Tafti & Ghyselinck, 2007).

This brain region is highly involved in neuronal processes [such as neuronal differentiation, synaptic plasticity, learning and memory] and all those processes are strongly affected by changes in vitamin A availability (Bremner & McCaffery, 2008). Several animal studies have shown the presence of retinoid-specific receptors in the hippocampus (Tafti & Ghyselinck, 2007) and have consistently demonstrated that vitamin A deprivation produces severe deficit in spatial learning and memory directly linked to proper hippocampal functioning (Cocco *et al.*, 2002; Hernandez-Pinto *et al.*,

2006; Ghenimi *et al.*, 2009); these deficits may, however, be corrected upon vitamin A repletion (Bonnet *et al.*, 2008).

2.6 MICRONUTRIENT INTERACTION IN CHILD NUTRITION

The concurrent co-existing high prevalence of multiple micronutrient deficiencies, particularly in low income populations necessitates the use of multiple micronutrient formulations as a micronutrient intervention strategy (Zimmermann *et al.*, 2000; Oelofse *et al.*, 2002; Zimmermann *et al.*, 2004; Lander *et al.*, 2008). However, nutrient-nutrient interactions are an important consideration for any multiple micronutrient formulation [such as MixmePlus[®] or Sprinkles[™]]. Theoretically, the effects of these interactions could be enhancing, impairing or unchanging (Table 2.3).

Table 2.3 Overview of the effects of selected micronutrient interactions (Adapted from Vanderpas, 2006; Tupe *et al.*, 2007; Zimmermann, 2007a, Jin *et al.*, 2009).

Micronutrient interaction	Effects
Mineral – Mineral interaction	
Zinc – iron	Concurrent zinc and iron supplementation may reduce the efficacy of iron to increase haemoglobin.
Iodine – iron	Iron supplementation may improve the efficacy of iodised salt in concurrent iron and iodine deficient individuals.
Calcium – iron	Addition of calcium to diet may interfere with dietary iron absorption and may even induce iron deficiency.
Mineral – Vitamin interaction	
Vitamin A – iron	Vitamin A repletion in combined iron and vitamin A deficient populations improves haemoglobin status.
Vitamin C – iron or zinc	Vitamin C is a strong promoter of iron or zinc such that it can improve the absorption and bioavailability of iron or zinc from the diet.
Vitamin A – zinc	Poor zinc status can negatively affect the utilisation of vitamin A or provitamin A carotenoids in plant based diets.
Folic acid – iron	Routine supplementation with iron-folic acid in preschool children in a population with high rates of malaria infection can increase risk of severe illness and death.
Mineral – Non-nutrient interaction	
Phytate – iron or zinc	Presence of phytate or phytic acid in staple crops (like maize) can lower the absorption and bioavailability of iron or zinc in the diet.
Polyphenols – iron or zinc	High polyphenol content in tea may reduce the absorption and bioavailability of iron or zinc in the diet.
Goitrogens – iodine	High goitrogen content in certain plants can inhibit the uptake of iodine from foods and may even induce iodine deficiency.

Additionally, the interactions could also occur between micronutrients and non-nutrient dietary anti-nutrient components [such as phytate, polyphenols and goitrogens]. It may be important, however, to consider the safety, benefit as well as harmful effects of these interactions when planning or implementing micronutrient control program in developing populations.

2.7 EFFECT OF MULTIPLE MICRONUTRIENTS ON COGNITION OF YOUNG CHILDREN IN DEVELOPING COUNTRIES

The roles of single micronutrients, such as iron, iodine, and zinc on cognitive performance of children have been investigated in many nutrition interventions (Bhatnagar & Taneja, 2001; Sachdev *et al.*, 2004, Zimmermann *et al.*, 2006). However, relatively few trials have studied the efficacy and effectiveness of multiple-micronutrient formulation on cognitive or psychomotor performance of young micronutrient deficient children from developing countries (Black, 2003b; Allen *et al.*, 2009).

Recent cross-sectional studies suggest that concurrent micronutrient deficiencies exist during pregnancy (Pathak *et al.*, 2007) or early childhood (Lander *et al.*, 2008; Anderson *et al.*, 2008) which can impair mental development and reduce cognitive function in later life (Black, 2003b; Black & Ackermann, 2008). Optimistically, several studies have also reported that micronutrient interventions with the use of multiple micronutrient formulations may be beneficial in improving several nutrition and health outcomes in initially micronutrient-deficient individuals (Zimmermann *et al.*, 2002; Winichagoon *et al.*, 2006; Manger *et al.*, 2008, Allen *et al.*, 2009). In view of this, randomised controlled trials investigating the effects of multiple-micronutrient treatment on the cognition of young children in developing countries were identified in a PUBMED (Medline) search. The aim of this was to summarise the main findings of several micronutrient interventions, give an indication of the variables investigated, as well as the cognitive assessment methods employed in each of the studies.

Details of the micronutrient intervention trials are summarised in **Table 2.4**. The effect of multiple micronutrients on haemoglobin concentrations and cognition of young anaemic children were investigated in most of the studies. Direct comparison of these studies is rather challenging because of the variability in the experimental designs, duration, age

group, multi-micronutrient composition and the cognitive assessment method used in each of the studies. However, for the purpose of this review, the studies will be categorised into three different age groups (**Table 2.4**) that is: (i) infants (6 to 24 months), (ii) preschool-age children (2 to 6 years), and (iii) school age children (6 to 15 years).

(i) **Micronutrient and cognition in infants 6 to 24 months**

The duration of the studies ranged from 6 to 12 mo (Oelofse *et al.*, 2003; Lind *et al.*, 2004; Faber *et al.*, 2005; Adu-Afarwuah *et al.*, 2007) and most of the studies included between 100 – 181 children per treatment group (Lind *et al.*, 2004; Faber *et al.*, 2005; Adu-Afarwuah *et al.*, 2007) which are sufficient to detect clinical significance for the outcome measures that is haemoglobin concentration, mental development as well as motor development or acquisition (Smuts *et al.*, 2005). The exception was the study by Oelofse *et al.* (2003) where only 46 children were recruited to participate in the trial. As shown in **Table 2.4**, the form and amount (supplying 8.0 to 12.5 mg per day) of iron in the multiple micronutrient formulations varied considerably across the studies. The multi-micronutrient interventions improved mean haemoglobin concentrations significantly in some studies (Faber *et al.*, 2005; Adu-Afarwuah *et al.*, 2008) but not in others (Oelofse *et al.*, 2003; Lind *et al.*, 2003). Additionally, there was a positive improvement in motor acquisition or development in the Ghana (Adu-Afarwuah *et al.*, 2007) and South Africa (Faber *et al.*, 2005) trials.

No significant improvement was observed in the developmental outcomes assessed in the other two studies after 12 months micronutrient intervention (Oelofse *et al.*, 2003; Lind *et al.*, 2004). In the two Ghana studies by Adu-Afarwuah *et al.* (2007 & 2008), the three multi-micronutrient supplements [Sprinkles (SP), crushable Nutritabs (NT) and fat based Nutributter (NB)] used in the intervention had similar positive effect on the mean haemoglobin concentration after 12 mo intervention. However, change in haemoglobin concentration from baseline was significantly higher in the NT and NB infants, but not in SP infants, when compared to no intervention (NI) control **Table 2.4**. The authors acknowledged, however, that the lack of significant difference in haemoglobin concentration in the SP group compared to NI control infants may possibly be as a result of micronutrient deficiencies in vitamins B6 and vitamin B12 which might have limited

Table 2.4 Summary of intervention trials investigating the effects of multiple-micronutrient formulations on haemoglobin concentration and cognition in children¹

Author and country	Sample size & study design	Age group	Duration of intervention	Intervention description ²	Mean Baseline Hb (g/dL)	Change in Hb (g/dL) ³	Cognitive measurements	Main findings
INFANTS (6 – 24 MONTHS)								
Adu-Afarwuah <i>et al.</i> (2007); Adu-Afarwuah <i>et al.</i> (2008), Ghana.	313 (Sprinkles, SP = 105; Nutritab, NT= 105; Nutributter, NB = 103; No intervention, NI = 170), Community-based randomised controlled trial	6-12 mo	12 mo	SP (12.5mg Fe as ferrous sulphate, 5 mg Zn, 300ug RE Vit.A, 80 mg Folic acid), NT & NB (9mg Fe as ferrous sulphate, 4mg Zn, 400ug Vit. A, 300mg Vit.C, 80ug Folic acid, 90ug Iodine) vs. NI.	SP = 10.7 ± 1.5 NT = 10.6 ± 1.4 NB = 10.8 ± 1.3 NI = 10.6 ± 1.4	+ 0.31 + 0.65 + 0.57 0.00	Four Gross motor milestones as described in the WHO MGRS.	Hb concentration significantly higher in NT and NB (p < 0.05) but not in SP compared to NI at 12 months. All 3 supplements had positive effects on motor acquisition compared to NI.
Faber <i>et al.</i> (2005), South Africa.	361 (Fortified porridge, FP = 180; Control group, CG = 181), Randomised controlled trial	6-12 mo	6 mo	FP (11mg Fe as ferrous fumarate, 3 mg Zn, 3mg B-carotene, 56mg Ascorbic acid) vs. CG.	FP = 11.1 ± 1.1 CG = 11.1 ± 1.1	+ 0.80 - 0.10	Mother's report for Gross Motor Milestones (Motor development)	Significant positive intervention effect (p<0.05) in Hb concentration and motor development score in FP compared to CG.
Lind <i>et al.</i> (2003); Lind <i>et al.</i> (2004), Indonesia.	333 (Treatment group, TG = 170; Placebo group, PG = 170), Community-based randomised controlled trial	6-12 mo	12 mo	TG (10mg Fe as ferrous sulphate, 10mg Zn, 30mg Ascorbic acid) vs. PG.	TG = 11.2 ± 1.2 PG = 11.4 ± 1.5	+ 0.32 - 0.06	Bayleys scales of Infant development	No significant change in Hb concentration, mental developmental index, psychomotor development index and behaviour rating scale in TG compared to PG.
Oelofse <i>et al.</i> (2003), South Africa.	46 (Treatment group, TG = 25; Control group, CG = 21), Randomised controlled trial	6-12 mo	12 mo	TG (8mg Fe as ferric pyrophosphate, 5.6mg Zn, 1200IU Vit.A, 17.6ug Folic acid, 26ug Iodine) vs. CG.	Baseline = NR (at 6 mo) TG = 10.8 ± 1.0 CG = 10.3 ± 1.0	(at 12 mo) 0.00 + 0.30	Denver Developmental Screening Test	No significant change in Hb concentration and psychomotor development score after 12 mo intervention.

Table 2.4 (Continued) Summary of intervention trials investigating the effects of multiple-micronutrient formulations on haemoglobin concentration and cognition in children¹

Author and country	Sample size & study design	Age group	Duration of intervention	Intervention description ²	Mean Baseline Hb (g/dL)	Change in Hb (g/dL) ³	Cognitive measurements	Main findings
PRESCHOOL-AGE CHILDREN (2 TO 6 YEARS)								
Chen <i>et al.</i> (2008), China.	282 (Treatment1, T1 = 85; Treatment2, T2 = 96; Treatment3, T3 = 101), Double blind, randomised controlled trial	2-6 y	6 mo	Treatment1 (500mg vit.A) vs. Treatment2 (12mg Fe as NaFeEDTA, 500mg Vit. A) vs. Treatment3 (12mg Fe as NaFeEDTA, 12mg Zn, 500mg Vit.A, 0.2mg Folic acid)	T1 = 11.7 T2 = 11.4 T3 = 11.5	+1.14 +1.16 +1.07	Not Assessed	Significant Increase (P<0.0001) in Hb from baseline to end within each of the three treatment groups but no significant differences between treatment groups. The effects of micronutrient intervention on cognition were not assessed.
Varma <i>et al.</i> (2007), India.	684 (Fortified group, FG) = 342; Nonfortified group, NFG) = 342), Double blind, cluster randomised controlled trial	3-5.5 y	6 mo	FG (14mg Fe as encapsulated ferrous fumarate, 500IU Vit.A, 0.05mg Folic acid) vs. NFG.	(Overall) FG = 12.4 ± 1.5 NFG = 12.0 ± 1.8 (Anaemic) FG = 9.99 ± 0.9 NFG = 9.89 ± 0.7	(Overall) + 0.40 + 0.40 (Anaemic) + 1.70 + 1.10	Not Assessed	No significant difference in mean Hb in both groups at baseline and end of intervention. However, Hb of anaemic children in fortified group significantly improved (P=0.04). The effects of micronutrient intervention on cognition were not assessed.
Lopriore <i>et al.</i> (2004), Algeria.	322 (Fortified spread, FS = 134; Unfortified spread, UFS = 130; Control group, CG = 58), Double blind, randomised placebo controlled trial	3-6 y	6 mo	FS (42mg Fe ⁴ , 41mg Zn, 2000ug VitA, 125mg Vit.C, 500ug Folate) vs. UFS (2mg Fe [∞]) vs. CG	FS = 9.00 ± 2.3 UFS = 9.00 ± 2.1 CG = 9.30 ± 2.3	+ 3.70 + 1.90 + 1.60	Not Assessed	Increase in Hb in the FS group was ~ 2-fold more than that of the US and control groups (p<0.001). The effects of micronutrient intervention on cognition were not assessed.

Table 2.4 (Continued) Summary of intervention trials investigating the effects of multiple-micronutrient formulations on haemoglobin concentration and cognition in children¹

Author and country	Sample size & study design	Age group	Duration of intervention	Intervention description ²	Mean Baseline Hb (g/dL)	Change in Hb (g/dL) ³	Cognitive measurements	Main findings
Sari <i>et al.</i> (2001), Indonesia.	117 (Fortified group, FG = 57; Placebo group, PG = 60), Double blind placebo-controlled	4-6 y	~ 3 mo (12 wk)	FG (4.3mg Fe as elemental Iron, 157 IU VitA, 1.3mg Vit.C, 0.10mg Folic acid) vs. PG.	FG = 11.1 ± 1.0 PG = 11.1 ± 0.9	+ 1.02 + 0.40	Not Assessed	Hb improved significantly in FG compared to PG (p<0.001). The effects of micronutrient intervention on cognition were not assessed.
SCHOOL-AGE CHILDREN (6 TO 15 YEARS)								
Muthaya <i>et al.</i> (2009), India.	598 (Treatment 1, T1 ^a = 150; Treatment 2, T2 ^b = 148; Treatment 3, T3 ^a = 149; Treatment 4, T4 ^b = 151), 2-by-2 factorial, double-blind, randomised controlled trial	6-10 y	6 mo & 12 mo	T1 ⁶ & T2 ⁷ (18mg Fe ⁴ , 10.5mg Zn, 500ug RE VitA, 300ug Folic acid, 227.1 Vit.C, 100ug I) vs. T3 ⁶ & T4 ⁷ (2.7mg Fe ⁴ , 2.7mg Zn, 75ug RE VitA, 45ug Folic acid, 5.25 Vit.C, 15ug Iodine)	T1 ⁶ = 12.7 ± 1.0 T2 ⁷ = 12.7 ± 1.0 T3 ⁶ = 12.9 ± 0.9 T4 ⁷ = 12.7 ± 1.0	+ 0.90 + 1.00 + 0.50 + 0.50	KABC-II, RAVLT, NEPSY, WISC-R	Significant increases in Hb in high micronutrient groups (T1 ⁶ &T2 ⁷) compared to low micronutrient groups (T3 ⁶ &T4 ⁷), (p<0.001). T1 ⁶ & T2 ⁷ were more beneficial than T3 ⁶ &T4 ⁷ for short term memory at 6 mo (p=0.025) while T3 ⁶ and T4 ⁷ were more beneficial than T1 ⁶ and T2 ⁷ for fluid reasoning at 6mo (p=0.004) and 12 mo (p=0.004).
Manger <i>et al.</i> (2008); Winichagoon <i>et al.</i> 2006, Thailand.	569 (Fortified group, FG = 285; Unfortified group, UFG = 284), Randomised controlled trial	5.5-13.4 y	~8 mo (31wks)	Fortified seasoning powder (5mg Fe as H-reduced elemental iron, 5mg Zn, 270ug Vit A and 50ug Iodine) vs. unfortified seasoning powder.	FG = 11.8 ± 1.1 UFG = 12.0 ± 0.9	+ 0.30 + 0.10	WISC-III and visual recall task.	Significantly higher Hb in fortified group compared to unfortified group (p=0.008). Micronutrient Intervention Improved the short-term cognitive function (indicated by visual recall test) of the fortified group compared to unfortified group (p=0.008).

Table 2.4 (Continued) Summary of intervention trials investigating the effects of multiple-micronutrient formulations on haemoglobin concentration and cognition in children¹

Author and country	Sample size & study design	Age group	Duration of intervention	Intervention description ²	Mean Baseline Hb (g/dL)	Change in Hb (g/dL) ³	Cognitive measurements	Main findings
Osendarp <i>et al.</i> (2007), Indonesia.	189 (Vitamin group1, VG1 = 94; Vitamin group2, VG2 ^c = 98; Placebo group, PG = 95), Randomised controlled trial	6-10 y	12 mo	VG1 & VG2 ^b (10mg Fe as NaFeEDTA, 5mg Zn, 400ug RE VitA, 150ug Folic acid, 45mg VitC) vs. PG.	VG1 = 12.9 ± 1.1 VG2 ^c = 12.7 ± 1.1 PG = 12.8 ± 1.0	+ 0.2 + 0.5 + 0.2	WISC-III, NEPSY, WAIS-III, RAVLT, WAIT-III.	Significant improvement in Hb in VG2 ^c compared to VG1 & PG. Cognitive test (indicated by VLM) significantly improved in girls only in VG1 & VG2 ^c compared to placebo.
Sivakumar <i>et al.</i> (2006a); Sivakumar <i>et al.</i> (2006b); Vazir <i>et al.</i> (2006), India.	814 (Micronutrient group, MG = 421; Control group, CG = 393), Matched pair, cluster randomised controlled trial	6-15 y	14 mo	MG (14mg Fe ⁴ , 2.3 mg Zn, 400ug VitA, 200ug Folic acid, 80mg VitC, 75ug Iodine) vs. CG.	MG = 11.8 CG = 11.7	No change No change	MISIC (Intelligence), PGI Memory Scale (Memory), Knox cube test score (Attention & Concentration).	Overall, micronutrient intervention had no significant effect in improving mean Hb concentration; however, mean Hb improved only in children who were anaemic at baseline in the treatment group compared to placebo. Significant statistical improvement in mean Knox cube test score (attention-concentration) but not in other cognitive tests.
Solon <i>et al.</i> (2003), Philippines.	831 (Fortified = 412; Unfortified = 419), Double-blind Randomised controlled trial	Grade 1-6 (~7-13 y)	~4 mo (16 wks)	Micronutrient fortified beverage (4.8mg Fe ⁴ , 3.75mg Zn, 700IU VitA, 0.06mg Folic acid, 75mg VitC, 48ug Iodine) vs. non-fortified beverage.	(Overall) FG = 11.9 ± 0.1 UFG = 12.0 ± 0.1 (Anaemic) FG = 10.24 ± 0.1 UFG = 10.25 ± 0.1	(Overall) + 0.3 + 0.2 (Anaemic) + 1.7 + 1.4	Primary Mental Abilities Test for Filipino Children	Non significant improvement (p=0.09) only among children with Hb<11g/dL at baseline in fortified compared with unfortified group. Mental ability (Indicated by non-verbal and verbal ability scores) improved among children with both Hb<11g/dL and MID (UIE>50to<100) at baseline.

Table 2.4 (Continued) Summary of intervention trials investigating the effects of multiple-micronutrient formulations on haemoglobin concentration and cognition in children¹

Author and country	Sample size & study design	Age group	Duration of intervention	Intervention description ²	Mean Baseline Hb (g/dL)	Change in Hb (g/dL) ³	Cognitive measurements	Main findings
Jinabhai <i>et al.</i> (2001b), South Africa.	470 (Micronutrient group1, MG1 = 145; Micronutrient group2, MG2 = 153; Control group, CG = 172), Randomised controlled trial	8-10 y	~4 mo (16 weeks)	MG1 (5mg Fe as NaFeEDTA, 2.5mg Zn, 350ug Vit.A, 17.6ug Folic acid) vs. MG2 (5mg Fe as NaFeEDTA, 350ug Vit.A) vs. CG.	MG1 = 12.9 ± 0.1 MG2 = 12.8 ± 0.1 CG = 12.8 ± 0.1	- 0.01 + 0.10 + 0.07	A devised test that measure a range of scholastic and cognitive functions.	No significant differences in Hb were found post-Intervention or across the intervention groups. No significant treatment effects on scholastic and cognitive scores.
Van Stuijvenberg <i>et al.</i> (1999), South Africa.	228 (Intervention group, IG = 115; Control group, CG = 113), Randomised controlled trial	6-11 y	6 mo & 12 mo	IG (5.9mg Fe as ferrous fumarate, 2.0mg B-carotene, 110mg Vit.C, 134.4ug Iodine) vs. CG.	IG ⁵ = 12.5 (11.7,13.5) CG ⁵ = 12.6 (11.5,13.6)	+ 0.40 + 0.10	Range of Tasks to assess cognitive functions.	Significant treatment effect in Hb in the treatment group compared with control group (p=0.0015). Significant between group treatment effect was found for cognitive score (digit span forward task: a measure of short-term memory and attention) in the treatment group compared to control (p=0.04). There was no significant treatment effect in the other cognitive tests

- Reduction

~ Approximately

+ Increment

¹Studies restricted to developing countries only,

²Selected micronutrient most closely associated with improvement of haemoglobin status and cognition,

³Mean change in Hb concentration after multiple micronutrient intervention except otherwise stated,

⁴Type of iron used in study not mentioned,

⁵Median (10th and 90th percentiles)

⁶Contains high concentration n-3 poly unsaturated fatty acids

⁷Contains low concentration n-3 poly unsaturated fatty acids

⁸Contains DHA and EPA

BVRT = Benton Visual Retention Test

CG = Control group

CG = Control group

CRT = Choice Reaction Time test

FG = Fortified group

FP = Fortified porridge

FS = Fortified spread

Hb = Haemoglobin concentration

IG = Intervention group

KABCII = Kauffman Assessment Battery for Children, second edition.

MG = Micronutrient group

MGRS = Multi-center Growth Reference Study

MID = Mild iodine deficiency

mo = month,

NB = Nutributter

NEPSY = Neuropsychological assessment test

NFG = Nonfortified group

NI = No intervention

NR = Not reported,

NT = Nutritab

PG = Placebo group

RAVLT = Rey Auditory Verbal Learning Test

SP = Sprinkles

T1 = Treatment1

T2 = Treatment2

T3 = Treatment3

T3 = Treatment3

T4 = Treatment4

TG = Treatment group

UFS = Unfortified spread

UIE = Urinary iodine excretion

VG1 = Vitamin group 1

VG2 = Vitamin group 2

VLM = Visual learning and memory

vs. = Versus

WAIS-III = Wechsler Adult Intelligence Scale, Third edition

WAIT-III = Wechsler Individual Achievement Test, Third edition

WISC-III = Wechsler's Intelligent Scale for Children III,

WISC-R = Wechsler Intelligence Scales for Children

y = Year

haemoglobin synthesis in the SP group, given that the two other intervention groups received these vitamins (Adu-Afarwuah *et al.*, 2008). Furthermore, compared to the NI group, the odds of being able to walk independently by 12 mo were ~2 fold greater in the SP and NT groups and 3.4 times those in the NB group (Adu-Afarwuah *et al.*, 2008). In the South African study by Faber *et al.* (2005) among 6 to 12 mo old infants, the mean haemoglobin concentration and motor development improved significantly after the consumption of a low cost fortified maize-meal porridge supplying 11 mg iron as ferrous fumarate per day for 6 months compared to control. The children in the fortified porridge group had on average a 1-point higher motor development score than did children in the control group (Faber *et al.*, 2005).

In contrast, the 12 months micronutrient intervention trial among Indonesian infants, did not significantly improve neither the haemoglobin concentration nor any of the measured developmental outcome in the intervention compared with placebo group (Lind *et al.*, 2003, Lind *et al.*, 2004). The author speculated that the higher frequency of vomiting (resulting in loss of an unknown proportion of the ingested supplement before absorption) coupled with the negative biochemical interaction between iron and zinc might have reduced the effectiveness of the intervention. Finally, in the small trial by Oelofse *et al.* (2001), the lack of significant intervention effect in haemoglobin concentration in the intervention group at the end of the intervention could possibly be ascribed to the less bioavailable iron fortificant [ferric pyrophosphate (supplying 8mg iron per day for 12 mo)] used in the study. Additionally, the relatively prevalence of undernutrition and low sample size as well as the high percentage (~ 35%) of dropouts in the study group may also explain the lack of difference in the psychomotor development score in the experimental compared to control group after 12 months intervention (Oelofse *et al.*, 2001).

(ii) Micronutrient and cognition in preschool children aged 2 to 6 years

Multiple micronutrient intervention among the preschool age group lasted for a period of 6 months for most studies (Lopriore *et al.*, 2004; Verma *et al.*, 2007; Chen *et al.*, 2008) with the exception of the study by Sari *et al.* (2001), which lasted for only 3 months as shown in **Table 2.4**. The studies recruited from 57 to 342 children per treatment group, which is sufficient to detect measurable changes in mean haemoglobin concentrations as well as developmental outcomes (. The dosage and type of iron in the multi-micronutrient

formulations varied considerably, supplying from 4.3 to 12 mg per day across the studies, however, one of the studies supplied as high as 42 mg iron per day (Lopriore *et al.*, 2004). Regrettably, in the Lopriore *et al.* (2004) trial, the type of iron used in the 6 months intervention trial was not reported (**Table 2.4**). Nevertheless, the micronutrient intervention enhanced haemoglobin concentrations significantly in most of the studies reviewed (Sari *et al.*, 2001, Lopriore *et al.*, 2004; Chen *et al.*, 2008) but not in all (Verma *et al.*, 2007) as shown in **Table 2.4**. The lack of significant improvement in the haemoglobin status in the Verma *et al.* (2007) study, was attributed to high dropout rate in the fortified (28%) compared with unfortified (21%) groups. Nevertheless, further analysis among anaemic children revealed that the mean haemoglobin concentration improved significantly in the fortified group compared to the unfortified group.

Although there was significant improvement in the haemoglobin concentrations as a result of the multiple micronutrient intervention in the studies reviewed, it is a missed opportunity as none of the studies assessed the effect of the improvement in haemoglobin concentration on the cognitive outcome of the children. Several studies within the preschool age group have, however, reported improvements in the cognitive function after single micronutrient intervention. Iron supplementation of initially iron deficient or anaemic children in several developing populations for instance, consistently showed improvements in intelligence quotient test (Seshadri & Gopaldas, 1989), learning process (Soewondo *et al.*, 1989) as well as language or motor developmental outcome (Stoltzfus *et al.*, 2001). In view of this, it is plausible that intervention with multiple micronutrient formulations might also positively influence the micronutrient status and cognitive function or development of preschool children that are initially micronutrient deficient.

(iii) Micronutrient and cognition in school children aged 6 to 15 years

The study duration ranged from 4 to 14 months and the sample size ranged from 189 to 831 school-age children. Most of the studies included between 94 to 421 children per treatment group (**Table 2.4**) which is sufficient to detect significant intervention effects in haemoglobin concentration as well as cognitive outcomes measured (Van Stuijvenberg *et al.*, 1999; Gera *et al.*, 2009). The trial among Indian (Muthaya *et al.*, 2009; Sivakumar *et al.*, 2006a & 2006b; Vazir *et al.*, 2006) and Filipino (Solon *et al.*, 2003) school-age children did not indicate the form of iron used in their studies, but, the dosage ranged from 4.8 to 18.0 mg; Iron per day.

The amount of iron used in the multiple micronutrient formulation in the other trials ranged from 5 to 10 mg iron per day as hydrogen-reduced elemental iron (Manger *et al.*, 2008), sodium iron ethylenediaminetetra-acetate (NaFeEDTA) (Jinabhai *et al.*, 2001b; Osendarp *et al.*, 2007) and ferrous fumarate (Van Stuijvenberg *et al.*, 1999). Haemoglobin concentration improved significantly as a result of the multiple micronutrient intervention in four (van Stuijvenberg *et al.*, 1999; Osendarp *et al.*, 2007; Manger *et al.*, 2008; Muthaya *et al.*, 2009) out of the seven studies reviewed.

Although the multiple micronutrient interventions improved some of the cognitive outcome measured, it is rather difficult to compare the cognitive results because of the different cognitive assessment methods used in each of the trials (**Table 2.4**). Nevertheless, a general trend across all the studies is that multiple micronutrient interventions improved the short-term memory and/or attention-concentration of Indian (Sivakumar *et al.*, 2006a & 2006b; Vazir *et al.*, 2006; Muthaya *et al.*, 2009), Thai (Manger *et al.*, 2008) and South African (Van Stuijvenberg *et al.*, 1999) school-age children. In the four months trial among Filipino children by Solon *et al.* (2003), the micronutrient intervention improved mental ability (indicated by nonverbal and verbal ability scores) as measured by the primary mental abilities test for Filipino children only in initially anaemic (haemoglobin < 11 g/dL) and iodine deficient (urinary iodine equivalent > 50 to < 100 µg/L) children. In contrast, Jinabhai *et al.* (2001b) did not show improvement on any of the scholastic and cognitive scores assessed after 16 weeks (~ 4 months) micronutrient intervention among South African school-age children. The lack of improvement as acknowledged by the authors is in part due to unsuitability of the cognitive test battery used in their study for the age or culture group.

Summary and conclusion

Taken together, multiple micronutrient intakes might be beneficial for improvement in cognitive outcomes in children from developing countries. In infants, there was an improvement in motor acquisition or motor development score in two (Faber *et al.*, 2005; Adu-Afarwuah *et al.*, 2007 & 2008) out of the four trials. For the preschool-age children, none of the studies assessed the cognitive outcomes as a result of the micronutrient intervention, this indeed is a missed opportunity, as it is expected that improved micronutrient status of these children might have also translated into improved cognitive outcome. The most rapid, distinct, spurts of growth occur in the frontal lobes (responsible for

executive functions) during the preschool-age years (Giedd *et al.*, 1999; Thompson *et al.*, 2000).

For the school-age children, there seems to be a trend towards improvement in short-term memory and/or attention-concentration in four (Van Stuijvenberg *et al.*, 1999; Sivakumar *et al.*, 2006a & 2006b; Vazir *et al.*, 2006; Manger *et al.*, 2008; Muthaya *et al.*, 2009) out of the seven studies as shown in **Table 2.4**. Certain brain regions (especially the frontal, temporal and parietal lobes) are not yet fully even after infancy, but continue to undergo less rapid anatomical changes throughout childhood (Thompson *et al.*, 2000; Thompson & Nelson, 2001; Lenroot & Giedd, 2006). However, cognitive or mental development of infants and young children is multidimensional (Connolly & Grantham-McGregor, 1993); such that, it can be influenced by several factors such as short-term hunger, neurological maturation, interaction with the environment and the integration of stimuli provided by mother or caregiver in a broader social or economic context (Connolly & Grantham-McGregor, 1993; Grantham-McGregor *et al.*, 2007). Nevertheless, to effectively interpret studies that measure the influence of micronutrient intervention on cognition in young children, it is crucial to employ cognitive tests that are suitable for the age group, cognitive domain of interest, as well as the cultural or environmental set-up.

2.8 STANDARDISED METHODS OF ASSESSING COGNITION IN PRESCHOOL CHILDREN

The term cognition refers to a broad range of high level physiological processes or brain functions, such as learning, memory, reasoning, attention and language. Cognitive development refers to the changes of the cognitive or brain processes observed over long periods of time (months or years) and is usually assessed in children by batteries of performance tests assessing specific cognitive abilities. Although there is considerable interest in the role of certain nutrients in the biochemistry and function of the developing brain (Fernstrom, 2000; Schmitt *et al.*, 2005), interpreting intervention studies that measure the influence of nutrients on cognition in young children are usually complicated by the wide range of cognitive assessment methods employed. Other factors associated with cognition include psychosocial stimulation provided by the mother as well as environmental stimuli (Connolly & Grantham-McGregor, 1993).

The assessment of cognitive abilities of preschool-age children requires the examination of multiple domains of cognitive functioning in order to be able to get a true reflection of the cognitive abilities of a child. Cognitive function is a term that is used to describe a vast variety of different brain-mediated functions/processes (Schmitt *et al.*, 2005), and can be clustered into six main domains [executive, memory, attention, perception and psychomotor functions as well as language skills]. Furthermore, selection of cognitive tests should be based on the suitability of such test to the age group, brain domain of interest, as well as the cultural or environmental set-up of the child. Recently, Lichtenberger (2005) and Isaacs *et al.* (2008) reviewed and compared several standardised methods of assessing cognition in several nutrition intervention trials in preschool-age children, this has been selectively summarised in **Table 2.5**.

Table 2.5 Summary of standardized methods of assessing cognition in preschool children (Adapted from Lichtenberger, 2005; Isaacs *et al.*, 2008)

Test	Age group	Test Scales	Time to administer	Cognitive domain assessed
Bayley Scales of Infant Development 3rd edition (BSID-III)	1 to 42 mo	Cognitive scale, motor scale, language scale, adaptive & behaviour scale and social-emotional scale.	30 to 90 min	Current level of cognitive, language, personal-social, gross / fine motor and adaptive development.
British Ability Scales, 2 nd edition (BAS2)	2:6 to 17:11 y	General conceptual ability score, verbal and non-verbal composite scores.	Varies	Language, visualization, fluid reasoning, learning and attention.
Differential Abilities Scales, 2 nd Edition (DAS-II)	2:6 to 17:11 y	General conceptual ability score, lower level composite score.	Diagnostics (30 min)	Language, visualization, fluid reasoning, learning and attention.
Kaufman Assessment Battery for Children, 2nd edition (KABC-II)	3:0 to 18:11 y	Mental processing index, fluid crystallize index, non-verbal index, sequential, learning, simultaneous	25 to 70 min	Learning ability (or long-term storage and retrieval), sequential processing (short-term memory) and simultaneous processing (or visual processing).

Table 2.5 (Continued) Summary of standardized methods of assessing cognition in preschool children (Adapted from Lichtenberger, 2005; Isaacs *et al.*, 2008)

Test	Age group	Test Scales	Time to administer	Cognitive domain assessed
Mullen Scales of Early Learning	Birth to 68 mo	Fine and gross motor function, visual reception, expressive and receptive language.	15 min to 1 y	General cognitive and motor abilities.
Neuropsychological assessment tool 2 nd edition (NEPSY-II)	3:0 to 16:0 y	Full assessment, general assessment (excluding social perception),	45 to 60 min	Attention & executive function, language, memory & learning, sensori-motor function and visuo-spatial processing.
Wechsler Preschool and Primary Scale of Intelligence, 3 rd edition (WPPSI-III)	2:6 to 7:3 y	Full scale intelligence quotient, performance intelligence quotient, general language composite, processing speed quotient.	30 to 60 min	Overall cognitive ability, acquired knowledge, verbal reasoning, fluid measuring, spatial processing, attention, visual motor co-ordination and planning ability.
Stanford-Binet Intelligence Scale, 5 th edition (SB5)	2:0 to 85:0 y	Full scale intelligent quotient, verbal and non-verbal intelligent quotient.	45 to 75 min	Fluid reasoning, knowledge, quantitative processing, visuo-spatial processing and working memory.

mo, month; y, year; min, minutes

Compared to other cognitive tests, the KABC-II contains several novel tasks with stimuli that are appealing to young children, making the test more play-like and capable of keeping a young child easily engaged. Additionally, the KABC-II test results can be more meaningfully interpreted because they are grounded on an empirically supported theoretical model (Lichtenberger, 2005).

2.8.1.1 STRUCTURE AND FUNCTION OF THE HUMAN BRAIN ACCORDING TO LURIA NEUROPSYCHOLOGICAL THEORY

The KABC-II (a revised and re-standardised second edition of the K-ABC) is an individually administered measure of cognitive ability that can be used for children from 3 to 18 years of age (Kaufman *et al.*, 2005). The test combines three characteristics that make it promising for nutrition research and applications in a multi-lingual, multicultural setting of various African populations: (i) the KABC-II is based on a theoretical model (the Luria neuropsychological model) that assesses general mental processing ability excluding measures of acquired knowledge, which is assumed to have a universal validity; (ii) the test

has been designed to minimize the influence of language and cultural knowledge on test results (Skuy *et al.*, 2000; Schmitt *et al.*, 2005) and (iii) the test contains teaching items, that ensure understanding of the task demands.

2.8.1.2 DEFINITIONS OF SOME KABC-II SCALES BASED ON THE LURIA NEUROPSYCHOLOGICAL MODEL

The Luria neuropsychological theory of brain function (**Figure 2.7 & 2.8**) proposes that observable behaviours are the result of molecular skills and invariably involve a coordination of several functionally inter-linked brain regions or structures (Mecacci, 2005).

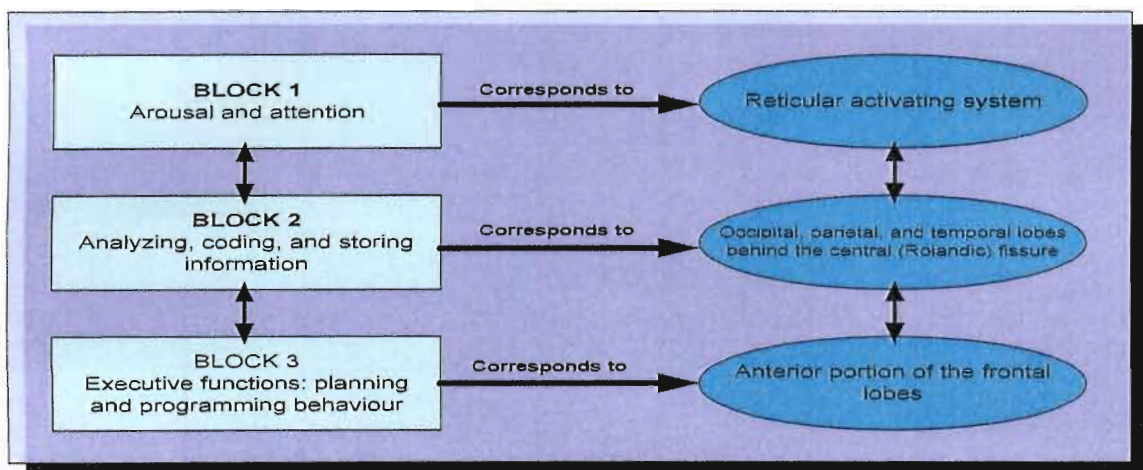


Figure 2.7 The human brain function based on the Luria neuropsychological theory (Adapted from Kaufman *et al.*, 2005)

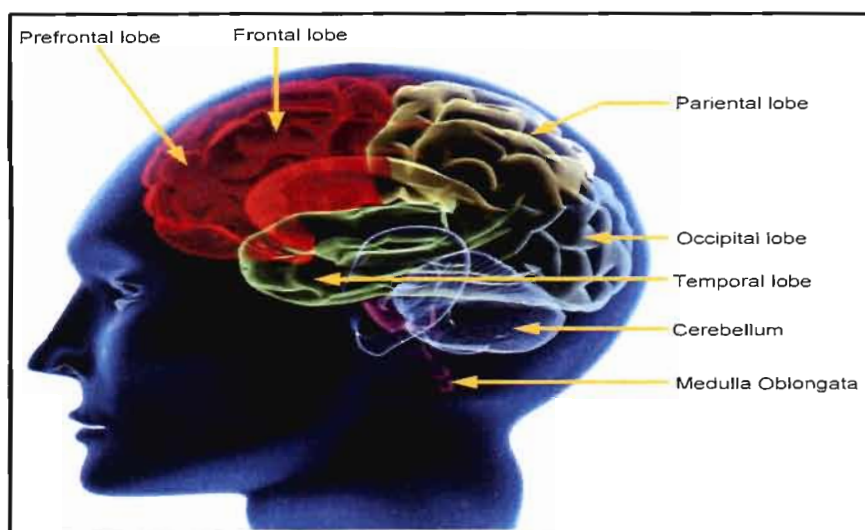


Figure 2.8 The human brain regions

Learning (Atlantis and Atlantis delayed)

The learning scale on the KABC-II assesses learning abilities (or long-term storage and retrieval). The two subtests used in the study reported in this dissertation were the Atlantis and Atlantis delayed (Kaufman *et al.*, 2005). The Atlantis measures the ability to learn new information, specifically associations between pictures and nonsense names. The child is taught names of pictures of imaginary fish, plants and shells. Then the assessor says a name and the child points to the correct picture. Atlantis delayed measures long-term storage and retrieval of information learned earlier in the testing session. About 20 minutes after the end of the Atlantis, and without being forewarned, the child is asked to point to pictures whose names were taught earlier. In general, the learning scale reflects an integration of the processes associated with all three Luria blocks (**Figure 2.7 & 2.8**), placing a premium on the attention-concentration processes that are in domain block 1 (Kaufman *et al.*, 2005).

Simultaneous (Conceptual thinking)

The simultaneous scale on the KABC-II assesses simultaneous processing (or visual processing) (Kaufman *et al.*, 2005). The subtest used in the study reported in the present dissertation was conceptual thinking. The conceptual thinking is a nonverbal measure of reasoning in which the child demonstrates classification ability; the child looks at a set of four or five pictures and points to the one that does not belong with others. For its tasks, the input has to be integrated and synthesised simultaneously (holistically), usually spatially, to produce the appropriate solution. It blends Luria block 2 and block 3 to enhance the complexity of the simultaneous syntheses that are required (**Figure 2.7 & 2.8**).

Sequential (Hand movement)

This scale measures sequential processing and short-term memory within the visual-motor modality (Kaufman *et al.*, 2005). The assessor makes a series of hand movements and the child repeats them in the same sequence, measures the kind of coding function that Luria labelled “successive” and involves arranging input in sequential or serial order to solve a problem, where each idea is linearly and temporally related to the preceding one. It is primarily associated with domain block 2 analysing, coding as well as storing information (**Figure 2.7 & 2.8**).

Mental processing index

The mental processing index (MPI) is a theory-based global measure of intellectual functioning which correlates well with other IQ measures (Kaufman *et al.*, 2005). It measures general mental processing ability on the KABC-II from the Luria neuropsychological perspectives and excludes measures of acquired knowledge. The MPI is the combination of the learning, simultaneous and sequential scales into a single global cognitive function index. The MPI produces a standardised score with a mean of 100 and a standard deviation of 15.

Nonverbal Index

The nonverbal index (NVI) is also a global (not theory based) measure of intellectual functioning and correlates well with other IQ measures (Kaufman *et al.*, 2005). The NVI is the combination of the learning, simultaneous and sequential scales into a single global cognitive function index. The NVI produces a standardised score with a mean of 100 and a standard deviation of 15.

2.9 CONCLUDING REMARKS

In most developing countries, undernutrition and micronutrient deficiencies are highly prevalent. Infants, young children and pregnant women are the most affected. Vitamin and mineral deficiencies affecting these high-risk groups includes deficiencies of iron, iodine, vitamin A, zinc and folate. Taken together, micronutrient malnutrition impairs early childhood developmental potentials as well as functional performance in adulthood; these impairments may be physical as well as cognitive. The effects of these impairments may have far-reaching detrimental effects on economic productivity which may eventually result into a never ending intergenerational vicious cycle of undernutrition and poverty (Vorster *et al.*, 1997).

Overcoming early childhood micronutrient deficiencies requires investing in targeted nutrition intervention with proven impact. The use of recently developed micronutrient powders may sustainably improve the micronutrient status of infants and young children. The first years of life (conception to 2 years) offer a critical window of opportunity for addressing childhood nutritional needs; the preschool-age years (especially 2 to 6 years) also represent a period of an extended window of opportunity during which targeted nutrition interventions may sustainably improve early childhood developmental potentials.

REFERENCES

- ADELEKAN, D.A. 2003. Multiple micronutrient deficiencies in developing countries. *Nutrition*, 19(5):473-474.
- ADU-AFARWUAH, S., LARTEY, A., BROWN, K.H., ZLOTKIN, S., BRIEND, A. & DEWEY, K.G. 2007. Randomized comparison of 3 types of micronutrient supplements for home fortification of complementary foods in Ghana: Effects on growth and motor development. *American journal of clinical nutrition*, 86(2):412-420.
- ADU-AFARWUAH, S., LARTEY, A., BROWN, K.H., ZLOTKIN, S., BRIEND, A. & DEWEY, K.G. 2008. Home fortification of complementary foods with micronutrient supplements is well accepted and has positive effects on infant iron status in Ghana. *American journal of clinical nutrition*, 87(4):929-38.
- AHMED, F. & DARNTON-HILL, I. 2004. Vitamin A deficiency. (In Gibney, M.J., Margetts, B.M., Kearney, J.M. & Arab, L., eds. Public health nutrition. Blackwell Publishing. p. 192-215.).
- AHMED, F., KHAN, M.R., BANU, C.P., QAZI, M.R. & AKHTARUZZAMAN, M. 2008. The coexistence of other micronutrient deficiencies in anaemic adolescent schoolgirls in rural Bangladesh. *European journal of clinical nutrition*, 62(3):365-372.
- AKINDAHUNSI, A.A., GRISSOM, F.E., ADEWUSI, S.R., AFOLABI, O.A., TORIMIRO, S.E. & OKE, O.L. 1998. Parameters of thyroid function in the endemic goitre of Akungba and Oke-agbe villages of Akoko area of Southwestern Nigeria. *African journal of medicine and medical sciences*, 27(3-4):239-242.
- ALDERMAN, H., BEHRMAN, J.R. & HODDINOTT, J. 2007. Economic and nutritional analyses offer substantial synergies for understanding human nutrition. *Journal of nutrition*, 137(3):537-544.
- ALLEN, L.H., ROSADO, J.L., CASTERLINE, J.E., LOPEZ, P., MUNOZ, E., GARCIA, O.P. & MARTINEZ, H. 2000. Lack of hemoglobin response to iron supplementation in anemic Mexican preschoolers with multiple micronutrient deficiencies. *American journal of clinical nutrition*, 71(6):1485-1494.
- ALLEN, L.H. 2002. Iron supplements: Scientific issues concerning efficacy and implications for research and programs. *Journal of nutrition*, 132(4 Suppl):813S-9S.
- ALLEN, L.H. 2005. Multiple micronutrients in pregnancy and lactation: An overview. *American journal of clinical nutrition*, 81(5):1206S-1212S.
- ALLEN, L., DE BENOIST, B., DARY, O. & HURRELL, R., eds. 2006. Guidelines on food fortification with micronutrients. Geneva, Switzerland: World Health Organisation. 341.
- ALLEN, L.H. 2008. Causes of vitamin B12 and folate deficiency. *Food and nutrition bulletin*, 29(2 Suppl):S20-34; discussion S35-7.

ALLEN, L., BLACK, R.E., BRANDES, N., BRITTENHAM, G., CHAZOT, G., CHUNMING, C., CRAWLEY, J., DE BENOIST, B., DALMIYA, N., DARNTON-HILL, I., DEWEY, K., EL-ARIFEEN, S., FONTAINE, O., GEISSLER, C., HABERLE, H., HARVEY, P., HASLER, J., HERSHKO, C., HURRELL, R., JUMA, M.A., LONNERDAL, B., LOZOFF, B., LYNCH, S., MARTINES SALGADO, H., MCLEAN, E., METZ, J., OPPENHEIMER, S., PREMJI, Z., PRENTICE, A., RAMSAN, M., RATLEDGE, C., STOLTZFUS, R., TIELSCH, J., WINACHAGOON, P. & SECRETARIAT DE L'ORGANISATION MONDIALE DE LA SANTE, AU NOM DES PARTICIPANTS A LA CONSULTATION. 2008. Conclusions and recommendations of a WHO expert consultation meeting on iron supplementation for infants and young children in malaria endemic areas. *Food and nutrition bulletin*, 68(2):182-188.

ALLEN, L.H. 2009. How common is vitamin B-12 deficiency? *American journal of clinical nutrition*, 89(2):693S-6S.

ALLEN, L.H., PEERSON, J.M. & OLNEY, D.K. 2009. Provision of multiple rather than two or fewer micronutrients more effectively improves growth and other outcomes in micronutrient-deficient children and adults. *Journal of nutrition*, 139(5):1022-1030.

ALONSO, P.L., LINDSAY, S.W., ARMSTRONG, J.R., CONTEH, M., HILL, A.G., DAVID, P.H., FEGAN, G., DE FRANCISCO, A., HALL, A.J. & SHENTON, F.C. 1991. The effect of insecticide-treated bed nets on mortality of Gambian children. *Lancet*, 337(8756):1499-1502.

AMOUZOU, E.K., CHABI, N.W., ADJALLA, C.E., RODRIGUEZ-GUEANT, R.M., FEILLET, F., VILLAUME, C., SANNI, A. & GUEANT, J.L. 2004. High prevalence of hyperhomocysteinemia related to folate deficiency and the 677C→T mutation of the gene encoding methylenetetrahydrofolate reductase in coastal West Africa. *American journal of clinical nutrition*, 79(4):619-624.

ANDERSON, V.P., JACK, S., MONCHY, D., HEM, N., HOK, P., BAILEY, K.B. & GIBSON, R.S. 2008. Co-existing micronutrient deficiencies among stunted Cambodian infants and toddlers. *Asia pacific journal of clinical nutrition*, 17(1):72-79.

ASHWORTH, A., MORRIS, S.S., LIRA, P.I. & GRANTHAM-MCGREGOR, S.M. 1998. Zinc supplementation, mental development and behaviour in low birth weight term infants in northeast Brazil. *European journal of clinical nutrition*, 52(3):223-227.

ASSEY, V.D., GREINER, T., MZEE, R.K., ABUU, H., MGOBA, C., KIMBOKA, S. & PETERSON, S. 2006. Iodine deficiency persists in the Zanzibar islands of Tanzania. *Food and nutrition bulletin*, 27(4):292-299.

ASSAF see ACADEMY OF SCIENCE OF SOUTH AFRICA.

ACADEMY OF SCIENCE OF SOUTH AFRICA. 2007. HIV / AIDS, TB AND NUTRITION. Academic of Science of South Africa. Pretoria: South Africa. P.143-151.

BALTUSSEN, R., KNAI, C. & SHARAN, M. 2004. Iron fortification and iron supplementation are cost-effective interventions to reduce iron deficiency in four subregions of the world. *Journal of nutrition*, 134(10):2678-2684.

BAUTISTA, A., BARKER, P.A., DUNN, J.T., SANCHEZ, M. & KAISER, D.L. 1982. The effects of oral iodized oil on intelligence, thyroid status, and somatic growth in school-age children from an area of endemic goiter. *American journal of clinical nutrition*, 35(1):127-134.

BAXTER, M.G. & MURRAY, E.A. 2002. The amygdala and reward. *Nature reviews neuroscience*, 3(7):563-573.

BEARD, J.L. 2001. Iron biology in immune function, muscle metabolism and neuronal functioning. *Journal of nutrition*, 131(2S-2):568S-579S; discussion 580S.

BEARD, J. 2007. Recent evidence from human and animal studies regarding iron status and infant development. *Journal of nutrition*, 137(2):524S-530S.

BEISEIGEL, J.M., HUNT, J.R., GLAHN, R.P., WELCH, R.M., MENKIR, A. & MAZIYA-DIXON, B.B. 2007. Iron bioavailability from maize and beans: A comparison of human measurements with caco-2 cell and algorithm predictions. *American journal of clinical nutrition*, 86(2):388-396.

BENTLEY, M.E., CAULFIELD, L.E., RAM, M., SANTIZO, M.C., HURTADO, E., RIVERA, J.A., RUEL, M.T. & BROWN, K.H. 1997. Zinc supplementation affects the activity patterns of rural Guatemalan infants. *Journal of nutrition*, 127(7):1333-1338.

BENTON, D. & ILSI EUROPE A.I.S.B.L. 2008. Micronutrient status, cognition and behavioral problems in childhood. *European journal of nutrition*, 47 Suppl 338-50.

BERKMAN, D.S., LESCANO, A.G., GILMAN, R.H., LOPEZ, S.L. & BLACK, M.M. 2002. Effects of stunting, diarrhoeal disease, and parasitic infection during infancy on cognition in late childhood: A follow-up study. *Lancet*, 359(9306):564-571.

BHATNAGAR, S. & TANEJA, S. 2001. Zinc and cognitive development. *British journal of nutrition*, 85 Suppl 2S139-45.

BHUTTA, Z.A., BIRD, S.M., BLACK, R.E., BROWN, K.H., GARDNER, J.M., HIDAYAT, A., KHATUN, F., MARTORELL, R., NINH, N.X., PENNY, M.E., ROSADO, J.L., ROY, S.K., RUEL, M., SAZAWAL, S. & SHANKAR, A. 2000. Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries: Pooled analysis of randomized controlled trials. *American journal of clinical nutrition*, 72(6):1516-1522.

BLACK, M.M. 2003a. Nutrition and brain development. (In Walker, W.A., Duggan, C. & Watkins, J.B., eds. *Nutrition in pediatrics*. 3rd ed. New York: BC Decker. p. 386-396.).

- BLACK, M.M. 2003b. Micronutrient deficiencies and cognitive functioning. *Journal of nutrition*, 133(11):3927S-3931S.
- BLACK, R. 2003c. Micronutrient deficiency—an underlying cause of morbidity and mortality. *Bulletin of the world health organization*, 81(2):79.
- BLACK, R.E., MORRIS, S.S. & BRYCE, J. 2003. Where and why are 10 million children dying every year? *Lancet*, 361(9376):2226-2234.
- BLACK, M.M., SAZAWAL, S., BLACK, R.E., KHOSLA, S., KUMAR, J. & MENON, V. 2004a. Cognitive and motor development among small-for-gestational-age infants: Impact of zinc supplementation, birth weight, and caregiving practices. *Pediatrics*, 113(5):1297-1305.
- BLACK, M.M., BAQUI, A.H., ZAMAN, K., PERSSON, L.A., EL ARIFEEN, S., LE, K., MCNARY, S.W., PARVEEN, M., HAMADANI, J.D. & BLACK, R.E. 2004b. Iron and zinc supplementation promote motor development and exploratory behavior among Bangladeshi infants. *American journal of clinical nutrition*, 80(4):903-910.
- BLACK, M.M. & ACKERMAN, J.P. 2008. Neuropsychological development (In Walker, W.A., Duggan, C. & Watkins, J.B., eds. *Nutrition in pediatrics*. 4th ed. New York: BC Decker. p. 273-282.)
- BLACK, R.E., ALLEN, L.H., BHUTTA, Z.A., CAULFIELD, L.E., DE ONIS, M., EZZATI, M., MATHERS, C., RIVERA, J. & MATERNAL AND CHILD UNDERNUTRITION STUDY GROUP. 2008. Maternal and child undernutrition: Global and regional exposures and health consequences. *Lancet*, 371(9608):243-260.
- BONNET, E., TOUYAROT, K., ALFOS, S., PALLET, V., HIGUERET, P. & ABROUS, D.N. 2008. Retinoic acid restores adult hippocampal neurogenesis and reverses spatial memory deficit in vitamin A deprived rats. *PLoS one*, 3(10):e3487.
- BOUIS, H.E. 2000. Enrichment of food staples through plant breeding: a new strategy for fighting micronutrient malnutrition. *Nutrition*, 16(7-8):701-704.
- BREMNER, J.D. & MCCAFFERY, P. 2008. The neurobiology of retinoic acid in affective disorders. *Progress in neuro-psychopharmacology & biological psychiatry*, 32(2):315-331.
- BROWN, K.H., PEERSON, J.M., BAKER, S.K. & HESS, S.Y. 2009. Preventive zinc supplementation among infants, preschoolers, and older prepubertal children. *Food and nutrition bulletin*, 30(1 Suppl):S12-40.
- BROWN, K.H., RIVERA, J.A., BHUTTA, Z., GIBSON, R.S., KING, J.C., LÖNNERDAL, B., RUEL, M.T., SANDTRÖM, B., WASANTWISUT, E. & HOTZ, C. 2004. International zinc nutrition consultative group (IZINCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. *Food and nutrition bulletin*, 25(1):S99-203.

BRYAN, J., OSENDARP, S., HUGHES, D., CALVARESI, E., BAGHURST, K. & VAN KLINKEN, J.W. 2004. Nutrients for cognitive development in school-aged children. *Nutrition reviews*, 62(8):295-306.

CAMPBELL, A.A., THORNE-LYMAN, A., SUN, K., DE PEE, S., KRAEMER, K., MOENCH-PFANNER, R., SARI, M., AKHTER, N., BLOEM, M.W. & SEMBA, R.D. 2009. Indonesian women of childbearing age are at greater risk of clinical vitamin A deficiency in families that spend more on rice and less on fruits/vegetables and animal-based foods. *Nutrition research*, 29(2):75-81.

CASEY, B.J., GIEDD, J.N. & THOMAS, K.M. 2000. Structural and functional brain development and its relation to cognitive development. *Biological psychology*, 54(1-3):241-257.

CASEY, B.J., TOTTENHAM, N., LISTON, C. & DURSTON, S. 2005. Imaging the developing brain: What have we learned about cognitive development? *Trends in cognitive sciences*, 9(3):104-110.

CASTEJON, H.V., ORTEGA, P., AMAYA, D., GOMEZ, G., LEAL, J. & CASTEJON, O.J. 2004. Co-existence of anemia, vitamin A deficiency and growth retardation among children 24-84 months old in Maracaibo, Venezuela. *Nutritional neuroscience*, 7(2):113-119.

CAULFIELD, L.E. & BLACK, R.E. 2004. Zinc deficiency. (In Ezzati, M., Lopez, A.D., Rodgers, A. & Murray, C.J.L., eds. *Comparative quantification of health risks: Global and regional burden of disease attributable to selected major risk factors*. Geneva: World Health Organization. p. 257-259.).

CHANDRA, A.K., SINGH, L.H., DEBNATH, A., TRIPATHY, S. & KHANAM, J. 2008. Dietary supplies of iodine & thiocyanate in the aetiology of endemic goitre in Imphal East district of Manipur, North East India. *The Indian journal of medical research*, 128(5):601-605.

CHEN, K., LI, T.Y., CHEN, L., QU, P. & LIU, Y.X. 2008. Effects of vitamin A, vitamin A plus iron and multiple micronutrient-fortified seasoning powder on preschool children in a suburb of Chongqing, China. *Journal of nutritional science and vitaminology*, 54(6):440-447.

CHERIAN, A., SEENA, S., BULLOCK, R.K. & ANTONY, A.C. 2005. Incidence of neural tube defects in the least-developed area of India: A population-based study. *Lancet*, 366(9489):930-931.

CHRISTOFIDES, A., ASANTE, K.P., SCHAUER, C., SHARIEFF, W., OWUSU-AGYEI, S. & ZLOTKIN, S. 2006. Multi-micronutrient sprinkles including a low dose of iron provided as microencapsulated ferrous fumarate improves haematologic indices in anaemic children: A randomized clinical trial. *Maternal & child nutrition*, 2(3):169-180.

COCCO, S., DIAZ, G., STANCAMPIANO, R., DIANA, A., CARTA, M., CURRELI, R., SARAIS, L. & FADDA, F. 2002. Vitamin A deficiency produces spatial learning and memory impairment in rats. *Neuroscience*, 115(2):475-482.

CONNOLLY, K.J. & GRANTHAM-MCGREGOR, S.M. 1993. Key issues in generating a psychological-testing protocol. *American journal of clinical nutrition*, 57(2 Suppl):317S-318S.

CONNOLLY, K.J. & GRANTHAM-MCGREGOR, S.M. 1993. Key issues in generating a psychological-testing protocol. *American journal of clinical nutrition*, 57(2 Suppl):317S-318S.

CONNOR, J.R. & MENZIES, S.L. 1996. Relationship of iron to oligodendrocytes and myelination. *Glia*, 17(2):83-93.

COPENHAGEN CONSENSUS. Copenhagen consensus 2008 - results. <http://www.copenhagenconsensus.com/Default.aspx?ID=953>. [Date of access: March 20, 2009].

CORNIOLA, R.S., TASSABEHJI, N.M., HARE, J., SHARMA, G. & LEVENSON, C.W. 2008. Zinc deficiency impairs neuronal precursor cell proliferation and induces apoptosis via p53-mediated mechanisms. *Brain research*, 123752-61.

CUNNINGHAM-RUNDLES, S., KIM, S.H., DNISTRIAN, A., NOROSKI, L., MENENDEZ-BOTET, C., GRASSEY, C.B., HINDS, G. & CERVIA, J.S. 1996. Micronutrient and cytokine interaction in congenital pediatric HIV infection. *Journal of nutrition*, 126(10 Suppl):2674S-2679S.

CURTIS, V. & CAIRNCROSS, S. 2003. Effect of washing hands with soap on diarrhoea risk in the community: A systematic review. *The lancet infectious diseases*, 3(5):275-281.

DANG, J., ARCOT, J. & SHRESTHA, A. 2000. Folate retention in selected processed legumes. *Food chemistry*, 68(3):295-298.

DARNTON-HILL, I., WEBB, P., HARVEY, P.W., HUNT, J.M., DALMIYA, N., CHOPRA, M., BALL, M.J., BLOEM, M.W. & DE BENOIST, B. 2005. Micronutrient deficiencies and gender: Social and economic costs. *American journal of clinical nutrition*, 81(5):1198S-1205S.

DEKABAN, A.S. 1978. Changes in brain weights during the span of human life: Relation of brain weights to body heights and body weights. *Annals of neurology*, 4(4):345-356.

DE BENOIST, B., MCLEAN, E., COGSWELL, M., EGLI, I. and WOJDYLA, D., 2008. Worldwide prevalence of anaemia 1993–2005: WHO Global Database on Anaemia. Geneva, Switzerland: World Health Organization.

DE BENOIST, B., MCLEAN, E., ANDERSSON, M. & ROGERS, L. 2008. Iodine deficiency in 2007: Global progress since 2003. *Food and nutrition bulletin*, 29(3):195-202.

DE PEE, S. & DARY, O. 2002. Biochemical indicators of vitamin A deficiency: Serum retinol and serum retinol binding protein. *Journal of nutrition*, 132(9 Suppl):2895S-2901S.

DE PEE, S., KRAEMER, K., VAN DEN BRIEL, T., BOY, E., GRASSET, C., MOENCH-PFANNER, R., ZLOTKIN, S., BLOEM, M.W., WORLD FOOD PROGRAMME & SPRINKLES GLOBAL HEALTH INITIATIVE. 2008. Quality criteria for micronutrient powder products: Report of a meeting organized by the world food programme and sprinkles global health initiative. *Food and nutrition bulletin*, 29(3):232-241.

DELANGE, F. 2005. Epidemiology and impact of iodine deficiency in pediatrics. *Journal of pediatric endocrinology & metabolism*, 18 Suppl 11245-1251.

DIJKHUIZEN, M.A., WIERINGA, F.T., WEST, C.E., MUHERDIYANTININGSIH & MUHILAL. 2001. Concurrent micronutrient deficiencies in lactating mothers and their infants in Indonesia. *American journal of clinical nutrition*, 73(4):786-791.

DIALLO, D.A. 2008. Sickle cell disease in africa: Current situation and strategies for improving the quality and duration of survival. *Bulletin de l'academie nationale de medecine*, 192(7):1361-72; discussion 1372-3.

DOBBING, J. & SANDS, J. 1979. Comparative aspects of the brain growth spurt. *Early human development*, 3(1):79-83.

DONG, J., YIN, H., LIU, W., WANG, P., JIANG, Y. & CHEN, J. 2005. Congenital iodine deficiency and hypothyroidism impair LTP and decrease C-fos and C-jun expression in rat hippocampus. *Neurotoxicology*, 26(3):417-426.

DREYFUSS, M.L. & FAWZI, W.W. 2002. Micronutrients and vertical transmission of HIV-1. *American journal of clinical nutrition*, 75(6):959-970.

DUQUE, X., FLORES-HERNANDEZ, S., FLORES-HUERTA, S., MENDEZ-RAMIREZ, I., MUNOZ, S., TURNBULL, B., MARTINEZ-ANDRADE, G., RAMOS, R.I., GONZALEZ-UNZAGA, M., MENDOZA, M.E. & MARTINEZ, H. 2007. Prevalence of anemia and deficiency of iron, folic acid, and zinc in children younger than 2 years of age who use the health services provided by the Mexican social security institute. *BMC public health*, 7:345.

EKSTROM, E.C., HYDER, S.M., CHOWDHURY, A.M., CHOWDHURY, S.A., LONNERDAL, B., HABICHT, J.P. & PERSSON, L.A. 2002. Efficacy and trial effectiveness of weekly and daily iron supplementation among pregnant women in rural Bangladesh: Disentangling the issues. *American journal of clinical nutrition*, 76(6):1392-1400.

ELEY, B.S., SIVE, A.A., ABELSE, L., KOSSEW, G., COOPER, M. & HUSSEY, G.D. 2002a. Growth and micronutrient disturbances in stable, HIV-infected children in Cape Town. *Annals of tropical paediatrics*, 22(1):19-23.

ELEY, B.S., SIVE, A.A., SHUTTLEWORTH, M. & HUSSEY, G.D. 2002b. A prospective, cross-sectional study of anaemia and peripheral iron status in antiretroviral naive, HIV-1 infected children in Cape Town, South Africa. *Biomedical central infectious diseases*, 2:3.

EL-SOHEMY, A. 2007. Nutrigenetics. *Forum of nutrition*, 60:25-30.

ENGLE, P.L., BLACK, M.M., BEHRMAN, J.R., CABRAL DE MELLO, M., GERTLER, P.J., KAPIRIRI, L., MARTORELL, R., YOUNG, M.E. & INTERNATIONAL CHILD DEVELOPMENT STEERING GROUP. 2007. Strategies to avoid the loss of developmental potential in more than 200 million children in the developing world. *Lancet*, 369(9557):229-242.

FABER, M., KVALSVIG, J.D., LOMBARD, C.J. & BENAIDE, A.J. 2005. Effect of a fortified maize-meal porridge on anemia, micronutrient status, and motor development of infants. *American journal of clinical nutrition*, 82(5):1032-1039.

FALCHUK, K.H. 1998. The molecular basis for the role of zinc in developmental biology. *Molecular and cellular biochemistry*, 188(1-2):41-48.

FAWZI, W.W., MSAMANGA, G.I., SPIEGELMAN, D., URASSA, E.J., MCGRATH, N., MWAKAGILE, D., ANTELMAN, G., MBISE, R., HERRERA, G., KAPIGA, S., WILLETT, W. & HUNTER, D.J. 1998. Randomised trial of effects of vitamin supplements on pregnancy outcomes and T cell counts in HIV-1-infected women in Tanzania. *Lancet*, 351(9114):1477-82.

FAWZI, W.W., MSAMANGA, G.I., SPIEGELMAN, D., WEI, R., KAPIGA, S., VILLAMOR, E., MWAKAGILE, D., MUGUSI, F., HERTZMARK, E., ESSEX, M. & HUNTER, D.J. 2004. A randomized trial of multivitamin supplements and HIV disease progression and mortality. *New England journal of medicine*, 351(1):23-32.

FERGUSON, E.L., GIBSON, R.S., OPARE-OBISAW, C., OUNPUU, S., THOMPSON, L.U. & LEHRFELD, J. 1993. The zinc nutriture of preschool children living in two African countries. *Journal of nutrition*, 123(9):1487-1496.

FERNSTROM, J.D. 2000. Can nutrient supplements modify brain function? *American journal of clinical nutrition*, 71(6 Suppl):1669S-75S.

FISCHER-WALKER, C.L., EZZATI, M. & BLACK, R.E. 2009. Global and regional child mortality and burden of disease attributable to zinc deficiency. *European journal of clinical nutrition*, 63(5):591-597.

FREDERICKSON, C.J. 1989. Neurobiology of zinc and zinc-containing neurons. *International review of neurobiology*, 31:145-238.

FREDERICKSON, C.J. & DANSCHER, G. 1990. Zinc-containing neurons in hippocampus and related CNS structures. *Progress in brain research*, 83:71-84.

FRIEL, J.K., ANDREWS, W.L., MATTHEW, J.D., LONG, D.R., CORNEL, A.M., COX, M., MCKIM, E. & ZERBE, G.O. 1993. Zinc supplementation in very-low-birth-weight infants. *Journal of pediatric gastroenterology and nutrition*, 17(1):97-104.

- GARZA, C. & DE ONIS, M. 2004. Rationale for developing a new international growth reference. *Food and nutrition bulletin*, 25(1 Suppl):S5-14.
- GBADEBO, A.M. & OYESANYA, T.M. 2005. Assessment of iodine deficiency and goitre incidence in parts of Yewa area of Ogun state, Southwestern Nigeria. *Environmental geochemistry and health*, 27(5-6):491-499.
- GEORGIEFF, M.K. 2008. The role of iron in neurodevelopment: Fetal iron deficiency and the developing hippocampus. *Biochemical society transactions*, 36(Pt 6):1267-1271.
- GERA, T., SACHDEV, H.P. & NESTEL, P. 2009. Effect of combining multiple micronutrients with iron supplementation on Hb response in children: Systematic review of randomized controlled trials. *Public health nutrition*, 12(6):756-773.
- GHENIMI, N., BEAUVIEUX, M.C., BIRAN, M., PALLET, V., HIGUERET, P. & GALLIS, J.L. 2009. Vitamin A deficiency in rats induces anatomic and metabolic changes comparable with those of neurodegenerative disorders. *Journal of nutrition*, 139(4):696-702.
- GIBSON, R.S. 1994. Zinc nutrition in developing countries. *Nutrition research reviews*, 7(1):151-173.
- GIBSON, R.S. & HOTZ, C. 2001. Dietary diversification/modification strategies to enhance micronutrient content and bioavailability of diets in developing countries. *British journal of nutrition*, 85 Suppl 2S159-66.
- GIBSON, R.S., PERLAS, L. & HOTZ, C. 2006. Improving the bioavailability of nutrients in plant foods at the household level. *The proceedings of the Nutrition Society*, 65(2):160-168.
- GIEDD, J.N., BLUMENTHAL, J., JEFFRIES, N.O., CASTELLANOS, F.X., LIU, H., ZIJDENBOS, A., PAUS, T., EVANS, A.C. & RAPOPORT, J.L. 1999. Brain development during childhood and adolescence: A longitudinal MRI study. *Nature neuroscience*, 2(10):861-863.
- GORDON, N. 2003. Iron deficiency and the intellect. *Brain & development*, 25(1):3-8.
- GORDON, R.C., ROSE, M.C., SKEAFF, S.A., GRAY, A.R., MORGAN, K.M.D. & RUFFMAN, T. 2009. Iodine supplementation improves cognition in mildly iodine-deficient children. *American journal of clinical nutrition*, 90(5):1264-1271.
- GRANTHAM-MCGREGOR, S.M., WALKER, S.P. & CHANG, S. 2000. Nutritional deficiencies and later behavioural development. *The proceedings of the nutrition society*, 59(1):47-54.
- GRANTHAM-MCGREGOR, S. & ANI, C. 2001. A review of studies on the effect of iron deficiency on cognitive development in children. *Journal of nutrition*, 131(2S-2):649S-666S; discussion 666S-668S.
- GRANTHAM-MCGREGOR, S. 2002. Linear growth retardation and cognition. *Lancet*, 359(9306):542.

GRANTHAM-MCGREGOR, S., CHEUNG, Y.B., CUETO, S., GLEWWE, P., RICHTER, L., STRUPP, B. & INTERNATIONAL CHILD DEVELOPMENT STEERING GROUP. 2007. Developmental potential in the first 5 years for children in developing countries. *Lancet*, 369(9555):60-70.

GREGORY, J.F. 1997. Bioavailability of folate. *European journal of clinical nutrition*, 51 Suppl 1S54-9.

GUEANT-RODRIGUEZ, R.M., GUEANT, J.L., DEBARD, R., THIRION, S., HONG, L.X., BRONOWICKI, J.P., NAMOUR, F., CHABI, N.W., SANNI, A., ANELLO, G., BOSCO, P., ROMANO, C., AMOUZOU, E., ARRIETA, H.R., SANCHEZ, B.E., ROMANO, A., HERBETH, B., GUILLAND, J.C. & MUTCHINICK, O.M. 2006. Prevalence of methylenetetrahydrofolate reductase 677T and 1298C alleles and folate status: A comparative study in Mexican, West African, and European populations. *American journal of clinical nutrition*, 83(3):701-707.

HAAS, J.D., BEARD, J.L., MURRAY-KOLB, L.E., DEL MUNDO, A.M., FELIX, A. & GREGORIO, G.B. 2005. Iron-biofortified rice improves the Iron stores of nonanemic Filipino women. *Journal of nutrition*, 135(12):2823-2830.

HAAS, J.D. & BROWNLIE, T. 2001. Iron deficiency and reduced work capacity: A critical review of the research to determine a causal relationship. *Journal of nutrition*, 131(2S-2):676S-688S; discussion 688S-690S.

HAIDER, B.A. & BHUTTA, Z.A. 2009. The effect of therapeutic zinc supplementation among young children with selected infections: A review of the evidence. *Food and nutrition bulletin*, 30(1 Suppl):S41-59.

HALL, A., HEWITT, G., TUFFREY, V. & DE SILVA, N. 2008. A review and meta-analysis of the impact of intestinal worms on child growth and nutrition. *Maternal & child nutrition*, 4 Suppl 1118-236.

HAMADANI, J.D., FUCHS, G.J., OSENDARP, S.J., KHATUN, F., HUDA, S.N. & GRANTHAM-MCGREGOR, S.M. 2001. Randomized controlled trial of the effect of zinc supplementation on the mental development of Bangladeshi infants. *American journal of clinical nutrition*, 74(3):381-386.

HAWDON, J.M. & HOTEZ, P.J. 1996. Hookworm: Developmental biology of the infectious process. *Current opinion in genetics & development*, 6(5):618-623.

HENDRICKS, M., BOURNE, L. & ELEY, B. 2006. Health related indicators. (In Ijuumba, P. & Padarath, A., eds. South African health review 2006. Durban: Health System Trust. p. 203-220.).

HENDRICKS, M.K., ELEY, B. & BOURNE, L.T. 2007. Nutrition and HIV/AIDS in infants and children in South Africa: Implications for food-based dietary guidelines. *Maternal & child nutrition*, 3(4):322-333.

HERNANDEZ-PINTO, A.M., PUEBLA-JIMENEZ, L. & ARILLA-FERREIRO, E. 2006. A vitamin A-free diet results in impairment of the rat hippocampal somatostatinergic system. *Neuroscience*, 141(2):851-861.

HESS, S.Y., LÖNNERDAL, B., HOTZ, C., RIVERA, J.A. & BROWN, K.H. 2009. Recent advances in knowledge of zinc nutrition and human health. *Food & nutrition bulletin*, 30(1):S5-11.

HODDINOTT, J., MALUCCIO, J.A., BEHRMAN, J.R., FLORES, R. & MARTORELL, R. 2008. Effect of a nutrition intervention during early childhood on economic productivity in Guatemalan adults. *Lancet*, 371(9610):411-416.

HORTON, S. & LEVIN, C. 2001. Commentary on "evidence that iron deficiency anemia causes reduced work capacity". *Journal of nutrition*, 131(2S-2):691S-696S.

HORTON, S. & ROSS, R.J. 2003. The economics of iron deficiency. *Food policy*, 28(1):51-75.

HORTON, S. 2006. The economics of food fortification. *Journal of nutrition*, 136(4):1068-1071.

HORTON, S., BEGIN, F., GREIG, A. & LAKSHMAN, A. 2009a. Best practice paper: Micronutrient supplements for child survival (vitamin a and zinc). Copenhagen consensus. p.5

HORTON, S., MANNER, V. & WESLEY, A. 2009b. Best practice paper: Food fortification with iron and iodine. Copenhagen consensus.

HOTZ, C. & GIBSON, R.S. 2007. Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. *Journal of nutrition*, 137(4):1097-1100.

HOTZ, C., GIBSON, R.S. & TEMPLE, L. 2001. A home-based method to reduce phytate content and increase zinc bioavailability in maize-based complementary diets. *International journal of food sciences and nutrition*, 52(2):133-142.

HAWDON, J.M. & HOTEZ, P.J. 1996. Hookworm: Developmental biology of the infectious process. *Current opinion in genetics & development*, 6(5):618-623.

HOWE, J.A., MAZIYA-DIXON, B. & TANUMIHARDJO, S.A. 2009. Cassava with enhanced beta-carotene maintains adequate vitamin A status in Mongolian gerbils (*Meriones unguiculatus*) despite substantial cis-isomer content. *British journal of nutrition*, 102(3):342-349.

HUDA, S.N., GRANTHAM-MCGREGOR, S.M. & TOMKINS, A. 2001. Cognitive and motor functions of iodine-deficient but euthyroid children in Bangladesh do not benefit from iodized poppy seed oil (lipiodol). *Journal of nutrition*, 131(1):72-77.

HURRELL, R.F., REDDY, M. & COOK, J.D. 1999. Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. *British journal of nutrition*, 81(4):289-295.

IZINGC see INTERNATIONAL ZINC NUTRITION CONSULTATIVE GROUP.

INTERNATIONAL ZINC NUTRITION CONSULTATIVE GROUP, BROWN, K.H., RIVERA, J.A., BHUTTA, Z., GIBSON, R.S., KING, J.C., LONNERDAL, B., RUEL, M.T., SANDTROM, B., WASANTWISUT, E. & HOTZ, C.

2004. International zinc nutrition consultative group (IZINCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. *Food and nutrition bulletin*, 25(1 Suppl 2):S99-203.

ISAACS, E., OATES, J. & ILSI EUROPE A.I.S.B.L. 2008. Nutrition and cognition: Assessing cognitive abilities in children and young people. *European journal of nutrition*, 47 Suppl 34-24.

JACKSON, M.J. 1997. The assessment of bioavailability of micronutrients: Introduction. *European journal of clinical nutrition*, 51 Suppl 1S1-2.

JERNIGAN, T.L. & TALLAL, P. 1990. Late childhood changes in brain morphology observable with MRI. *Developmental medicine and child neurology*, 32(5):379-385.

JIN, F., FROHMAN, C., THANNHAUSER, T.W., WELCH, R.M. & GLAHN, R.P. 2009. Effects of ascorbic acid, phytic acid and tannic acid on iron bioavailability from reconstituted ferritin measured by an in vitro digestion-Caco-2 cell model. *British journal of nutrition*, 101(7):972-81.

JINABHAI, C.C., TAYLOR, M., COUTSODIS, A., COOVADIA, H.M., TOMKINS, A.M. & SULLIVAN, K.R. 2001a. Epidemiology of helminth infections: Implications for parasite control programmes, a South African perspective. *Public health nutrition*, 4(6):1211-1219.

JINABHAI, C.C., TAYLOR, M., COUTSODIS, A., COOVADIA, H.M., TOMKINS, A.M. & SULLIVAN, K.R. 2001b. A randomized controlled trial of the effect of antihelminthic treatment and micronutrient fortification on health status and school performance of rural primary school children. *Annals of tropical paediatrics*, 21(4):319-333.

JONES, G., STEKETEE, R.W., BLACK, R.E., BHUTTA, Z.A., MORRIS, S.S. & BELLAGIO CHILD SURVIVAL STUDY GROUP. 2003. How many child deaths can we prevent this year? *Lancet*, 362(9377):65-71.

JOOSTE, P.L. & ZIMMERMANN, M.B. 2008. Progress towards eliminating iodine deficiency in South Africa. *South African journal of clinical nutrition*, 21(1):8-14.

KAISER, J.D., CAMPA, A.M., ONDERCIN, J.P., LEOUNG, G.S., PLESS, R.F. & BAUM, M.K. 2006. Micronutrient supplementation increases CD4 count in HIV-infected individuals on highly active antiretroviral therapy: a prospective, double-blinded, placebo-controlled trial. *Journal of Acquired Immune Deficiency Syndrome*, 42(5):523-528.

KAUFMAN, A.S., LICHTENBERGER, E.O., FLETCHER-JANZEN, E. & KAUFMAN, N.L. 2005. Essentials of KABC-II assessment. Hoboken, New Jersey: John Wiley & Sons, Inc.

KESSLER, L., DALEY, H., MALENGA, G. & GRAHAM, S. 2000. The impact of the human immunodeficiency virus type 1 on the management of severe malnutrition in Malawi. *Annals of tropical paediatrics*, 20(1):50-56.

KESTER, M.H., MARTINEZ DE MENA, R., OBREGON, M.J., MARINKOVIC, D., HOWATSON, A., VISSER, T.J., HUME, R. & MORREALE DE ESCOBAR, G. 2004. Iodothyronine levels in the human developing brain: Major regulatory roles of iodothyronine deiodinases in different areas. *The journal of clinical endocrinology and metabolism*, 89(7):3117-3128.

KHIN-MAUNG-NAING, CHO-NWE-OO, TIN-TIN-OO & THANE-TOE. 1989. A study on the aetiology of endemic goitre in lowland Burma. *European journal of clinical nutrition*, 43(10):693-698.

KIM, J., PAIK, H.Y., JOUNG, H., WOODHOUSE, L.R., LI, S. & KING, J.C. 2007. Effect of dietary phytate on zinc homeostasis in young and elderly Korean women. *Journal of the American College of Nutrition*, 26(1):1-9.

KING, J.C., SHAMES, D.M. & WOODHOUSE, L.R. 2000. Zinc homeostasis in humans. *Journal of nutrition*, 130(5S Suppl):1360S-6S.

KLOKA, D. 2003. Integrated nutrition programme. <http://www.doh.gov.za/programmes/nutrition-f.html> [Date of access: October 16. 2008].

KRITTAPHOL, W., BAILEY, K.B., PONGCHAROEN, T., WINICHAGOON, P. & GIBSON, R.S. 2006. Low zinc, iron, and calcium intakes of northeast Thai school children consuming glutinous rice-based diets are not exacerbated by high phytate. *International journal of food sciences and nutrition*, 57(7-8):520-528.

KUMAR, M.V. & RAJAGOPALAN, S. 2007. Multiple micronutrient fortification of salt and its effect on cognition in Chennai school children. *Asia pacific journal of clinical nutrition*, 16(3):505-511.

KUMAR, M.V. & RAJAGOPALAN, S. 2007. Multiple micronutrient fortification of salt and its effect on cognition in Chennai school children. *Asia Pacific journal of clinical nutrition*, 16(3):505-511.

KURAWIGE, J.B., GATSINZI, T., KLEINFELDT, V., REHLE, T. & BULTERYS, M. 1993. HIV-1 infection among malnourished children in Butare, Rwanda. *Journal of tropical pediatrics*, 39(2):93-96.

LABADARIOS, D., STEYN, N.P., MAUNDER, E., MACINTYRE, U., SWART, R. and GERICKE, G. 2000. The national food consumption survey (NFCS): Children aged 1-9 years, South Africa, 1999. Pretoria: Department of Health.

LABADARIOS, D., STEYN, N.P., MAUNDER, E., MACINTYRE, U., GERICKE, G., SWART, R., HUSKISSON, J., DANNHAUSER, A., VORSTER, H.H., NESMVUNI, A.E. & NEL, J.H. 2005. The national food consumption survey (NFCS): South Africa, 1999. *Public health nutrition*, 8(5):533-543.

LABADARIOS, D., SWART, R., MAUNDER, E., KRUGER, H.S., GERICKE, G., KUZWAYO, P.M.N., NTSIE, P.R., STEYN, N.P., SCHLOSS, I., DHANSAY, M.A., JOOSTE, P.L., DANNHAUSER, A., NELL, J.H., MOLEFE, D. & KOTZE, T.J.W. 2008. Executive summary of the national food consumption survey-fortification baseline (NFCS-FB-I): South Africa, 2005. *South African journal of clinical nutrition*, (supplement 2). 21(3):245-300.

- LAMBERG, B.A. 1993. Iodine deficiency disorders and endemic goitre. *European journal of clinical nutrition*, 47(1):1-8.
- LAMIKANRA, A.A., BROWN, D., POTOCHNIK, A., CASALS-PASCUAL, C., LANGHORNE, J. & ROBERTS, D.J. 2007. Malarial anemia: Of mice and men. *Blood*, 110(1):18-28.
- LANDER, R.L., ENKHJARGAL, T., BATJARGAL, J., BAILEY, K.B., DIOUF, S., GREEN, T.J., SKEAFF, C.M. & GIBSON, R.S. 2008. Multiple micronutrient deficiencies persist during early childhood in Mongolia. *Asia pacific journal of clinical nutrition*, 17(3):429-440.
- LENROOT, R.K. & GIEDD, J.N. 2006. Brain development in children and adolescents: Insights from anatomical magnetic resonance imaging. *Neuroscience and biobehavioral reviews*, 30(6):718-729.
- LICHTENBERGER, E.O. 2005. General measures of cognition for the preschool child. *Mental retardation and developmental disabilities research reviews*, 11(3):197-208.
- LIND, T., LÖNNERDAL, B., STENLUND, H., ISMAIL, D., SESWANDHANA, R., EKSTRÖM, E.C. & PERSSON, L.A. 2003. A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: interactions between iron and zinc. *American journal of clinical nutrition*, 77(4):883-90.
- LIND, T., LONNERDAL, B., STENLUND, H., GAMAYANTI, I.L., ISMAIL, D., SESWANDHANA, R. & PERSSON, L.A. 2004. A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: Effects on growth and development. *American journal of clinical nutrition*, 80(3):729-736.
- LIU, Z. 1994. The soil zinc distribution in China. *Chinese agricultural science*, 27, 30-37.
- LIU, H.L., LAM, L.T., ZENG, Q., HAN, S.Q., FU, G. & HOU, C.C. 2009. Effects of drinking water with high iodine concentration on the intelligence of children in Tianjin, China. *Journal of public health*, 31(1):32-38.
- LOPRIORE, C., GUIDOUM, Y., BRIEND, A. & BRANCA, F. 2004. Spread fortified with vitamins and minerals induces catch-up growth and eradicates severe anemia in stunted refugee children aged 3-6 y. *American journal of clinical nutrition*, 80(4):973-981.
- LOZOFF, B. 1998. Explanatory mechanisms for poorer development in iron deficient anemic infants. (In Anon. *Nutrition, health, and child development: Research advances and policy recommendations*. Washington, DC: Pan American Health Organization. p. 162-178.).
- LOZOFF, B. 2007. Iron deficiency and child development. *Food and nutrition bulletin*, 28(4 Suppl):S560-71.
- LOZOFF, B. & GEORGIEFF, M.K. 2006. Iron deficiency and brain development. *Seminars in pediatric neurology*, 13(3):158-165.

LUKACIK, M., THOMAS, R.L. & ARANDA, J.V. 2008. A meta-analysis of the effects of oral zinc in the treatment of acute and persistent diarrhea. *Pediatrics*, 121(2):326-336.

MANGER, M.S., MCKENZIE, J.E., WINICHAGOON, P., GRAY, A., CHAVASIT, V., PONGCHAROEN, T., GOWACHIRAPANT, S., RYAN, B., WASANTWISUT, E. & GIBSON, R.S. 2008. A micronutrient-fortified seasoning powder reduces morbidity and improves short-term cognitive function, but has no effect on anthropometric measures in primary school children in northeast Thailand: A randomized controlled trial. *American journal of clinical nutrition*, 87(6):1715-1722.

MANNAR, V. & GALLEG0, E.B. 2002. Iron fortification: Country level experiences and lessons learned. *Journal of nutrition*, 132(4 Suppl):856S-8S.

MARILL, J., IDRES, N., CAPRON, C.C., NGUYEN, E. & CHABOT, G.G. 2003. Retinoic acid metabolism and mechanism of action: A review. *Current drug metabolism*, 4(1):1-10.

MCCALL, K.A., HUANG, C. & FIERKE, C.A. 2000. Function and mechanism of zinc metalloenzymes. *Journal of nutrition*, 130(5S Suppl):1437S-46S.

MCCANN, J.C. & AMES, B.N. 2007. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. *American journal of clinical nutrition*, 85(4):931-945.

MCKILLOP, D.J., PENTIEVA, K., DALY, D., MCPARTLIN, J.M., HUGHES, J., STRAIN, J.J., SCOTT, J.M. & MCNULTY, H. 2002. The effect of different cooking methods on folate retention in various foods that are amongst the major contributors to folate intake in the UK diet. *British journal of nutrition*, 88(6):681-688.

MCLAREN, D.S. & FRIGG, M. 2001. Sight and life manual on vitamin A deficiency disorders (VADD). 2nd Edition ed. Basel, Switzerland: SIGHT AND LIFE.

MCLEAN, E., DE BENOIST, B. & ALLEN, L.H. 2008. Review of the magnitude of folate and vitamin B12 deficiencies worldwide. *Food and nutrition bulletin*, 29(2 Suppl):S38-51.

MCLEAN, E., COGSWELL, M., EGLI, I., WOJDYLA, D. & DE BENOIST, B. 2009. Worldwide prevalence of anaemia, WHO vitamin and mineral nutrition information system, 1993-2005. *Public health nutrition*, 12(4):444-454.

MECACCI, L. 2005. Luria: A unitary view of human brain and mind. *Cortex*, 41(6):816-822.

MEENAKSHI, J.V., JOHNSON, N., MANYONG, V.M., DE GROOTE, H., JAVELOSA, J., YANGGEN, D., NAHER, F., GONZALEZ, C., GARCIA, J. & MENG, E. 2007. How cost-effective is biofortification in combating micronutrient malnutrition? an *ex-ante* assessment. Washington D.C. and Cali: the International Food Policy Research Institute (IFPRI) and International Center for Tropical Agriculture (CIAT). 34p.

MOLLOY, A.M., DALY, S., MILLS, J.L., KIRKE, P.N., WHITEHEAD, A.S., RAMSBOTTOM, D., CONLEY, M.R., WEIR, D.G. & SCOTT, J.M. 1997. Thermolabile variant of 5,10-methylenetetrahydrofolate reductase associated with low red-cell folates: Implications for folate intake recommendations. *Lancet*, 349(9065):1591-1593.

MOLLOY, A.M., KIRKE, P.N., BRODY, L.C., SCOTT, J.M. & MILLS, J.L. 2008. Effects of folate and vitamin B12 deficiencies during pregnancy on fetal, infant, and child development. *Food and nutrition bulletin*, 29(2 Suppl):S101-11; discussion S112-5.

MORREALE DE ESCOBAR, G., OBREGON, M.J. & ESCOBAR DEL REY, F. 2004. Role of thyroid hormone during early brain development. *European journal of endocrinology*, 151 Suppl 3U25-37.

MOSHA, T.C., PACE, R.D., ADEYEYE, S., LASWAI, H.S. & MTEBE, K. 1997. Effect of traditional processing practices on the content of total carotenoid, beta-carotene, alpha-carotene and vitamin A activity of selected Tanzanian vegetables. *Plant foods for human nutrition*, 50(3):189-201.

MOSLEY, W.H. & CHEN, L.C. 2003. An analytical framework for the study of child survival in developing countries. *Bulletin of the World Health Organization*, 81(2):140-145.

MOTULSKY, A.G. 1996. Nutritional ecogenetics: Homocysteine-related arteriosclerotic vascular disease, neural tube defects, and folic acid. *American journal of human genetics*, 58(1):17-20.

MUOKI, M.A., TUMUTI, D.S. & ROMBO, G.O. 2008. Nutrition and public hygiene among children under five years of age in Mukuru slums of Makadara division, Nairobi. *East African medical journal*, 85(8):386-397.

MURRAY, C.J. and LOPEZ, A.D., 1994. Global Comparative Assessments in the Health Sector: Disease Burden, Expenditures and Intervention Packages. Geneva: World health organization.

MUTHAYYA, S., EILANDER, A., TRANSLER, C., THOMAS, T., VAN DER KNAAP, H.C., SRINIVASAN, K., VAN KLINKEN, B.J., OSENDARP, S.J. & KURPAD, A.V. 2009. Effect of fortification with multiple micronutrients and n-3 fatty acids on growth and cognitive performance in Indian schoolchildren: the CHAMPION (Children's Health and Mental Performance Influenced by Optimal Nutrition) Study. *American journal of clinical nutrition*, 89(6):1766-75.

MUZHINGI, T., YEUM, K.J., RUSSELL, R.M., JOHNSON, E.J., QIN, J. & TANG, G. 2008. Determination of carotenoids in yellow maize, the effects of saponification and food preparations. *International journal for vitamin and nutrition research*, 78(3):112-120.

NANNAN, N., NORMAN, R., HENDRICKS, M., DHANSAY, M.A., BRADSHAW, D. & SOUTH AFRICAN COMPARATIVE RISK ASSESSMENT COLLABORATING GROUP. 2007. Estimating the burden of disease attributable to childhood and maternal undernutrition in South Africa in 2000. *South African medical journal*, 97(8 Pt 2):733-739.

NELSON, C., ERIKSON, K., PINERO, D.J. & BEARD, J.L. 1997. In vivo dopamine metabolism is altered in iron-deficient anemic rats. *Journal of nutrition*, 127(12):2282-2288.

NESTEL, P., BOUIS, H.E., MEENAKSHI, J.V. & PFEIFFER, W. 2006. Biofortification of staple food crops. *Journal of nutrition*, 136(4):1064-1067.

NEWELL, M.L. 1998. Mechanisms and timing of mother-to-child transmission of HIV-1. *AIDS*, 12(8):831-837.

NOJILANA, B., NORMAN, R., DHANSAY, M.A., LABADARIOS, D., VAN STUIJVENBERG, M.E., BRADSHAW, D. & SOUTH AFRICAN COMPARATIVE RISK ASSESSMENT COLLABORATING GROUP. 2007a. Estimating the burden of disease attributable to iron deficiency anaemia in South Africa in 2000. *South African medical journal*, 97(8 Pt 2):741-746.

NOJILANA, B., NORMAN, R., BRADSHAW, D., VAN STUIJVENBERG, M.E., DHANSAY, M.A., LABADARIOS, D. & SOUTH AFRICAN COMPARATIVE RISK ASSESSMENT COLLABORATING GROUP. 2007b. Estimating the burden of disease attributable to vitamin A deficiency in South Africa in 2000. *South African medical journal*, 97(8 Pt 2):748-753.

NIENABER, C., PISA, P.T. KRUGER, A. HOEKSTRA, T. VENTER, C.S. MOSS, H.S.J. MELSE-BOONSTRA A. & TOWERS. G.W. Homocysteine, alcohol consumption and the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism. Manuscript in preparation.

OELOFSE, A., VAN RAAIJ, J.M., BENADE, A.J., DHANSAY, M.A., TOLBOOM, J.J. & HAUTVAST, J.G. 2002. Disadvantaged black and coloured infants in two urban communities in the Western Cape, South Africa differ in micronutrient status. *Public health nutrition*, 5(2):289-294.

OELOFSE, A., VAN RAAIJ, J.M., BENADE, A.J., DHANSAY, M.A., TOLBOOM, J.J. & HAUTVAST, J.G. 2003. The effect of a micronutrient-fortified complementary food on micronutrient status, growth and development of 6- to 12-month-old disadvantaged urban South African infants. *International journal of food sciences and nutrition*, 54(5):399-407.

OSENDARP, S.J., BAGHURST, K.I., BRYAN, J., CALVARESI, E., HUGHES, D., HUSSAINI, M., KARYADI, S.J., VAN KLINKEN, B.J., VAN DER KNAAP, H.C., LUKITO, W., MIKARSA, W., TRANSLER, C., WILSON, C. & NEMO STUDY GROUP. 2007. Effect of a 12-mo micronutrient intervention on learning and memory in well-nourished and marginally nourished school-aged children: 2 parallel, randomized, placebo-controlled studies in Australia and Indonesia. *American journal of clinical nutrition*, 86(4):1082-1093.

OUÉDRAOGO, H.Z., DRAMAIX-WILMET, M., ZEBA, A.N., HENNART, P. & DONNEN, P. 2008. Effect of iron or multiple micronutrient supplements on the prevalence of anaemia among anaemic young children of a malaria-endemic area: A randomized double-blind trial. *Tropical medicine & international health*, 13(10):1257-1266.

OWINO, V.O., KASONKA, L.M., SINKALA, M.M., WELLS, J.K., EATON, S., DARCH, T., COWARD, A., TOMKINS, A.M. & FILTEAU, S.M. 2007. Fortified complementary foods with or without alpha-amylase

treatment increase hemoglobin but do not reduce breast milk intake of 9-mo-old Zambian infants. *American journal of clinical nutrition*, 86(4):1094-1103.

PALAFOX, N.A., GAMBLE, M.V., DANCHECK, B., RICKS, M.O., BRIAND, K. & SEMBA, R.D. 2003. Vitamin A deficiency, iron deficiency, and anemia among preschool children in the republic of the Marshall Islands. *Nutrition*, 19(5):405-408.

PATHAK, P., KAPIL, U., YAJNIK, C.S., KAPOOR, S.K., DWIVEDI, S.N. & SINGH, R. 2007. Iron, folate, and vitamin B12 stores among pregnant women in a rural area of Haryana state, India. *Food and nutrition bulletin*, 28(4):435-438.

PENLAND, J.G. 2000. Behavioral data and methodology issues in studies of zinc nutrition in humans. *Journal of nutrition*, 130(2S Suppl):361S-364S.

PERIQUET, B.A., JAMMES, N.M., LAMBERT, W.E., TRICOIRE, J., MOUSSA, M.M., GARCIA, J., GHISOLFI, J. & THOUVENOT, J. 1995. Micronutrient levels in HIV-1-infected children. *AIDS*, 9(8):887-893.

PINEDA-LUCATERO, A., AVILA-JIMENEZ, L., RAMOS-HERNANDEZ, R.I., MAGOS, C. & MARTINEZ, H. 2008. Iodine deficiency and its association with intelligence quotient in schoolchildren from Colima, Mexico. *Public health nutrition*, 11(7):690-698.

POLLITT, E. 1996. Timing and vulnerability in research on malnutrition and cognition. *Nutrition reviews*, 54(2 Pt 2):S49-55.

PRASAD, A.S. 1988. Zinc in growth and development and spectrum of human zinc deficiency. *Journal of the American College of Nutrition*, 7(5):377-384.

PRENTICE, A.M., GHATTAS, H., DOHERTY, C. & COX, S.E. 2007. Iron metabolism and malaria. *Food and nutrition bulletin*, 28(4 Suppl):S524-39.

PRINJA, S., THAKUR, J.S. & BHATIA, S.S. 2009. Pilot testing of WHO child growth standards in Chandigarh: Implications for India's child health programmes. *Bulletin of the World Health Organization*, 87(2):116-122.

RAMAKRISHNAN, U. 2002. Prevalence of micronutrient malnutrition worldwide. *Nutrition reviews*, 60(5):S46-S52.

RAMAKRISHNAN, U., ABURTO, N., MCCAULE, G. & MARTORELL, R. 2004. Multimicronutrient interventions but not vitamin A or iron interventions alone improve child growth: Results of 3 meta-analyses. *Journal of nutrition*, 134(10):2592-2602.

RAMAKRISHNAN, U., NGUYEN, P. & MARTORELL, R. 2009. Effects of micronutrients on growth of children under 5 y of age: Meta-analyses of single and multiple nutrient interventions. *American journal of clinical nutrition*, 89(1):191-203.

REDDI, A., LEEPER, S.C., GROBLER, A.C., GEDDES, R., FRANCE, K.H., DORSE, G.L., VLOK, W.J., MNTAMBO, M., THOMAS, M., NIXON, K., HOLST, H.L., KARIM, Q.A., ROLLINS, N.C., COOVADIA, H.M. & GIDDY, J. 2007. Preliminary outcomes of a paediatric highly active antiretroviral therapy cohort from KwaZulu-Natal, South Africa. *BMC pediatrics*, 713.

REITAN, R.M. 1988. Integration of neuropsychological theory, assessment, and application. *The clinical neuropsychologist*, 2(4):331-349.

REISS, A.L., ABRAMS, M.T., SINGER, H.S., ROSS, J.L. & DENCKLA, M.B. 1996. Brain development, gender and IQ in children. A volumetric imaging study. *Brain*, 119 (Pt 5):1763-1774.

RICE, A.L., WEST, K.P. & BLACK, R.E. 2004. Vitamin A deficiency. (In Ezzati, M., Lopez, A.D., Rodgers, A. & Murray, E.L.J., eds. Comparative quantification of health risks: Global and regional burden of disease attributable to selected major risk factors. Geneva: World Health Organization. p. 211-256.).

RIVERA, J.A., HOTZ, C., GONZALEZ-COSSIO, T., NEUFELD, L. & GARCIA-GUERRA, A. 2003. The effect of micronutrient deficiencies on child growth: A review of results from community-based supplementation trials. *Journal of nutrition*, 133(11 Suppl 2):4010S-4020S.

ROBBERSTAD, B., STRAND, T., BLACK, R.E. & SOMMERFELT, H. 2004. Cost-effectiveness of zinc as adjunct therapy for acute childhood diarrhoea in developing countries. *Bulletin of the World Health Organization*, 82(7):523-531.

RODRIGUEZ-AMAYA, D.B. 2003. Food carotenoids: Analysis, composition and alterations during storage and processing of foods. *Forum of nutrition*, 5635-37.

ROSS, A.C. 2003. Retinoid production and catabolism: Role of diet in regulating retinol esterification and retinoic acid oxidation. *Journal of nutrition*, 133(1):291S-296S.

SACHDEV, H., GERA, T. & NESTEL, P. 2005a. Effect of iron supplementation on mental and motor development in children: Systematic review of randomised controlled trials. *Public health nutrition*, 8(2):117-132.

SACHDEV, P.S., PARSLow, R.A., LUX, O., SALONIKAS, C., WEN, W., NAIDOO, D., CHRISTENSEN, H. & JORM, A.F. 2005b. Relationship of homocysteine, folic acid and vitamin B12 with depression in a middle-aged community sample. *Psychological medicine*, 35(4):529-538.

SACHDEV, H., GERA, T. & NESTEL, P. 2006. Effect of iron supplementation on physical growth in children: Systematic review of randomised controlled trials. *Public health nutrition*, 9(7):904-920.

SANDSTEAD, H.H. 1985. W.O. Atwater memorial lecture. Zinc: Essentiality for brain development and function. *Nutrition reviews*, 43(5):129-137.

SANDSTEAD, H.H., PENLAND, J.G., ALCOCK, N.W., DAYAL, H.H., CHEN, X.C., LI, J.S., ZHAO, F. & YANG, J.J. 1998. Effects of repletion with zinc and other micronutrients on neuropsychologic performance and growth of Chinese children. *American journal of clinical nutrition*, 68(2 Suppl):470S-475S.

SANGHVI, T., VAN AMERINGEN, M., BAKER, J., FIEDLER, J., BORWANKAR, R., PHILLIPS, M., HOUSTON, R., ROSS, J., HEYMANN, H. & DARY, O. 2007. Vitamin and mineral deficiencies technical situation analysis: A report for the ten year strategy for the reduction of vitamin and mineral deficiencies. *Food and nutrition bulletin*, 28(1 Suppl Vitamin):S160-219.

SANTIAGO-FERNANDEZ, P., TORRES-BARAHONA, R., MUELA-MARTINEZ, J.A., ROJO-MARTINEZ, G., GARCIA-FUENTES, E., GARRIGA, M.J., LEON, A.G. & SORIGUER, F. 2004. Intelligence quotient and iodine intake: A cross-sectional study in children. *The journal of clinical endocrinology and metabolism*, 89(8):3851-3857.

SARI, M., BLOEM, M.W., DE PEE, S., SCHULTINK, W.J. & SASTROAMIDJOJO, S. 2001. Effect of iron-fortified candies on the iron status of children aged 4-6 y in east Jakarta, Indonesia. *American journal of clinical nutrition*, 73(6):1034-1039.

SAVACG see SOUTH AFRICAN VITAMIN A CONSULTATIVE GROUP.

SOUTH AFRICAN VITAMIN A CONSULTATIVE GROUP. 1996. Anthropometric, vitamin A, iron and immunisation coverage status in children aged 6-71 months in South Africa, 1994. *South African medical journal*, 86(4):354-357.

SAZAWAL, S., BENTLEY, M., BLACK, R.E., DHINGRA, P., GEORGE, S. & BHAN, M.K. 1996. Effect of zinc supplementation on observed activity in low socioeconomic Indian preschool children. *Pediatrics*, 98(6 Pt 1):1132-1137.

SAZAWAL, S., BLACK, R.E., RAMSAN, M., CHWAYA, H.M., STOLTZFUS, R.J., DUTTA, A., DHINGRA, U., KABOLE, I., DEB, S., OTHMAN, M.K. & KABOLE, F.M. 2006. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: Community-based, randomised, placebo-controlled trial. *Lancet*, 367(9505):133-143.

SCHAEFER, G.B., THOMPSON, J.N., JR, BODENSTEINER, J.B., HAMZA, M., TUCKER, R.R., MARKS, W., GAY, C. & WILSON, D. 1990. Quantitative morphometric analysis of brain growth using magnetic resonance imaging. *Journal of child neurology*, 5(2):127-130.

SCHMITT, J.A., BENTON, D. & KALLUS, K.W. 2005. General methodological considerations for the assessment of nutritional influences on human cognitive functions. *European journal of nutrition*, 44(8):459-464.

SESHADRI, S. & GOPALDAS, T. 1989. Impact of iron supplementation on cognitive functions in preschool and school-aged children: The Indian experience. *American journal of clinical nutrition*, 50(3 Suppl):675-84; discussion 685-6.

SESHADRI, S. 2001. Prevalence of micronutrient deficiency particularly of iron, zinc and folic acid in pregnant women in south East Asia. *British journal of nutrition*, 85 Suppl 2S87-92.

SHARIEFF, W., ZLOTKIN, S.H., UNGAR, W.J., FELDMAN, B., KRAHN, M.D. & TOMLINSON, G. 2008. Economics of preventing premature mortality and impaired cognitive development in children through home-fortification: A health policy perspective. *International journal of technology assessment in health care*, 24(3):303-311.

SHET, A., MEHTA, S., RAJAGOPALAN, N., DINAKAR, C., RAMESH, E., SAMUEL, N.M., INDUMATHI, C.K., FAWZI, W.W. & KURPAD, A.V. 2009. Anemia and growth failure among HIV-infected children in India: A retrospective analysis. *Biomed central pediatrics*, 937.

SIMEON, D.T., GRANTHAM-MCGREGOR, S.M., CALLENDER, J.E. & WONG, M.S. 1995. Treatment of *Tichuris trichiura* infections improves growth, spelling scores and school attendance in some children. *Journal of nutrition*, 125(7):1875-1883.

SIVAKUMAR, B., VIJAYARAGHAVAN, K., VAZIR, S., BALAKRISHNA, N., SHATRUGNA, V., SARMA, .K.V., NAIR, K.M., RAGHURAMULU, N. & KRISHNASWAMY, K. 2006a. Effect of micronutrient supplement on health and nutritional status of schoolchildren: study design. *Nutrition*, 22(1 Suppl):S1-7.

SIVAKUMAR, B., NAIR, K.M., SREERAMULU, D., SURYANARAYANA, P., RAVINDER, P., SHATRUGNA, V., KUMAR, P.A., RAGHUNATH, M., RAO, VV., BALAKRISHNA, N., KUMAR, P.U. & RAGHURAMULU, N. 2006b. Effect of micronutrient supplement on health and nutritional status of schoolchildren: biochemical status. *Nutrition*, 22(1 Suppl):S15-25.

SKUY, M., TAYLOR, M., O'CARROLL, S., FRIDJHON, P. & ROSENTHAL, L. 2000. Performance of black and white South African children on the Wechsler intelligence scale for children-revised and the Kaufman assessment battery. *Psychological reports*, 86(3 Pt 1):727-737.

SMUTS, C.M., DHANSAY, M.A., FABER, M., VAN STUIJVENBERG, M.E., SWANEVELDER, S., GROSS, R. & BENADÉ, A.J. 2005. Efficacy of multiple micronutrient supplementation for improving anemia, micronutrient status, and growth in South African infants. *Journal of nutrition*, 135(3):653S-659S

SNOW, R.W., BASTOS DE AZEVEDO, I., LOWE, B.S., KABIRU, E.W., NEVILL, C.G., MWANKUSYE, S., KASSIGA, G., MARSH, K. & TEUSCHER, T. 1994. Severe childhood malaria in two areas of markedly different *Falciparum* transmission in East Africa. *Acta tropica*, 57(4):289-300.

SNOW, R.W., CRAIG, M., DEICHMANN, U. & MARSH, K. 1999. Estimating mortality, morbidity and disability due to malaria among Africa's non-pregnant population. *Bulletin of the World Health Organization*, 77(8):624-640.

SOEMANTRI, A.G., POLLITT, E. & KIM, I. 1985. Iron deficiency anemia and educational achievement. *American journal of clinical nutrition*, 42(6):1221-1228.

SOEWONDO, S., HUSAINI, M. & POLLITT, E. 1989. Effects of iron deficiency on attention and learning processes in preschool children: Bandung, Indonesia. *American journal of clinical nutrition*, 50(3 Suppl):667-73; discussion 673-4.

OLON, F.S., SAROL, J.N.,JR, BERNARDO, A.B., OLON, J.A., MEHANSHO, H., SANCHEZ-FERMIN, L.E., WAMBANGCO, L.S. & JUHLIN, K.D. 2003. Effect of a multiple-micronutrient-fortified fruit powder beverage on the nutrition status, physical fitness, and cognitive performance of schoolchildren in the Philippines. *Food and nutrition bulletin*, 24(4 Suppl):S129-40.

SOMMER, A. & WEST, K.P.J. 1996. Vitamin A deficiency: Health, survival, and vision. New York: Oxford University Press.

STEIN, A.J., MEENASHI, J.V., QAIM, M., NESTEL, P., SACHDEV, H.P.S. & BHITTA, Z.A. 2005. Analyzing the health benefits of biofortified staple crops by means of the daily-adjusted life years approach: A handbook focusing on iron, zinc and vitamin A. Washington D.C. and Cali: The International Food Policy Research Institute (IFPRI) and International Center for Tropical Agriculture (CIAT). 32p.

STEIN, A.D., WANG, M., DIGIROLAMO, A., GRAJEDA, R., RAMAKRISHNAN, U., RAMIREZ-ZEA, M., YOUNT, K. & MARTORELL, R. 2008. Nutritional supplementation in early childhood, schooling, and intellectual functioning in adulthood: A prospective study in Guatemala. *Archives of pediatrics & adolescent medicine*, 162(7):612-618.

STEPHENSON, L.S., LATHAM, M.C., ADAMS, E.J., KINOTI, S.N. & PERTET, A. 1993. Weight gain of Kenyan school children infected with hookworm, trichuris trichiura and ascaris lumbricoides is improved following once- or twice-yearly treatment with albendazole. *Journal of nutrition*, 123(4):656-665.

STEVENSON, R.E., SCHWARTZ, C.E., DU, Y.Z. & ADAMS, M.J.,JR. 1997. Differences in methylenetetrahydrofolate reductase genotype frequencies, between whites and blacks. *American journal of human genetics*, 60(1):229-230.

STEYN, N.P. & MARAIS, D. 2002. Children have a right to meals of good nutritional quality at state-subsidised crèches. *South African journal of clinical nutrition*, 15(2):3.

STOLTZFUS, R.J., DREYFUSS, M.L., CHWAYA, H.M. & ALBONICO, M. 1997. Hookworm control as a strategy to prevent iron deficiency. *Nutrition reviews*, 55(6):223-232.

STOLTZFUS, R.J. & DREYFUSS, M.L. 1998. Guidelines for the use of iron supplements to prevent and treat iron deficiency anaemia. Washington, D. C.: ILSI Press. 30p.

STOLTZFUS, R.J., KVALSVIG, J.D., CHWAYA, H.M., MONTRESOR, A., ALBONICO, M., TIELSCH, J.M., SAVIOLI, L. & POLLITT, E. 2001. Effects of iron supplementation and anthelmintic treatment on motor and language development of preschool children in Zanzibar: Double blind, placebo controlled study. *British medical journal*, 323(7326):1389-1393.

- STOLTZFUS, R.J. 2003. Iron deficiency: Global prevalence and consequences. *Food and nutrition bulletin*, 24(4 Suppl):S99-103.
- STOLTZFUS, R.J., MULLANY, L. & BLACK, R.E. 2004a. Iron deficiency anaemia. (In Ezzati, M., Lopez, A.D., Rodgers, A. & Murray, E.L.J., eds. Comparative quantification of health risks: Global and regional burden of disease attributable to selected major risk factors. Geneva: World Health Organization. p. 163-209.).
- STOLTZFUS, R.J., CHWAY, H.M., MONTRESOR, A., TIELSCH, J.M., JAPE, J.K., ALBONICO, M. & SAVIOLI, L. 2004b. Low dose daily iron supplementation improves iron status and appetite but not anemia, whereas quarterly anthelmintic treatment improves growth, appetite and anemia in Zanzibar preschool children. *Journal of nutrition*, 134(2):348-356.
- SWART, R., SANDERS, D. & MCLACHLAN, M. 2008. Nutrition: A primary health care perspective. (In: South African health review 2006. BARRON, P. & ROMA-REARDON, J. eds. Durban: Health System Trust. 134 p.)
- TAFTI, M. & GHYSELINCK, N.B. 2007. Functional implication of the vitamin A signaling pathway in the brain. *Archives of neurology*, 64(12):1706-1711.
- TAKO, E., LAPARRA, J.M., GLAHN, R.P., WELCH, R.M., LEI, X.G., BEEBE, S. & MILLER, D.D. 2009. Biofortified black beans in a maize and bean diet provide more bioavailable iron to piglets than standard black beans. *Journal of nutrition*, 139(2):305-309.
- THANKACHAN, P., MUTHAYYA, S., WALCZYK, T., KURPAD, A.V. & HURRELL, R.F. 2007. An analysis of the etiology of anemia and iron deficiency in young women of low socioeconomic status in Bangalore, India. *Food and nutrition bulletin*, 28(3):328-336.
- THOMPSON, P.M., GIEDD, J.N., WOODS, R.P., MACDONALD, D., EVANS, A.C. & TOGA, A.W. 2000. Growth patterns in the developing brain detected by using continuum mechanical tensor maps. *Nature*, 404(6774):190-193.
- THOMPSON, R.A. & NELSON, C.A. 2001. Developmental science and the media: Early brain development. *The American psychologist*, 56(1):5-15.
- THORNTON, I. & ALLOWAY, B.J. 1974. Geochemical aspects of the soil-plant-animal relationship in the development of trace element deficiency and excess. *Proceedings of the Nutrition Society*, 33(3): 257-266.
- THURLOW, R.A., WINICHAGOON, P., PONGCHAROEN, T., GOWACHIRAPANT, S., BOONPRADERM, A., MANGER, M.S., BAILEY, K.B., WASANTWISUT, E. & GIBSON, R.S. 2006. Risk of zinc, iodine and other micronutrient deficiencies among school children in North East Thailand. *European journal of clinical nutrition*, 60(5):623-632.

THURNHAM, D.I., MCCABE, G.P., NORTHROP-CLEWES, C.A. & NESTEL, P. 2003. Effects of subclinical infection on plasma retinol concentrations and assessment of prevalence of vitamin A deficiency: Meta-analysis. *Lancet*, 362(9401):2052-2058.

THURNHAM, D.I. & NORTHROP-CLEWES, C.A. 2007. Infection and the etiology of anemia. (In Kraemer, K. & Zimmermann, M.B., eds. Nutritional anemia. Switzerland: SIGHT AND LIFE Press. p. 231-256.)

TIEMEIER, H., VAN TUIJL, H.R., HOFMAN, A., MEIJER, J., KILIAAN, A.J. & BRETELER, M.M. 2002. Vitamin B12, folate, and homocysteine in depression: The Rotterdam study. *The American journal of psychiatry*, 159(12):2099-2101.

TIWARI, B.D., GODBOLE, M.M., CHATTOPADHYAY, N., MANDAL, A. & MITHAL, A. 1996. Learning disabilities and poor motivation to achieve due to prolonged iodine deficiency. *American journal of clinical nutrition*, 63(5):782-786.

TROESCH, B., EGLI, I., ZEDER, C., HURRELL, R.F., DE PEE, S. & ZIMMERMANN, M.B. 2009. Optimization of a phytase-containing micronutrient powder with low amounts of highly bioavailable iron for in-home fortification of complementary foods. *American journal of clinical nutrition*, 89(2):539-544.

TSARAS, G., OWUSU-ANSAH, A., BOATENG, F.O. & AMOATENG-ADJEPONG, Y. 2009. Complications associated with sickle cell trait: A brief narrative review. *American journal of medicine*, 122(6):507-512.

TUPE, R.S., CHIPLONKAR, S.A., AGTE, V.V. 2007. Changes in zinc uptake in response to ascorbic acid and folic acid in rat liver slices under normal and oxidative stress conditions. *Biofactors*, 30(1):27-34.

UNICEF see UNITED NATION'S CHILDREN FUNDS.

UNITED NATION'S CHILDREN FUNDS, 2008. *The State of the World's Children 2009: Maternal and Newborn Health*. New York: UNICEF.

VALLEE, B.L. & FALCHUK, K.H. 1993. The biochemical basis of zinc physiology. *Physiological reviews*, 73(1):79-118.

VAN DEN BRIEL, T., WEST, C.E., BLEICHRODT, N., VAN DE VIJVER, F.J., ATEGBO, E.A. & HAUTVAST, J.G. 2000. Improved iodine status is associated with improved mental performance of schoolchildren in Benin. *American journal of clinical nutrition*, 72(5):1179-1185.

VAN DER PUT, N.M., GABREELS, F., STEVENS, E.M., SMEITINK, J.A., TRIJBELS, F.J., ESKES, T.K., VAN DEN HEUVEL, L.P. & BLOM, H.J. 1998. A second common mutation in the methylenetetrahydrofolate reductase gene: An additional risk factor for neural-tube defects? *American journal of human genetics*, 62(5):1044-1051.

- VAN STUIJVENBERG, M.E., KVALSVIG, J.D., FABER, M., KRUGER, M., KENOYER, D.G. & BENADE, A.J. 1999. Effect of iron-, iodine-, and beta-carotene-fortified biscuits on the micronutrient status of primary school children: A randomized controlled trial. *American journal of clinical nutrition*, 69(3):497-503.
- VAN JAARSVELD, P.J., FABER, M., TANUMIHARDJO, S.A., NESTEL, P., LOMBARD, C.J. & BENADE, A.J. 2005. Beta-carotene-rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. *American journal of clinical nutrition*, 81(5):1080-1087.
- VAN STUIJVENBERG, M.E. 2005. Using the school feeding system as a vehicle for micronutrient fortification: Experience from South Africa. *Food and nutrition bulletin*, 26(2 Suppl 2):S213-9.
- VAN STUIJVENBERG, M.E., SMUTS, C.M., LOMBARD, C.J. & DHANSAY, M.A. 2008. Fortifying brown bread with sodium iron EDTA, ferrous fumarate, or electrolytic iron does not affect iron status in South African schoolchildren. *Journal of nutrition*, 138(4):782-786.
- VANDERPAS, J. 2006. Nutritional epidemiology and thyroid hormone metabolism. *Annual review of nutrition*, 26:293-322.
- VARMA, J.L., DAS, S., SANKAR, R., MANNAR, M.G., LEVINSON, F.J. & HAMER, D.H. 2007. Community-level micronutrient fortification of a food supplement in India: A controlled trial in preschool children aged 36-66 mo. *American journal of clinical nutrition*, 85(4):1127-1133.
- VAZIR, S., NAGALLA, B., THANGIAH, V., KAMASAMUDRAM, V. & BHATTIPROLU, S. 2006. Effect of micronutrient supplement on health and nutritional status of schoolchildren: Mental function. *Nutrition*, 22(1 Suppl):S26-32.
- VERMIGLIO, F., SIDOTI, M., FINOCCHIARO, M.D., BATTIATO, S., LO PRESTI, V.P., BENVENGA, S. & TRIMARCHI, F. 1990. Defective neuromotor and cognitive ability in iodine-deficient schoolchildren of an endemic goiter region in Sicily. *The journal of clinical endocrinology and metabolism*, 70(2):379-384.
- VICTORA, C.G., ADAIR, L., FALL, C., HALLAL, P.C., MARTORELL, R., RICHTER, L., SACHDEV, H.S. & MATERNAL AND CHILD UNDERNUTRITION STUDY GROUP. 2008. Maternal and child undernutrition: Consequences for adult health and human capital. *Lancet*, 371(9609):340-357.
- VILLAMOR, E., SAATHOFF, E., BOSCH, R.J., HERTZMARK, E., BAYLIN, A., MANJI, K., MSAMANGA, G., HUNTER, D.J. & FAWZI, W.W. 2005. Vitamin supplementation of HIV-infected women improves postnatal child growth. *American journal of clinical nutrition*, 81(4):880-888.
- VINOD KUMAR, M. & RAJAGOPALAN, S. 2008. Trial using multiple micronutrient food supplement and its effect on cognition. *Indian journal of pediatrics*, 75(7):671-678.

- VITERI, F.E., ALI, F. & TUJAGUE, J. 1999. Long-term weekly iron supplementation improves and sustains nonpregnant women's iron status as well or better than currently recommended short-term daily supplementation. *Journal of nutrition*, 129(11):2013-2020.
- VORSTER, H.H., OOSTHUIZEN, W., JERLING, J.C., VELDMAN, F.J. & BURGER, H.M. 1997. The nutritional status of South Africans - A review of the literature from 1975-1996. Durban: Health System Trust, 1:1-48.
- WALKER, S.P., CHANG, S.M., POWELL, C.A. & GRANTHAM-MCGREGOR, S.M. 2005. Effects of early childhood psychosocial stimulation and nutritional supplementation on cognition and education in growth-stunted Jamaican children: Prospective cohort study. *Lancet*, 366(9499):1804-1807.
- WALKER, S.P., WACHS, T.D., GARDNER, J.M., LOZOFF, B., WASSERMAN, G.A., POLLITT, E., CARTER, J.A. & INTERNATIONAL CHILD DEVELOPMENT STEERING GROUP. 2007. Child development: Risk factors for adverse outcomes in developing countries. *Lancet*, 369(9556):145-157.
- WALSH, C.T., SANDSTEAD, H.H., PRASAD, A.S., NEWBERNE, P.M. & FRAKER, P.J. 1994. Zinc: Health effects and research priorities for the 1990s. *Environmental health perspectives*, 102 Suppl 25-46.
- WAMBUA, S., MWACHARO, J., UYOGA, S., MACHARIA, A. & WILLIAMS, T.N. 2006a. Co-inheritance of alpha+-thalassaemia and sickle trait results in specific effects on haematological parameters. *British journal of haematology*, 133(2):206-209.
- WAMBUA, S., MWANGI, T.W., KORTOK, M., UYOGA, S.M., MACHARIA, A.W., MWACHARO, J.K., WEATHERALL, D.J., SNOW, R.W., MARSH, K. & WILLIAMS, T.N. 2006b. The effect of alpha+-thalassaemia on the incidence of malaria and other diseases in children living on the coast of Kenya. *PLoS medicine*, 3(5):e158.
- WELCH, R.M. 2002. Breeding strategies for biofortified staple plant foods to reduce micronutrient malnutrition globally. *Journal of nutrition*, 132(3):495S-499S.
- WEST, K.P. 2002. Extent of vitamin A deficiency among preschool children and women of reproductive age. *Journal of nutrition*, 132(9):2857S.
- WEST, K.P. & DARNTON-HILL, I. 2001. Vitamin A deficiency. (In Semba, R.D. & Bloem, M.W., eds. *Nutrition and health in developing countries*. Totowa, New Jersey: Humana Press. p. 267-306.).
- WEST, K.P. 2003. Vitamin A deficiency disorders in children and women. *Food and nutrition bulletin*, 24(4 Suppl):S78-90.
- WINICHAGOON, P., MCKENZIE, J.E., CHAVASIT, V., PONGCHAROEN, T., GOWACHIRAPANT, S., BOONPRADERM, A., MANGER, M.S., BAILEY, K.B., WASANTWISUT, E. & GIBSON, R.S. 2006. A multimicronutrient-fortified seasoning powder enhances the hemoglobin, zinc, and iodine status of primary school children in North East Thailand: a randomized controlled trial of efficacy. *Journal of nutrition*, 136(6):1617-23.

WILLIAMS, T.N., WAMBUA, S., UYOGA, S., MACHARIA, A., MWACHARO, J.K., NEWTON, C.R. & MAITLAND, K. 2005. Both heterozygous and homozygous alpha+ thalassemias protect against severe and fatal *Plasmodium falciparum* malaria on the coast of Kenya. *Blood*, 106(1):368-371.

WORLD BANK. 1993. *World development report 1993*. (In Anon. *Investing in health*). New York: Oxford University Press.

WHITE, J. G. & ZASOSKI, R. J. 1999. Mapping soil micronutrients. *Field crops research*, 60:11-26.

WHO see WORLD HEALTH ORGANIZATION.

WORLD HEALTH ORGANIZATION. 1993. Global prevalence of iodine deficiency disorders. Micronutrient deficiency information system (MDIS) working paper no. 1. Geneva: World Health Organization.

WORLD HEALTH ORGANIZATION, UNITED NATIONS CHILDREN'S FUND and UNITED NATIONS UNIVERSITY, 2001a. Iron deficiency anemia: Assessment, prevention, and control. WHO/NHD/01.3. Geneva: World Health Organization.

WORLD HEALTH ORGANIZATION, INTERNATIONAL COUNCIL FOR THE CONTROL OF THE IODINE DEFICIENCY DISORDERS, UNITED NATIONS CHILDRENS' FUNDS (WHO/ICCIDD/UNICEF). 2001b. Assessment of the iodine deficiency disorders and monitoring their elimination. WHO/NHD/01.1. Geneva: World Health Organization.

WORLD HEALTH ORGANIZATION. 2002. *World health report: Reducing risks, promoting healthy life*. Geneva: World Health Organization.

WORLD HEALTH ORGANIZATION, 2008. Worldwide prevalence of anaemia 1993-2005: WHO global database on anaemia. Geneva: World Health Organization.

WORLD HEALTH ORGANIZATION. 2009. Global prevalence of vitamin A deficiency in populations at risk 1995-2005: WHO global database on vitamin A deficiency. Geneva: World Health Organization.

WHO / FAO see WORLD HEALTH ORGANIZATION / FOOD AND AGRICULTURE ORGANIZATION.

WORLD HEALTH ORGANIZATION / FOOD AND AGRICULTURE ORGANIZATION. 2006. Guidelines on food fortification with micronutrients. (In Allen, L., de Benoist, B., Dary, O. & Hurrell, R. eds.). Geneva: World health organization.

YADAV, S.K. & SEHGAL, S. 1997. Effect of home processing and storage on ascorbic acid and beta-carotene content of Bathua (*Chenopodium album*) and Fenugreek (*Trigonella foenum graecum*) leaves. *Plant foods for human nutrition*, 50(3):239-247.

- YANG, X-E., CHEN, W-R. & FENG, Y. 2006. Improving human micronutrient nutrition through biofortification in the soil-plant system: China a case study. *Environmental geochemistry & health*, 29(5): 413-428.
- YAGER, J.Y. & HARTFIELD, D.S. 2002. Neurologic manifestations of iron deficiency in childhood. *Pediatric neurology*, 27(2):85-92.
- ZIMMERMANN, M., ADOU, P., TORRESANI, T., ZEDER, C. & HURRELL, R. 2000. Persistence of goiter despite oral iodine supplementation in goitrous children with iron deficiency anemia in Cote d'Ivoire. *American journal of clinical nutrition*, 71(1):88-93.
- ZIMMERMANN, M.B., ZEDER, C., CHAOUKI, N., TORRESANI, T., SAAD, A. & HURRELL, R.F. 2002. Addition of microencapsulated iron to iodized salt improves the efficacy of iodine in goitrous, iron-deficient children: A randomized, double-blind, controlled trial. *European journal of endocrinology*, 147(6):747-753.
- ZIMMERMANN, M.B., WEGMUELLER, R., ZEDER, C., CHAOUKI, N., BIEBINGER, R., HURRELL, R.F. & WINDHAB, E. 2004. Triple fortification of salt with microcapsules of iodine, iron, and vitamin A. *American journal of clinical nutrition*, 80(5):1283-1290.
- ZIMMERMANN, M.B., CHAOUKI, N. & HURRELL, R.F. 2005a. Iron deficiency due to consumption of a habitual diet low in bioavailable iron: A longitudinal cohort study in Moroccan children. *American journal of clinical nutrition*, 81(1):115-121.
- ZIMMERMANN, M.B., MOLINARI, L., STAUBLI-ASOBAYIRE, F., HESS, S.Y., CHAOUKI, N., ADOU, P. & HURRELL, R.F. 2005b. Serum transferrin receptor and zinc protoporphyrin as indicators of iron status in African children. *American journal of clinical nutrition*, 81(3):615-623.
- ZIMMERMANN, M.B., CONNOLLY, K., BOZO, M., BRIDSON, J., ROHNER, F. & GRIMCI, L. 2006. Iodine supplementation improves cognition in iodine-deficient schoolchildren in Albania: A randomized, controlled, double-blind study. *American journal of clinical nutrition*, 83(1):108-114.
- ZIMMERMANN, M.B. 2007a. Interaction between iron and vitamin A, riboflavin, copper, and zinc in the etiology of anemia. (In Kraemer, K. & Zimmermann, M.B., eds. Nutritional anemia. Switzerland: SIGHT AND LIFE Press. p. 199-213.)
- ZIMMERMANN, M.B. 2007b. The adverse effects of mild-to-moderate iodine deficiency during pregnancy and childhood: A review. *Thyroid*, 17(9):829-835.
- ZIMMERMANN, M.B. 2007c. The impact of iodised salt or iodine supplements on iodine status during pregnancy, lactation and infancy. *Public health nutrition*, 10(12):1584-1595.
- ZIMMERMANN, M.B. & HURRELL, R.F. 2007. Nutritional iron deficiency. *Lancet*, 370(9586):511-520.

ZIMMERMANN, M.B., JOOSTE, P.L. & PANDAV, C.S. 2008. Iodine-deficiency disorders. *Lancet*, 372(9645):1251-1262.

ZIMMERMANN, M.B. 2008a. Iodine requirements and the risks and benefits of correcting iodine deficiency in populations. *Journal of trace elements in medicine and biology*, 22(2):81-92.

ZIMMERMANN, M.B. 2008b. Methods to assess iron and iodine status. *British journal of nutrition*, 99S2-9.

ZIMMERMANN, M.B. 2009a. Iodine deficiency in pregnancy and the effects of maternal iodine supplementation on the offspring: A review. *American journal of clinical nutrition*, 89(2):668S-672.

ZIMMERMANN, M.B. 2009b. Iodine deficiency. *Endocrine reviews*, 30(4):376-408.

ZLOTKIN, S.H. & TONDEUR, M. 2007. Successful approaches: Sprinkles. (In Kraemer, K. & Zimmermann, M.B., eds. Nutritional anemia. Switzerland: SIGHT AND LIFE Press. p. 269-284.).

ZOELLER, T.R., DOWLING, A.L., HERZIG, C.T., IANNAcone, E.A., GAUGER, K.J. & BANSAL, R. 2002. Thyroid hormone, brain development, and the environment. *Environmental health perspectives*, 110 Suppl 3355-361.

CHAPTER 3

POINT-OF-USE MICRONUTRIENT FORTIFICATION: LESSONS LEARNED IN IMPLEMENTING A PRESCHOOL-BASED PILOT TRIAL IN SOUTH AFRICA

OGUNLADE, A.O., KRUGER, H.S. & JERLING, J.C.

**Centre of Excellence for Nutrition, North-West University, Potchefstroom
Campus, South Africa.**

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ABSTRACT

Background: The use of micronutrient powders also known as “in-home or point-of-use “PoU” fortification” is a promising new approach for improving early childhood nutrition and developmental potentials in a sustainable way.

Objective: The aim of this pilot study was to assess the feasibility of implementing a PoU micronutrient fortification in preschool settings.

Design: Preschool children (n = 151), aged 36 – 79 months with haemoglobin concentration (Hb) $\leq$ 12.5 g/dL, from 8 schools in a low socio-economic community were randomly assigned to intervention (n = 76) and control (n = 75) groups, both receiving breakfast maize-meal porridge either with added micronutrient or placebo powder for 52 school days. Several process evaluation indicators (fidelity, dose delivered, dose received, reach, recruitment and context) were used to assess trial feasibility. Selected indicators of early childhood development (Hb concentration, anthropometric indices and cognitive function) were used to evaluate the outcome of the intervention within the context of a pilot study.

Results: The process evaluation results showed that the implementation components were feasible and could be delivered with high fidelity. The PoU fortificant was well accepted, and the mean percentage of days that it was consumed (~85%) did not differ between groups. There were significant increases in Hb concentration ($p < 0.05$) from baseline to follow-up in both the intervention [mean change: 0.38 g/dL (95% CI: 0.14, 0.61 g/dL)] and control [mean change: 0.57 g/dL (95% CI: 0.35, 0.80 g/dL)] groups, however, this did not differ significantly between the groups ($p = 0.250$). The intervention did not improve any of the anthropometric indices measured in the intervention group compared to control. However, there was a medium likelihood for clinical/practical significance for the two global cognitive scores assessed, nonverbal index [intervention effects: 7.20 (95% CI: 2.60, 11.81); $p = 0.002$, effect size: 0.55] and mental processing index [intervention effects: 2.73 (95% CI: 0.25, 5.70); $p = 0.072$, effect size: 0.36] on the Kaufman Assessment Battery for Children, Second Edition.

Conclusion: The feasibility of implementing a PoU micronutrient fortification trial was demonstrated among South African preschool children with potential benefits of improving their cognitive function. The lessons from this trial could help in planning/implementing future PoU micronutrient fortification trial among South African preschool children.

Introduction

In spite of continued concerted efforts, early childhood micronutrient malnutrition remains a global public health burden (Sanghvi et al. 2007; Black et al. 2008). In developing countries for instance, poor nutrition (especially micronutrient deficiencies) is not only responsible for over 10 million preventable child deaths yearly (Black et al. 2003), it is also a risk factor preventing an additional estimated 200 million children under five years from attaining their full cognitive developmental potential (Grantham-McGregor et al. 2007). Like many other developing populations, report of a recent national survey conducted among South African children 1 to 9 years as reviewed by Swart et al. (2008) revealed that in 2005, 63.6% and 13.7% had inadequate vitamin A status (serum retinol $<0.70 \mu\text{mol/L}$) and severe vitamin A deficiency (serum retinol $<0.35 \mu\text{mol/L}$) respectively. Additionally, 27.9% had low haemoglobin (Hb) status (Hb $<11 \text{ g/dL}$ for children ≤ 5 years and Hb $<11.5 \text{ g/dL}$ for children >5 years) while 45.3% were zinc deficient (serum zinc $<65 \mu\text{g/dL}$). This recent national survey report clearly shows that micronutrient deficiencies are still a problem of public health importance among South African children.

Although several micronutrient intervention strategies have been evaluated among South African children; infants (Oelofse et al. 2003; Faber et al. 2005; Smuts et al. 2005) and school-age children (Van Stuijvenberg et al. 1999; Van Stuijvenberg et al. 2001a; Van Stuijvenberg et al. 2001b; Van Jaarsveld et al. 2005) have been the major beneficiaries, while only a few studies were conducted among preschool-age children. In view of this, micronutrient malnutrition control targeting South African preschool children at home or in school should be intensified. Optimistically, the use of recently developed micronutrient powders also known as “in-home fortification” or “point-of-use (PoU) fortification” can be an effective long-term, sustainable approach (Menon et al. 2007; Zlotkin & Tondeur, 2007; De Pee et al. 2008) and may not only significantly improve their micronutrient status and development in the short-term (Varma et al. 2007), but also their cognitive skills (Stein et al. 2008) and productivity (Hodinott et al. 2008) in adulthood, which may also possibly result in substantial economic benefits (Alderman et al. 2007).

However, experience with the use of PoU fortificants [such as “MixMe plus[®]” (DSM Nutritional Products Ltd, Basel, Switzerland) and “Sprinkles[™]” (Sprinkles Global Health Initiative, Toronto, Canada)] in larger-scale trials and in preschool settings in many developing populations for instance is limited (De Pee et al. 2008). Therefore, before

embarking on large-scale, long-term PoU fortification trials, it is imperative to conduct external pilot trials in which the feasibility of implementing full-scale studies can be effectively assessed (Gardner et al. 2003; Lancaster et al. 2004). Pilot studies, however, are relevant to best practice in research, but their potential and usefulness in nutrition intervention trials appears to be underutilised (Van Teijlingen et al. 2001; Lancaster et al. 2004). Most of the pilot studies reported in the literature have focused mainly on reporting the results of the pilot trials in terms of outcomes (Colangelo et al. 2006; Williams et al. 2007; Kalman et al. 2009) and not on the implementation process *per se* (Van Teijlingen et al. 2001; Kong et al. 2009) which may also provide valuable insights on the design and operational aspects, as well as readiness for implementing full-scale randomised controlled trials (Gardner et al. 2003; Lancaster et al. 2004).

In this present pilot trial, the process evaluation approach was employed to help assess the possibility of implementing the intervention in preschool settings, under normal field conditions and program constraints. The motivation for using this approach was based on its successful usage in assessing the feasibility of other pilot (Kong et al. 2009), small (Toroyan et al. 2004; Robert et al. 2006) and large (Loechl et al. 2009) scale nutrition intervention trials. Therefore, the aim of this pilot study was to assess the feasibility of implementing a PoU micronutrient fortification trial. Selected process indicators (namely: reach, recruitment, dose delivered, dose received, context and fidelity), as well as indicators of early childhood physical and cognitive development (Hb concentration, anthropometric indices and cognitive function) were used to assess the intervention process and outcome. These were interpreted within the context of a pilot study.

Subjects and methods

Study site

This pilot study was conducted in the North West Province, South Africa, in eight privately owned preschools serving a low socio-economic community where young children represent the most vulnerable population group at risk of micronutrient deficiencies. The eight schools had a combined population of about 408 children aged 36 to 80 months. Although the schools usually provide the children with breakfast and lunch, preliminary analysis of crèche menus showed that the school meals were predominantly based on maize, with low content of vegetables or animal products. During meetings at the schools, the aims and procedure of the study were explained to the parents or legal guardians (caregivers), who subsequently signed the informed consent forms before the inclusion of their children in the study; each

caregiver had the option to withdraw his/her child at any stage of the study. The preschool proprietors and principals also gave permission for the study to be undertaken at their schools.

Pilot study design and intervention

The pilot study design was a randomised, parallel-controlled, single-blind intervention (the groups and schools were blinded) of 52 school days duration (11 weeks; 5d/week). One hundred and fifty one preschool children aged between 36 and 79 months were randomly allocated to either the intervention or control group within each of the preschools. The intervention group (n = 76) received stiff maize-meal porridge with added micronutrient powder (~8 g) (containing amylase-rich light malted barley flour) while those in the control group (n = 75) received soft maize-meal porridge with added placebo powder (~8 g) containing only maize maltodextrin. Both powders were obtained from DSM Nutritional Products South Africa (Pty) Ltd. Between 6 to 21 kg (depending on the school population) of raw maize-meal flour was provided to the preschools per week to ensure that all children receive standard portion sizes of porridge. This was based on estimation of a daily breakfast consumption of at least 28 g raw maize-meal flour for children consuming the soft porridge and 35 g raw maize-meal flour for children consuming the stiff porridge.

The porridge for the intervention group had to be stiff before the addition of the amylase-rich micronutrient powder. Although the hydrolytic effect of the enzyme on maize starch gel structure makes the stiff porridge less viscous, but still culturally acceptable. By design, it was expected that the children in the intervention group would be able to consume more of the porridge without the diluting effect of adding water to change its texture. All participating children received antihelminthic treatment (500 mg mebendazole) before the start of the intervention. The separate breakfast meals containing two types of porridge were prepared daily with the help of the dedicated study assistants. The assistants were responsible for preparing and cooking the food ingredients according to standard recipes. The maize porridge breakfast meals were then served in colour-coded plastic plates according to the different groups between 09H00 and 09H30.

The daily portion sizes of the maize-meal porridge corresponded to the group allocation. Compliance (used as an indirect measure of acceptability in this present study) was monitored on a daily basis using colour coded record sheets with a different colour for each of the two groups. When the child received his/her porridge on a specific day, they were ticked off against his/her name for that day by the study assistant. The assistants also

checked if the full serving of porridge was consumed, if a child did not eat the porridge, a reason had to be given and recorded on the compliance form. A new form was used every week and the investigators checked these forms on a weekly basis.

Selected indicators of early childhood development as indicated by Behrman et al. (2007) were assessed. These included: (1) physical development indicators: assessed using Hb concentration and several anthropometric indices (weight, height, mid-upper arm circumference (MUAC), triceps-skinfold thickness (TSF), height-for-age z score, weight-for-age z score and body mass index z-score) and (2) cognitive development indicators: assessed using the Kaufman Assessment Battery for Children version 2 (KABC-II); these indicators were evaluated at baseline and end of the pilot trial. Additionally, socio-demographic data as well as dietary intakes by 24h recall were measured for all children from each group. The trial was approved by the Ethics Committee of the North-West University, South Africa. The study period was from September to November, 2008.

Process evaluation assessment

The development of the process evaluation methodology for the pilot trial began by creating a model as described by Rossi et al. (2004), illustrating the expected implementation pathway leading to the anticipated outcomes (as assessed using selected indicators of early childhood development) as shown in Figure 1.

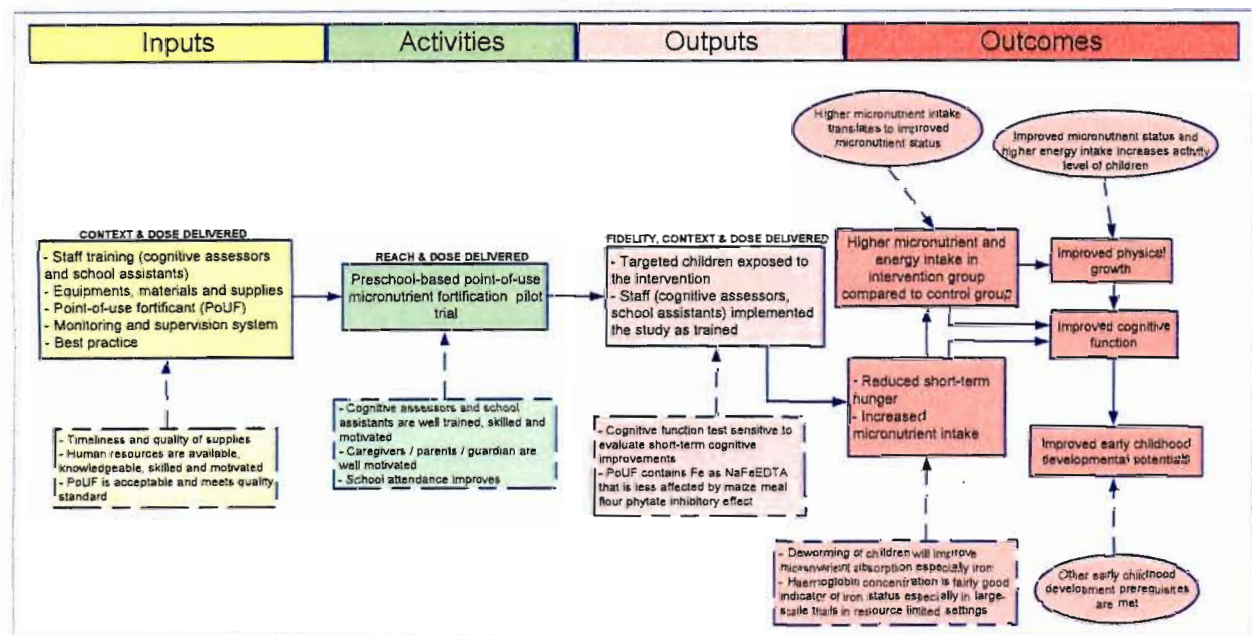


Figure 1 Model of the implementation pathway of the pilot trial including indicators of process evaluation (in capital). Theoretical (circles) and trial (dashed boxes) assumptions are shown.

The various process evaluation indicators were then linked to important points of the model which included: 'recruitment', procedure used to approach and attract participants or target audience; 'reach', the number of target group that participates in the intervention; 'dose delivered', the number of units of each intervention or component delivered or provided by the interventionists; 'dose received', the extent to which target audience actively engages in and receives intervention activities; 'context', aspects of the environment that may influence intervention implementation or study outcomes (includes contamination or the extent to which the control group was exposed to the intervention), 'fidelity', the quality of intervention delivery and extent to which intervention was implemented as planned (Linnan & Steckler, 2002). Data for the process indicators as shown in Table 1 were obtained from questionnaires, semi-structured observations, record reviews, interviews and investigator or study assistances' field-notes.

Recruitment and screening of at-risk children

Eight (8) preschools were selected based on preliminary menu analysis of poor dietary intakes with respect to the school meals, which were below the estimated average requirement (EAR) for preschool children 36 to 79 months. All the children aged >36 months at the preschools were invited to participate and only children with signed informed consent forms were screened for eligibility. Out of the initial pool of 408 children attending the preschools, a total of 279 consenting children were screened for low Hb concentrations and those with $Hb \leq 12.5$ g/dL ($n = 151$) were selected participate in the pilot study. The only exclusion criteria were major chronic illnesses and recent consumption of micronutrient supplements. The sample size was calculated on the basis of the primary hypothesis that the children consuming the highly digestible, micronutrient-dense maize-meal breakfast porridge would have higher total energy and micronutrient intakes, and that the intervention group would, therefore, have better physical growth and micronutrient status as well as improved cognitive function than the control group. Therefore, the sample size was estimated at 54 children per treatment group to enable detection of clinically relevant difference of 7 points and standard deviation (SD) of 13 for the global cognitive function indices (mental processing index and non-verbal index) of the KABC-II (Kaufman et al. 2005; Fletcher-Janzen & Daniel, 2006), assuming a significance level of 0.05 and a power of 80%. The sample size was increased to 75 per group, in anticipation of a dropout rate of $\approx 25\%$.

Table 1 Process evaluation indicators and outputs of the preschool-based pilot trial

Process indicators	Process outputs
Recruitment (procedure used to approach and attract participants or target audience)	- Ethics approval was obtained from the university as well as permission from the school principals. - Letter of support from the project co-ordinator was delivered to the key stakeholders (school principal, parents, guardian and care-givers). - Meetings with parents and caregiver explaining the study procedure and important trial concepts in the local language (<i>Setswana</i>), informed consent form in both English and <i>Setswana</i>. - Twelve cognitive assessors were recruited and adequately trained by a psychologist for 2 weeks; 8 were finally selected (66.67%).
Reach (the number of target group that participates in the intervention)	- Out of a total population of 408 children 36-80 months in the 8 preschools, only 279 (68%) children with signed informed consent were screened. - A total of 151 (54.12%) children at-risk of micronutrient deficiencies as assessed by their haemoglobin status $\leq 12.5\text{g/dL}$ were selected to participate in the pilot trial, however, 143 (94.70%) completed the study, only 8 (5.30%) dropped out before the end of the study. - A total of 35 applicants were screened for eligibility for the cognitive assessment, 8 cognitive assessors were finally selected - A total of 35 applicants were screened for eligibility as a school assistant, 18 School assistants were finally selected.
Dose delivered (the number of units of each intervention or component delivered or provided by the interventionists)	- Feeding of at-risk children was in operation 5 days per week for 11 weeks (100%). - 8 cognitive assessors were recruited and adequately trained by an experienced psychologist. - 18 front-line school assistants were recruited and trained to assist in cooking and in the implementation of the point-of-use fortification (100%).
Dose received (the extent to which target audience actively engages in and receives intervention activities)	- Recruited children consumed breakfast porridge with added micronutrient or placebo powder 5 days per week for 11 weeks (Intervention group: 79.7% compliance, Control group: 83.0% compliance). Overall compliance in all the children combined is 81.3%. - Adequately trained cognitive assessors accurately evaluated the cognitive functions of children in the local language (<i>Setswana</i>) before and after trial implementation (100%). - Cognitive assessors and school assistants implemented study protocol as instructed during the training process.
Contexts (aspects of the environment that may influence intervention implementation or study outcomes)	- Participation limited only to those living and attending the selected preschools which may have affected our population pool. - HIV/AIDS research stigmatization due to blood drawing. - School principals, parents and care-givers were more comfortable in communicating in the local language. Requiring translation from bilingual members 2 of the research team to explain and translate responses during the meetings.
Fidelity (the quality of intervention delivery)	- Point-of-use micronutrient fortification can be successfully implemented among at-risk African children in preschool settings.

Capacity development of front-line staff

Recruitment and training of cognitive assessors: Using the school principals as the main contact point, eligible front-line staff were contacted, interviewed and recruited from the community, based on a set of stringent criteria such as availability throughout the training and study periods, ability to communicate in the local language and minimum Grade 12 senior certificate holders.

A total of 12 cognitive assessors selected from an initial pool of 38 applicants from the community were comprehensively and adequately trained by an experienced psychologist (J.D. Kvalsvig) using a training manual specifically developed for this purpose. The duration of the training was 2 weeks. Components of the training included best practice, as well as how to administer the selected battery of cognitive tests using KABC-II to assess cognitive function of preschool-age children. As an integral part of the training process, the inter-assessor reliability of the trainees was assessed in a three day field trial among children aged 36 to 60 months in a preschool that was not part of the study. Finally, eight cognitive assessors with acceptable intraclass correlation co-efficient (ICC) values >0.60 (Cicchetti, 1994) were selected to administer the cognitive tests in the main pilot trial as shown in Figure 2.

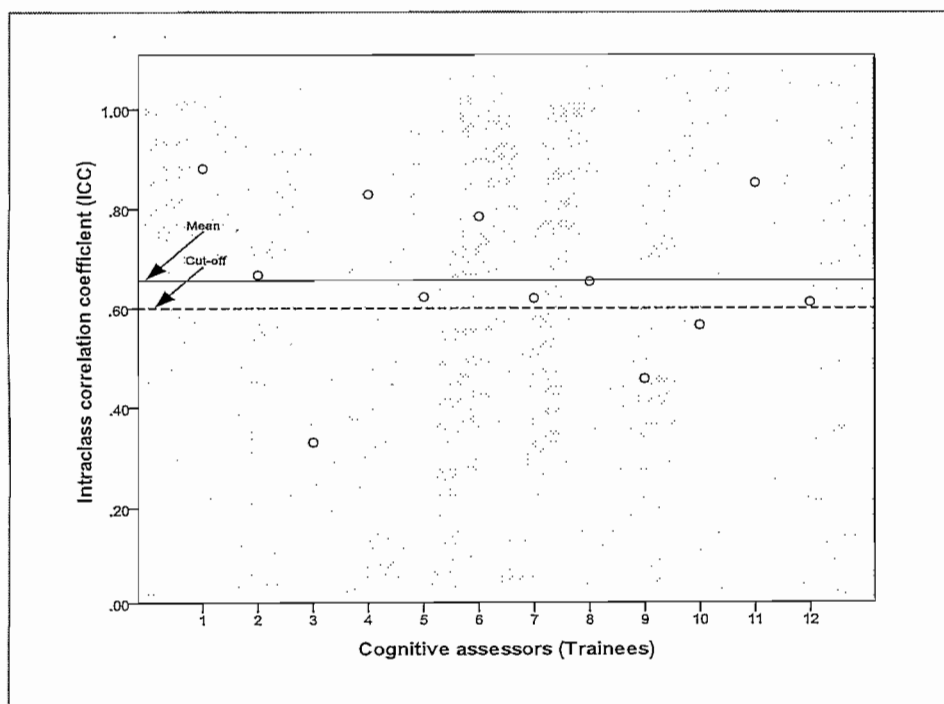


Figure 2 Scatter plot showing the inter-assessor reliability from a three day field trial.

Recruitment and training of study assistants: A comprehensive three day training program was organised to explain in detail what was expected of the 18 study assistants (2 assistants per preschool). A training manual was developed and demonstration on how to administer the PoU fortificant was conducted in the experimental kitchen of the Centre of Excellence for Nutrition, North-West University (Potchefstroom Campus), South Africa. Issues such as hygiene, best practice and maize-meal porridge standard recipe preparation as well as cooking were the focal points of the training. Field demonstrations were also conducted using two preschools selected to be part of the trial, one with worst and another with best cooking facilities and infrastructure, to see how well the study assistants could adapt their initial training to the real field situation.

Haemoglobin concentration measurements

Finger-prick blood samples were used to measure Hb concentrations in the field by use of the HemoCue system (HemoCue Angelholm, Sweden) at baseline and after 11 weeks of intervention.

Dietary intake assessment

Dietary intakes were assessed with an interactive 24-hour recall performed for all children from each of the intervention and control groups at three different time-points; this corresponded to once every four weeks during the study period. The 24-hour recall was completed for each child during a personal interview with the parents or care-giver of the child during home visits and with school cooks during school visits. Food portion sizes were estimated using a validated food photo book (Venter et al. 2000). The 24-hour recalls were conducted by previously trained and experienced fieldworkers in the local language.

Socio-demographic assessment

Structured questionnaires were used to obtain the household resources information from the parent or care-giver during a personal interview at the home. Household resources include, for example, structure of the house, household size, availability of electricity and tap water, energy used for food preparation and type of toilet. Interviews were conducted in the local language by previously trained field workers who were familiar with the community.

Anthropometric assessment

Anthropometric assessment were performed at baseline and at the end of the study according to standard procedures described by the International Society for the Advancement of Kinanthropometry (ISAK, 2001). The following measurements were taken in duplicate using calibrated equipment and standardised techniques with the children wearing light clothing and no shoes: standing height, weight, (MUAC) and (TSF). Each measurement was taken by the same trained anthropometrist (level 2) to eliminate inter-tester variation. Height was measured to the nearest 0.1 cm using a calibrated portable stadiometer; weight was measured to the nearest 0.01 kg on a portable electronic scale (Masskot, UC-300 Precision Health Scale; A&D Co Ltd, Tokyo, Japan). MUAC was measured to the nearest 0.1 cm with a Lufkin steel measuring tape (Cooper Tools, Apex, NC, USA) and TSF to the nearest 0.1 mm with Harpenden skin fold calliper (Baty International, West Sussex, UK). The electronic scale and stadiometer were calibrated before measurements using a calibration weight and steel tape, respectively.

Cognitive function assessment

A battery of cognitive tests was selected to assess learning abilities (or long-term storage and retrieval), sequential processing (or short-term memory) and simultaneous processing (or visual processing) from the KABC-II (Kaufman et al. 2005). The KABC-II has been designed and is appropriate for this age and culture (multilingual and multicultural setting) group. The test procedures were translated into the local language of the community, pre-tested, standardised and then tested again in children in a preschool not involved in the study. All assessors were blinded to the group allocation.

Learning scale: The learning scale on the KABC-II assesses learning abilities (or long-term storage and retrieval). The two subtests used in this study were the Atlantis and Atlantis delayed tests (Kaufman et al. 2005). The Atlantis test measures the ability to learn new information, specifically associations between pictures and nonsense names. The child is taught names of pictures of imaginary fish, plants, and shells. Then the assessor says a name and the child points to the correct picture. Atlantis delayed test measures long-term storage and retrieval of information learned earlier in the testing session. About 20 minutes after the end of the Atlantis, and without being forewarned, the child is asked to point to pictures whose names were taught earlier.

Sequential scale: The sequential scale on the KABC-II assesses sequential processing (or short-term memory) (Kaufman et al. 2005). The subtest used in this study was the hand movement test. The hand movement test measures sequential processing and short-term memory within the visual-motor modality. The assessor makes a series of hand movements, and the child repeats them in the same sequence.

Simultaneous scale: The simultaneous scale on the KABC-II assesses simultaneous processing (or visual processing) (Kaufman et al. 2005). The subtest used in this study was conceptual thinking test. The conceptual thinking test is a nonverbal measure of reasoning in which the child demonstrate classification ability; the child looks at a set of four or five pictures and points to the one that does not belong with others.

Mental processing index: The mental processing index (MPI) is a global measure of intellectual functioning which correlates well with other Intelligence Quotient (IQ) measures (Kaufman et al. 2005). It measures general mental processing ability on the KABC-II from the Luria neuropsychological perspectives and excludes measures of acquired knowledge. The MPI is the combination of the learning, simultaneous and sequential scales into a single global cognitive function index.

Nonverbal Index: The nonverbal index (NVI) is also a global (not theory based) measure of intellectual functioning and correlates well with other IQ measures (Kaufman et al. 2005). The NVI is the combination of the simultaneous and sequential scales into a single global cognitive function index.

Data interpretation

Data were initially entered and managed using Microsoft Office Access (2008; Microsoft Corporation, Redmond, WA). Data processing and statistical analysis were performed with Microsoft Office Excel (2008; Microsoft Corporation, Redmond, WA) and SPSS software (version 17.0, 2008; SPSS Inc, Chicago, IL). For the anthropometric calculations, the WHOAnthro Version 2 and WHOAnthroplus were used (WHO, 2007; WHO, 2009). The analyses of the cognitive function test scores were done using KABC-II Assist software (Kaufman et al. 2005). The dietary data were analysed, using the Medical Research Council (MRC) Food Finder software (Food Finder, version 3, MRC, Tygerberg, South Africa). Inter-assessor reliabilities were analysed using a two-way mixed model ICC. For the intervention

outcome data, normality for continuous variables was explored visually (histogram and Q-Q plots) and numerically (Kolmogorov-Smirnov and Shapiro-Wilk tests). The multiple imputation procedure was followed for missing data (Kenward & Carpenter, 2007). The within group differences were tested using a one-way analysis of variance (ANOVA). For inference on the intervention effects, analysis of covariance (ANCOVA) was done on the change from baseline to end after 11 weeks of intervention. Statistical significance was set at $p < 0.05$. In order to determine whether statistically significant intervention effects are likely to be relevant in practice, effect sizes were determined. The effect size is an objective measure of the likelihood of a difference having a practical or clinical significance and is independent of the sample size. Effect sizes (Cohen's d-value) were calculated according to the following formula: $d = |X_i - X_j|$ divided by $\sqrt{MSE}$; where $|X_i - X_j|$ is the absolute difference between the means of change from baseline in the intervention minus control groups and MSE is the mean square error of the ANCOVA (Cohen, 1988; Ellis & Steyn, 2003). The likelihood of statistically significant differences being practically relevant, is reported as effect size (d) and can be interpreted as follows; $d = 0.2$ is a small likelihood, $d = 0.5$ is a medium likelihood and $d = 0.8$ is a large likelihood (Cohen, 1988).

RESULTS

The implementation process pathway of the present study is laid out in the trial model as illustrated in Figure 1, which contributed to the interpretation of the process outcomes as summarised in **Table 1**. There were a total of 52 feeding days over the 11 weeks study period, excluding a 5 day school holiday. The recruited children consumed breakfast porridge with either added micronutrient or placebo powders during the 52 feeding days. The comparison of the dietary intakes in the intervention and control groups during the study period as well as the contribution of the PoU fortificant to the EAR for children 12 to 96 months are shown in **Table 2**. Of the 151 children that were initially recruited to take part in the study, 143 children completed the trial (5 [6.6%] and 3 [4.0%] children were lost to follow-up in the intervention and control groups, respectively). Additionally, 12 children that completed follow-up were excluded from the final analyses because of recurrent absenteeism (<60% compliance to study regimen) during the pilot study (**Figure 3**).

For the 131 children that were included in the final analysis ($\geq 60\%$ compliance to the study regimen), compliance with feeding was $84.42 \pm 10.18\%$ and $85.18 \pm 10.18\%$ in the intervention and control groups, respectively. As shown in **Tables 3** and **4**, the baseline characteristics of the children included in the final analysis (**Figure 3**) revealed that there

were no clinically important differences in Hb concentration, anthropometric or socio-demographic variables. At baseline, 15.9% versus 19.1% were stunted in the intervention versus control groups respectively, while the proportions of underweight children were small and similar in both groups (7.9% versus 10.3%). The mean Hb concentration (11.55 ± 0.85 g/dL versus 11.55 ± 0.79 g/dL), mean body mass index z-score (0.20 ± 0.82 versus 0.13 ± 0.82), MUAC (15.92 ± 1.20 cm versus 15.97 ± 1.15 cm) and mean TSF (8.65 ± 2.28 mm versus 8.63 ± 2.09 mm) were similar in both groups and within normal range in the intervention versus the control groups, respectively.

The socio-demographic data showed that, the majority of the children spoke *Setswana* (78.7% versus 72.9%, equal proportion in the intervention and control groups) as their first language; almost all had access to tap water (100% versus 97.8%), flush toilet (68.1% versus 66.73%), access to electricity in the house (97.8% versus 95.8%), with electricity as the main cooking fuel or heating energy source (74.0% versus 59.2%) (**Table 4**). At baseline, the cognitive function test scores for most of the variables were not significantly different as shown in **Table 3**. However, despite randomisation, there were trends towards significant differences between the study groups at baseline on the NVI ($p = 0.103$) and sequential processing (hand movement) ($p = 0.063$). To adjust for baseline differences between groups, the test data were analysed using ANCOVA with relevant baseline values prior to intervention as covariates.

There were significant increases in Hb concentration ($p < 0.05$) from baseline to end in both the intervention (mean change: 0.38 g/dL [95% CI: 0.14, 0.61 g/d]) versus control (mean change: 0.57 g/dL [95% CI: 0.35, 0.80 g/d]) groups. As shown in **Table 5**, the intervention effects on Hb concentration as a result of the treatment were not significantly different ($p = 0.250$) between the study groups. Furthermore, the micronutrient intervention, did not improve any of the anthropometric indices or variables (**Table 5**). For the cognitive function tests, the 11 weeks PoU micronutrient fortification resulted in significant improvements for simultaneous processing (conceptual thinking) (intervention effect: 1.02 [95%CI: 0.28, 1.77]; $p = 0.008$, effect size: 0.480) and NVI (intervention effects: 7.20 [95% CI: 2.60, 11.81]; $p = 0.002$, effect size: 0.55) while there was a marginal significant improvement for MPI (intervention effects: 2.73 [95% CI: -0.25, 5.70]; $p = 0.072$, effect size: 0.36). For the other cognitive function tests (learning abilities [atlantis, atlantis delayed] and sequential

processing [hand movement]) there were no significant micronutrient intervention effects in the intervention compared to control groups as shown in **Table 6**.

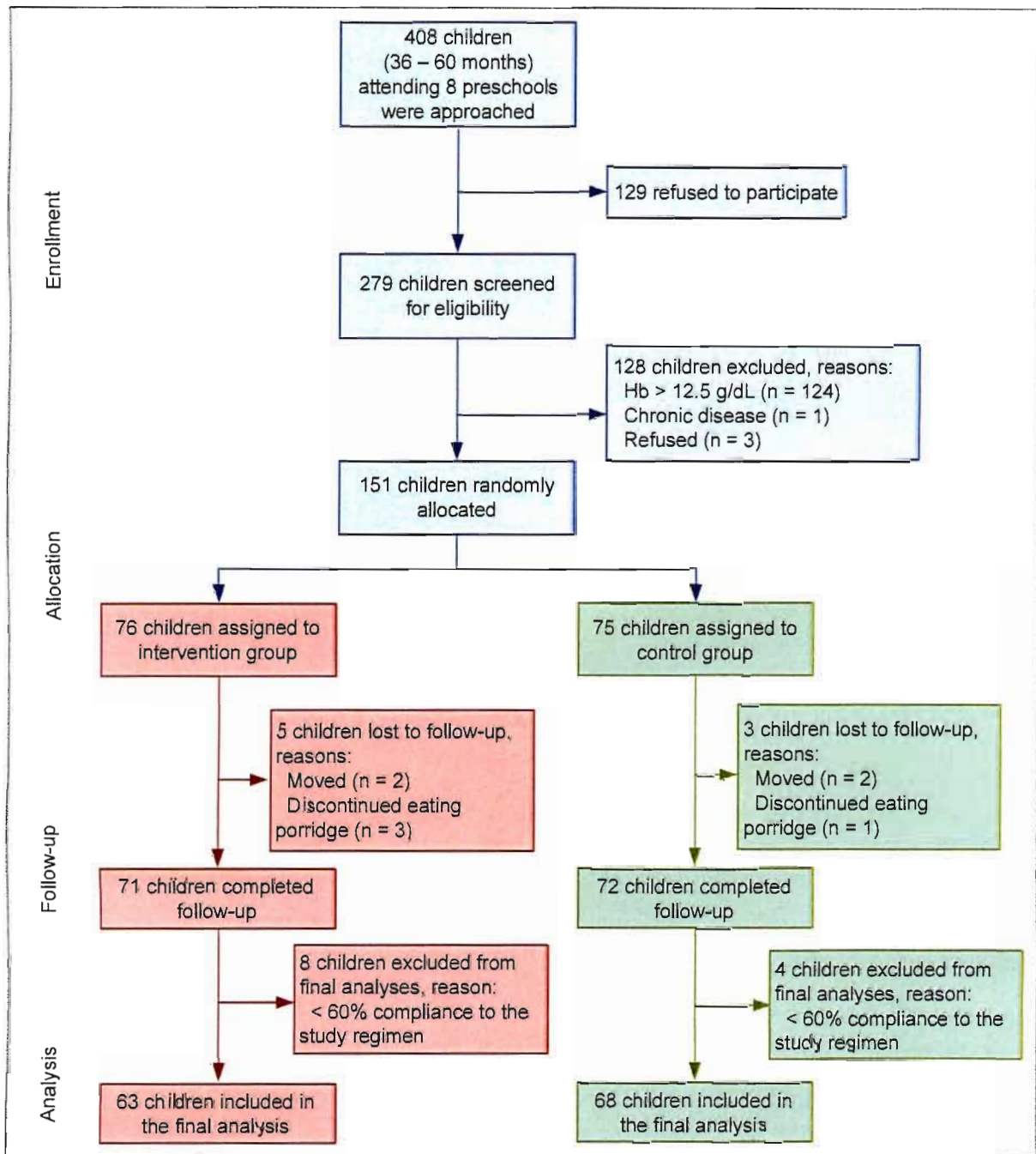


Figure 3 Pilot study participant flow chart according to the Consolidated Standards of Reporting Trials (CONSORT) (Moher et al. 2001).

Table 2 Dietary intake comparison between intervention and control groups during the pilot trial¹

Nutrient	Intervention group		Control group		Point-of-use fortificant ²		
	Percentile		Percentile		Nutritional composition	EER ³ / EAR ⁴ / AI ⁵	EER ³ / EAR ⁴ / AI ⁵
	Median	(25, 75)	Median	(25, 75)		3 y	4 - 6 y
Energy intake (kJ / d)	5746	(4829, 6377)	5413	(4519, 6249)	—	5561 ^{3,b} 5221 ^{3,g}	5838 - 6447 ^{3,b} 5502 - 6094 ^{3,g}
Total fat (g)	34.2	(25.2, 39.1)	30.7	(23.2, 40.4)	—	—	—
Carbohydrate (g)	201	(178, 234)	207	(170, 229)	—	—	—
Iron (mg)	1.35	(0.76, 1.90)	1.63	(0.86, 2.51)	2.86 ⁵	3.00 ⁴	4.10 ⁴
Heme iron (mg)	0.20	(0.05, 0.20)	0.21	(0.07, 0.48)	—	—	—
Non-heme iron (mg)	1.15	(0.71, 1.70)	1.42	(0.79, 2.03)	—	—	—
Zinc (mg)	4.61	(3.86, 6.02)	4.72	(3.64, 6.09)	2.86	2.20 ⁴	4.00 ⁴
Iodine (µg)	14.4	(9.69, 22.2)	16.43	(8.47, 24.09)	34.3	65.0 ⁴	65.0 ⁴
Calcium (mg)	240	(173, 363)	307	(187, 405)	457	500 ⁵	800 ⁵
Vitamin A (RE)	147	(86.8, 218)	150	(92.1, 219)	457	210 ⁴	275 ⁴
Vitamin C (mg)	16.4	(11.8, 30.6)	15.8	(9.09, 32.5)	68.6	13.0 ⁴	22.0 ⁴
Vitamin E (mg)	3.56	(2.45, 4.94)	3.18	(2.71, 5.15)	5.71	5.00 ⁴	6.00 ⁴
Vitamin B12 (µg)	1.42	(0.75, 2.19)	1.36	(0.94, 2.64)	1.03	0.70 ⁴	1.00 ⁴
Thiamine (mg)	0.81	(0.66, 0.96)	0.77	(0.68, 1.00)	0.57	0.40 ⁴	0.60 ⁴
Niacin (mg)	9.65	(6.90, 12.75)	8.04	(6.60, 12.23)	6.86	5.00 ⁴	6.00 ⁴
Riboflavin (mg)	0.69	(0.56, 1.26)	0.89	(0.56, 1.43)	0.57	0.40 ⁴	0.50 ⁴
Folate (µg)	104	(74.03, 130)	94.0	(77.1, 144.8)	103	120 ⁴	160 ⁴
	Mean	95% CI	Mean	95% CI			
Total protein (g)	40.9	(37.4, 44.4)	40.9	(37.1, 44.8)	—	13.0 ⁵	19.0 ⁵
Vitamin B6 (mg)	0.99	(0.87, 1.12)	0.91	(0.81, 1.02)	0.57	0.40 ⁴	0.50 ⁴

¹ Available for children that completed the intervention.² Daily micronutrient contribution of ~8.0 g point-of-use fortificant (containing ~ 1.0 g amylase-rich light malted barley) to the intervention group.³ EER = Estimated energy requirement by gender and age, low active physical activity level.⁴ EAR = Estimated average requirement by gender and age.⁵ AI = Adequate intake.⁶ Iron from highly bioavailable sodium iron ethylenediaminetetra-acetate (NaFeEDTA).^b Estimated energy requirement for boys.^g Estimated energy requirement for girls.

Table 3 Baseline anthropometric and cognitive function characteristics of the intervention and control groups that completed the pilot trial¹

Characteristics	Intervention group		Control group	
Compliance category	≥ 60%	< 60%	≥ 60%	< 60%
n	63	8	68	4
<i>Compliance with feeding (%)</i> ^{2,3}	84.44 ± 10.18	42.23 ± 9.15	85.18 ± 10.01	45.11 ± 10.58
Selected early childhood developmental indicators				
<i>Haemoglobin concentration (g/dL)</i> ³	11.55 ± 0.85	11.72 ± 0.94	11.51 ± 0.79	11.75 ± 0.81
Anthropometric indices				
Weight (kg) ³	16.00 ± 2.57	15.97 ± 1.36	16.22 ± 2.81	16.40 ± 1.89
Height (cm) ³	101.2 ± 7.14	104.2 ± 6.32	102.1 ± 7.60	104.9 ± 6.54
Height for age (Z score) ^{3,4}	-1.09 ± 1.17	-0.75 ± 1.20	-1.19 ± 1.15	-1.05 ± 0.59
Weight for age (Z score) ^{3,4}	-0.57 ± 0.95	-0.62 ± 1.01	-0.68 ± 0.95	-0.80 ± 0.58
Body mass index (Z score) ^{3,4}	0.20 ± 0.82	-0.19 ± 0.64	0.13 ± 0.82	-0.25 ± 1.24
Stunting [n (%)]	10.0 (15.9)	1.00 (12.5)	13 (19.1)	0.0 (0.0)
Underweight [n (%)]	5.00 (7.9)	0.0 (0.0)	7.0 (10.3)	0.0 (0.0)
Mid upper-arm circumference (cm) ³	15.92 ± 1.20	15.62 ± 1.11	15.97 ± 1.15	16.08 ± 1.72
Triceps-skinfold thickness (mm) ³	8.65 ± 2.28	7.80 ± 2.03	8.63 ± 2.09	7.80 ± 2.06
Cognitive function tests⁵				
Learning Abilities				
Atlantis ⁶	7.14 ± 2.70	8.17 ± 1.74	7.32 ± 2.55	6.75 ± 2.22
Atlantis delayed ⁷	8.52 ± 1.76	8.12 ± 2.95	8.13 ± 1.98	7.50 ± 2.12
Simultaneous processing				
Conceptual thinking ⁸	7.25 ± 2.65	7.34 ± 2.38	6.91 ± 2.80	4.25 ± 2.99
Sequential processing				
Hand movement ⁹	8.79 ± 2.37	9.33 ± 2.18	8.03 ± 2.26	8.75 ± 1.50
Mental processing index ¹⁰	81.14 ± 8.99	83.06 ± 8.47	82.08 ± 10.52	79.50 ± 7.72
Nonverbal index ¹¹	86.27 ± 15.73	88.69 ± 14.98	81.88 ± 14.86	75.80 ± 11.35

¹ Children that completed the trial categorised into < 60% and ≥ 60% compliance categories.

² The actual amount of the point-of use fortificant ingested, expressed as a percentage of the total amount of fortificant provided during the 52 d of the pilot trial.

³ Mean ± Standard deviation.

⁴ Determined by using the World Health Organization Child Growth Standards (WHO, 2007; WHO, 2009).

⁵ Measure of cognitive function assessed using the Kaufman Assessment Battery for children, Second Edition.

⁶ Atlantis measured on a scale ranging from 1 to 19.

⁷ Atlantis delayed measure on a scale ranging from 2 to 16.

⁸ Conceptual thinking measured on a scale ranging from 2 to 19.

⁹ Hand movement measured on a scale ranging from 2 to 23.

¹⁰ Mental processing index measured on a scale from 40 to 160.

¹¹ Nonverbal index measured on a scale ranging from 40 to 160.

Table 4 Baseline socio-demographic characteristics of the intervention and control groups that completed the pilot trial¹

Characteristics	Intervention group		Control group	
	≥ 60%	< 60%	≥ 60%	< 60%
Compliance category				
Selected socio-demographic characteristic				
Age (months) ²	54.2 ± 11.0	56.2 ± 15.0	56.5 ± 10.8	60.8 ± 10.2
Boys : Girls (%) ³	50.8 : 49.2	50.0 : 50.0	51.5 : 48.5	25.0 : 75.0
Home language				
Setswana ⁴	37 (78.7)	3 (37.5)	35 (72.9)	0 (0.0)
Sesotho	9 (19.1)	0 (0.0)	9 (14.6)	1 (25.0)
Xhosa	1 (2.1)	0 (0.0)	5 (10.4)	0 (0.0)
Afrikaans	0 (0.0)	0 (0.0)	1 (2.1)	0 (0.0)
Type of dwelling				
Brick, concrete	14 (29.8)	0 (0.0)	14 (29.2)	0 (0.0)
Tin	24 (51.1)	2 (25.0)	24 (50.0)	0 (0.0)
Brick, concrete and tin combined	9 (19.1)	1 (12.5)	10 (20.8)	1 (25.0)
No of people in household				
< 4 persons	12 (25.5)	0 (0.0)	9 (18.8)	1 (25.0)
4 – 6 persons	23 (48.9)	0 (0.0)	31 (64.6)	2 (50.0)
> 7 persons	11 (23.4)	1 (25.0)	7 (14.6)	0 (0.0)
Do not know	1 (2.1)	0 (0.0)	1 (2.1)	0 (0.0)
Source of drinking water				
Own tap	47 (100)	3 (37.5)	47 (97.9)	1 (25)
Others	0 (0.0)	0 (0.0)	1 (2.1)	0 (0)
Type of toilet				
Flush	32 (68.1)	2 (25.0)	32 (66.7)	2 (25)
Pit	15 (31.9)	0 (0.0)	16 (33)	0 (0)
Assess to electricity in the house				
Yes	46 (97.9)	3 (37.5)	46 (95.8)	1 (25)
No	1 (2.1)	0 (0.0)	2 (4.2)	0 (0)
Cooking fuel and heating energy source				
Electricity	35 (74.5)	2 (25.0)	28 (58.3)	1 (25)
Others: gas / paraffin / wood	12 (25.5)	1 (12.5)	20 (41.7)	0 (0)
Working refrigerator				
Yes	39 (83.0)	2 (25.0)	41 (85.4)	1 (12.5)
No	8 (17.0)	0 (0.0)	7 (14.6)	0 (0)
Working microwave				
Yes	23 (48.9)	2 (25.0)	24 (50.0)	1 (25)
No	24 (51.1)	1 (12.5)	24 (50.0)	0 (0)
Working washing machine				
Yes	22 (46.8)	2 (25.0)	21 (43.8)	1 (25)
No	25 (53.2)	1 (12.5)	27 (56.3)	0 (0)

Table 4 (Continued) Baseline socio-demographic characteristics of the intervention and control groups that completed the pilot trial¹

Characteristics Compliance category	Intervention group		Control group	
	≥ 60%	< 60%	≥ 60%	< 60%
Selected socio-demographic characteristics				
<i>Working television</i>				
Yes	41 (87.2)	2 (25)	42 (87.5)	1 (25)
No	6 (12.8)	1 (12.5)	6 (12.5)	0 (0)
<i>Working radio</i>				
Yes	39 (83.0)	3 (37.5)	41 (85.4)	1 (25)
No	8 (17.0)	0 (0)	7 (14.6)	0 (0)

¹ Children that completed the trial categorised into < 60% and ≥ 60% compliance categories, data available from 50 children randomly selected from each group.

² Mean ± Standard deviation.

³ Percentage (%)

⁴ [n (%)] (all such values).

Table 5 Effect of 11 weeks point-of-use micronutrient fortification on the haemoglobin concentration and anthropometric indices in the intervention and control groups¹

Variables	n	Intervention group	Control group
		63	68
<i>Haemoglobin concentration (g/dL)</i>			
Change from baseline ²		0.38 (0.14, 0.61)	0.57 (0.35, 0.80)
Estimated intervention effects ³		-0.19 (-0.53, 0.14)	—
p-Value ⁴		0.250	
<i>Anthropometric indices</i>			
<i>Weight (kg)</i>			
Change from baseline ²		0.22 (0.11, 0.33)	0.34 (0.23, 0.45)
Intervention effects ³		-0.12 (-0.27, 0.04)	—
p-Value ⁴		0.135	
<i>Height (cm)</i>			
Change from baseline ²		1.60 (1.47, 1.74)	1.65 (1.51, 1.78)
Intervention effects ³		-0.04 (-0.24, 0.15)	—
p-Value ⁴		0.654	
<i>Height for age (Z score)⁵</i>			
Change from baseline ²		0.08 (0.04, 0.13)	0.11 (0.07, 0.15)
Intervention effects ³		-0.02 (-0.08, 0.04)	—
p-Value ⁴		0.468	
<i>Weight for age (Z score)⁵</i>			
Change from baseline ²		-0.14 (-0.20, -0.07)	-0.09(-0.15,-0.22)
Intervention effects ³		-0.05 (-0.14, 0.04)	—
p-Value ⁴		0.267	
<i>Body mass index (Z score)⁵</i>			
Change from baseline ²		-0.29 (-0.39, -0.19)	-0.23 (-0.33, -0.13)
Intervention effects ³		-0.06 (-0.20, 0.08)	—
p-Value ⁴		0.421	
<i>Mid upper-arm circumference (cm)</i>			
Change from baseline ²		0.13 (-0.01, 0.24)	0.26 (0.15, 0.38)
Intervention effects ³		-0.14 (-0.30, 0.03)	—
p-Value ⁴		0.099	
<i>Triceps-skinfold thickness (mm)</i>			
Change from baseline ²		-0.49 (-0.74, -0.23)	-0.35(-0.59,-0.10)
Intervention effects ³		-0.14 (-0.50, 0.22)	—
p-Value ⁴		0.442	

¹ Intervention effect determined from analysis of covariance

² Expressed as a difference between baseline and end of intervention. Interpretation example: 0.38 represents an increase of 0.38 units from baseline to 11 weeks.

³ Expressed as a difference between the intervention and control groups. Interpretation example: -0.19 represents a 0.19 unit lower biochemical outcome by 11 weeks.

⁴ Statistical difference between intervention and control group: Statistical significance set at $p < 0.05$.

⁵ Determined by using the World Health Organization Child Growth Standards (WHO, 2007; WHO, 2009).

Table 6 Effect of 11 weeks point-of-use micronutrient fortification on the cognitive function in the intervention and control groups: change from baseline and effect size¹

Variables	n	Intervention group	Control group	Effect size ²
		63	68	
Cognitive function tests³				
Learning Abilities				
<i>Atlantis⁴</i>				
Change from baseline ⁵		1.32 (0.76, 1.88)	0.87 (0.33, 1.41)	0.11
Intervention effects ⁶		0.45 (-0.34, 1.23)	—	
p-Value ⁷		0.261		
<i>Atlantis delayed⁸</i>				
Change from baseline ⁵		1.55 (0.66, 2.44)	1.03 (0.25, 1.81)	0.20
Estimated intervention effects ⁶		0.52 (-0.66, 1.70)	—	
p-Value ⁷		0.382		
Simultaneous processing				
<i>Conceptual thinking⁹</i>				
Change from baseline ⁵		0.75 (0.21, 1.28)	-0.28 (-0.79, 0.24)	0.48
Intervention effects ⁶		1.02 (0.28, 1.77)	—	
p-Value ⁷		0.008		
Sequential processing				
<i>Hand movement¹⁰</i>				
Change from baseline ⁵		0.05 (-0.50, 0.60)	-0.20 (-0.73, 0.33)	0.11
Intervention effects ⁶		0.25 (-0.52, 1.01)	—	
p-Value ⁷		0.530		
Mental processing index¹¹				
Change from baseline ⁵		4.14 (1.97, 6.32)	1.42 (-0.60, 3.43)	0.36
Intervention effects ⁶		2.73 (-0.25, 5.70)	—	
p-Value ⁷		0.072		
Nonverbal index¹²				
Change from baseline ⁵		3.40 (0.01, 6.70)	-3.80 (-6.98, -0.63)	0.55
Intervention effects ⁶		7.20 (2.60, 11.81)	—	
p-Value ⁷		0.002		

¹ Intervention effect determined from analysis of covariance adjusted for baseline age and individual baseline values.

² Expressed as Cohen d-value which gives an indication whether the statistically significant intervention effect will likely be relevant in practice: Practical / clinical significance set at 0.2 = small likelihood; 0.50 = medium likelihood; 0.80 = large likelihood.

³ Measure of cognitive function assessed using the Kaufman Assessment Battery for children, Second Edition.

⁴ Atlantis measured on a scale ranging from 1 to 19.

⁵ Expressed as a difference between baseline and end of intervention. Interpretation example: 1.32 represents an increase of 1.32 points from baseline to 11 weeks.

⁶ Difference in change between the intervention group and control group. Illustration example: 0.45 represent a 0.45 points higher cognitive function by 11 weeks.

⁷ Statistical difference between intervention and control group: Statistical significance set at $p < 0.05$.

⁸ Atlantis delayed measure on a scale ranging from 2 to 18.

⁹ Conceptual thinking measured on a scale ranging from 2 to 19.

¹⁰ Hand movement measured on a scale ranging from 2 to 23.

¹¹ Mental processing index measured on a scale from 40 to 160.

¹² Nonverbal index measured on a scale ranging from 40 to 160.

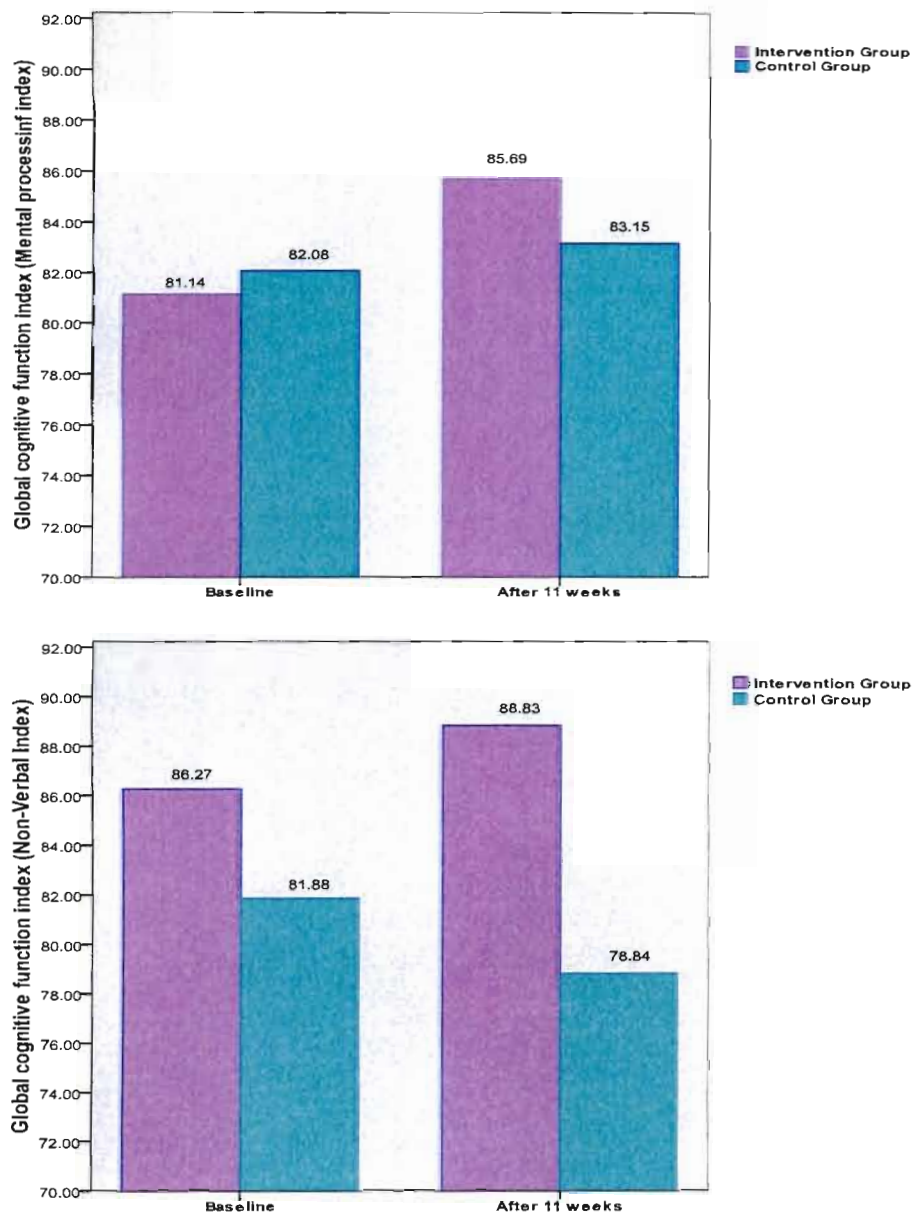


Figure 4 Effect of 11 weeks point-of-use micronutrient fortification on the global cognitive function indices.

DISCUSSION

The present study was designed to assess the feasibility of implementing a preschool-based, PoU micronutrient fortification among African children. To our knowledge, the study is the first to implement the use of micronutrient powders among South African children at-risk of micronutrient deficiencies in preschool settings. Evidently, the trial has been able to demonstrate the value of conducting pilot studies prior to the main trial. The present pilot trial

has identified preschools as a potential setting for the implementation of a PoU micronutrient fortification program as clearly illustrated in **Figure 1**. Additionally, the likelihood of the intervention in improving some early childhood development indicators based on some of the outcome measures (Hb concentration, cognitive function tests) was also demonstrated. However, the outcome of the present study should be considered preliminary and interpreted within the context of a pilot trial and its intended purposes, which is to test the study design, logistics and implementation process as well as reveal limitations or implementation challenges which can be addressed before the main trial (Gardner et al. 2003; Lancaster et al. 2004).

Reach and recruitment

At the inception of the present pilot trial, preschools were identified as the most appropriate avenue to deliver a PoU micronutrient intervention for children >36 months. School-based interventions have the potential to make valuable contributions to both the prevention and treatment of early childhood micronutrient deficiencies (Steyn & Marais 2002). Contact was therefore, initiated with care-givers through the various school principals, however, it was identified early that parents or caregivers' willingness to participate in the study can be greatly influenced by the school principals understanding of the study benefits to the children. Additionally, in recruiting front-line staff (cognitive assessors and study assistants) the school principals also helped initiate contact with the most eligible and reliable individuals within the community. Considerable effort was centred towards the training and retaining of all the selected cognitive assessors. The success of the training was judged based on the mean inter-tester reliability of the 12 cognitive assessors as shown in Figure 2, the final selection was based on the number of assessors above the ICC cut-off point (0.6) (Cicchetti, 1994). Two cognitive assessors out of nine that were selected disengaged from the study either because of a new job or personal reasons; but not as a result of their involvement in the study. Because it was envisaged that the front-line staff (cognitive assessors and the study assistants) should be kept motivated throughout the duration of the study, a monthly stipend was provided to augment their transportation costs. Other incentives were also provided as motivational factors which were feasible and sustainable such as provision of special colour bags and files.

Dose delivered and received

The delivery of the PoU fortificant during the intervention period was ensured, and compliance was actively promoted and monitored according to best practice but not strictly ensured (Victora *et al.*, 2004). This is in contrast to a strict and ideal efficacy trial in which all the children are strictly monitored to consume the test product. This recent study simulated an ideal scenario of what might have happened if a PoU fortificant is implemented when

several other socio-political and environmental factors or activities are ongoing. It is proposed that the study design will better inform intervention trials in which the design is close to that of an effectiveness study (Hurrell, 2007). Typical examples include two recent PoU fortification trials conducted among Chinese preschool-age (Sharieff et al. 2006) and Indian school-age (Andersson et al. 2008) children.

Context and Fidelity

During the recruitment phase, care-givers of the preschool children were not comfortable with blood drawing. The main reason for this could have been that caregivers immediately associated the study with other Human Immunodeficiency Virus (HIV) trials and they do not want their children to be involved in this kind of study because of stigmatization issues: *“the parents are concerned that HIV screening will be done on their children...I think you will need to convince them that your study will not involve HIV assessment” (School principal)*. Due to the sensitive nature of this within the community where this study took place it was, therefore, opted to use a field-friendly or simple finger prick method to measure and collect blood samples for micronutrient status determination. This was implemented after careful explanation of the process and commitment on the part of the investigators that HIV status of the children will not be assessed. Additionally, because of the anticipated potential impacts of language barrier on health care programs (Schenker et al. 2007), two members of our research group familiar with *Setswana* (the local language) acted as the major link with regards to communicating/explaining the aims and objectives of the study to the school principals and care-givers during the recruitment process as well as throughout the duration of the study. Care-givers were also invited to observe the baseline and end measurements at the various preschools.

The lack of significant intervention effect on Hb concentration in the study was surprising. Evidence from other studies within similar age groups showed that the use of multiple micronutrient intervention led to improvement in Hb concentration in some (Sari et al. 2001; Lopriore et al. 2004; Ayoya et al. 2009) but not in all (Stoltzfus et al. 2004; Sharieff et al. 2006; Wegmuller et al. 2006). However, it is very difficult to explain the observed difference in Hb concentration in the experimental and control groups. It is speculated that the abnormal reverse result for the Hb concentrations as shown in **Table 5**, could be attributed to several interlinked factors. First, one cannot rule out the possibility that the more energy dense, micronutrient-rich breakfast porridge provided to the children in the intervention group could have possibly partially replaced the participants' usual meals at home. This phenomenon had been clearly demonstrated by Murphy et al. (2003). In their study they showed convincing evidence that there was a reduction in at-home consumption of children participating in a school-feeding program. Possible reasons could be that foods are redistributed within the

household or that participating children are not hungry and as a result chose to eat less. Secondly, the iron form in the PoU fortificant was highly bioavailable iron (supplying 2.86 mg per day) from sodium iron ethylenediaminetetra-acetate (NaFeEDTA). It is speculated that this iron dose might have been too low to have any significant intervention effect for the period of the intervention in this current study. It is, therefore, envisaged that a slightly higher iron dose for a longer period will probably have a more beneficial effect on Hb concentration for this type of study. A recent study by Van Stuijvenberg et al. (2008) revealed that iron (2.35 mg per day) as NaFeEDTA was not effective in improving Hb concentration or iron status of school-age children after six months' micronutrient intervention using bread as the carrier. Thirdly, as shown in Table II, the PoU fortificant used in the current study contained other nutrients; the negative interactions among these micronutrients might have potentially interfered with the utilization of iron and other nutrients such as zinc (Lind et al. 2003; Kordas & Stoltzfus, 2004) and/or calcium (Hallberg et al. 1991). As a result, their presence might have affected the absorption of iron explaining in part the outcome. Fourthly, the PoU fortificant might have enabled the uptake of iron into the tissue pools not necessarily reflected by the measurement in Hb concentration because Hb levels are known to have a very low specificity and sensitivity when used alone to assess iron status (Wegmuller et al. 2006; Zimmermann et al. 2008). Other measures of iron status such as serum transferrin receptor, erythrocyte zinc protoporphyrin and serum ferritin might have shown a different result. However, all explanations mentioned earlier are possible reasons and speculative as none of them were assessed in this current pilot trial.

Despite all these study limitations, evidence was found that implementing a PoU fortification among African preschool children in preschool settings improved some of the cognitive function test scores after 11 weeks intervention. In the present study, it was observed that there was a 2.73 points ($p = 0.072$, $d = 0.36$) and 7.20 points ($p = 0.002$, $d = 0.55$) improvement in the global cognitive function indices on the MPI and NVI, respectively (**Table 6**). The NVI measures non-verbal intelligence, which reflects the ability to analyze information and solve problems (using visual or hands-on reasoning) while the MPI measures general mental processing abilities. These domains of cognitive functioning assessed are known to be influenced by nutritional intervention (Isaacs et al. 2008) and at age 36 to 72 months, the most rapid, distinct, spurts of growth occur in several brain regions, especially the frontal lobes (responsible for executive functions) that are expressed in the cognitive domains measured in the present study (Giedd et al. 1999; Thompson et al. 2000). Several previous intervention trials showed that cognitive function of children can be improved by short-term hunger alleviation (Simeon & Grantham-McGregor, 1989), antihelminthic treatment (Stoltzfus et al. 2001) as well as improved micronutrient intake (Van Stuijvenberg et al. 1999). Hence, the improvement in the cognitive function in this present

study would not have been possible without all the different aspects of the intervention. By design, all children included in our study received antihelminthic treatment (500 mg mebendazole) before the start of the intervention. Additionally, the intervention group received ~7.0 g more raw maize flour (providing ~100kJ) with the PoU fortificant containing both multiple micronutrient formulation and amylase-rich light malted barley (**Table 2**). Therefore, the higher energy, highly digestible, micronutrient-dense maize breakfast porridge received by the intervention group could have therefore modulated cognitive function through several mechanisms. Firstly, glucose is primarily the breakdown product of carbohydrate and the main source of energy for the brain essential for brain functioning (Gilsenan et al. 2009). Therefore, the effect of glucose absorbed from the ingested breakfast meal on short-term cognitive improvement could not be ruled out (Benton et al. 1987; Kaplan et al. 2000; Benton et al. 2003; Mahoney et al. 2005). Secondly, brain development and function are known to be altered by several micronutrients such as iron (Metallinos-Katsaras et al. 2004; Sachdev et al. 2005; Lozoff & Georgieff, 2006), zinc (Frederickson, 1989; Corniola et al. 2008), iodine (Zimmermann et al. 2006; Gordon et al. 2009) and vitamin A (Bonnet et al. 2008) as well as the B-vitamins (Bourre, 2006); all these nutrients were present in the fortificant (**Table 2**).

However, because of cost constraints and the fact that the present study was a preliminary trial other biomarkers of micronutrient status (except iron status as Hb concentration) were not assessed. One, therefore, cannot attribute the improvement in the cognitive score in the intervention group to any particular nutrient, but to a combination of the several micronutrients contained in the PoU fortificant. The results are consistent with the findings from Haskell et al. (2008); they showed that 12 weeks multi-vitamin/minerals supplementation had the potential to improve brain function among healthy 8- to 14-year-old children. Other recent longer-term multiple micronutrient intervention trials within slightly different contexts also reported similar trends for improvement in cognitive outcome among children (Van Stuijvenberg et al. 1999; Solon et al. 2003; Kumar & Rajagopala, 2007; Manger et al. 2008; Muthayya et al. 2009).

Within the context of this preschool-based intervention, changes in anthropometric indices were expected due to normal growth of children, as well as increased energy intake leading to weight and/or height gain and increased micronutrient intake which could help contribute to growth and muscle gains. However, no significant improvement was observed as a result of the intervention in any of the anthropometric indices measured. This is not surprising because of the short duration of the study (11 weeks) which may have been too short to detect any measurable changes. These results are consistent with findings from other multiple-micronutrient intervention trials (van Stuijvenberg et al. 1999; Giovannini et al. 2006;

Manger et al. 2008). This study had several strengths worth noting. It was a well controlled randomised trial, the breakfast porridge with micronutrient or placebo powder was eaten under supervision and consumption recorded daily, the dropout rate was low, and compliance to the intervention was good (~85%) and similar for both groups; and the study population came from the same community with similar background in terms of socio-demographic status (**Table 3**).

Some key lessons that can help improve similar future large-scale trials are (1) recruiting and training of the most suitable and qualified front-line staff (study assistant and cognitive assessors) are vital to successful implementation; (2) fieldworkers from the same background as the children help to overcome the language barrier to bridge communication gaps; (3) quality and timeliness of the delivery of the PoU fortificants help to streamline the implementation; (4) use of a sensitive cognitive test battery that is appropriate for the age and culture group in an African setting is necessary to show an effect of the intervention and (5) the use of a simple field-friendly finger prick method to measure Hb concentration may not be sufficiently sensitive to show differences in iron status after the intervention. This is a valuable pilot study for the use of micronutrient powders among young children, which has used the preschool setting in an innovative way in which to improve the cognitive function of preschool-age children through the implementation of a PoU fortification trial in preschool settings.

Implications for research and practice

The United Nations World Food Program is one of the food aid agencies that has included micronutrient powders or PoU fortificants in its food basket. Currently, most micronutrient interventions using micronutrient powders have focused mainly on its use at home among infants 6 to 24 months (Adu-Afarwuah et al. 2007; Sharieff et al. 2008) in resource poor settings, which is commendable. However, this type of intervention can be further extended to preschool-age children 24 to 72 months in school settings; this creates an extended window of opportunity to target and improve early developmental potentials of children at-risk of micronutrient deficiencies. In this present study, it was demonstrated that the implementation of PoU micronutrient fortification is a feasible and acceptable strategy that can be used to effectively improve the physical and cognitive developmental potential of preschool-age children. Nevertheless, future research should focus on assessing the long-term benefits of adding micronutrient powders to commonly consumed staple foods from various developing countries.

REFERENCES

- Adu-Afarwuah S, Lartey A, Brown KH, Zlotkin S, Briend A, Dewey KG. 2007. Randomized comparison of 3 types of micronutrient supplements for home fortification of complementary foods in Ghana: Effects on growth and motor development. *Am J Clin Nutr* 86(2):412-420.
- Alderman H, Behrman JR, Hoddinott J. 2007. Economic and nutritional analyses offer substantial synergies for understanding human nutrition. *J Nutr* 137(3):537-44.
- Anderson GL, Prentice RL. 1999. Individually randomized intervention trials for disease prevention and control. *Stat Methods Med Res* 8(4):287-309.
- Andersson M, Thankachan P, Muthayya S, Goud RB, Kurpad AV, Hurrell RF, Zimmermann MB. 2008. Dual fortification of salt with iodine and iron: a randomized, double-blind, controlled trial of micronized ferric pyrophosphate and encapsulated ferrous fumarate in southern India. *Am J Clin Nutr* 88(5):1378-87.
- Ayoya MA, Spiekermann-Brouwer GM, Traoré AK, Stoltzfus RJ, Habicht JP, Garza C. 2009. Multiple micronutrients including iron are not more effective than iron alone for improving hemoglobin and iron status of Malian school children. *J Nutr* 139(10):1972-9.
- Beard J. 2007. Recent evidence from human and animal studies regarding iron status and infant development. *J Nutr* 137(2):524S-530S.
- Behrman JR, Glewwe P, Miguel E, editors. 2007. Methodologies to evaluate early childhood development programs. Washington D.C.: World Bank.
- Benton D, Brett V, Brain P. 1987. Glucose improves attention and reaction to frustration in children. *Biol Psychol* 24(2):95-100.
- Benton D, Ruffin MP, Lassel T, Nabb S, Messaoudi M, Vinoy S, Desor D, Lang V. 2003. The delivery rate of dietary carbohydrates affects cognitive performance in both rats and humans. *Psychopharmacology* 166(1): 86– 90.
- Black RE, Morris SS, Bryce J. 2003. Where and why are 10 million children dying every year? *Lancet* 361(9376):2226-34.
- Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, Mathers C, Rivera J, Maternal and Child Undernutrition Study Group. 2008. Maternal and child undernutrition: Global and regional exposures and health consequences. *Lancet* 371(9608):243-60.
- Bonnet E, Touyarot K, Alfos S, Pallet V, Higuieret P, Abrous DN. 2008. Retinoic acid restores adult hippocampal neurogenesis and reverses spatial memory deficit in vitamin A deprived rats. *PLoS one*, 3(10):e3487.
- Bourre JM. 2006. Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirements for brain. part 1: micronutrients. *J Nutr Health Aging* 10(5):377-85.

- Bremner JD, McCaffery P. 2008. The neurobiology of retinoic acid in affective disorders. *Prog Neuropsychopharmacol Biol Psychiatry* 32(2):315-31.
- Cicchetti DV. 1994. Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology. *Psychol Assess* 6(4): 284–90.
- Cohen J. 1988. *Statistical power analysis for behavioural sciences*. 2nd ed. Hillsdale, NJ: Lawrence Earlbaum Associates.
- Colangelo LA, Chiu BC, Lopez P, Scholtens D, Willis LC, Hendrick RE, Gapstur SM. 2006. A pilot study of vitamin D, calcium, and percent breast density in hispanic women. *Nutrition Research* 26(1):11-5.
- Corniola RS, Tassabehji NM, Hare J, Sharma G, Levenson CW. 2008. Zinc deficiency impairs neuronal precursor cell proliferation and induces apoptosis via p53-mediated mechanisms. *Brain Res* 27;1237:52-61
- De Pee S, Kraemer K, van den Briel T, Boy E, Grasset C, Moench-Pfanner R, Zlotkin S, Bloem MW, World Food Programme, Sprinkles Global Health Initiative. 2008. Quality criteria for micronutrient powder products: Report of a meeting organized by the world food programme and sprinkles global health initiative. *Food Nutr Bull* 29(3):232-41.
- Ellis SM, Steyn HS. 2003. Practical significance (effect sizes) versus or in combination with statistical significance (p-values). *Management dynamics*, 12(4): 51-3.
- Faber M, Kvalsvig JD, Lombard CJ, Benade AJ. 2005. Effect of a fortified maize-meal porridge on anemia, micronutrient status, and motor development of infants. *Am J Clin Nutr* 82(5):1032-9.
- Fletcher-Janzen E, Daniel MH. 2006. *Socio-economic status research*. AGS Publishing: Pearson Assessments. [Available on internet:] <http://www.ags.pearsonassessments.com> [Date of access: 6 March 2008].
- Frederickson CJ. 1989. Neurobiology of zinc and zinc-containing neurons. *Int Rev Neurobiol* 31:145-238.
- Gardner G, Gardner A, MacLellan L, Osborne S. 2003. Reconceptualising the objectives of a pilot study for clinical research. *Int J Nurs Stud* 40(7):719-24.
- Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, Paus T, Evans AC, Rapoport JL. 1999. Brain development during childhood and adolescence: A longitudinal MRI study. *Nat Neurosci* 2(10):861-3.
- Gilsenan MB, De Bruin EA, Dye L. 2009. The influence of carbohydrate on cognitive performance: a critical evaluation from the perspective of glycaemic load. *Br J Nutr* 101(7):941-9.
- Giovannini M, Sala D, Usielli M, Livio L, Francescato G, Braga M, Radaelli G, Riva E. 2006. Double-blind, placebo-controlled trial comparing effects of supplementation with two different combinations of micronutrients delivered as sprinkles on growth, anemia, and iron deficiency in Cambodian infants. *J Pediatr Gastroenterol Nutr*. 42(3):306-12.

- Gordon RC, Rose MC, Skeaff SA, Gray AR, Morgan KMD, Ruffman T. 2009. Iodine supplementation improves cognition in mildly iodine-deficient children. *Am J Clin Nutr* 90(5):1264-71.
- Grantham-McGregor S, Cheung YB, Cueto S, Glewwe P, Richter L, Strupp B, International Child Development Steering Group. 2007. Developmental potential in the first 5 years for children in developing countries. *Lancet* 369(9555):60-70.
- Hall A, Hewitt G, Tuffrey V, De Silva N. 2008. A review and meta-analysis of the impact of intestinal worms on child growth and nutrition. *Matern Child Nutr* 4 Suppl 1:118-236.
- Hallberg L, Brune M, Erlandsson M, Sandberg AS, Rossanderhulten L. 1991. Calcium—effect of different amounts on nonheme-iron and heme-iron absorption in humans. *Am J Clin Nutr* 53:112–9.
- Haskell CF, Scholey AB, Jackson PA, Elliot JM, Defeyter MA, Greer J, Robertson BC, Baughan T, Tiplady B, Kennedy DO. 2008. Cognitive and mood effects in healthy children during 12 weeks' supplementation with multi-vitamin/minerals. *Br J Nutr* 100(5):1086-96.
- Hoddinott J, Maluccio JA, Behrman JR, Flores R, Martorell R. 2008. Effect of a nutrition intervention during early childhood on economic productivity in guatemalan adults. *Lancet* 371(9610):411-6.
- Hunt MK, Lobb R, Delichatsios HK, Stone C, Emmons K, Gillman MW. 2001. Process evaluation of a clinical preventive nutrition intervention. *Prev Med* 33(2 Pt 1):82-90.
- Hurrell RF, Reddy MB, Burri J, Cook JD. 2000. An evaluation of EDTA compounds for iron fortification of cereal-based foods. *Br J Nutr*. 84(6): 903-10.
- Hurrell RF. 2007. Iron fortification: its efficacy and safety in relation to infections. *Food Nutr Bull*. 28(4 Suppl): S585-94.
- ISAK see International Society for Kinanthropometry.
- International Society for Kinanthropometry. 2001. International standards for anthropometric assessment. Potchefstroom, South Africa.
- Isaacs E, Oates J. & ILSI Europe a.i.s.b.l. 2008. Nutrition and cognition: Assessing cognitive abilities in children and young people. *Eur J Nutr* 47 Suppl 3:4-24.
- Jinabhai CC, Taylor M, Coutoudis A, Coovadia HM, Tomkins AM, Sullivan KR. 2001. A randomized controlled trial of the effect of antihelminthic treatment and micronutrient fortification on health status and school performance of rural primary school children. *Ann Trop Paediatr* 21(4):319-333.
- Jooste PL, Weight MJ, Lombard CJ. 2000. Short-term effectiveness of mandatory iodization of table salt, at an elevated iodine concentration, on the iodine and goiter status of schoolchildren with endemic goiter. *Am J Clin Nutr* 71(1):75-80.

- Kalman DS, Feldman S, Feldman R, Schwartz HI, Krieger DR, Garrison R. 2008. Effect of a proprietary magnolia and phellodendron extract on stress levels in healthy women: A pilot, double-blind, placebo-controlled clinical trial. *Nutr J* 7:11.
- Kalman DS, Lou L, Schwartz HI, Feldman S, Krieger DR. 2009. A pilot trial comparing the availability of vitamins C, B(6), and B(12) from a vitamin-fortified water and food source in humans. *Int J Food Sci Nutr* :1-11.
- Kaplan RJ, Greenwood CE, Winocur G, Wolever TMS. 2000. Cognitive performance is associated with glucose regulation in healthy elderly persons and can be enhanced with glucose and dietary carbohydrates. *Am J Clin Nutr* 72(3):825-36.
- Kaufman AS, Lichtenberger EO, Fletcher-Janzen E, Kaufman NL. 2005. *Essentials of KABC-II assessment*. Hoboken, New Jersey: John Wiley & Sons, Inc.
- Kenward MG and Carpenter J. 2007. Multiple imputation: Current perspectives. *Stat Methods Med Res* 16(3):199-218.
- Kloka D, 2003. Integrated nutrition programme. <http://www.doh.gov.za/programmes/nutrition-f.html> [Date of access: October 16. 2008].
- Kong AS, Süssman AL, Negrete S, Patterson N, Mittleman R, Hough R. 2009. Implementation of a walking school bus: Lessons learned. *J Sch Health* 79(7):319,25; quiz 333-4.
- Kordas K, Stoltzfus RJ. 2004. New evidence of iron and zinc interplay at the enterocyte and neural tissues. *J Nutr* 134(6):1295-8.
- Kumar MV, Rajagopalan S. 2007. Multiple micronutrient fortification of salt and its effect on cognition in Chennai school children. *Asia Pac J Clin Nutr* 16(3):505-511.
- Labadarios D, Swart R, Maunder E, Kruger HS, Gericke G, Kuzwayo PMN, Ntsie PR, Steyn NP, Schloss I, Dhansay MA, Jooste PL, Dannhauser A, Nell JH, Molefe D, Kotze TJW. 2008. Executive summary of the national food consumption survey-fortification baseline (NFCS-FB-I): South Africa, 2005. *S Afr J Clin Nutr* (Suppl 2):245-300.
- Lancaster GA, Dodd S, Williamson PR. 2004. Design and analysis of pilot studies: Recommendations for good practice. *J Eval Clin Pract* 10(2):307-12.
- Lichtenberger EO, 2005. General measures of cognition for the preschool child. *Ment Retard Dev Disabil Res Rev*
- Linnan L, Steckler A. 2002. Process evaluation for public health intervention and research: an overview. In: *Process evaluation for public health intervention and research*. Steckler AB and Linnan L, editors. San Francisco, CA: Jossey-Bass. 12 p.
- Lind T, Lönnerdal B, Stenlund H, Ismail D, Seswandhana R, Ekström EC, Persson LA. 2003. A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: interactions between iron and zinc. *Am J Clin Nutr* 77(4):883-90.

- Loechl CU, Menon P, Arimond M, Ruel MT, Peltó G, Habicht JP, Michaud L. 2009. Using programme theory to assess the feasibility of delivering micronutrient sprinkles through a food-assisted maternal and child health and nutrition programme in rural haiti. *Matern Child Nutr* 5(1):33-48.
- Lopriore C, Guidoum Y, Briand A, Branca F. 2004. Spread fortified with vitamins and minerals induces catch-up growth and eradicates severe anemia in stunted refugee children aged 3-6 y. *Am J Clin Nutr* 80(4):973-81.
- Lozoff B, Georgieff MK. 2006. Iron deficiency and brain development. *Seminars in pediatric neurology*, 13(3):158-165.
- Mahoney CR, Taylor HA, Kanarek RB, Samuel P. 2005. Effect of breakfast composition on cognitive processes in elementary school children. *Physiol Behav* 7;85(5):635-45.
- Manger MS, McKenzie JE, Winichagoon P, Gray A, Chavasit V, Pongcharoen T, Gowachirapant S, Ryan B, Wasantwisut E, Gibson RS. 2008. A micronutrient-fortified seasoning powder reduces morbidity and improves short-term cognitive function, but has no effect on anthropometric measures in primary school children in northeast Thailand: A randomized controlled trial. *Am J Clin Nutr* 87(6):1715-1722.
- Mendoza C, Pearson JM, Brown KH, Lonnerdal B. 2004. Effect of a micronutrient fortificant mixture and 2 amounts of calcium on iron and zinc absorption from processed food supplement. *Am J Clin Nutr*. 79: 244-50
- Menon P, Ruel MT, Loechl CU, Arimond M, Habicht JP, Peltó G, Michaud L. 2007. Micronutrient sprinkles reduce anemia among 9- to 24-mo-old children when delivered through an integrated health and nutrition program in rural haiti. *J Nutr* 137(4):1023-30.
- Metallinos-Katsaras E, Valassi-Adam E, Dewey KG, Lonnerdal B, Stamoulakatou A, Pollitt E. 2004. Effect of iron supplementation on cognition in Greek preschoolers. *Eur J Clin Nutr* 58(11):1532-42.
- Moher D, Schulz KF, Altman DG. 2001. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. *Lancet* 357(9263):1191-94.
- Murphy SP, Gewa C, Liang L, Grillenberger M, Bwibo NO, Neumann CG. 2003. School Snacks Containing Animal Source Foods Improve Dietary Quality for Children in Rural Kenya. *J Nutr* 133(11 Suppl 2):3950S-3956S.
- Muthayya S, Eilander A, Transler C, Thomas T, van der Knaap HC, Srinivasan K, van Klinken BJ, Osendarp SJ, Kurpad AV. 2009. Effect of fortification with multiple micronutrients and n-3 fatty acids on growth and cognitive performance in Indian schoolchildren: the CHAMPION (Children's Health and Mental Performance Influenced by Optimal Nutrition) Study. *Am J Clin Nutr* 89(6):1766-75.
- Oelofse A, Van Raaij JM, Benade AJ, Dhansay MA, Tolboom JJ, Hautvast JG. 2003. The effect of a micronutrient-fortified complementary food on micronutrient status, growth and development of 6- to 12-month-old disadvantaged urban south african infants. *Int J Food Sci Nutr* 54(5):399-407.

- Robert RC, Gittelsohn J, Creed-Kanashiro HM, Penny ME, Caulfield LE, Narro MR, Black RE. 2006. Process evaluation determines the pathway of success for a health center-delivered, nutrition education intervention for infants in trujillo, peru. *J Nutr* 136(3):634-41.
- Robert RC, Gittelsohn J, Creed-Kanashiro HM, Penny ME, Caulfield LE, Narro MR, Steckler A, Black RE. 2007. Implementation examined in a health center-delivered, educational intervention that improved infant growth in trujillo, peru: Successes and challenges. *Health Educ Res* 22(3):318-31.
- Rossi PH, Lipsey MW, Freeman HE. 2004. *Evaluation: A systematic approach*. 7th ed. Thousand Oaks, CA.: Sage.
- Sachdev H, Gera T, Nestel P. 2005. Effect of iron supplementation on mental and motor development in children: Systematic review of randomised controlled trials. *Public Health Nutr* 8(2):117-32.
- Sanghvi T, Van Ameringen M, Baker J, Fiedler J, Borwankar R, Phillips M, Houston R, Ross J, Heymann H, Dary O. 2007. Vitamin and mineral deficiencies technical situation analysis: A report for the ten year strategy for the reduction of vitamin and mineral deficiencies. *Food Nutr Bull* 28(1 Suppl Vitamin):S160-219.
- Sari M, Bloem MW, De Pee S, Schultink WJ, Sastroamidjojo S. 2001. Effect of iron-fortified candies on the iron status of children aged 4-6 y in east Jakarta, Indonesia. *Am J Clin Nutr* 73(6):1034-1039.
- Schenker Y, Wang F, Selig SJ, Ng R, Fernandez A. 2007. The impact of language barriers on documentation of informed consent at a hospital with on-site interpreter services. *J Gen Intern Med* 22 Suppl 2:294-9.
- Sharieff W, Yin SA, Wu M, Yang Q, Schauer C, Tomlinson G, Zlotkin S. 2006. Short-term daily or weekly administration of micronutrient Sprinkles has high compliance and does not cause iron overload in Chinese schoolchildren: a cluster-randomised trial. *Public Health Nutr* 9(3):336-44.
- Sharieff W, Zlotkin SH, Ungar WJ, Feldman B, Krahn MD, Tomlinson G. 2008. Economics of preventing premature mortality and impaired cognitive development in children through home-fortification: A health policy perspective. *Int J Technol Assess Health Care* 24(3):303-311.
- Simeon D, Grantham-McGregor S. 1989. Effects of missing breakfast on the cognitive functions of school children of differing nutritional status. *Am J Clin Nutr* 49(4):646-53.
- Simeon DT, Grantham-McGregor SM, Callender JE, Wong MS. 1995. Treatment of trichuris trichiura infections improves growth, spelling scores and school attendance in some children. *J Nutr* 125(7):1875-83.
- Smuts CM, Dhansay MA, Faber M, Van Stuijvenberg ME, Swanevelder S, Gross R, Benadé AJ. 2005. Efficacy of multiple micronutrient supplementation for improving anemia, micronutrient status, and growth in South African infants. *J Nutr* 135(3):653S-659S
- Solon FS, Sarol JN Jr, Bernardo AB, Solon JA, Mehansho H, Sanchez-Fermin LE, Wambangco LS, Juhlin KD. 2003. Effect of a multiple-micronutrient-fortified fruit powder beverage on the nutrition status, physical fitness, and cognitive performance of schoolchildren in the Philippines. *Food Nutr Bull* 24(4 Suppl):S129-40.

- Stein AD, Wang M, DiGirolamo A, Grajeda R, Ramakrishnan U, Ramirez-Zea M, Yount K, Martorell R. 2008. Nutritional supplementation in early childhood, schooling, and intellectual functioning in adulthood: A prospective study in Guatemala. *Arch Pediatr Adolesc Med* 162(7):612-8.
- Stephenson LS, Latham MC, Adams EJ, Kinoti SN, Pertet A. 1993. Weight gain of Kenyan school children infected with hookworm, trichuris trichiura and ascaris lumbricoides is improved following once- or twice-yearly treatment with albendazole. *J Nutr* 123(4):656-65.
- Steyn NP, Marais D. 2002. Children have a right to meals of good nutritional quality at state-subsidised crèches. *South African J Clin Nutr* 15(2):3.
- Stoltzfus RJ, Dreyfuss ML, Chwaya HM, Albonico M. 1997. Hookworm control as a strategy to prevent iron deficiency. *Nutr Rev* 55(6):223-32.
- Stoltzfus RJ, Kvalsvig JD, Chwaya HM, Montresor A, Albonico M, Tielsch JM, Savioli L, Pollitt E. 2001. Effects of iron supplementation and anthelmintic treatment on motor and language development of preschool children in Zanzibar: Double blind, placebo controlled study. *BMJ* 323(7326):1389-93.
- Stoltzfus RJ, Chwaya HM, Montresor A, Tielsch JM, Jape JK, Albonico M, Savioli L. 2004. Low dose daily iron supplementation improves iron status and appetite but not anemia, whereas quarterly anthelmintic treatment improves growth, appetite and anemia in Zanzibari preschool children. *J Nutr*. 134(2):348-56..
- Swart R, Sanders, D. McLachlan, M. 2008. Nutrition: A primary health care perspective. In: South African health review. Barron P and Roma-Reardon J, editors. Durban: Health System Trust. 134 p.
- Thompson PM, Giedd JN, Woods RP, MacDonald D, Evans AC, Toga AW. 2000. Growth patterns in the developing brain detected by using continuum mechanical tensor maps. *Nature* 404(6774):190-3.
- Toroyan T, Oakley A, Laing G, Roberts I, Mugford M, Turner J. 2004. The impact of day care on socially disadvantaged families: An example of the use of process evaluation within a randomized controlled trial. *Child Care Health Dev* 30(6):691-8.
- Van Jaarsveld PJ, Faber M, Tanumihardjo SA, Nestel P, Lombard CJ, Benade AJ. 2005. Beta-carotene-rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. *Am J Clin Nutr* 81(5):1080-7.
- Van Stuijvenberg ME, Kvalsvig JD, Faber M, Kruger M, Kenoyer DG, Benade AJ. 1999. Effect of iron-, iodine-, and beta-carotene-fortified biscuits on the micronutrient status of primary school children: A randomized controlled trial. *Am J Clin Nutr* 69(3):497-503.
- Van Stuijvenberg ME, Dhansay MA, Lombard CJ, Faber M, Benade AJ. 2001a. The effect of a biscuit with red palm oil as a source of beta-carotene on the vitamin A status of primary school children: A comparison with beta-carotene from a synthetic source in a randomised controlled trial. *Eur J Clin Nutr* 55(8):657-62.
- Van Stuijvenberg ME, Dhansay MA, Smuts CM, Lombard CJ, Jogessar VB, Benade AJ. 2001b. Long-term evaluation of a micronutrient-fortified biscuit used for addressing micronutrient deficiencies in primary school children. *Public Health Nutr* 4(6):1201-9.

- Van Stuijvenberg ME, Smuts CM, Lombard CJ, Dhansay MA. 2008. Fortifying brown bread with sodium iron EDTA, ferrous fumarate, or electrolytic iron does not affect iron status in South African schoolchildren. *J Nutr* 138(4):782-6.
- Van Teijlingen ER, Rennie AM, Hundley V, Graham W. 2001. The importance of conducting and reporting pilot studies: The example of the Scottish births survey. *J Adv Nurs* 34(3):289-95.
- Venter CS, MacIntyre UE, Vorster HH. 2000. The development and testing of a food portion photograph book for use in an African population. *J Hum Nutr Diet* 13(3):205-218.
- Victora CG, Habicht JP, Bryce J. 2004. Evidence-based public health: moving beyond randomized trials. *Am J Public Health* 94(3): 400-5.
- Wegmuller R, Camara F, Zimmermann MB, Adou P, Hurrell F. 2006. Salt dual-fortified with iodine and micronized ground ferric pyrophosphate affects iron status but not haemoglobin in children in Cote d'Ivoire. *J Nutr* 136(7):1814-20.
- Williams CL, Strobino BA, Brotanek J. 2007. Weight control among obese adolescents: A pilot study. *Int J Food Sci Nutr* 58(3):217-30.
- World Health Organization. 2007. WHO Anthro for personal computers, version 2, 2007 Manual: Software for assessing growth and development of the world's children. Geneva: WHO. (<http://www.who.int/childgrowth/software/en/>).
- World Health Organization. 2009. WHO AnthroPlus for personal computers Manual: Software for assessing growth of the world's children and adolescents. Geneva: WHO. (<http://www.who.int/growthref/tools/en/>).
- Zimmermann MB, Connolly K, Bozo M, Bridson J, Rohner F, Grimci L. 2006. Iodine supplementation improves cognition in iodine-deficient schoolchildren in Albania: A randomized, controlled, double-blind study. *Am J Clin Nutr* 83(1):108-114.
- Zimmermann MB. 2008. Methods to assess iron and iodine status. *Br J Nutr* 99, Suppl. 3, S2–S9
- Zlotkin SH, Tondeur M. 2007. Successful approaches: Sprinkles. In: Nutritional anemia. Kraemer K and Zimmermann MB, editors. Switzerland: SIGHT AND LIFE Press. 269 p.

CHAPTER 4

GENERAL SUMMARY, CONCLUSION AND RECOMMENDATIONS

CHAPTER 4: GENERAL SUMMARY, CONCLUSION AND RECOMMENDATIONS

4.1 INTRODUCTION

The aim of the study described in this dissertation was to pilot-test the study design, methodology and implementation of a point-of-use micronutrient intervention among African preschool children from a low socio-economic background. The planning, implementation and interpretation of the pilot study in this dissertation stretched over a period of 18 months. The research study comprised of two major phases. The first phase (prior to implementation) of the pilot study involved the planning, recruitment (children and front-line staff) and various capacity strengthening activities which took a period of 8 months (appendix 1 – 13). In the second phase of this study, the feasibility of implementing a point-of-use fortificant among African preschool children was assessed, in a randomised, single-blinded controlled trial for a period of ~ 3 months using maize-meal porridge as a carrier (Chapter 3).

The purpose of this final chapter is to summarize the main findings in the pilot study in terms of the salient factors that are critical to the successful implementation of a point-of-use micronutrient fortification trial in a preschool setting. However, since the results of the study are already discussed, interpreted and compared to the relevant literature in the preceding chapter, only a general conclusion will subsequently be made. This will be followed by general recommendations regarding this study as deduced from the findings.

4.2 MAIN FINDINGS

The study described in this dissertation illustrates the value of attention to process within a logical framework of process evaluation incorporated in a randomised controlled pilot trial. The findings from this pilot study revealed that the preschool setting proffers an excellent opportunity for targeted intervention in this segment of the population group with regard to point-of-use fortification. The result from the pilot study showed that it is feasible to implement point-of-use micronutrient fortification in preschool settings. Acceptability of the point-of-use fortificant was measured only indirectly through children's compliance which revealed that the intervention was well accepted among the children. No significant intervention effects on the haemoglobin concentration as well as the anthropometric indices assessed were observed. However, the 11 weeks intervention had a medium likelihood of having clinical or practical likelihood for improving the cognitive function of African preschool

children as assessed by the two global cognitive function indices (Mental processing index and Nonverbal index).

4.3 CONCLUSION

The high prevalence of micronutrient deficiencies among South African preschool-age children reinforces the need for an intensified micronutrient malnutrition control strategy targeting children at-home or in-school. Micronutrient powders also known as “in-home fortification” or “point-of-use fortification” can be an effective long-term, sustainable approach in improving early childhood nutrition and cognitive developmental potential. The feasibility of implementing a point-of-use micronutrient fortification was demonstrated among African preschool children with potential benefits of improving their micronutrient status as well as cognitive function. The lessons from this trial could help in planning and/or implementing future point-of-use micronutrient fortification trial among South African children in preschool settings.

4.4 RECOMMENDATIONS

- This is the first study to implement the use of micronutrient powders among South African children at-risk of micronutrient deficiencies in preschool settings. At present there is a national school feeding program being implemented for primary school children. It is recommended that similar initiative should be extended towards preschool children.
- It is recommended that a full scale (longer duration with larger sample size) point-of-use micronutrient fortification trial should be conducted among children at-risk of developing micronutrient deficiencies. This could be done among preschool and school-age children in school settings to explore the acceptability and effectiveness of this micronutrient control strategies further.
- It is recommended that the cost-benefit analysis of implementing a point-of-use micronutrient fortification among the preschool children be determined. This will help demonstrate the sustainability of this approach in comparison to other strategies. Furthermore, it will facilitate and enable national governments to make informed decisions in adopting this micronutrient control measure.

APPENDICES

Appendix 1

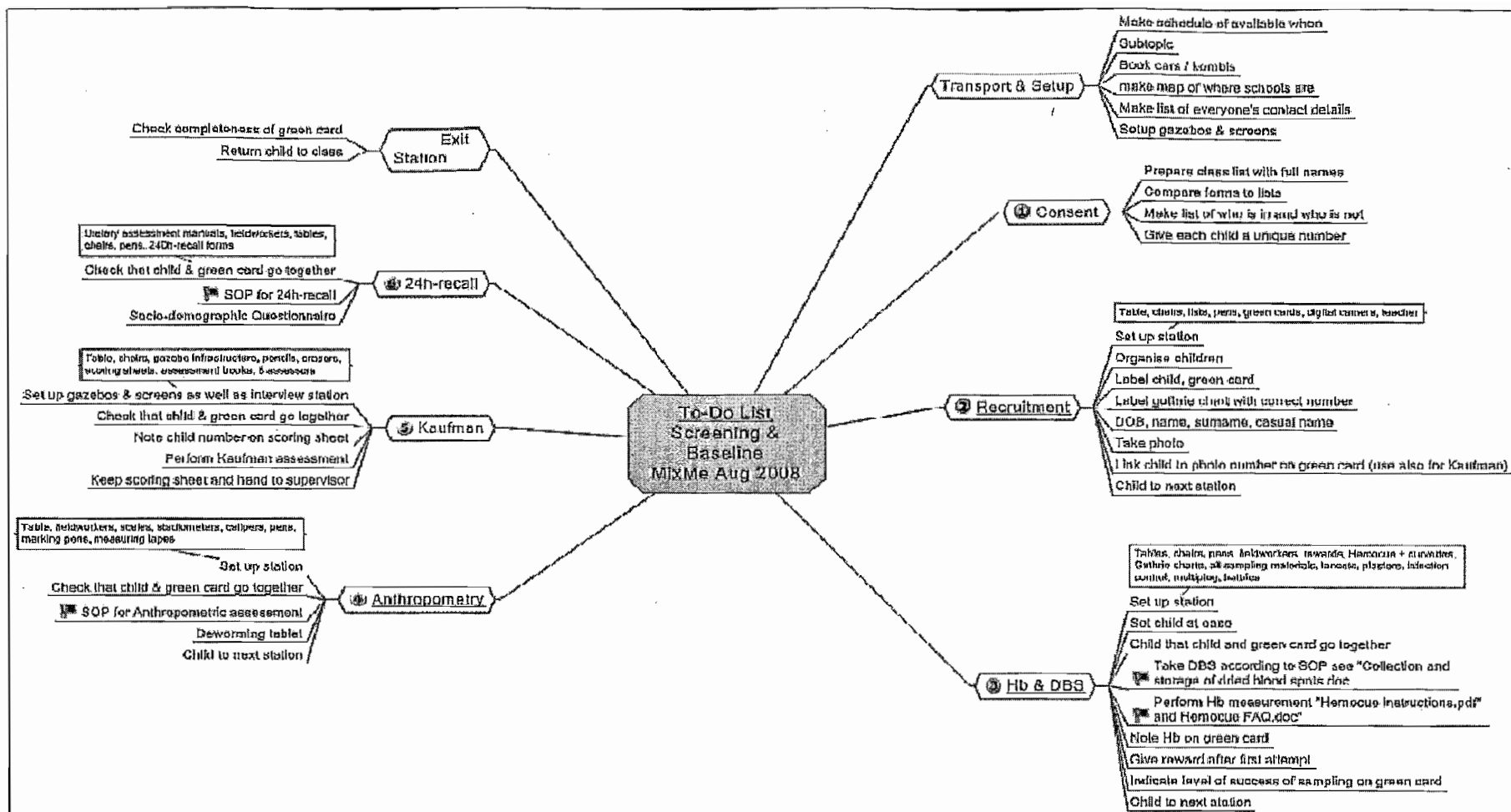
Schedule and planning of the pilot trial before the study using the Gantt chart

Phase 1: Planning, recruitment and capacity strengthening activities

ID	Task Name	Duration	Start	March	April	May	June	July	August	September
1	Protocol development	10 days	Wed 3/5/08							
2	NWU ethics application preparation	7 days	Thu 3/6/08							
3	Submission of ethics application	1 day?	Mon 3/17/08							
4	Ethics evaluation	45 days	Tue 3/18/08							
5	Budget approval	10 days	Wed 5/21/08							
6	Setting up Study	31 days?	Tue 5/20/08							
7	? Apply for additional funds (Prof. Johann & Klaus)	30 days	Wed 5/21/08							
8	Organise micronutrient powder (Prof. Johann & Klaus)	5 days	Wed 5/21/08							
9	Organise placebo powder	5 days	Wed 5/21/08							
10	Cognitive assessment	30 days?	Tue 5/20/08							
11	Decide on cognitive markers	30 days	Tue 5/20/08							
12	Phone conference with dr. J. Kvalsvig (11.00-1.00pm)	8 days?	Tue 5/20/08							
13	Finalizing cognitive markers to be used	1 day	Thu 5/29/08							
14	What cognitive scales to use?	1 day?	Tue 5/20/08							
15	How many field workers needed?	1 day?	Tue 5/20/08							
16	When to come for training?	1 day?	Tue 5/20/08							
17	Schedule training on the cognitive markers	1 day	Thu 5/29/08							
18	Other logistics: field workers,num.,duration etc	1 day	Thu 5/29/08							
19	dr. J. Kvalsvig's visit (Mid or late June)	2 days?	Thu 6/12/08							
20	Training of fieldworkers	2 days?	Thu 6/12/08							
21	Visiting of Site (preschools) if possible?	2 days?	Thu 6/12/08							
22	Other logistics / final decisions	2 days?	Thu 6/12/08							
23	Contact preschools	5 days?	Mon 5/26/08							
24	Get in touch with preschools' heads	5 days	Mon 5/26/08							
25	Phoning to arrange visit with school heads (Fixing a suitable date)	1 day?	Mon 5/26/08							
26	Arrange visitation	5 days	Mon 5/26/08							
27	Hand-in papers for the kids (One box per preschool)	1 day?	Mon 5/26/08							
28	Expectations from school heads / preschoolers / parents / guardians	5 days	Mon 5/26/08							
29	Negotiate cooperation	5 days	Mon 5/26/08							
30	Informed consent from parents / guardians	14 days?	Mon 5/26/08							
31	Informed consent forms	1 day?	Mon 5/26/08							
32	? Informed consent forms. How many? (App. 500 forms needed)	1 day	Mon 5/26/08							
33	Do we need translation to other languages? (Setswana)	1 day	Mon 5/26/08							
34	Consent forms (1 page summary + Translate to other languages if necessary)	14 days	Mon 5/26/08							
35	People that might help with translation (Maemo, Stokey, Noyoliso, Lesley)	1 day?	Mon 5/26/08							
36	When? and where? will the form be signed?	1 day?	Mon 5/26/08							
37	Possibility of signing the informed consent forms during :-	1 day?	Mon 5/26/08							
38	1. Health awareness day some-times around mid-june	1 day?	Mon 5/26/08							
39	2. Parents meeting (When? To ask school heads?)	1 day?	Mon 5/26/08							
40	3a. Information section for parents (when? To ask school heads?)	1 day?	Mon 5/26/08							
41	3b. Information section on SA FBDG in Tswana (Short talk).	1 day?	Mon 5/26/08							
42	Schedule dates for Screening / intervention	3 days	Tue 5/20/08							
43	Screening	5 days?	Mon 6/2/08							
44	Order screening materials	1 day?	Mon 6/2/08							
45	Portable HEMOCUE (Already ordered)	1 day?	Mon 6/2/08							
46	EMLA cream (Buy from Pharmacists)	1 day?	Mon 6/2/08							
47	Train screeners (Train / Figure out how to use the Hemocue)	1 day	Mon 6/2/08							
48	Postgraduate students will be asked to help out during the screening.	1 day?	Mon 6/2/08							
49	Discussion on When? to involve Sister Christein during the next meeting.	1 day?	Tue 6/3/08							
50	SCREENING OF CHILDREN	3 days	Wed 6/4/08							
51	Things to note :-	1 day?	Mon 6/2/08							
52	1. Prepare Log book containing(Project ID, Class list, Photograph, DOB & Hb value)	1 day?	Mon 6/2/08							
53	2. Marginally anaemic & Anaemic children will participate in the study.	1 day?	Mon 6/2/08							
54	3a. Severely Anaemic will be referred for treatment.	1 day?	Tue 6/3/08							
55	3b. Draft letter referring severely anaemic children to the clinic for treatment.	1 day?	Mon 6/2/08							
56	Analysing screening results and decision on the Cut-off points.	2 days	Mon 6/2/08							

Phase 2: Pilot study implementation

ID	Task Name	Duration	Start	Finish	May	July	September	November	January	March	May	July
1	Intervention study	5 mons	Mon 7/14/08	Fri 11/28/08								
2	Order supplies ?	4 days	Tue 7/1/08	Fri 7/4/08								
3	Things to note :-	1 day?	Mon 5/19/08	Mon 5/19/08								
4	1. Stationery ??	1 day?	Mon 5/19/08	Mon 5/19/08								
5	2. Programme of the day in each Creche (time of Opening / Closing)	1 day?	Mon 5/19/08	Mon 5/19/08								
6	2. What are the children feeding from presently? / Daily menu	1 day?	Mon 5/19/08	Mon 5/19/08								
7	3. Possibilities of getting new Plates/cups.	1 day?	Mon 5/19/08	Mon 5/19/08								
8	3. Need to know their daily menu / state of the kitchen.	1 day?	Mon 5/19/08	Mon 5/19/08								
9	4. Who will administer the Food Supplement?	1 day?	Mon 5/19/08	Mon 5/19/08								
10	Baseline	9 days?	Tue 7/1/08	Fri 7/11/08								
11	Basic info?	5 days	Tue 7/1/08	Mon 7/7/08								
12	Cognitive measures	4.81 days	Tue 7/1/08	Mon 7/7/08								
13	Day 1: 15 children @ 30 mins/child	7.5 hrs	Tue 7/1/08	Tue 7/1/08								
14	Day 2: 15 children @ 30 mins/child	7.5 hrs	Wed 7/2/08	Wed 7/2/08								
15	Day 3: 15 children @ 30 mins/child	7.5 hrs	Thu 7/3/08	Thu 7/3/08								
16	Day 4: 15 children @ 30 mins/child	7.5 hrs	Fri 7/4/08	Fri 7/4/08								
17	Day 5: 15 children @ 30 mins/child	7.5 hrs	Fri 7/4/08	Mon 7/7/08								
18	Anthropometry and body composition measure	3 days?	Fri 7/4/08	Wed 7/9/08								
19	Anthropometry and Body composition (using Bodpod) measures.	3 days	Fri 7/4/08	Wed 7/9/08								
20	Will be done at the Nutrition department, NWU.	1 day?	Fri 7/4/08	Mon 7/7/08								
21	Children will have to be transported from their schools.	1 day?	Fri 7/4/08	Mon 7/7/08								
22	Anthropometry measures.	1 day?	Fri 7/4/08	Mon 7/7/08								
23	Day 1: 50 children @ 4 mins/child	3.5 hrs	Fri 7/4/08	Mon 7/7/08								
24	Day 2: 50 children @ 4 mins/child	3.5 hrs	Mon 7/7/08	Mon 7/7/08								
25	Day 3: 50 children @ 4 mins/child	3.5 hrs	Mon 7/7/08	Tue 7/8/08								
26	Body composition measures using Bodpod.	1 day?	Fri 7/4/08	Mon 7/7/08								
27	5 children / hour.	1 day?	Fri 7/4/08	Mon 7/7/08								
28	Blood measure (Haemoglobin & DBS (sTfR, RBP))	3 days	Tue 7/8/08	Thu 7/10/08								
29	Day 1: 50 children @ 1 min/child	50 mins	Wed 7/9/08	Wed 7/9/08								
30	Day 2: 50 children @ 1 min/child	50 mins	Thu 7/10/08	Thu 7/10/08								
31	Day 3: 50 children @ 1 min/child	50 mins	Thu 7/10/08	Thu 7/10/08								
32	Deworming after blood measure	3 days	Tue 7/8/08	Thu 7/10/08								
33	Dietary assessment	4 days?	Tue 7/8/08	Fri 7/11/08								
34	How? Home visit?	3 days	Tue 7/8/08	Thu 7/10/08								
35	24 hour recall will be used (To be taken 3 times) :-	1 day?	Tue 7/8/08	Tue 7/8/08								
36	1. During home visit on a Sunday.	1 day?	Wed 7/9/08	Wed 7/9/08								
37	2. During home visit during the week to gather the evening meals.	1 day?	Thu 7/10/08	Thu 7/10/08								
38	3. During the week in the creche.	1 day?	Fri 7/11/08	Fri 7/11/08								
39	Sprinkles intervention	100 days	Mon 7/14/08	Fri 11/28/08								
40	End	9 days?	Mon 12/1/08	Thu 12/11/08								
41	Basic info?	3 days	Mon 12/1/08	Wed 12/3/08								
42	Cognitive measures	4.94 days	Mon 12/1/08	Fri 12/5/08								
43	? Day 1: 15 children @ 30 mins/child	7.5 hrs	Mon 12/1/08	Mon 12/1/08								
44	? Day 2: 15 children @ 30 mins/child	7.5 hrs	Tue 12/2/08	Tue 12/2/08								
45	? Day 3: 15 children @ 30 mins/child	7.5 hrs	Wed 12/3/08	Wed 12/3/08								
46	? Day 4: 15 children @ 30 mins/child	7.5 hrs	Thu 12/4/08	Thu 12/4/08								
47	? Day 5: 15 children @ 30 mins/child	7.5 hrs	Fri 12/5/08	Fri 12/5/08								
48	Anthropometry measure	4 days?	Mon 12/8/08	Thu 12/11/08								
49	Day 1: 50 children @ 4 mins/child	3.5 hrs	Mon 12/8/08	Mon 12/8/08								
50	Day 2: 50 children @ 4 mins/child	3.5 hrs	Tue 12/9/08	Tue 12/9/08								
51	Day 3: 50 children @ 4 mins/child	3.5 hrs	Wed 12/10/08	Wed 12/10/08								
52	Anthropometry and body composition measure	1 day?	Mon 12/8/08	Mon 12/8/08								
53	Anthropometry and Body composition (using Bodpod) measures.	1 day?	Mon 12/8/08	Mon 12/8/08								
54	Will be done at the Nutrition department, NWU.	1 day?	Mon 12/8/08	Mon 12/8/08								
55	Children will have to be transported from their schools.	1 day?	Mon 12/8/08	Mon 12/8/08								
56	Anthropometry measures.	1 day?	Mon 12/8/08	Mon 12/8/08								
57	Day 1: 50 children @ 4 mins/child	1 day?	Tue 12/9/08	Tue 12/9/08								
58	Day 2: 50 children @ 4 mins/child	1 day?	Wed 12/10/08	Wed 12/10/08								
59	Day 3: 50 children @ 4 mins/child	1 day?	Thu 12/11/08	Thu 12/11/08								
60	Body composition measures using Bodpod.	3 days?	Mon 12/8/08	Wed 12/10/08								
61	5 children / hour.	3 days?	Mon 12/8/08	Wed 12/10/08								
62	Blood measure (Haemoglobin & DBS (sTfR, RBP))	2.1 days	Mon 12/8/08	Wed 12/10/08								
63	Day 1: 50 children @ 1 min/child	50 mins	Mon 12/8/08	Mon 12/8/08								
64	Day 2: 50 children @ 1 min/child	50 mins	Tue 12/9/08	Tue 12/9/08								
65	Day 3: 50 children @ 1 min/child	50 mins	Wed 12/10/08	Wed 12/10/08								
66	Dietary assessment	3 days?	Mon 12/8/08	Wed 12/10/08								
67	How? Home visit? or ?ion Kids or ?	3 days	Mon 12/8/08	Wed 12/10/08								
68	How? Home visit?	1 day?	Mon 12/8/08	Mon 12/8/08								
69	24 hour recall will be used (To be taken 3 times) :-	1 day?	Mon 12/8/08	Mon 12/8/08								
70	1. During home visit on a Sunday.	1 day?	Mon 12/8/08	Mon 12/8/08								
71	2. During home visit during the week to gather the evening meals.	1 day?	Mon 12/8/08	Mon 12/8/08								
72	3. During the week in the creche.	1 day?	Mon 12/8/08	Mon 12/8/08								
73	Data analysis	5 days?	Wed 12/10/08	Tue 12/16/08								
74	Data computerisation	4 days	Wed 12/10/08	Mon 12/15/08								
75	Data Cleaning	2 days?	Wed 12/10/08	Thu 12/11/08								
76	Statistical analysis	3 days?	Fri 12/12/08	Tue 12/16/08								
77	Reporting	15 days?	Tue 12/16/08	Mon 1/5/09								



Appendix 2

A sample copy of the letter of ethical approval



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

Private Bag X6001, Potchefstroom
South Africa 2520

Tel: (018) 299-4900
Faks: (018) 299-4910
Web: <http://www.nwu.ac.za>

Ethics Committee

Tel +27 18 299 4850
Fax +27 18 293 5329
Email Ethics@nwu.ac.za

Prof J C Jerling

11 July 2008

Dear Prof Jerling

ETHICS APPROVAL OF PROJECT

The North-West University Ethics Committee (NWU-EC) hereby approves your project as Indicated below. This implies that the NWU-EC grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the project may be initiated, using the ethics number below.

Project title: Multi-micronutrient sprinkles and the cognitive development of anaemic pre-school children

Ethics number:

N W U - 0 0 1 9 - 0 8 - S 1

Indicator: Project Number Year Status
Sign: S = Submission R = Re-Submission P = Project not authorised A = Authorisation

Approval date: 11 July 2008

Expiry date: 10 July 2013

Special conditions of the approval (if any): None

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- The project leader (principal investigator) must report in the prescribed format to the NWU-EC:
 - annually (or as otherwise requested) on the progress of the project;
 - without any delay in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.
- The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the NWU-EC. Would there be deviation from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date, a new application must be made to the NWU-EC and new approval received before or on the expiry date.
- In the interest of ethical responsibility the NWU-EC retains the right to:
 - request access to any information or data at any time during the course or after completion of the project;
 - withdraw or postpone approval if:
 - any unethical principles or practices of the project are revealed or suspected,
 - it becomes apparent that any relevant information was withheld from the NWU-EC or that information has been false or misrepresented,
 - the required annual report and reporting of adverse events was not done timely and accurately,
 - new institutional rules, national legislation or international conventions deem it necessary.

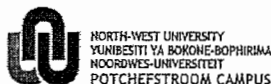
The Ethics Committee would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the Ethics Committee for any further enquiries or requests for assistance.

Yours sincerely

Prof MMJ Lowes
(chair NWU Ethics Committee)

Appendix 3

A sample copy of the information sheets for parents and guardian containing informed consent form



INFORMATION TO PARENT OR LEGAL GUARDIAN

Dear Parent / legal guardian

The North West University wants to undertake a study to determine the effect of a multi-micronutrient food supplement on the cognitive development of anaemic to marginally anaemic preschool children. The learning ability of a child can be improved through supplementary feeding during the school day. We need your consent for your child to take part in this study. Participation is completely voluntarily. We want to take pictures of each child to be sure that we compare measurements of the same child before and after the study. During the study your child will be asked questions in a personal interview by a trained adult lady.

This information is about the child's ability to learn in school and will be regarded as confidential. Your child's height, weight, arm circumference and fat skinfolds will also be measured. The skinfold measurements are pinched and will cause slight discomfort, but will not hurt the child. The child will be transported to the university in a minibus driven by a university staff member for special measurements in a BODPOD machine that measures body fat and muscle in a safe way that does not hurt the child. It is essential for a nutrition study of this kind to analyse the blood of the children biochemically. For this reason, about 2-3 drops of blood will be obtained from a simple finger prick from your child at the beginning and at the end of the study to assess the level of nutritional improvement in your child.

This procedure will be carried out by a qualified nursing sister / scientist using sterile equipment (only used once and then discarded). It is completely safe and the slight discomfort experienced will be minimized by the use of a local anaesthetic (EMLA cream) 20 minutes prior to the finger prick. Your child will be dewormed during the course of the study, a therapy which is not associated with serious side effects. Worms may, however, appear in the stools of your child.

Benefits of the study to your child:

- 1) All crèche children will receive a multi-micronutrient food supplement for a period of 6 months after completion of the experimental phase.
- 2) Children who are severely anaemic will be identified during the screening phase and will be referred to receive treatment.
- 3) De-worming medication will be provided before the study.

TSHEDIMOSETSO GO MOTSADI KGOTSA MOTLAMEDI

(Translation in Setswana).

Motsadi / Motlamedi

Unibesiti ya Bokone-bophirima e batla go dira dipatlisiso tse di riling mo go itemogeleng gore sejo se se riling sa dikotla tse di Farologaneng mo baneng ba dikreche tse di Farologaneng sepheto sa sona e ka nna eng. Go ithuta ga ngwana go ka tokafala ka ona mokgwa O, wa gore bana na Fiwe dijo mo nakong ya sekoko. Ka jaalo re kopa tetla ya gago jaaka motsadi gore ngwana wa gago a tseye karolo mo dipatlisisong getse. Go tsaya karolo ga ngwana wa gago a gapeletswa, ke tshoetso ee tswang mo motsading gore o a dumela kgo tsaya. Re ka rata le go tsaya setshwantsho sa ngwana mongwe le mongwe go dira bo-nnete ba gore re bapisa ngwana lefa a ne a simolola le fa a fetsa ka dipatlisiso tse.

Nakong ya dipatlisiso se, ngwana wa gago o tla botsiwa dipotso mo bonngweng jwa gagwe ke motho o rutilweng go dira tiro e, mme e tla nna mosadi. Kitso eo ke ka ga bokgoni jwa ngwana jwa go ithuta mo sekolong mme e tla tsholwa jaaka sephiri. Bokima / boleele, mafura a letlalo, matsogo a tla lekgwa (mejamente), mme go dira seo letlalo le tsa tsipiwa, mme go tla nna eketse go botlhoko, mme ngwana ga a na go utlwa botlhoko. Ngwana o tla isiwa kwa unibesiting, go dira diteko tsa (BODPOD) motshini oo metang mafura a mmele le ditshika ka mokgwa oo sirele tsegileng, o o sa utlwiseng ngwana botlhoko.

Go botlhokwa go dira serutwa se, go lebelela madi a ngwana gore a santse a le mo maemong aa siameng. Tiro e, e tla diriwa ke mooki yo o nang le dipampiri tsa thuto tse, mme e bile o tla dirisa dilo tse sireletsegileng, tse di dirisiwang gangwe fela. E sireletsegile e bile botlhoko bo tla Fokodiwa ka sengwe se se tshasiwang (EMLA cream) metsotso e le 20 pele ga go tlhabiwa monwana. Ngwana wa gago o tla ntshiwa le manyowa mo serutweng se, mme ga se tsamaiso e e tla nnang le kotsi epe mo ngwaneng wa gago. Manyowa a ka bonagala mo mantleng a ngwana.

Masola a Serutwa se mo ngwaneng wag gago:

- 1) Bana botlhe ba kretche bat-la amogela sejo se sa dikotla tse di farologaneng bakeng sa dikgwedi tse di thataro, morago ga go dira diteko tse.
- 2) Bana ba ba nang le bokowa, batla itsisiwe gore ba kgone go bona thuso e e tsamaelanang le bolwetse jo.
- 3) Moriyangwa wa manyowa o tla fiwa bana pele ga dipatlisiso tse di simolola.

INFORMED CONSENT

THE EFFECT OF A MULTI-MICRONUTRIENT FOOD SUPPLEMENT ON THE COGNITIVE DEVELOPMENT OF PRESCHOOL CHILDREN.

I have been informed about the purpose and nature of the study and that all information will be regarded as confidential *[Ke itsesitswe ka morero le mokgwa tsa dipatlisiso tse, le gore kitso yotlhe e tla tshwolwa ja ka sephiri]*.

I have been informed about the advantages and possible adverse effects that may result from procedures and/or treatment, and I understand what it says *[Ke itsisitswe le ka mosola le dikgonagalo tsa dipheto tse di rileng tsamaiso-ng le egore ke tlhaloganya gore go raya eng]*.

I understand that participation is voluntary and that I can recall my consent at anytime without forfeiting the availability of any future routine medical care *[Ke tlhaloganya gore gotsaya karolo ke ga go ithaopilweng, e bile nka gogela kwa morago go tsaya karolo ga ngwana wa me nako ngwe le nngwe kwantle le go senya]*.

Nutritional status will be assessed by means of the measurement of height and weight, and analysis of blood sample. About 2 to 3 drops of blood will be obtained by a simple finger prick by a nursing sister / scientist *[Maemo a dijo a tla tlhokomelwa ka go lekgwa ka boima le boleele patlisiso ya madi a a lekanang le didropo tse 2-3 di tla fitlhelwa ka go tlhabiwa mo monwaneng ke mooki / kgotsa setsibi sa tsa mahlale]*.

Name of Volunteer:

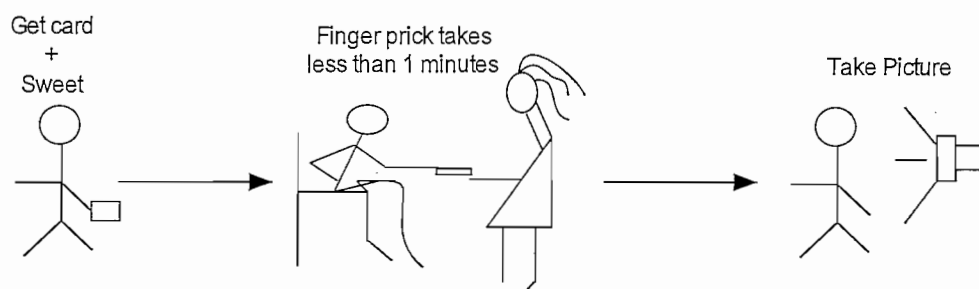
Address:

.....
Parent or Legal Guardian
Motsadi / Motlamedi

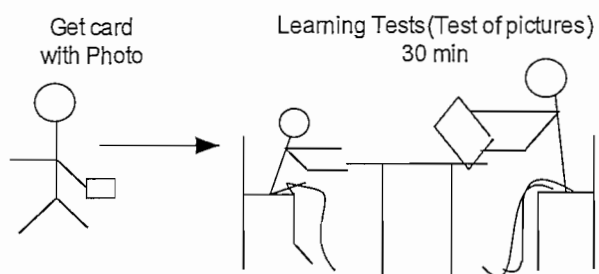
Signed this.....Day of.....2008
at.....

THE MIXME STUDY

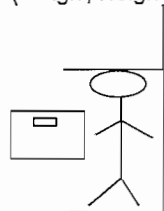
SCREENING



LEARNING TESTS



MEASUREMENTS (Weight, Height etc)



BLOOD SPOT (Finger prick)



Appendix 4

A sample copy of the standard operation procedure for haemoglobin assessment

STANDARD OPERATION PROCEDURE: HEMOCUE HAEMOGLOBIN METER

Operating the Hemocue

Step 1: Press the start button to ON the Hemocue (it will automatically perform a self test after which 3 dashes will appear on the screen indicating that it's ready).

Step 2: Hold the arm sideways while using the thumb to press the finger to be pricked gently.

Step 3: Wipe the finger to be pricked at the thumb side with soft tissue and disinfectant.

Step 4: Prick at the thumb side of the finger.

Step 5: The first drop of blood should be wiped off in the direction of the finger tip.

Step 6: Fill the cuvette by placing the tip of the cuvette in the drop of blood.

Step 7: Place the cuvette in the cuvette-holder of the Hemocue and close.

Step 8: Result will be displayed after 15 – 60 seconds.

Step 9: Remove cuvette and dispose immediately.

Cleaning the Hemocue

Step 1: Remove the black removable part : wipe with alcohol swab or cotton wool + alcohol.

Step 2: For the inside of the Hemocue, use the cleaning rod provided by the manufacturer.

Important information

Keep the cuvettes sealed until needed and always check the expiry date before tests are done.

Appendix 5

A sample copy of the standard operation procedure for anthropometry assessment

STANDARD OPERATION PROCEDURE: ANTHROPOMETRIC MEASUREMENTS

The following anthropometric measurements should be done:

- Weight (W)
- Height (H)
- Midupperarm circumference (MUAC)
- Triceps skinfold (TSF)

In order to do these measurements you will need with the following equipment:

- An electronic scale
- A stadiometer
- A steel measuring tape
- A "Harpender" skinfold caliper

The scale must be calibrated before-hand with a 2kg calibration weight

If measurements are done in the field, take a wooden board to get an even surface for weighing.

Weight

The scale should be placed on an even, uncarpeted area and levelled with the aid of its in-built spirit level.

- After the scale is switched on, wait for the zero indication (0,0), as well as the stable indicator (0 on the display panel) to appear.
- The child should be weighed (preferably after emptying his/her bladder) and with the minimum of clothing or in underclothes for older children
- The child is placed on the scale, standing still and upright in the middle of the platform, facing the field worker, looking straight ahead. If standing, his/her feet should be flat and slightly apart until the reading is taken.
- After the reading is recorded in the space provided in the questionnaire, the child is removed from the scale. The weight is recorded to the nearest 0.01kg.
- After the child steps down from the scale, wait for the zero reading to appear on the digital display before repeating the procedure.
- The two readings should not vary by more than 10g. If they do, the scale has to be checked for accuracy, and the procedure has to be repeated until two similar weight readings are obtained.

In extreme cases, when the child is not able to stand alone on the scale, the following method is employed:

- The mother/caregiver is weighed first (without heavy clothing and shoes). The weighing should be done according to the discussed procedures.
- Then the zero/reset button is pressed and the field worker has to wait for the zero reading (0.0) to appear on the digital display.
- The child is then placed in the mother's arms and the reading taken and recorded.
- The mother and child are then taken off the scale, and when the zero reading appears again on the display, the procedure is repeated.

Height (stature)

The standing height of these children is taken by means of a stadiometer. Two readings are taken and the measurement is repeated if the two readings vary by more than 0.5cm.

- The stadiometer should be wall-mounted or placed on an even, uncarpeted area.
- The subject's shoes and hat or cap are removed
- If the hair is tied up on the top of the head, it should be released
- The subject is positioned as follows:
 - Facing the field worker, shoulders relaxed, with shoulder blades, buttocks and heels touching the measuring board
 - Arms relaxed at sides, legs straight and knees together
 - Feet flat, heels touching together, with the subject looking straight ahead (Frankfurt plane), the headpiece is slid down until it touches the crown of the head
 - The subject should stretch, but the feet should not come off the floor, the reading is taken at the end of a deep inward breath
 - The hair should be crushed as much as possible, in order to measure height on the hard flat surface of the head, the reading is taken at the bottom of the head piece to the nearest 0.1cm
 - The measurement is recorded in the space provided in the questionnaire and repeated at least once.

Triceps skinfold

Stand at the back of the subject with the child sitting or standing and locate the acromial and radial landmarks at the right side of the body. Palpate along the straight part of the scapula (shoulder bone) to the most lateral point of the shoulder and mark the lateral end of the scapula as the acromial landmark. The radial landmark is at the top of the radius (bone in the fore-arm), in the dimple of the elbow, in the space between the humerus (bone in the upper-arm) and the radius. Measure the straight distance between these two points, without the tape following the curves on the surface of the arm. Mark the midpoint between these two points with a horizontal mark and then project this mark to the back-side of the arm as a horizontal line. The triceps skinfold is measured at a vertical line parallel to the humerus. The measurement is taken to the nearest 1mm. Take two measurements and record them in the appropriate section of the questionnaire. If the two measurements differ by more than 0.5mm, take a third measurement and select the two measurements that are nearest to each other.

Mid-upperarm circumference

For measurement of the arm circumference the child needs to undress, or lift her/his sleeve of the right arm, so that the measurement can be made on the bare skin. Stand on the right side of the child. The measurement is made at the midpoint between the acromial (shoulder tip bone) and radial (tip of the right-sided bone of the elbow) landmarks. The fieldworker should ensure that the tape is in the horizontal position. The subject is instructed to lower her/his arms to the relaxed position and to breathe normally. The fieldworker needs to readjust the tape as necessary to ensure that it does not indent the skin. The measurement is taken to the nearest 0.1cm. Take two measurements and record them in the appropriate section of the questionnaire. If the two measurements differ by more than 0.2cm, take a third measurement and select the two measurements that are nearest to each other. (Clean the tape with a wet wipe after a day's measurements.)

References

LEE RD & NIEMAN DC. 2003. Nutritional assessment. 2nd Mosby. St Louis.

INTERNATIONAL SOCIETY FOR THE ADVANCEMENT OF KINANTHROPOMETRY (ISAK). 2001. International Standards for Anthropometric Assessment. Potchefstroom. ISAK.

WHO. The WHO Child Growth Standards (2005) and the WHO Anthro 2005 software and macros. <http://www.who.int/childgrowth/software/en/>

Appendix 6

A sample copy of the standard operation procedure for 24-hour dietary recall

STANDARD OPERATION PROCEDURE: 24-HOUR DIETARY RECALL

Introduction

The 24-hour recall is based on foods and amounts actually consumed by an individual on one specific day. If a number of recalls are collected over a long period, these recalls can be used to estimate usual intakes in research studies.

Method

The interviewer must take time to explain:

- the purpose and
- importance of the research and
- to establish a friendly but business-like relationship with the interviewees.

Create an atmosphere of trust and motivate the interviewee to provide accurate information. The purpose of the study is to assess the health and nutritional status of children at the creche. If the caregivers can report accurately on what the child ate the previous day, the results of the study can be used to make appropriate recommendations to improve nutrition education at the clinic.

The 24-hour dietary recall is based on an in-depth interview conducted by a trained dietary interviewer. The interviewee is the mother or caregiver. The interviewer solicits detailed information about everything the child had to eat and drink from midnight to midnight of the previous day (over the past 24-hour period). The accuracy of the dietary intake is dependent on the subject's short-term memory. It is important to probe for additional foods and food preparation methods.

Ask questions in a manner designed to put the respondent at ease and to facilitate his or her ability to recall the previous day's intake. The interviewer must provide a relaxed and unhurried atmosphere to give the subject a chance to carefully reflect on what he or she ate the previous day. *Collecting a brief history of the previous day's activities* prior to beginning to ask questions about food intake may facilitate memory. Begin with the first thing the subject had to eat or drink on the previous day and then proceed forward to cover 24 hours. Ask questions in a non-judgmental manner and maintain a neutral attitude toward all responses. Avoid asking questions in a way that might influence the subject's responses.

The amounts of food consumed must be quantified accurately. To help the subject to report the amount of food or drink accurately, tools such as photo books, food models, household cups and spoons or packets of commercial foods may be used. For example Marie biscuits of different brand names are in the same packet size and the most brands of cookies come in the same size per cookie. Foods that are commonly forgotten are snacks and beverages, or foods that are less frequently consumed.

Detailed information about:

- food preparation methods,
- recipe ingredients and
- brand names is necessary

For the necessary level of detail: Ask about *possible additions, such as butter or margarine* added to vegetables. Information about vitamins and other dietary supplements must also be collected.

Number of days and which days

A single 24-hour recall per individual may be sufficient if estimates of group means are of interest and when a large sample is studied. Ideally all 7 days of the week should be equally represented, since there may be differences in dietary intake on different days of the week. The number of days required depends on the day-to-day variability of the nutrients of interest and the precision desired. For practical reasons collection of multiple days of intake is not feasible for epidemiological studies involving large numbers of individuals.

If the distribution of individual intakes within the group is of importance, it is necessary to collect more than one recall per individual, at least in a sub-sample of the subjects. The minimum number of days of intake required for description of usual intake ranges from 3-10 days. Participant motivation falls off with increasing number of days, especially if the days are consecutive. If an estimate of long-term usual intake is required, collection of 3-4 days of recalls in each of four seasons of the year is ideal.

Summary

Follow the following steps:

- Fill in the subject number, your own initials (interviewer) and the date.
- Tick what the day was yesterday
- Ask the caregiver if yesterday was like most days, that is: did the child eat like most other days?
- Put the caregiver at ease, act friendly
- Explain the purpose of the study
- Motivate the caregiver to report accurately
- Ask about the previous day's programme, when did he/she go where.
- Ask about what time the child woke up.
- Ask what the child's first food or drink was.
- Proceed over the rest of the day, as shown on the questionnaire
- Write only one item per line, for example brown bread on one line and margarine on the next and jam on the next.
- Write down details on each item, for example: Brown bread or fresh full-cream milk.
- Explain the cooking method, for example spinach with potatoes and onions and margarine. Remember always to ask if there is extra margarine, sugar or oil added to food.
- Select the amount eaten from the photograph book or models and write it in for EACH food item. We cannot go back later for missed information.
- Remember to record if it was a level (L) or heaped (H) spoon of for example sugar.
- Leave the last two columns open for coding.
- Review the full day with the caregiver to check for any foods or drinks that he/she may have forgotten at first.
- Ask if the child take any vitamin tablets or syrup and try to record as accurately as possible the brand name and how much of it is taken, for example one tablet or one medicine spoonful, or the teaspoon size.
- Thank the caregiver for his/her cooperation in the study.

References

- Lee D & Nieman DC. Nutritional assesment. Boston, McGraw-Hill, 2003. pp 73-79.
- Buzzard M. 24-Hour dietary recall and food record methods. In Willet WC, ed. Nutritional epidemiology. New York, Oxford University Press, 1998. pp 50-73.

Appendix 7

A sample copy of the standard operation procedure for breakfast maize meal porridge recipe preparation

STANDARD OPERATION PROCEDURE: RECIPE PREPARATION

Important points to remember:

1. It is important to follow the recipe for stiff and soft porridge.
2. Please indicate to us if you need any measuring equipment, for example, jugs to measure the water with.
3. Mix the micronutrient powder properly with the porridge so that the child gets ALL of it in the porridge.
4. Milk and/or sugar may be added to the porridge.
5. Serve the porridge while still warm. It should be eaten as soon as possible after the supplement has been added to the porridge.
6. Be careful not to mix the supplement containers' content. The yellow and green marked containers are different from each other.
7. Please follow the hygiene rules.
8. Only use the maize meal marked for the specific day of the week. There should be enough maize meal to make the recipes, as it was calculated from the numbers given by the school and the specific recipe.
9. Please store the maize meal in a safe place.
10. Maize meal will be delivered weekly to each school.

Please contact Mrs Noloyiso Matiwane at _____ when you encounter problems in connection with the food preparation.

Proper hand washing procedures

Personal Hygiene

It is vital that good standards of personal hygiene are maintained by food handlers. Contaminated hands will spread bacteria around a kitchen very quickly.

To prevent cross contamination of food it is essential to wash your hands frequently. Examples include:

- Before starting work.
- Before handling food.
- Between handling raw and ready to eat foods.
- After going to the toilet.
- After handling raw foods.
- After handling waste.
- After eating, drinking or smoking, coughing, sneezing or touching your face.
- After taking a break.
- After handling chemicals.
- After handling money.

Maize meal porridge, stiff (orange group)

5 – 45 portions

	Portions	Portions	Portions	Portions	Portions	Portions	Portions	Portions	Portions	
Ingredient	5	10	15	20	25	30	35	40	45	Method
Water, cups	4	8	12	16	20	24	28	32	36	Measure correctly in a pot.
Salt, teaspoon	½	1	1½	2	2½	3	3½	4	4½	Add to the water in the pot. Bring water to boil.
Maize meal, cups*	1½	3	4½	6	7½	9	10½	12	13½	Add maize meal to boiling water and stir until all the lumps are gone. Cook over low heat for 25-30 minutes, stirring a few times.
Dish up with the 118ml spoon (green handle) in individual yellow plates. The serving must be level to the spoon. Allow to cool for a minute.										
Mix (make sure to use the mix from the container with the yellow sticker).	1 level spoon per dish									Start with the first plate that was dished up and add the mix to the porridge. Use <u>only</u> the wooden spatula and spoon in the container and scrape the mix level with the spoon. Do not add more or less than a level spoon to each bowl of porridge. Mix in with the porridge and serve while still warm.
IF available, sugar and milk may be added to the porridge.										

It is very important to always use the **SAME CUP** for measuring the water **AND** the maize meal.

*Note: One standard portion size of stiff maize-meal porridge translates to approximately 35 grams of raw maize-meal flour.

Maize meal porridge, stiff (orange group)

50-90 portions

	Portions	Portions	Portions	Portions	Portions	Portions	Portions	Portions	Portions	
Ingredient	50	55	60	65	70	75	80	85	90	Method
Water, cups	40	44	48	52	56	60	64	68	72	Measure correctly in a pot.
Salt, teaspoon	5	5½	6	6½	7	7½	8	8½	9	Add to the water in the pot. Bring water to boil.
Maize meal, cups	15	16½	18	19½	21	22½	24	25½	27	Add maize meal to boiling water and stir until all the lumps are gone. Cook over low heat for 25-30 minutes, stirring a few times.
Dish up with the 118ml spoon (green handle) in individual yellow plates. The serving must be level to the spoon. Allow to cool for a minute.										
Mix (make sure to use the mix from the container with the yellow sticker).	1 level spoon per dish									Start with the first plate that was dished up and add the mix to the porridge. Use <u>only</u> the wooden spatula and spoon in the container and scrape the mix level with the spoon. Do not add more or less than a level spoon to each bowl of porridge. Mix in with the porridge and serve while still warm.
IF available, sugar and milk may be added to the porridge.										

It is very important to always use the **SAME CUP** for measuring the water **AND** the maize meal.

Maize meal porridge, soft (green group)

5-45 portions

	Portions	Portions	Portions	Portions	Portions	Portions	Portions	Portions	Portions	
Ingredient	5	10	15	20	25	30	35	40	45	Method
Water, cups	2	4	6	8	10	12	14	16	18	Measure correctly in a pot.
Salt, teaspoon	½	1	1½	2	2½	3	3½	4	4½	Add to the water in the pot. Bring water to boil.
Water, cold, cups	2	4	6	8	10	12	14	16	18	Mix the maize meal with the cold water and stir into boiling water. Cover and simmer over low heat until done, stirring a few times.
Maize meal, cups*	1	2	3	4	5	6	7	8	9	
Dish up with the 118ml spoon (green handle) in individual green plates. The serving must be level to the spoon. Allow to cool for a minute.										
Mix (make sure to use the mix from the container with the green sticker).	1 level spoon per dish									Start with the first plate that was dished up and add the mix to the porridge. Use <u>only</u> the wooden spatula and spoon in the container and scrape the mix level with the spoon. Do not add more or less than a level spoon to each bowl of porridge. Mix in with the porridge and serve while still warm.
IF available, sugar and milk may be added to the porridge.										

*Note: One standard portion size of soft maize-meal porridge translates to approximately 28 grams of raw maize-meal flour.

Maize meal porridge, soft (green group)

50-90 portions

	Portions	Portions	Portions	Portions	Portions	Portions	Portions	Portions	Portions	
Ingredient	50	55	60	65	70	75	80	85	90	Method
Water, cups	20	22	24	26	28	30	32	34	36	Measure correctly in a pot.
Salt, teaspoon	5	5½	6	6½	7	7½	8	8½	9	Add to the water in the pot. Bring water to boil.
Water, cold, cups	20	22	24	26	28	30	32	34	36	Mix the maize meal with the cold water and stir into boiling water. Cover and simmer over low heat until done, stirring a few times.
Maize meal, cups	10	11	12	13	14	15	16	17	18	
Dish up with the 118ml spoon (green handle) in individual green plates. The serving must be level to the spoon. Allow to cool for a minute.										
Mix (make sure to use the mix from the container with the green sticker).	1 level spoon per dish									Start with the first plate that was dished up and add the mix to the porridge. Use only the wooden spatula and spoon in the container and scrape the mix level with the spoon. Do not add more or less than a level spoon to each bowl of porridge. Mix in with the porridge and serve while still warm.
IF available, sugar and milk may be added to the porridge.										

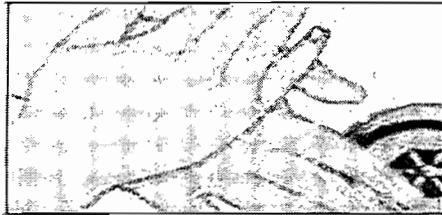
IF available, sugar and milk may be added to the porridge.

It is very important to always use the **SAME CUP** for measuring the water **AND** the maize meal.

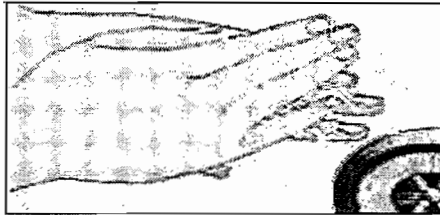
How to Wash Your Hands.

Use warm water and preferably antibacterial soap. After wetting hands, apply soap and use the following procedure to clean your hands thoroughly:

Six stage handwashing technique



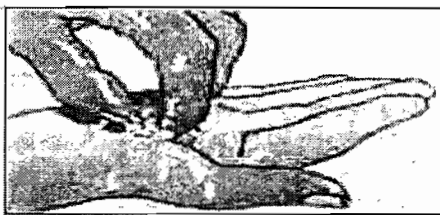
1. Palm to palm



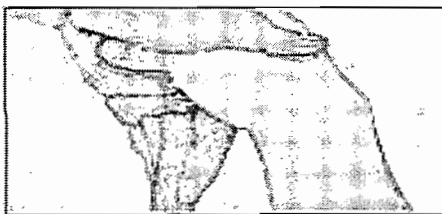
2. Backs of hands



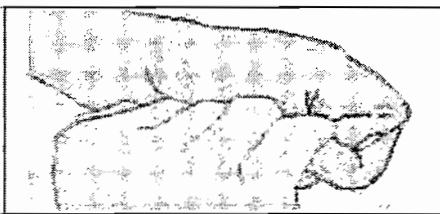
3. Interdigital spaces



4. Fingertips



5. Thumbs and wrists



6. Nails

Then rinse thoroughly with water.

PROPER HAND WASHING PROCEUDRES

Personal Hygiene

It is vital that good standards of personal hygiene are maintained by food handlers. Contaminated hands will spread bacteria around a kitchen very quickly.

To prevent cross contamination of food it is essential to wash your hands frequently. Examples include:

- Before starting work.
- Before handling food.
- Between handling raw and ready to eat foods.
- After going to the toilet.
- After handling raw foods.
- After handling waste.
- After eating, drinking or smoking, coughing, sneezing or touching your face.
- After taking a break.
- After handling chemicals.
- After handling money.

In addition, it is important that staff maintain a high degree of personal hygiene with regard their personal habits. For example:

- No smoking in food areas.
- No coughing, sneezing, spitting over food.
- No strong smelling perfumes should be worn when handling foods.
- No nail varnish should be worn when handling food.
- No jewellery other than a plain wedding band or sleeper earrings should be worn.

All cuts, wounds, sores should be covered with a waterproof dressing.

Overclothing should be clean and present no risk of contamination to food.

Hair should be tidy and covered where necessary to prevent the risk of it falling into food.

Staff should report to their supervisor if they have had symptoms of diarrhoea, vomiting, nausea, abdominal cramps or fever. These may be indications that they have or have had food poisoning. They should also inform their supervisor if they have infected cuts or wounds, boils or sores that may lead to the contamination of foods.

Reference

Personal hygiene http://www.eastdevon.gov.uk/google/personal_hygiene.htm

Appendix 8

A sample copy of the standard operation procedure for cognitive function assessment using the
Kauffman Assessment Battery for Children version 2

STANDARD OPERATION PROCEDURE: COGNITIVE FUNCTION ASSESSMENT

A. Ethical considerations in the field

- All work that is done with children or anyone else for that matter must be done with utmost care.
- You are expected to work with everyone with respect even the little ones.
- All records and results of the assessments to be done are to be maintained confidential and can only be discussed with the university staff on the project.
- You may not discuss how a child has done even among yourselves.
- Parents must not know how the child did.
- You must protect the identity of the child at all times.
- If something unusual happens discuss it with a member of the university research team.

B. Assessment Process

1. Introduction yourself to the child

- Tell the child your name and ask the child for his or her name.
- Let the child feel they are helping you and talk to the child in a manner that makes them feel they are doing something worthwhile.

2. Complete the child's details assessment form

Enter the following details:

Name of the child:

Study ID of child:

Child date of birth:

Name of preschool:

Date:

Name of assessor:

NB: If the child has not been assessed give a reason in the space provided.

3. Prepare for the assessment

- The sitting position should allow you to see where the child is pointing without disturbing the concentration of the child.
- If the child does not look well or extremely tired indicate that you will try another day and make the child feel comfortable about not doing the assessment today.
- If the child is very frightened do not force the assessment work with another child and then try again later.

4. During the assessment process

- Do not expect the children to go too far on the tests as these are very young children.
- The younger the children the less likely they are to get very far on the assessments.
- Organize yourself well so that you do not waste time unnecessarily.
- Avoid taking a break in the middle of a test especially Atlantis 1 which is the teaching test all through.
- When a child gets distracted keep reminding the child that you need to continue with the task in mind.
- Reassure the child that it will not take too long and you will soon be finished if he / she concentrates on what you are doing.
- Stick to the instructions as they are as they are given and do not give any clues.....
what does this mean? Any descriptions that would help the child decide on what the answer should be are not allowed.

5. Order of the testing

- Atlantis
- Conceptual thinking
- Hand movements
- Atlantis delayed

NB: You may not change this order!!

Appendix 9

Questionnaire for 24 hour dietary recall



NORTH-WEST UNIVERSITY
YUNIBESITHI YA BOKONE-BOPHIRIMA
HOOROWES-UNIVERSITEIT

THE MIXMe STUDY

24-hour dietary recall

(All information in this questionnaire is confidential and for research purposes only).

Participant number: _____ Interviewer: _____

Crèche: _____ Date: ___/___/200__

Tick what day was yesterday:

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
--------	--------	---------	-----------	----------	--------

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

Yes	1	No	2
-----	---	----	---

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

Time (Approximately)	Place (Home, school, etc)	Description of food and preparation method	Amount	Amount in g (office use only)	Code (office use only)
From waking up to going to crèche, or starting day's activities					
During the morning at the crèche					
Middle of the day (Lunch time)					
During the afternoon					
At night (dinner time)					
After dinner, before going to sleep					
Do you take any vitamins (tablet or syrup)?			Yes	1	No 2
Give the brand name and dose of the vitamin/tonic:					

Appendix 10

Questionnaire for Socio-demographic status



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

THE MIXMe STUDY

Socio-demographic questionnaire

(All information in this questionnaire is confidential and for research purposes only)

Interviewers Name: _____

Interview Date: _____

Subject Number: _____

Age: _____

Name of Preschool: _____

1. Home language	1 Zulu	2 English	3 Sesotho	4 Setswana	5 Xhosa	6 Other specify
2. Type of dwelling: (You can tick more than one block if necessary)	1 Brick, Concrete	2 Traditiona l mud	3 Tin	4 Plank, Wood	5 Other, specify	
3. Number of people living in the your household (Tick one)	1 <4 persons	2 4-6 persons	3 7-8 persons	4 >8 persons	5 Don't know	
4. Where do you get drinking water most of the time (Tick one)	1 Own Tap	2 Commun al Tap	3 River, Dam	4 Borehole, Well	5 Other: Specify	
5. What type of toilet does your household have? (Tick one)	1 Flush	2 Pit	3 Bucket, Pot	4 Ventilated Improved Pit latrine (VIP)	5 Other (Specify)	
6. What fuel is used for cooking most of the time in your household? (You can tick more than one)	1 Electric	2 Gas	3 Paraffin	4 Wood/ coal	5 Sun	6 Open Fire
						7 Don't know
7. Do you have access to electricity inside your house?	1 Yes	2 No				
8. Does your household have a working:	1	2	3	4		
1. Refrigerator / Freezer	Fridge	Freezer	Fridge/freezer combination	Don't know		
2. Stove	1 Yes	2 No	If yes, choose one Coal Paraffin		Gas	Electric
3. Washing machine	1 Yes	2 No				
4. Microwave	1 Yes	2 No				
5. Television	1 Yes	2 No				
6. Radio	1 Yes	2 No				

Appendix 11

A sample copy of the compliance register (orange sheets were used for the intervention group; green sheets were used for the control group)

Name of preschool:

Class list

Orange group

First name	Family name	Casual name	Gender

Instructions:

- Children not part of the study (purple group) should also receive soft porridge but without added micronutrient powder
- Read the child's name and indicate presence on the applicable form. The two groups (Green and Orange) should sit apart from each other.
- Dish up the porridge as indicated on the recipe:
 - Orange group: Stiff porridge with micronutrient powder; and
 - Green group: Soft porridge with placebo powder.

Make sure that:

- The child does not exchange his/her porridge with someone else.
- Complete the form everyday for each child. If a child is absent and does not eat the porridge, mark with an "A" for absent for that specific date on the applicable form. If the child does not want to eat the porridge, mark the reason for this, for example, "B" child not hungry (See attached list for applicable codes).

Name of preschool:

LIST WITH APPLICABLE CODES

Reason why food has not been eaten		Reason for absence			
Code	Reason*	Code	Reason*	Code	Reason*
A	Child absent	1	Influenza / lung infection	10	Skin infection
B	Child has no appetite for food, for example feels ill	2	Diarrhea / stomach / vomiting	11	Injury or broken arm
C	Food not available today	3	Measles	12	Not ill, another reason
D	Child late for school	4	German measles	13	Not in school anymore
E	Too much food for the child	5	Chicken pox	14	No reason
F		6	Mumps	15	Other reasons
G		7	Headache	16	
H		8	Ear infection	17	
I		9	Eye infection	18	

*Add any other reasons (not found in the list) for not eating the food F, G, H, I.

*Add any other reasons for absence (not found in the list 16, 17, 18.

Name of preschool:

Study week:

First name	Family name	Casual name	Gender	Monday 15/9	Tuesday 16/9	Wednesday 17/9	Thursday 18/9	Friday 19/9

Notes for study leader's attention:

Appendix 12

Selected pictures during the training of the front-line staff



Appendix 13

Selected pictures during the implementation of the pilot study

