

# **Effect of a micronutrient-fortified beverage on cognition and nutritional status of primary school children**

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**Thesis submitted for the degree *Philosophiae Doctor* in Nutrition at the Potchefstroom campus of the North-West University**



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**December 2012**

To my Shepherd, Savior, Helper and Friend

To the One who was, and is and is to come.

To the One who set my feet on a solid rock and gave me a firm place to stand.

To the One who gave me a new song to sing, a hymn of praise to my God.

To the One whose ways are higher than my ways, and whose thoughts are higher than my thoughts.

By Your mighty power that works within me, I was able to accomplish infinitely more than I would ever have dared to ask or **hope** for.

May this work reflect Your name and Your name alone, may Your kingdom come, and Your will be done.



In loving memory of my sister, Elzanne Taljaard

1985-02-06 to 2012-01-25

Thank you for showing me the meaning of life

## ACKNOWLEDGEMENTS

**May God bless those listed below with this blessing, for they have truly been a blessing to me:**

The **LORD** your **God** is with you, he is mighty to save. **He** will take great delight in you, he will quiet you with his love, he will rejoice over you with singing.

The **LORD** bless you and keep you; the **LORD** make his face shine on you and be gracious to you; the **LORD** turn his face toward you and give you peace.

**Zephaniah 3 & Numbers 6**



### **My Beloved Parents**

Ian and Charlotte Taljaard

### **My Beautiful Sister**

Carla Taljaard

### **My Dear Friends**

Yolandi Yssel

Zelda de Lange

### **My Respected Mentors**

Averalde van Graan

Namukolo Covic

Salome Kruger

Johann Jerling

Marius Smuts

Jeannine Baumgartner

## ABSTRACT

Childhood micronutrient deficiencies have negative effects on cognition. Little is known about the effects of combined consumption of micronutrients and sugar on growth and cognitive function.

The aim of this thesis was to 1) investigate the effects of micronutrients and sugar, alone and in combination, in a beverage, on growth and cognition in South African children and 2) review recent evidence on iron status and anaemia prevalence in South African children since the National Food Consumption Survey-Fortification Baseline-2005 (NFCS-FB-2005).

Children (n = 408, 6–11 years) were randomly allocated to a beverage containing 1) micronutrients with sugar, 2) micronutrients with non-nutritive sweetener, 3) no micronutrients with sugar, or 4) no micronutrients with non-nutritive sweetener for 8.5 months. Cognition was assessed using sub-tests from the Kaufman Assessment Battery for Children-II. Growth was assessed as weight-for-age (WAZ), height-for-age and body-mass-index-for-age z-scores.

Relevant internet search engines identified studies reporting iron status of South African children after 2005. Secondary analysis was conducted on NFCS-FB-2005 provincial data for children 7–9 years old.

Positive intervention effects were observed for micronutrients (0.76; 95% CI: 0.10, 1.42) and sugar (0.71; 95% CI: 0.05, 1.37) on Atlantis (measure learning ability), and sugar on Rover (measure simultaneous processing) (0.72; 96% CI: 0.08, 1.35) test scores. Attenuating micronutrient x sugar interactions were observed on Atlantis, Number Recall (measure sequential processing) and Rover test performance. Micronutrients or sugar alone lowered WAZ. In combination, this effect was attenuated (significant micronutrient x sugar interaction).

Four studies from four different provinces were identified. All reported lower anaemia prevalence than the NFCS-FB-2005 (KwaZulu-Natal (11.5% vs 14.4%), North West (6.9% vs 27%) Western Cape (17.2% vs 18.8%) and Northern Cape (5.4% vs 22.2%).

A beverage fortified with micronutrients or added sugar had beneficial effects on cognition, but a lowering effect on WAZ in the children. Unexpectedly, the combination of micronutrients and sugar attenuated these effects. In the identified studies, anaemia prevalence in school-aged children was lower than reported in the NFCS-FB-2005.

**KEYWORDS:** anaemia, cognition, growth, micronutrient status, micronutrient fortification

## OPSOMMING

Mikronutriënttekorte in kinders het negatiewe gevolge op kognisie. Tans is min bekend oor die gesamentlike effek van mikronutriënte en suiker op groei en kognitiewe funksie.

Die doelwit van hierdie proefskrif is om 1) die effek van mikronutriënte en suiker in 'n koeldrank, afsonderlik en gekombineerd op groei en kognisie te ondersoek in Suid-Afrikaanse kinders en 2) 'n oorsig te gee oor onlangse bewyse van die voorkoms van anemie in Suid-Afrikaanse kinders sedert die "Nasionale Voedselverbruiksopname-Fortifiserings Basislyn-2005 (NVVO-FB-2005)."

Kinders (n = 408, 6-11 jaar) is ewekansig aangewys om 'n koeldrank in te neem oor 'n tydperk van agt en 'n half maande wat bevat: 1) mikronutriënte met suiker, 2) mikronutriënte met kunsmatige versoeter, 3) geen mikronutriënte maar met suiker en 4) geen mikronutriënte maar met kunsmatige versoeter. Geselekteerde kognitiewetoetse van die Kaufman-assesseringsbattery vir kinders-II is gebruik. Z-tellings vir gewig-vir-ouderdom, lengte-vir-ouderdom, en liggaamsmassa-indeks-vir-ouderdom is bepaal.

Relevante internetsoekenjins is gebruik om studies te identifiseer wat ysterstatus van Suid-Afrikaanse kinders sedert 2005 rapporteer. Sekondêre data-analise van die NVVO-FB-2005 se provinsiale data vir kinders 7-9 jaar is uitgevoer.

Positiewe intervensie-effekte is gevind vir mikronutriënte (0.76; 95% VI: 0.10, 1.42) en suiker (0.71; 95% VI: 0.05, 1.37) op Atlantis (maatstaf vir leervermoë) asook vir suiker op Rover (maatstaf vir opeenvolgende-verwerking) (0.72; 96% VI: 0.08, 1.35). Betekenisvolle interaksies tussen mikronutriënte en suiker is opgemerk vir Atlantis, Syferherroep (maatstaf vir sekweniële-verwerking) en Rover, maar die voordelige effek was laer wanneer dit vergelyk is met die afsonderlike effekte van mikronutriënte en suiker. Mikronutriënte en suiker afsonderlik het z-tellings vir gewig-vir-ouderdom verlaag. In kombinasie was die effek weereens verder verlaag.

Vier studies van vier verskillende provinsies is geïdentifiseer. Die studies het 'n laer voorkoms van anemie gerapporteer as die NVVO-FB-2005 (KwaZulu-Natal (11.5% vs. 14.4%), Noord-Wes (6.9% vs. 27%) Wes-Kaap (17.2% vs. 18.8%) en Noord-Kaap (5.4% vs. 22.2%).

Mikronutriënte en suiker het voordelige effekte op kognisie en 'n verlaagde effek op z-tellings vir gewig-vir-ouderdom in kinders. In kombinasie word hierdie effekte verder verlaag. Anemie vir die spesifieke ouderdomskategorie is laer as gerapporteer in die NVVO-FB-2005.

**SLEUTELWOORDE:** anemie, kognisie, groei, mikronutriëntstatus, mikronutriëntfortifisering

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**ABBREVIATIONS**

ANCOVA:	analysis of covariance
ANOVA:	analysis of variance
BeForMi:	beverage fortified with micronutrients
BAZ:	body mass index-for-age z-score
CEN:	Centre of Excellence for Nutrition
CI:	confidence interval
CNS:	control beverage with non-nutritive sweetener
CRP:	c-reactive protein
CS:	control beverage with sugar
EAR:	estimated average requirements
EER:	estimated energy requirements
ELISA:	enzyme-linked immunosorbent assays
DOH:	Department of Health
DNA:	deoxyribonucleic acid
FAO:	Food and Agriculture Organization
FCDA:	Foodstuffs, cosmetics and disinfectants act
HAZ:	height-for-age z-score
Hb:	haemoglobin
ID:	iron deficiency
IDA:	iron deficiency anaemia
INP:	Integrated Nutrition Programme

## ABBREVIATIONS

IQ:	intelligence quotient
ISAK:	International Standards for Anthropometric Assessment
IU:	international units
IVACG:	International Vitamin A Consultative Group
IZINCG:	International Zinc Nutrition Consultative Group
KABC-II:	Kaufman assessment battery for children II
MDG:	millennium development goals
mg:	milligram
MPI:	mental processing index
MNNS:	beverage fortified with micronutrients and non-nutritive sweetener
MNS:	beverage fortified with micronutrients and sugar
NWU:	North-West University
NFCS:	National Food Consumption Survey
NFCS-FB:	National Food Consumption Survey-Fortification Baseline
NFFP:	National Food Fortification Programme
NHANES:	National Health and Nutritional Examination Survey
NCHS:	National Center for Health Statistics
NSNP:	National School Nutrition Programme
PSNP:	Primary School Nutrition Programme
RDA:	recommended dietary allowance
RNA:	ribonucleic acid
SAVACG:	the South African vitamin A consultative group

## ABBREVIATIONS

SD:	standard deviation
SF:	serum ferritin
SR:	serum retinol
SZn:	serum zinc
SUN:	scaling-up nutrition
TfR:	transferrin receptor
WAZ:	weight-for-age z-score
WHO:	World Health Organization
UNICEF:	The United Nations Children's Fund
USD:	United States dollar
WISC:	Wechsler intelligence scale for children
µg:	microgram
ZnPP:	zinc protoporphyrin

## GLOSSARY

<b>Anaemia</b>	In clinical terms <i>anaemia</i> is an insufficient mass of red blood cells circulating in the blood; in public health terms <i>anaemia</i> is defined as a haemoglobin concentration below a specific threshold that varies by age and gender.
<b>Anthropometric index</b>	An international reference that includes standardized age- and sex-specific growth reference to calculate height-for-age Z-scores (HAZ), weight-for-age Z-scores (WAZ), and body-mass-index for-age Z-scores (BAZ).
<b>Cognition</b>	High level physiological processes involved in perception, attention, memory, language, problem solving, reasoning, and making decisions.
<b>Cognitive development</b>	The construction of thought processes, including remembering, problem solving, and decision-making beginning in infancy and continuing to change or progressively improve through adolescent and adulthood.
<b>Cognitive function</b>	The process of taking information from the environment, processing this information internally and finally, responding to the information through specific behavior. Intellectual/mental processes involving symbolic operations such as learning, memory, thinking, movement, reasoning, attention and language. Cognitive function can be divided into executive, memory, attention, perception and psychomotor functions and language skills.
<b>Cognitive performance</b>	An expression of a desired result of a learning experience according to specific cognitive tests conducted.
<b>Dietary diversification</b>	Improving the availability, accessibility and utilisation of foods high in bio-available micronutrients throughout the year.
<b>Estimated average requirements</b>	The average daily nutrient intake level estimated to meet the requirements of half of the healthy individuals in a group.

<b>Estimated energy requirements</b>	The average dietary energy intake that is predicted to maintain energy balance in healthy, normal weight individuals of a defined age, gender, weight, height, and level of physical activity consistent with good health.
<b>Food Fortification</b>	The addition of one or more essential nutrients to a food, whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups
<b>Iron Deficiency Anaemia</b>	The final stage of the development of iron deficiency, when iron stores are exhausted, circulating iron is very low, red cell production is drastically reduced and anaemia develops. Indicated by age appropriate haemoglobin and serum ferritin values.
<b>Iron deficiency</b>	A state in which there is insufficient iron to maintain the normal physiological function of tissues such as the blood, brain, and muscles. Iron deficiency can exist in the absence of anaemia if it has not lasted long enough or if it has not been severe enough to cause the haemoglobin concentration to fall below the threshold for the specific gender and age group.
<b>Malnutrition</b>	Malnutrition is a broad term commonly used as an alternative to undernutrition but also refers to overnutrition.
<b>Market-driven fortification</b>	The situation where the food manufacturer takes the initiative to add one or more micronutrients to processed foods, usually within regulatory limits, in order to increase sales and profitability.
<b>Mass fortification</b>	The addition of micronutrients to foods commonly consumed by the general public, such as cereals, condiments and milk.
<b>Nutritional status</b>	The state of a person's health in terms of the nutrients in his or her diet and the extent to which nutrients are available to meet metabolic needs.
<b>Obesity</b>	Abnormal or excessive fat accumulation that may impair health. Children: Body mass index at or above the 95 <sup>th</sup> percentile for children of

the same age and sex (as defined by the World Health Organization)

**Overweight**

Abnormal or excessive fat accumulation that may impair health.

Children: Body mass index at or above the 85<sup>th</sup> percentile and lower than the 95<sup>th</sup> percentile for children of the same age and sex (as defined by the World Health Organization)

**Stunting**

The result of long-term nutritional deprivation that reflects on a process of failure to reach linear growth potential.

Height-for-age < -2 standard deviations of the WHO Child Growth Standards median

**Sugar-sweetened beverage**

Sugar-sweetened beverages contain added, naturally-derived caloric sweeteners such as sucrose (table sugar), high-fructose corn syrup, or fruit juice concentrates.

These include soft drinks (soda or pop), fruit drinks, sports drinks, tea and coffee drinks, energy drinks, sweetened milk or milk alternatives, and any other beverages to which sugar, typically high fructose corn syrup or sucrose (table sugar), has been added

**Supplementation**

The periodic administration of pharmacological preparations of nutrients, either in the form of capsules and tablets.

**Targeted fortification**

The fortification of foods designed for specific population subgroups, such as complementary weaning foods for infants.

**Underweight**

Reflecting on body mass relative to chronological age.

BMI-for-age < -2 standard deviations of the World Health Organization Child Growth Standards median

**Wasting**

Wasting or thinness indicates in most cases a recent and severe process of weight loss, often associated with acute starvation and/or severe disease.

Weight-for-height < -2 standard deviations of the WHO Child Growth Standards median

# CHAPTER 1: INTRODUCTION



## 1.1 Background and motivation

Large parts of the world are still burdened by hunger and inadequate food supply, with sixteen percent of people in the developing world suffering from hunger (United Nations, 2011; United Nations system & Standing committee on nutrition, 2010). The consequences of this burden on health and well-being are far-reaching, especially in children. Lives are being compromised through the effect of malnutrition on physical and mental development (United Nations system & Standing committee on nutrition, 2010, Victora *et al.*, 2008).

The cost of undernutrition worldwide is astonishing. More than 3.5 million children die each year because of undernutrition (Horton *et al.*, 2010). Besides the cost in human lives, the economic burden of malnutrition has recently been recognised by the World Bank, which urges organisations and governments to acknowledge the importance of nutrition (The World Bank, 2006). More recently, a panel of economic experts was invited to address ten challenge areas and to answer the following question:

*“What are the best ways of advancing global welfare, and particularly the welfare of developing countries, illustrated by supposing that an additional \$75 billion of resources were at their disposal over a 4-year initial period?”* (Copenhagen Consensus Centre, 2012).

Several research papers were commissioned, forming the background information considered by the panellists in providing their recommendations. From the ten Challenge Papers that were commissioned, 39 proposals were set before the panel. Challenges that were examined included armed conflict, biodiversity, chronic disease, climate change, education, hunger and malnutrition, infectious disease, natural disasters, population growth and water and sanitation. The primary consideration was the economic costs and benefits of the proposals. The panel concluded that fighting malnourishment should be the top priority for policy makers (Copenhagen Consensus Centre, 2012).

Malnutrition (macronutrient and micronutrient deficiencies) leads to decreased productivity primarily through a direct loss of physical ability, indirect loss from poor cognitive performance and loss of resources from increased healthcare costs (Hoddinott *et al.*, 2012). Undernutrition refers not only to chronic and acute malnutrition (stunting and wasting) but also to micronutrient deficiencies. Micronutrient deficiencies can be described as “hidden hunger”. Although the symptoms of micronutrient deficiencies are easily overlooked, they negatively affect cognitive development and function (Black *et al.*, 2008; Victora *et al.*, 2008).

Undernutrition is a problem worldwide, but even more so in developing countries. In South Africa, acute and chronic malnutrition contribute to poor nutritional status among children. For country representative data, national food surveys have been conducted. Three such surveys have been conducted over the last fifteen years. These include the South African Vitamin A Consultative Group (SAVACG-1994) (SAVACG, 1995), National Food Consumption Survey (NFCS-1999) (Labadarios, 2000) and the National Food Consumption Survey-Fortification Baseline (NFCS-FB-2005) (Labadarios, 2007). The last survey served as baseline measurements for the National Food Fortification Programme (NFFP) that was implemented in 2003.

In a comparison of the above-mentioned surveys, the following observations can be made for South African children from one to five years of age. Between 1994 and 2005, the prevalence of wasting seemingly remained the same, at about 9%. Stunting rates declined from 22.9% to 18% between 1994 and 2005. The prevalence of anaemia and iron deficiency anaemia increased from 21.4% to 28.9% and from 5.0% to 11.3%, respectively (Labadarios, 2007).

While there seems to be a promising decline in stunting prevalence, some trends observed, such as the increased prevalence of anaemia, are matters of greater concern. It is important to note that the latest data mentioned above are now almost ten years old. The lack of recent up-to-date data prevents nutritionists, academics and the government from evaluating the efficacy of a programme such as the NFFP, and from defining the current challenges that need to be addressed.

The nutrition transition that South Africa is currently undergoing is characterised by a change in dietary patterns and physical activity levels, which contributes to a double burden of malnutrition (Vorster, 2010). This means that undernutrition and overnutrition are now commonly found within the same households. In South Africa in 2006, 13% of girls and almost 11% of boys were reported to be overweight. Furthermore, 5% of girls and 3.2% of boys were obese (Armstrong *et al.*, 2006).

While some research indicates that sugar-sweetened beverages may cause weight gain and obesity in children (Cassady *et al.*, 2012; Malik *et al.*, 2010), others believe that the results are inconclusive (Bachman *et al.*, 2006). Although consumption of a sugar-sweetened beverage may have an effect on anthropometric indicators, there is also the possible effect on cognition. The rate of glucose consumption between the ages of four and ten years is double the glucose consumption of the adult brain (Chugani, 1998). Therefore, when glucose, as the primary fuel

of the brain, fluctuates, cognitive function may be negatively affected (Bellisle, 2004). Several studies have reported that administration of glucose *via* a beverage improves some aspects of cognition such as memory and attention (Benton *et al.*, 1987; Benton & Stevens, 2008).

It is clear that malnutrition is a reality in the daily lives of South African children and that it cannot be ignored. The dietary intake of South African children has been shown to be below the recommended daily allowance values for macronutrients and micronutrients (MacKeown *et al.*, 2007; NFCS, 1999). Inadequate dietary intake plays a key role in malnutrition (Black *et al.*, 2008). Furthermore, a diet that is deficient in one micronutrient is likely to be deficient in others too (Benton, 2008). Iron, zinc, iodine and vitamin A have all been shown to have an impact on cognition (Eilander *et al.*, 2010; Hubbs-Tait *et al.*, 2005). Extensive research has been done on the mechanisms by which certain micronutrients may influence cognition, but these mechanisms remain mostly unclear.

Brain development, especially with regard to the frontal lobe, continues throughout childhood, and nutrition, including micronutrients and macronutrients, is likely to impact cognitive function (Bryan *et al.*, 2004). Cognitive function may be negatively affected through acute and chronic malnutrition. Acute malnutrition presents as wasting, which causes a child to be more apathetic, exploring the environment less and being less active (Grantham-McGregor, 1995). Long-term, chronic malnutrition, presenting as stunting, may retard cognitive development (Jukes, 2006; Victora *et al.*, 2008) and it is also possible that once this retardation of cognitive development takes place reversal may be less likely to occur with increasing age (McKay *et al.*, 1978).

The failure to find positive effects on cognitive performance with the administration of a single micronutrient could be due to a state of multi-micronutrient deficiencies. Therefore, it is important to investigate the effects on cognitive performance of providing a multi-micronutrient beverage.

In the words of Nobel laureate, economist Vernon Smith “*One of the most compelling investments is to get nutrients to the world’s undernourished. The benefits from doing so – in terms of increased health, schooling, and productivity – are tremendous*” (Copenhagen Consensus Centre, 2012).

Sustainable intervention programmes targeting children suffering from either acute or chronic malnutrition are necessary. For this reason the **Beverage Fortified with Micronutrients (BeForMi)** study was an intervention study conducted to investigate the effects of a multi-micronutrient-fortified beverage, with or without sugar, on the micronutrient status, cognitive function and growth of primary school children in South Africa aged 6 to 11 years.

Observations on anaemia prevalence and iron status from the BeForMi study contributed to the second article included in this thesis. The unexpectedly low prevalence of anaemia in the province led the researchers to investigate the anaemia prevalence that was reported by independent studies in other provinces as well.

## **1.2 Title of PhD thesis**

Effect of a micronutrient-fortified beverage on cognition and nutritional status of primary school children.

## **1.3 Aim, hypothesis and objectives**

### **1.3.1 Aim**

The primary aim of the thesis was to investigate the effects of a beverage fortified with micronutrients, with or without sugar, on the micronutrient status, cognitive performance and growth of South African primary school children between the ages of 6 and 11 years.

The secondary aim was to review the iron status and the anaemia prevalence in South African primary school children as observed by independent intervention and cross-sectional studies conducted in different parts of the country since the latest National Food Consumption Survey-Fortification Baseline in 2005 (NFCS-FB-2005) and to compare the findings reported by these independent studies with the results of the NFCS-FB-2005.

### **1.3.2 Objectives**

**1.3.2.1 Objectives for primary aim:** To investigate the effects of a beverage fortified with micronutrients, with or without sugar, on micronutrient status, cognitive performance and growth in South African primary school children between the ages of 6 and 11 years.

- a. To determine micronutrient intake in children consuming a beverage fortified with micronutrients and in children receiving a beverage without micronutrients. In addition, to determine the micronutrient dietary intake with and without the micronutrient-fortified beverage in the group of children that consumed the fortified beverage.
- b. To determine and compare end biochemical indicators of the four treatment groups and to determine the treatment effect of micronutrients on biochemical indicators. These biochemical indicators include:

Zinc protoporphyrin (ZnPP):	µmol/mol heme
Serum transferrin receptor (TfR):	mg/L
Haemoglobin (Hb):	g/dL
Serum ferritin (SF):	µg/L
Serum zinc (SZn):	µg/dL
Serum retinol (SR):	µg/dL

- c. To determine the treatment effects of micronutrients and sugar on cognitive test performance.
- d. To determine the treatment effects of micronutrients and sugar on weight-for-age (WAZ), height-for-age (HAZ) and body-mass-index-for-age (BAZ) z-scores.
- e. To determine potential interaction effects of sugar and micronutrients on biochemical indicators (ZnPP, TfR, Hb, SF, SZn, SR).
- f. To determine potential interaction effects of sugar and micronutrients on the selected KABC-II tests
- g. To determine potential interaction effects of sugar and micronutrients on the WAZ, HAZ and BAZ.

**1.3.2.2 Objectives for secondary aim:** To review the iron status of South African primary school children as observed by independent studies conducted in different parts of the country since the latest NFCS-FB-2005.

- a. To review iron status and anaemia by conducting a literature search and selecting studies that reported on Hb, SF, ZnPP and TfR after the NFCS-FB-2005 in primary school children.

### 1.3.3 Hypotheses:

This study tested the following hypotheses:

**Hypothesis 1:** Administration of a micronutrient-fortified beverage for a period of 8.5 months in apparently healthy schoolchildren between the ages of 6 and 11 years

- a. improves dietary intake of iron, zinc and vitamin A, as measured by repeated 24-hour recall questionnaires, to the extent that the estimated average requirements (EAR) are reached.
- b. improves iron (based on the iron status indicators ZnPP, SF and TfR), zinc (based on SZn) and vitamin A (based on SR) status and anaemia prevalence (based on Hb).
- c. improves cognitive performance as assessed with the Kaufman Assessment Battery for Children, Second Edition (KABC-II).
- d. improves growth as indicated by increases in WAZ, HAZ and BAZ.

**Hypothesis 2:** Administration of a beverage with added sugar, for a period of 8.5 months in apparently healthy schoolchildren between the ages of 6 and 11 years

- a. improves energy intake to reach the estimated energy requirements (EER).
- b. improves cognitive performance as assessed with the KABC-II.
- c. improves growth as indicated by increases in WAZ, HAZ and BAZ.

**Hypothesis 3:** Administration of a beverage fortified with micronutrients and added sugar in combination

- a. improves cognitive performance assessed with the KABC-II to a greater extent than administration of micronutrients and sugar alone.
- b. improves growth as indicated by WAZ, HAZ and BAZ to a greater extent than administration of micronutrients and sugar alone.

**Hypothesis 4:** South African independent studies report a lower prevalence of iron deficiency and anaemia compared with the NFCS-FB-2005.

## 1.4 Research team and authors' contributions

**Table 1.1** Research team of the BeForMi study 2010

Team member	Institution	Role
Miss Christine Taljaard	CEN, NWU Potchefstroom Campus	<b>Key role in day-to-day administration of project intervention, assistance in baseline and end anthropometric, biochemical and cognitive measurements and statistical analysis</b>
Dr Namukolo Covic	CEN, NWU Potchefstroom Campus	Project Director: directing all aspects of the project and stakeholder communications; training of cognitive assessors and school assistants
Prof. Johann Jerling	CEN, NWU Potchefstroom Campus	Project leader: all aspects of the project. Data base manager
Dr Jane Kvalsvig	Child Development Research Unit Kwazulu Natal	Overseeing cognitive assessor training. Guidance on all aspects of cognitive assessment
Prof. Marius Smuts	CEN, NWU Potchefstroom Campus	Intervention process logistical advice
Prof. Salome Kruger	CEN, NWU Potchefstroom Campus	Advisory role on body composition and on anthropometric measurements. Processing of dietary data
Sr Chrissie Lessing	CEN, NWU Potchefstroom Campus	Blood sampling logistical process and blood sampling
Mrs Noloyiso Matiwane	CEN, NWU Potchefstroom Campus	Assistance in training of field assistants, coordination of study participants
Dr Averalda van Graan Dr Hattie Wright	CEN, NWU Potchefstroom Campus	Assistance in anthropometric measurements
Mrs Ellenor Rossouw, Dr Seye Onabanjo Dr Wayne Tower Dr Karin Conradie	CEN, NWU Potchefstroom Campus	Laboratory analysis
Mr Thabang Phinda	CEN, NWU Potchefstroom Campus	Data input Day-to-day project logistics
Mrs Sarie Lee	CEN, NWU Potchefstroom Campus	Dietary data input

CEN, Centre of Excellence for Nutrition, NWU, North-West University

**Table 1.2** Level of involvement of the student, and authors' contributions to the article: "Effects of a multi-micronutrient-fortified beverage, with and without sugar, on growth and cognition in South African schoolchildren: a randomised, double-blind, controlled intervention".

<b>Team member</b>	<b>Institution</b>	<b>Role</b>
<b>Miss Christine Taljaard</b>	<b>CEN, NWU, Potchefstroom Campus</b>	<b>Full-time PhD student</b> <b>Protocol writing</b> <b>Statistical analysis</b> <b>Article writing</b>
Dr Namukolo Covic	CEN, NWU Potchefstroom Campus	Project Director and Promoter of PhD. dissertation Provided guidance to the student at all stages of the project
Dr Averalda van Graan	CEN, NWU Potchefstroom Campus	Co-Promoter of PhD dissertation Provided guidance to the student at all stages of the project
Prof. Salome Kruger	CEN, NWU Potchefstroom Campus	Co-Promoter of PhD. dissertation Provided guidance to the student at all stages of the project
Prof. Marius Smuts	CEN, NWU Potchefstroom Campus	Scientific input
Dr. Jeannine Baumgartner	CEN, NWU Potchefstroom Campus	Guidance on statistical analysis and scientific input
Dr Jane Kvalsvig	Departments of Public Health Medicine and Psychology, University of KwaZulu-Natal, Howard College campus, Durban	Scientific input
Dr. Lize van Stuijvenberg	Medical Research Council	Scientific input
Dr Hattie Wright	CEN, NWU Potchefstroom Campus	Scientific input
Prof. Johann Jerling	CEN, NWU Potchefstroom Campus	Project leader; scientific input

CEN, Centre of Excellence for Nutrition, NWU, North-West University

Included is a statement from the co-authors, confirming their role in the article and providing permission for the inclusion of the article in this dissertation.

I declare that I have approved the above-mentioned article, 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 PhD thesis of Miss C. Taljaard.

Dr. N.M Covic

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Dr. A van Graan

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Prof. H.S Kruger

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Dr. H.H Wright

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Dr. J.D Kvalsvig

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Prof. J.C Jerling

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Prof. C.M Smuts

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Dr. L van Stuijvenberg



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Dr. J. Baumgartner

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**Table 1.3** Level of involvement of the student, and authors' contributions to the review: "Studies of South African schoolchildren since 2005 suggest lower anaemia prevalence in some regions".

<b>Team member</b>	<b>Institution</b>	<b>Role</b>
<b>Miss Christine Taljaard</b>	<b>CEN, NWU Potchefstroom Campus</b>	<b>Full-time PhD student Statistical analysis Data-extraction Article writing</b>
Dr Namukolo Covic	CEN, NWU Potchefstroom Campus	Scientific input
Dr Averalda van Graan	CEN, NWU Potchefstroom Campus	Scientific input
Prof. Salome Kruger	CEN, NWU Potchefstroom Campus	Scientific input
Prof. Johann Jerling	CEN, NWU Potchefstroom Campus	Data extraction Scientific input

CEN, Centre of Excellence for Nutrition, NWU, North-West University

Included is a statement from the co-authors, confirming their role in the article and providing permission for the inclusion of the article in this dissertation.

I declare that I have approved the above-mentioned article, 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 PhD thesis of Miss C. Taljaard.

Dr. N.M Covic

Dr. A van Graan

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Prof. H.S Kruger

Prof. J.C Jerling

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## **1.5 Other study contributors**

The following persons served as school assistants and cognitive assessors and their hard work and contribution to the BeForMi intervention study are hereby acknowledged:

School assistants: Thandiwe Ntjengela, Mmamusi Montsho, Itumeleng Ramorou, Puleng Mokoena, Mmasabata Tsolo, Moipone Mbipha, Refilwe Mokgothu, Mpho Paraffin, Nosiphe Marhawule, Matshidiso Mokgosi, Innocent Tshiloane and Mpho Chauke.

Cognitive assessors: Victoria Nobatana, Gladys Couter, Joseph Dithipe, Lerato Kenosi, Nonasonto Mngxongo, Thabang Phinda, Lydia Mogapi and Alinah Tlhale, as well as Namapolise Mildred Thomas, who helped with logistics during the assessment period.

To Sr Chrissie Lessing and her team who conducted all the blood sampling and the logistics of the process, heartfelt thanks for all the work done.

## **1.6 Structure of this thesis**

This thesis is presented in article format. The technical aspects of this thesis follow the guidelines in the postgraduate manual of the North-West University (font Arial, size 11). For chapter three, which includes two articles, the authors' guidelines of the respective journals have been used for technical aspects and referencing.

Chapter 2 provides background information for the thesis and puts the research topic into perspective. It includes the aim, objectives and hypothesis, the authors' contributions and the thesis outline. Chapter 1 is followed by the literature review (chapter 2).

In this chapter, available literature has been reviewed with regard to malnutrition (including under- and overnutrition) and its far-reaching consequences, with the focus on children of primary school age. Malnutrition as a public health problem and the nutritional intervention strategies used to address undernutrition are discussed. Current nutrition intervention programmes in South Africa are highlighted. The link between overweight and obesity and the consumption of a sugar containing beverage is discussed as well as the role of glucose in the brain. Possible mechanisms through which iron, vitamin A, iodine and zinc could affect cognitive function are addressed. Furthermore, the effects of micronutrient malnutrition on cognitive function and development are discussed. The chapter closes with a brief discussion on the cognitive assessment kit used for this research project.

Chapter 3 contains two articles:

- Effects of a multi-micronutrient-fortified beverage, with and without sugar, on growth and cognition in South African schoolchildren: a randomised, double-blind, controlled intervention
- Studies of South African schoolchildren since 2005 suggest lower anaemia prevalence in some regions

The first article mentioned has been prepared for submission to the British Journal of Nutrition (font Times New Roman, size 12, 1.5 line spacing). Based on observations made in the BeForMi intervention study with regards to the prevalence of micronutrient deficiencies, the second article was done. The second article has been prepared for submission to the South African Journal of Clinical Nutrition (font Arial, size 11, single spacing). For the purpose of the thesis, tables and figures for both articles have been placed within the text at the appropriate points, rather than at the end of the article.

Chapter 4 is the final chapter, in which the main findings have been summarised and which concludes with some recommendations for further work to be done.

A combined reference list for chapters 1, 2 and 4 has been compiled. Referencing in chapter three has been done according to the instructions to authors of the specific journal. The references are followed by the addenda. Included in the addenda are the 24-hour recall questionnaire (addendum A), socio-demographic questionnaire (addendum B), identity card (addendum C) and informed consent form (addendum D). The authors' guidelines for the British Journal of Nutrition (addendum E) and for the South African Journal of Clinical Nutrition are included (addendum F) as well as the confirmation email from the specific journal that the article has been submitted (addendum G and H respectively).

## CHAPTER 2: LITERATURE REVIEW



## 2.1 Introduction: The public health significance of undernutrition

Religion, human rights, ethical opinions and national security are all reasons why people devote time to and conduct research on actions that will ultimately lead to improved nutrition across the globe. Until recently, the economic influence of undernutrition has been in the background and largely overlooked. However, strong economic arguments have lately been made that emphasise the importance of alleviating undernutrition and encourage nutrition interventions (Copenhagen Consensus Centre, 2012). Improved nutrition increases productivity and reduces economic costs, whereas overlooking the significance of malnutrition leads to higher budget outlays and losses in Gross Domestic Product. The returns of programmes aimed at improving nutrition outweigh the original cost (The World Bank, 2006). In 2006, the World Bank released the document “Repositioning nutrition as central to development”, which listed three reasons why interventions should be implemented to reduce malnutrition. The reasons are the following:

1. High economic returns, high impact on economic growth and poverty reduction
2. The alarming shape and scale of the malnutrition problem
3. Failing markets

In 2012, the Copenhagen Consensus was held in order to evaluate proposals commissioned to address ten of the most important challenges the world is facing (Copenhagen Consensus Centre, 2012). The strengths and weaknesses of each proposal were evaluated. These challenges included armed conflict, chronic disease, education, infectious disease, population growth, biodiversity, climate change, hunger and malnutrition, natural disasters, as well as water and sanitation (Copenhagen Consensus Centre, 2012).

According to the Copenhagen panel, among 30 challenges listed in order of costs and benefits, interventions bundled together to reduce undernutrition in pre-school children to address hunger and education were listed first (**Table 2.1**). The panel stated that for \$100 ( $\pm$ 835 South African Rand) per child, a bundle of interventions implemented in developing countries could reduce chronic undernutrition by 36 percent (Copenhagen Consensus Centre, 2012). These intervention bundles include micronutrient provision, complementary foods, treatments for hookworms and diarrheal diseases and programmes aimed at behaviour change that would influence nutrition. More impressive, however, is the fact that even poor countries could

achieve at least a \$30 ( $\pm 250$  South African Rand) pay-off for every dollar spent to reduce chronic undernutrition (Copenhagen Consensus Centre, 2012).

**Table 2.1** Challenges list based on the costs and benefits of the solution

	Challenge	Solution
1	Hunger and Education	Bundled interventions to reduce undernutrition in pre-schoolers
2	Infectious disease	Subsidy for malaria combination treatment
3	Infectious disease	Expanded childhood immunisation coverage
4	Infectious disease	De-worming of school children
5	Infectious disease	Expanding tuberculosis treatment
6	Hunger and biodiversity and climate change	R & D to increase yield enhancements
7	Natural disasters	Investing in effective early-warning systems
8	Infectious disease	Strengthening surgical capacity
9	Chronic disease	Hepatitis B immunisation
10	Chronic disease	Acute heart attack low-cost drugs

Adapted from Copenhagen Consensus Centre, 2012

This review will give an overview of the scale of the malnutrition problem (including under- and overnutrition) together with the nutrition intervention strategies that are known to work in addressing malnutrition. Since micronutrients have been identified as key interventions, the possible mechanisms through which iron, vitamin A, iodine and zinc might affect cognition and the consequence of micronutrient malnutrition on cognitive function and development will be discussed. Furthermore, the link between a sugar containing beverage and obesity is discussed as well as the role of glucose in the brain. Attention will also be given to the assessment of cognitive abilities in children. Throughout this chapter prominence will be given to literature which has relevance to primary school children.

## 2.2 Scaling-up of nutrition with interventions that work

More than a decade has passed since the Millennium Declaration was compiled, laying the foundation for freeing humanity from poverty, hunger, illiteracy and diseases (United Nations, 2011). The Millennium Development Goal (MDG) framework included eight goals and several targets, many of them relating to nutrition. Since nutrition plays a major role in enabling countries to reach the MDGs, it seems ironic that for many years nutrition had not featured on the political agenda of many governments. Undernutrition, as one of the world's most serious problems, has enormous economic cost, yet little attention has been given to this problem (Anon., 2011). In 2008, the publication of a series of articles in *The Lancet*, emphasised the impact of child and infant undernutrition on health as well as on cognitive and physical development (Bhutta *et al.*, 2008; Black *et al.*, 2008; Bryce *et al.*, 2008; Victora *et al.*, 2008). Even though a substantial amount of literature has been published on nutrition and child cognition prior to 2008, much needed attention was shifted to this phenomenon.

In 2010, the MDG roadmap was compiled, stating clearly what needed to be done in order to reach the goals by 2015; if this were to be followed diligently, the MDGs would be within reach for every country. The Secretary-General of the United Nations, Ban Ki-Moon, stated in the last MDG Report (2011) that millions of people had already been lifted out of poverty, that lives had been saved and that more children were attending school (United Nations, 2011). In addition, maternal deaths had been reduced, new opportunities for women had been created, many people had been freed from deadly diseases and more people now had access to clean water. The progress that had been made was duly recognised but at the time of publication it was cautioned that there was still a long way to go, especially with regard to poorer communities.

Effective interventions for addressing undernutrition are well known and many countries have intervention policies. Still, many countries, including South Africa, have not risen to the challenge of reaching the desired targets to reduce the prevalence of micronutrient deficiencies, stunting, underweight and wasting. Governments and development partners under-prioritise nutrition for several reasons (The World Bank, 2006). Firstly, malnutrition is largely invisible, and communities rarely demand nutrition services. The effect that moderate and mild malnutrition can have on intelligence, disease and mortality is mostly unknown or unappreciated in affected communities. The communities most affected are often poor and do not voice their concern. This point of “unknowing ignorance” is a thread throughout this chapter and is an stumbling block in many intervention strategies. Secondly, governments and development

partners have been unaware of the economic cost of malnutrition, and have not recognised that failing to reach the MDGs for malnutrition would compromise progress in reaching other MDGs. Finally, while nutrition has been the partial responsibility of several departments, agencies and ministries, it has been the main responsibility of none. This has also influenced the allocation of funds and, while there is no one sector to make it a priority, the desired action is unlikely to follow (The World Bank, 2006).

In April 2010, the Scaling-Up Nutrition (SUN) framework was compiled (Anon., 2011). SUN encouraged the converting of *nutrition talk* to *nutrition action* that is known to work. David Nabarro (2011), *Special Representative of the United Nations Secretary-General for Food Security and Nutrition*, describes SUN as follows:

*“SUN is not a new institution, initiative or financial mechanism. It is a movement that brings organizations together across sectors to support national plans to scale up nutrition by helping to ensure that financial and technical resources are accessible, coordinated, predictable and ready to go to scale.”*

The need to achieve the MDGs contributed to putting nutrition in the spotlight, and moving the focus to the reducing of undernutrition. The SUN Road Map followed in September 2010, and has been endorsed by many national governments, the United Nations system, civil society organisations, development agencies, academia, philanthropic bodies and the private sector (Anon., 2010). Investments were identified that had been proved to work when implemented correctly within the nutrition-focused development policies. The main elements of the framework for SUN action are as follows:

- 1) “Start from the principle that what ultimately matters is what happens at the country level.”
- 2) “Sharply scale up evidence-based, cost-effective interventions to prevent and treat undernutrition, with highest priority to the minus 9- to 24-month (1000 days) window of opportunity where the highest returns from investments are expected.”
- 3) “Take a multi-sector approach that includes integrating nutrition in related sectors and using indicators of undernutrition as one of the key measures of overall progress in these sectors.”
- 4) “Provide substantially scaled-up domestic and external assistance for country-owned nutrition programmes and capacity” (Anon., 2011).

Furthermore, the Road Map gives guidance for country, regional and international stakeholders to work together towards scaling up nutrition. It is unfortunate that South Africa has not yet grabbed this opportunity, while 22 of the 30 countries that have already signed up are from Africa. This includes countries such as Ghana, Malawi, Mozambique, Zambia and Zimbabwe (SUN, 2012).

While the World Bank emphasised the high economic returns of programmes aimed at improving undernutrition in 2006, the more recent Copenhagen Consensus document has affirmed the high economic pay-off for every dollar spent on interventions that improve nutritional status.

Nutrition intervention strategies that are known to be effective to alleviate micronutrient deficiencies are discussed in the following section. Interventions that will be discussed in the following section may include some, but not all of the strategies included in the SUN initiative. Nutrition intervention strategies discussed will focus on the alleviation of micronutrient deficiencies for school age children while other strategies in the SUN roadmap include programmes aimed at mothers and infants, such as complementary feeding and low birth weight of babies, which are not included in the scope of this review.

## **2.3 Nutrition intervention strategies aimed at reducing micronutrient deficiencies**

Strategies to decrease the prevalence of poor micronutrient status in populations include fortification, supplementation, and bio-diversification/modification and nutrition education. These nutrition intervention strategies will be dealt with in this section.

### **2.3.1 Fortification**

**Food fortification** is the action of adding nutrients to food vehicles to provide them at higher than natural amounts of nutrients found in a food (Thompson, 2007). Fortification with multiple micronutrients is a cost-effective and sustainable method of improving poor micronutrient status (Gibson & Ferguson, 1998). The benefit of food fortification lies in the fact that it is a food-based approach. It is important that food fortification should not be seen as a replacement or an alternative strategy for dietary improvement strategies (to be discussed in section 2.3.2) to improve micronutrient status, but rather as a supporting strategy (Tontisirin *et al.*, 2002). Food fortification is therefore a link supporting sustainable long term dietary change in populations

(Tontisirin *et al.*, 2002). Advantages of food fortification include the following (WHO & FAO, 2006):

- Body stores of nutrients can be maintained efficiently and effectively if fortified foods are consumed frequently. The risks of multiple deficiencies that are often the result of seasonal deficits or a poor quality diet are reduced.
- Fortified staple foods contain near natural levels of micronutrients.
- Both the poor and wealthy communities in a population are reached. Widely distributed and consumed fortified staple foods have the potential to target and improve the nutritional status of a large proportion of the population.
- Fortification does not require changes in the existing food pattern of a population.
- The delivery system for fortified foods is in most settings already functional.
- Because micronutrient deficiencies often coexist, multiple micronutrient fortification is ideal.
- The addition of one or more micronutrients to a food product does not add substantially to the total manufacturing cost. The burden on the health sector is generally low since the cost is carried mostly by the industry and the consumer (Gibson & Ferguson, 1998)

While there are numerous advantages to multiple food fortification there is however, also some limitations to this approach (WHO & FAO, 2006):

- Food fortification does not address the cause of poor micronutrient intake because it does not require a change in behaviour.
- Fortified foods increase the amounts of only those micronutrients that the food product is fortified with, but do not necessarily provide adequate energy, protein, and essential fats.
- A specific fortified product might not be consumed by all the members in a population. Furthermore, regardless of a person's current nutritional status, everyone in a population is exposed to increased levels of micronutrients in food products.
- It is possible that, due to the small amounts of food that children consume, they are less likely to obtain the recommended intake for a specific age group due to fortified foods.
- The poorest in a community often rely on own-grown or locally produced food and due to low purchasing power, do not have access to fortified foods.
- Technological issues such as appropriate levels of nutrients, cooking properties, taste, physical properties, nutrient interactions and the stability of some fortificants have not yet fully been resolved.

- The nature of the food fortificant and/or the food vehicle may limit the amount of fortificant that may be added.
- This approach is often viewed to be more cost-effective than other strategies but there are still significant costs associated with food fortification. These costs may limit the implementation and effectiveness of such programmes.

Foods that are fortified are edible, usually staple foods, processed foods, condiments or products for special groups. These products are manufactured by the food industry with a specific food composition and fortified with specific amounts of minerals and vitamins (Dary, 2007). Traditionally-eaten staple foods are excellent vehicles for fortification because of the potential for high coverage rates. The World Health Organization (WHO) recognises four categories of food fortification, namely mass fortification, targeted fortification, market-driven fortification and other types of fortification (WHO & Food and Agriculture Organization (FAO), 2006).

**Mass fortification** is described as the addition of micronutrients to edible foods that are regularly consumed by the general public (Dary, 2007). The government usually initiates, mandates and may regulate the process (WHO & FAO, 2006). Wheat flour, maize flour, rice, salt, sugar, cookies and soy sauce are all different foods that have been used as vehicles for mass fortification (Thompson, 2007). Together with non-government organizations certain states of India is targeting micronutrient malnutrition in school children with fortified biscuits (Global Alliance for Improved Nutrition, 2012) While relatively low intakes of the fortification vehicle should still be able to provide adequate amounts of micronutrients, high usage of the fortification vehicle should not produce the risk of toxicity. The need to ensure that the upper limits of micronutrient intake is not exceeded means that people with irregular intake will inevitably fail to reach desired intakes. **Targeted fortification** refers to fortification of food for specific groups and increasing the intake of a sub-group of the population (WHO & FAO, 2006), such as fortification of infant foods, while **market-driven fortification** is dependent on business-orientated initiatives of food manufacturers who add micronutrients to their products with the final aim of increasing sales (WHO & FAO, 2006). Other types of fortification include **household and community fortification**, where micronutrients are added to foods at household level and **bio-fortification**. Household fortification is often referred to as point-of-use fortification. Bio-fortification of foods includes the breeding and genetic modification of plants in order to improve the nutrient content and/or absorption.

For all fortification approaches, the vehicle chosen for fortification should preferably be temperature-stable, technologically and economically fortifiable, and should undergo no change in appearance, taste and texture during storage (Gibson & Ferguson, 1998). While fortification does not require change in the behaviour, food beliefs and practices of the current population, dietary modification/diversification together with education remains the most sustainable long-term nutritional strategy.

## **2.3.2 Dietary modification/diversification and nutrition education**

### **2.3.2.1 Dietary modification/diversification**

Dietary diversification/modification aims at improving the availability, accessibility and utilisation of foods high in bio-available micronutrients throughout the year, which simultaneously alleviates multiple micronutrient deficiencies (Gibson & Hotz, 2001; Gibson & Ferguson, 1998). This strategy is central to food-based approaches and needs to be supported by nutrition education (Tontisirin *et al.*, 2002). Dietary diversification/modification could be more sustainable, feasible and acceptable on a cultural level than supplementation and fortification (Gibson & Ferguson, 1998). Food-based approaches are more commonly employed in order to achieve long-lasting benefits for the control of micronutrient deficiencies (Thompson, 2007).

In order to achieve effective implementation of dietary diversification, a thorough knowledge of local dietary patterns, beliefs and preferences is needed, together with the ability to change community practices (Gibson & Hotz, 2001). Intervention strategies need to be economically feasible, sustainable, culturally acceptable and integrated with already existing national programmes related to agriculture, food nutrition and health education. The community needs to be committed to the programme and to be involved in the assessment and analysis of the programme and related monitoring actions (Thompson, 2007). Governments need to be aware of the consequences of micronutrient deficiencies and, having acknowledged these consequences, should take the lead in bringing them to the attention of the larger public (Tontisirin *et al.*, 2002). Change on many levels is required, including food production practices, food selection patterns and the manner in which food is prepared and processed to ensure adequate nutrition through dietary diversity.

One of the limiting factors to attaining adequate micronutrients through dietary diversity is the low bioavailability of some micronutrients from plant-based staple sources (Gibson & Hotz, 2001). Strategies to improve the bio-availability and content of micronutrients in staple foods include: genetic engineering of staple foods, increased production of animal source foods, and

changes in food preparation and processing methods at household level, such as fermentation and germination (Gibson & Hotz, 2001).

### **2.3.2.2 Nutrition education**

Nutrition education can be defined as any set of learning experiences that is designed to facilitate the voluntary adoption of new eating and other nutrition-related behaviours (Contento *et al.*, 1995). To bring about behavioural changes in populations, extensive efforts are needed. Nutrition education may include person-to-person communication, group talks, slide shows, street plays, and radio and television programmes as well as socio-marketing (Vijayaraghavan, 2004).

Extensive research has been done on nutrition education. In Peru, key nutrition messages were given to children's caregivers through education materials such as flipcharts and recipe fliers, with children younger than two years being the target group. Furthermore, the use of growth monitoring cards was promoted (Waters *et al.*, 2006). Clear positive impacts were reported on children's growth outcomes, with those children included in the intervention being 0.33 times less likely to be stunted ( $p < 0.05$ ) than the control group. In addition, nutrition-related services for the children in the intervention were extended to 17.6 visits *versus* 14.1 visits during the first 18 months of life for children in control area ( $p < 0.005$ ) (Waters *et al.*, 2006). Similar results were reported by Guldán *et al.* (2000) when nutrition education in rural Chinese townships improved mothers' knowledge and feeding practices to the extent that breastfeeding rates increased and infant growth improved.

Faber *et al.*, (2002) established home-gardening programmes in rural villages in South Africa with the aim to determine whether yellow and dark-green leafy vegetables dietary intake and serum retinol concentration would improve. After the 20 month intervention period, serum retinol concentration in the experimental village increased significantly. Consumption of yellow and dark-green leafy vegetables was more often consumed in the experimental group. Furthermore the maternal knowledge regarding vitamin A improved significantly in the experimental village. The authors concluded that home-gardening programmes that was integrated within a primary health care activity, and linked to nutrition education, while focusing on the production of yellow and dark-green leafy vegetables significantly improved the vitamin A status of children between the ages of 2-5 years. In Idaho (United States), the consumption of fruit and vegetables of adolescents increased through garden-based nutrition education (McAleese & Rankin, 2007).

Unfortunately, increased knowledge through nutrition education does not always bring the desired change. In a recent study conducted by Prelip *et al.* (2012) in a large urban school in the United States, change in knowledge was not enough to bring about change in behaviour in increasing fruit and vegetable intake in children between the age of 8 and 11 years, which underlines the fact that behaviour is difficult to change, for it is not only knowledge and attitude that influence behaviour. The study of Prelip *et al.* (2012) furthermore underlines the importance of parent involvement and the need to involve parents in interventions such as these. A review by Silveira *et al.* (2011) on school-based interventions indicated that those interventions where the education programme was introduced into the regular school activities and those with duration of more than a year tended to be the most effective. Furthermore, as mentioned in the study of Prelip *et al.* (2012) the involvement of parents contributed to the effectiveness of nutrition education.

Nutrition education programmes need to be multifaceted. The process also needs to be long-term in order to have the desired impact on behaviour change. An ongoing effort is needed to facilitate the evolution of the process from awareness, motivation, to enabling activities, and the ultimate goal, which is the maintenance of dynamic change (Contento *et al.*, 1995). The importance of nutrition education should therefore not be underestimated. Because of new knowledge that is continuously generated, now, more than ever, the emphasis should include nutrition education programmes that draw from research and practise in order to address nutrition problems effectively.

### **2.3.3 Supplementation**

Supplementation refers to periodic administration of pharmacological preparations of nutrients, either in the form of capsules and tablets or by injection (Thompson, 2007). This short-term strategy is aimed at emergency situations where immediate relief is needed or at populations with a high prevalence of a specific deficiency, e.g. vitamin A deficiency. Nutritional supplementation is aimed solely at vulnerable population groups who are unable to meet nutritional needs through daily food intake (Thompson, 2007).

The effectiveness of supplementation is determined by various factors and is often counteracted by inefficient and irregular supply, poor procurement and distribution, low accessibility, inadequate training of health workers and low compliance. While clinical trials have shown an impact of supplementation on micronutrient deficiencies they are often unsuccessful in the real

world where the situation is much more complex and compliance as well as coverage is not always guaranteed (Allen & Gillepsie, 2001).

Using vitamin A supplementation as an example, multiple doses of the supplement are needed for vitamin A-deficient children and follow-up visits are required. However, if a mother does not fully comprehend the nature and impact of vitamin A deficiency on the health of her child she is less likely to bring the child for follow-up visits (Allen & Gillepsie, 2001).

Well trained, motivated workers who understand the nature of the problem and who are able to communicate it effectively to the community are of major importance, for they are the link between important knowledge and the community. Furthermore, sufficient population coverage should be obtained and supplements that are of high quality, stable, and have a long shelf-life, as well as being acceptable to the local community, should be provided (Allen & Gillepsie, 2001).

The nutrition intervention strategies that have been discussed, namely supplementation, fortification, dietary modification/diversification and education, have the potential to combat undernutrition and micronutrient deficiencies in children. The next section will focus on programmes that are currently operational in South Africa. Mandatory fortification in South Africa will be discussed, followed by the two main nutrition intervention programmes of the Integrated Nutrition Programme (INP) of South Africa. These programmes are the National School Nutrition Programme (NSNP) and the Vitamin A Supplementation Programme.

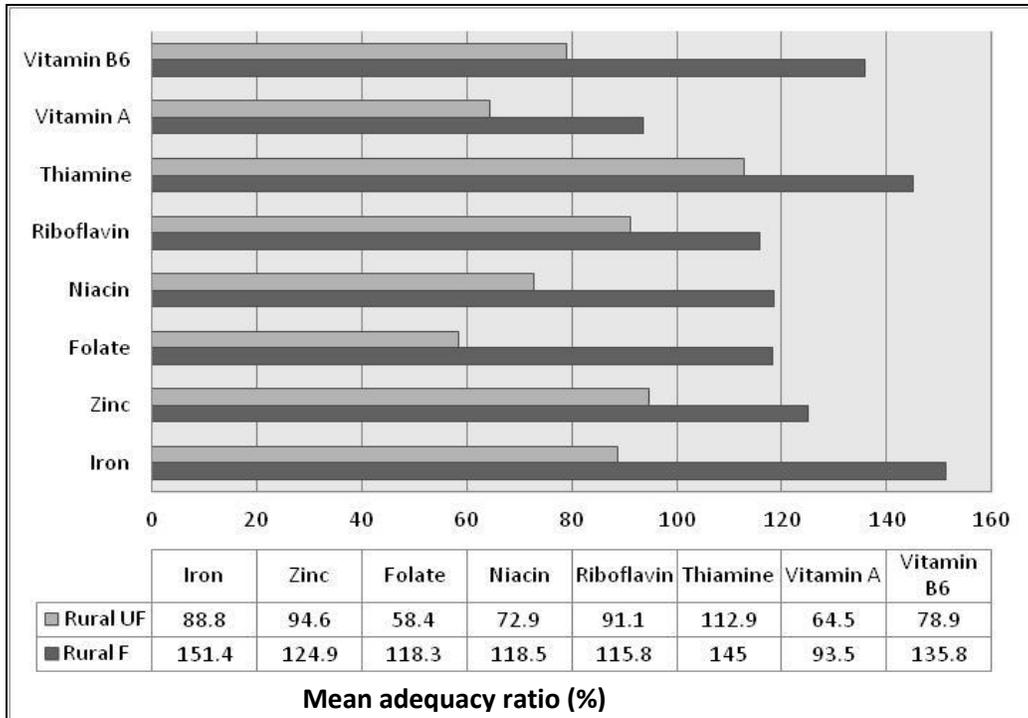
## **2.4 Nutrition interventions in South-Africa**

### **2.4.1 Multiple-micronutrient fortification initiatives in South Africa**

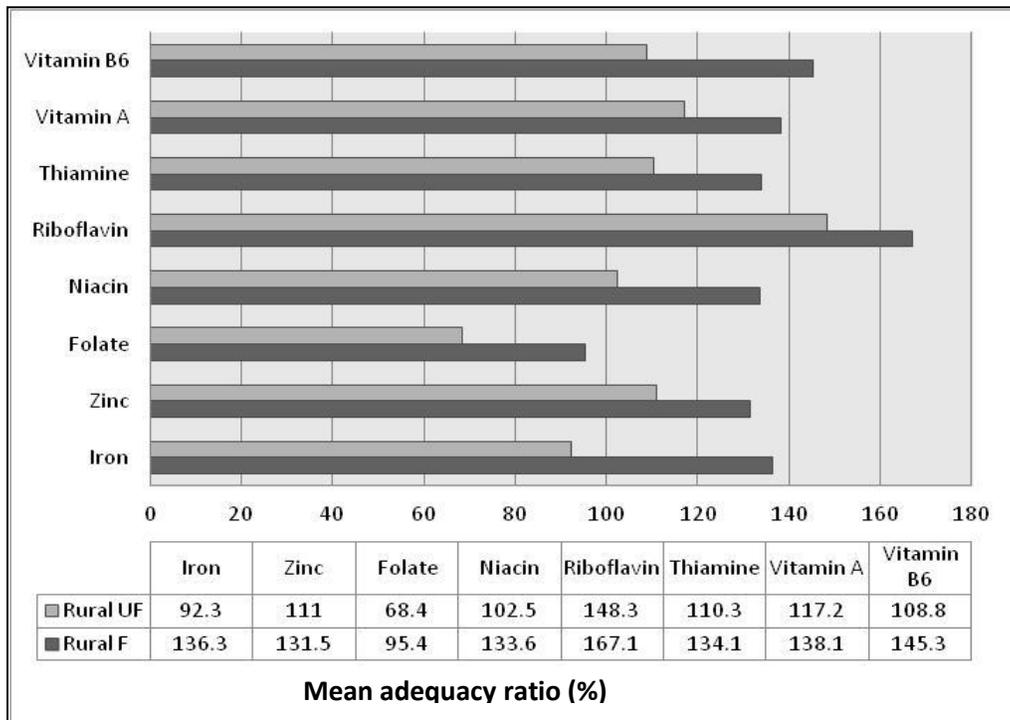
Fortification is an intervention for combating micronutrient deficiencies, and, with regard to timeframe, falls between supplementation, which is a direct short-term intervention, and dietary diversification, which is a long-term intervention. With the legislation of the South African National Food Fortification Programme (NFFP) in 2003 (Foodstuffs, Cosmetics and Disinfectants Act (FCDA), 54/1972), it is now mandatory that all maize meal and wheat flour be fortified. Affordability and access to fortified foods are important factors that influence micronutrient programmes (Steyn *et al.*, 2008). Furthermore, the success of such programmes also depends on continued dialogue between the various sectors that have to collaborate closely on issues relating to the production, promotion, distribution and consumption of fortified foods.

The ultimate question is whether national fortification is implemented in such a manner that micronutrient deficiency prevalence is reduced. Steyn *et al.* (2008) addressed this question by recalculating documented micronutrient intakes of children substituting the micronutrient values for maize meal, porridge and bread in the non-fortified products with fortified porridge, maize and bread (white and brown). **Figure 2.1** and **Figure 2.2** represent the mean adequacy ratio calculated for a child's diet in the study by Steyn *et al.* (2008). The authors concluded that if two of the most commonly consumed staple foods in South Africa were fortified, the micronutrient intake of children aged 1–9 years would significantly improve, as would the overall micronutrient density of the diet.

While in theory it seems that the fortification legislation might lead to increased overall micronutrient intake, this will be of no worth if the wrong iron fortification compound is used and the biochemical status of children is not affected. The choice of the iron fortification compound that is used in a fortification programme will determine whether the target iron deficient group is benefitting from the programme. Van Stuijvenberg *et al.* (2006) examined the efficacy of ferrous bisglycinate and electrolytic iron as fortificants in brown bread. They reported that no response was found in haemoglobin or ferritin concentrations in a group of iron deficient school children in the Northern Cape who consumed brown bread (four slices/day) fortified with electrolytic iron for seven and a half months. It was concluded that ferrous bisglycinate performed better as an iron fortification compound in brown bread. Even though only brown bread was investigated, the authors suggested that due to the high phytate of maize meal, the findings are likely to be applicable in a maize diet as well. Similar results was found in Kenyan children, aged 3-8 years old, where no improvement in iron status with whole maize meal that was fortified with electrolytic iron at 56mg/kg was reported (Andangó *et al.*, 2007) From current literature it is uncertain whether electrolytic iron is effective and without efficacy, it is difficult to expect effectiveness of a programme.



**Figure 2.1** Mean nutrient intake adequacy ratios of children in rural areas of South Africa before (UF) and after food fortification (F) of staple foods according the South African government regulations (Source: Steyn *et al.*, 2008)



**Figure 2.2** Mean nutrient intake adequacy ratios of children in urban areas of South Africa before (UF) and after food fortification (F) of staple foods according the South African government regulations (Source: Steyn *et al.*, 2008)

### 2.4.2 School feeding in South Africa

School feeding systems, if implemented and monitored correctly, have the potential to serve as practical platforms through which nutritious meals or snacks, micronutrient supplements, on-site fortification and education can successfully be administered (Best *et al.*, 2010). When school feeding programmes are considered, the following are key elements. The goals should be clear, whether it is to alleviate short-term hunger or to improve micronutrient status. Furthermore, the target population should be identified, with clear reasons why the specific group was chosen. The time at which school feeding will occur should be considered, because breakfast or a morning snack is usually better for alleviating hunger, if that is the primary goal (Del Rosso & Marek, 1996). The lowest rations for achieving the goals as well as the funds available per person should be calculated. Finally, all the additional services that are required to improve nutrition should be included. These include parental education, de-worming, water supply and sanitation at school (Del Rosso & Marek, 1996).

In 1994, the government established the Primary School Nutrition Programme (PSNP) in South African schools (The Public Service Commission (PSC), 2008). This programme was later renamed the National School Nutrition Programme (NSNP). In 2010, the programme provided one meal daily to more than six million learners (Department of Basic Education, 2010). The aim of the programme was to enhance the educational experience of needy primary school learners through promoting punctual school attendance, alleviating short-term hunger, improving concentration and contributing to general health (Department of Basic Education, 2008).

The contribution of energy by the school feeding programme to the children's recommended daily allowance (RDA) has been changed over time. In 1995, costs needed to be reduced, which led to the national reduction of the targeted contribution of the RDA to be reached, (**Table 2.2**) (Child Health Unit (CHU) & Health System Trust (HST), 1997). The percentage energy contributed to the RDA for the school meal was reduced from 30% to 25% for children of 7–10 years of age and to 20% for children of 11–14 years of age (CHU & HST, 1997). In 1997, an evaluation of the NSNP reported that out of 33 meals analysed, only six managed to provide more than 25% of the RDA for energy for children of 7–10 years of age (CHU & HST, 1997). This indicated the existence of challenges in the implementation of the programme as regards adhering to the intended RDA contribution for energy.

A report on the Evaluation of the NSNP compiled in March 2008 included only two of the nine provinces (PSC, 2008). This report noted that, as possible outcomes of the impact of the programme, there was increased enrolment of learners at schools, increased school attendance and improved participation by the learners in the classrooms (PSC, 2008). It seems that even though there were difficulties in reaching the goal of the prescribed RDA contribution for energy, some benefits at school level were nevertheless observed. Concern was expressed that reducing the contribution of energy to the RDA would have less of an impact on a child's nutritional needs and there could even be a minimum point at which no further impact on the improvement of school performance could be observed (CHU & HST, 1997).

The menus in current use in primary schools have been approved in 2010/2011. If these newly developed menus are to be implemented correctly, more of the goals that have been set by the NSNP might be met. These menus include the following guidelines (Department of Basic Education, 2010/2011):

- Repetition and menu fatigue should be avoided and therefore starch should be alternated. This includes samp, maize pap, rice, maize rice, pasta, potatoes, sweet potatoes and flour products.
- Fresh vegetables or fruit should be served daily alternating different colour vegetables or fruit such as green and yellow or red. Where possible both green and yellow or red can be served in one meal.
- Fresh fruit must be served on days that vegetables are not on the menu.
- High quality protein such as pilchards and milks should be served at least once a week.
- Soya products should not be served more than twice a week.
- Legumes such as dried beans, split peas and lentils should not be served more than twice a week.
- Traditional food is acceptable as long as it is properly placed within the food groups.

**Table 2.2** Change in specifications for the South African Primary School Nutrition Programme

Original objective 1994	1995
<ul style="list-style-type: none"> <li>• To meet 30% of the RDA for energy of primary school children</li> </ul>	<ul style="list-style-type: none"> <li>• To provide not less than 25% of the RDA for energy for 7- to 10-year-old children</li> <li>• To provide not less than 20% of the RDA for energy for 11- to 14-year-old children</li> </ul>

RDA, recommended dietary allowance

Adapted from CHU & HST (1997)

School children receive additional micronutrients as a result of fortified maize meal and flour (FCDA, 54/1972). Even though the programme does not target improvement of micronutrient intake as a goal, the NSNP would be indirectly addressing micronutrient intake through the fortification legislation. Micronutrient fortification includes vitamin A, thiamine, riboflavin, niacin, folic acid, pyridoxine, zinc and iron (FCDA, 54/1972). Wheat flour and maize meal are fortified with 35 mg/kg, wheat flour with 43 mg/kg, wheat bread (white) with 32 mg/kg and wheat bread (brown) with 34 mg/kg. The iron used for fortification is electrolytic iron, which, unfortunately, has low bioavailability in a maize or wheat diet (WHO & FAO, 2006).

### 2.4.3 Vitamin A supplementation programme

The South African Vitamin A Consultative Group (SAVACG) study conducted in 1994 reported that one in every three South African children of 6–71 months of age had marginal vitamin A status (serum retinol < 20 µg/dl) (SAVACG, 1995). These findings underlined the magnitude of the problem in South Africa and formed the basis on which the vitamin A supplementation programme was implemented in South Africa in 2002.

The International Vitamin A Consultative Group (IVACG) was established in 1975 and has guided international activities ever since with the aim of reducing vitamin A deficiency across the globe (IVACG, 2002). The schedule for routine high-dose vitamin A supplementation in vitamin A-deficient populations, as recommended by the International Vitamin A Consultative Group, is as follows:

**Infants 0–5 months:** 150,000 IU as three doses of 50,000 IU with an interval of at least 1 month between doses.

**Infants 6–11 months:** 100,000 IU as a single dose once

**Children 12 months and older:** 200,000 IU as a single dose every 4–6 months (WHO, 2011)

The INP stated in their strategic plan (2002/03-2006/07) that by 2007 they aimed at achieving 80% vitamin A supplementation coverage of children from 1–5 years (South Africa, 2002). Few studies have been conducted to measure supplementation coverage. However, a study conducted in the Boland district in 2005 found that vitamin A supplementation coverage of only 75% for eligible children was achieved (du Plessis *et al.*, 2007). The authors commented that if the high rate of missed opportunity and the practices observed in their study were representative of larger areas and the country as a whole, large numbers of children are still not receiving vitamin A supplementation. Another disquieting fact is that the current provincial

vitamin A supplementation protocol stipulates a once-off dose of 50 000 IU that should be administered at 6 weeks (Dhansay, 2007), in comparison with the three doses as indicated in the IVACG statement (IVACG, 2002), raising the question of what the effect of lower-than-recommended protocols combined with poor coverage might be.

One of the main barriers to sustainable programming is the lack of recognition by the communities of the need for vitamin A supplementation. “Dalmiya *et al.* (2006) emphasis in *The Lancet*, with a commentary titled “Sustaining vitamin A supplementation requires a new vision” that the importance of vitamin A supplementation should be communicated in such a way that a transition is reached from a push-driven to a demand-driven intervention (Dalmiya *et al.*, 2006). This transition is critical if policies are to succeed.

A lack of staff and mothers or care takers knowledge, remain to be fundamental when a programme is implemented (Hendricks *et al.*, 2003). In an observational study by Iverson *et al.* (2011) the perceptions of mothers who attended primary health clinics with regards to the purpose, management and eligibility of the vitamin A were evaluated. The mothers had little knowledge on the vitamin A programme and only a few could remember that they were briefed about the vitamin A capsules. Staff members on the other hand, overall felt that they were implementing the programme well and that the problem with implementation lay with the mothers.

While vitamin A supplementation has previously been viewed as a short-term deficiency control strategy, it is not integrated as a central component in the broader package for child survival (Dalmiya *et al.*, 2006). Special efforts need to be made to extend Vitamin A supplementation to those children that have not been reached in order to achieve the ultimate goal of universal coverage.

## **2.5 Malnutrition in South African children**

### **2.5.1 Undernutrition in South African children**

The United Nations Children’s Fund (UNICEF) framework underlines the basic and underlying causes of undernutrition. These include environmental, socio-political, contextual and economic factors. Of these, poverty stands out as one of the basic causes (**Figure 2.3**).

By addressing causes of undernutrition (e.g. deprivation and inequity) as a global priority, undernutrition can be reduced. However, through programmatic health and nutrition

interventions, additional reductions in undernutrition can be achieved (Black *et al.*, 2008). **Figure 2.3** illustrates the UNICEF conceptual framework together with the latest prevalences obtained from the NFCS-FB-2005 with regard to stunting, wasting and anaemia, and deficiencies of vitamin A, iodine and zinc. From this it is clear that undernutrition needs to be addressed from various angles, and that it is important to acknowledge the many contributing factors. In South Africa, inadequate dietary intake is a major contributing factor to micronutrient and macronutrient undernutrition.

In South Africa, poverty is a possible major factor in food deprivation among a large number of young children who, therefore, are not able to participate fully in their own educational development (Wildeman & Mbebetho, 2005). Hunger in children may have significant implications for cognitive performance, and remains a problem in both developed and developing nations (Fanjiang & Kleinman, 2007), but even more so in developing countries. Alleviation of hunger in school children improves school performance (Allen & Gillespie, 2001).

Sufficient data on the nutritional status of school children are still lacking in developing countries and countries currently in transition (Best *et al.*, 2010). This is largely due to the fact that the majority of research and national surveys is aimed at children younger than five years, while school children are often left out. In South Africa, as in the rest of the world, national data for school going children are scarce. Almost ten years have passed since national data for school going children in South Africa were collected (included in **Figure 2.3**), which stresses the need for up-to-date data.

In the following section overnutrition of South African children will be discussed. Some literature will also be reviewed on the possible effect that a micronutrient drink with or without sugar may have on growth of school children.

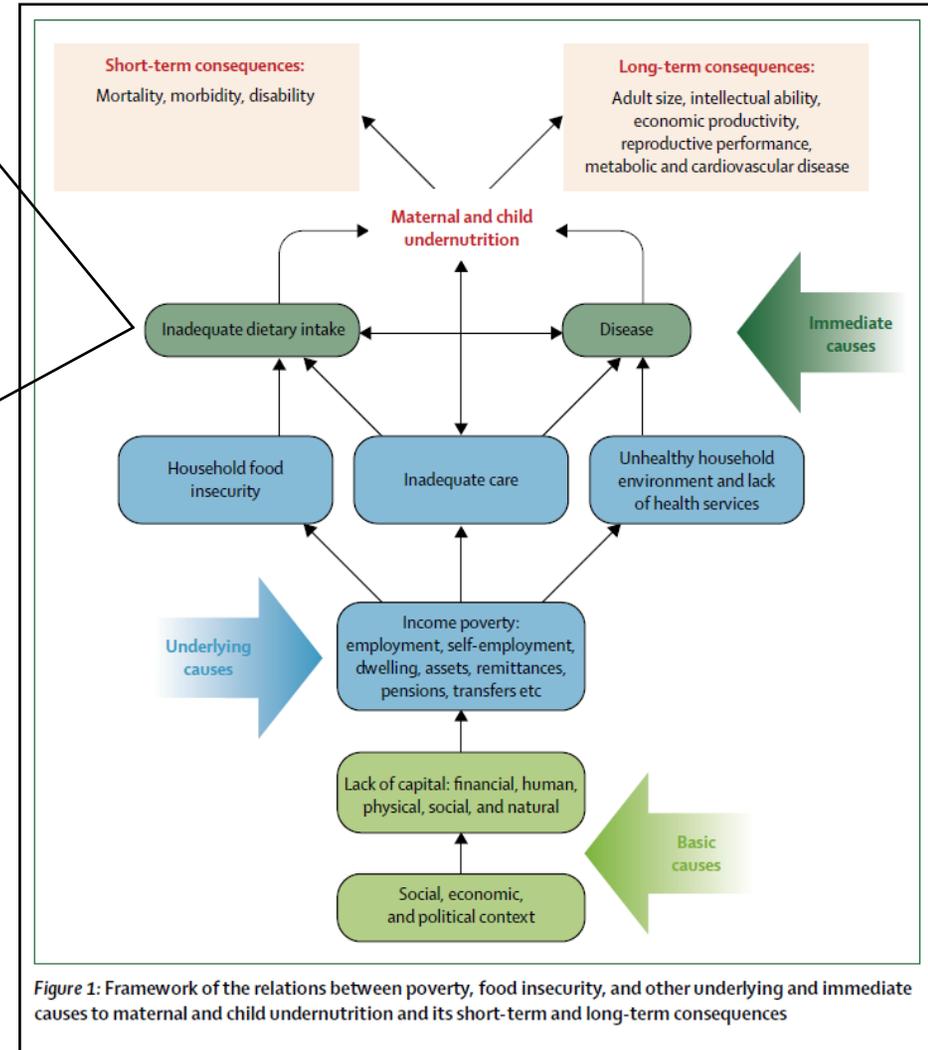
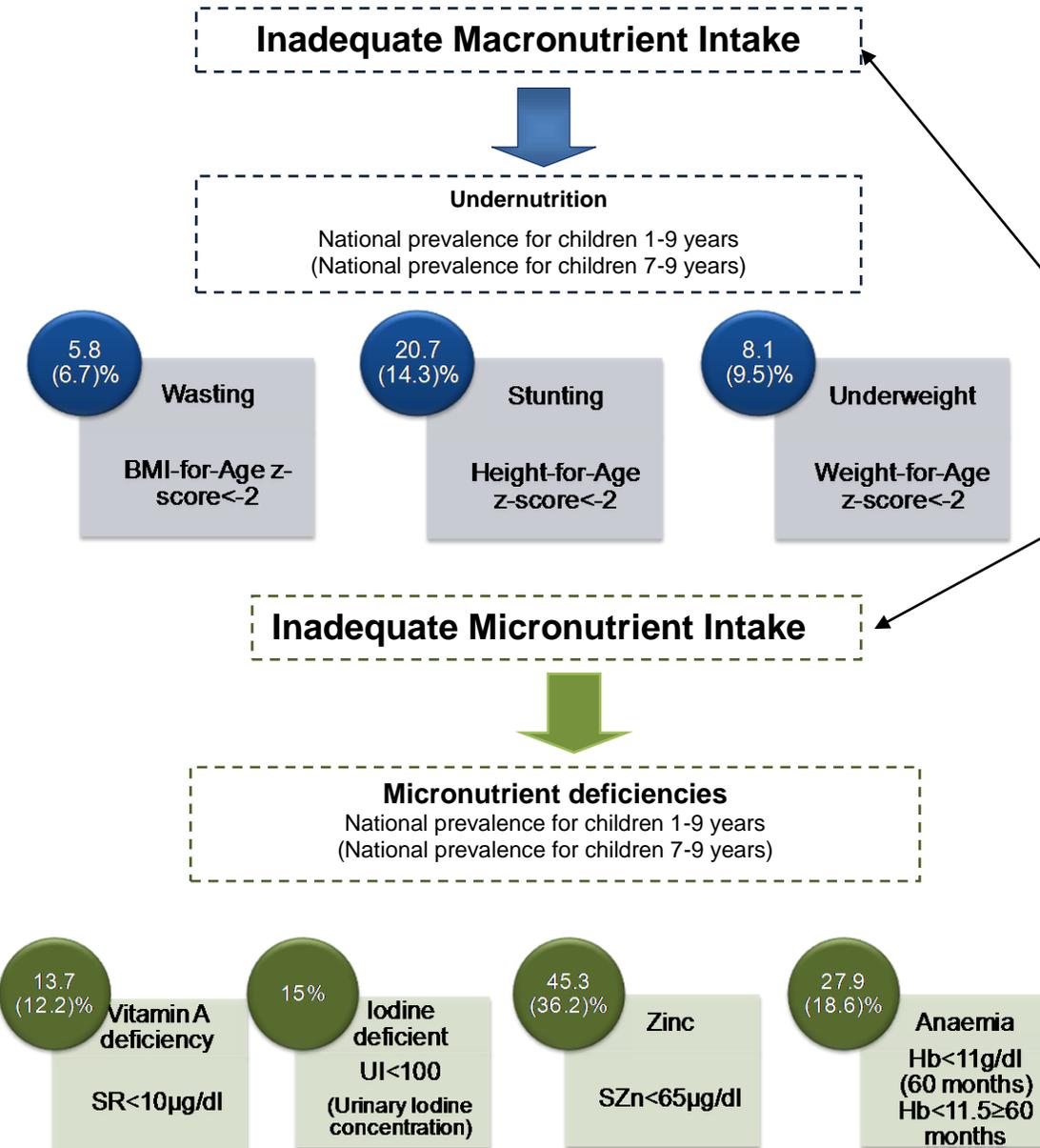


Figure 1: Framework of the relations between poverty, food insecurity, and other underlying and immediate causes to maternal and child undernutrition and its short-term and long-term consequences

Figure 2.3: The current undernutrition situation in South Africa juxtaposed with the UNICEF conceptual framework. (Source: Labadarios, 2007, Black *et al.*, 2008)

### 2.5.2 Obesity in South African children

South Africa is in the midst of the nutrition transition, a process where a large part of the population is turning away from a traditional rural lifestyle towards a westernised, modern lifestyle (Vorster, 2010). The transition is characterised by change in dietary patterns, physical activity levels, alcohol consumption and nutrient intakes, as well as by changes in education and socioeconomic status (Vorster, 2010). At the same time, the country is also faced with a double burden of malnutrition: undernutrition and overnutrition are now found simultaneously in the same household or community.

Ten percent of South African children in the NFCS-FB-2005 (n=2157) between the ages of one and nine years are overweight and 4% are obese (Kruger *et al.*, 2007). Armstrong *et al.* (2006) reported on 10 195 children between the ages of six and thirteen years. Thirteen percent of girls and 10.8% of boys were classified as overweight, whereas 5% of girls and 3.2% of boys were obese. Caucasian girls and boys had a higher prevalence of overweight and obesity than children of black or mixed ancestry. In 2004, the occurrence of overweight in South African children was found to be similar to that in developed countries about a decade earlier (Armstrong *et al.*, 2006). Many factors contribute to the rising obesity problem. It is important to single out these factors in order to target effective interventions at the correct groups.

In South Africa, girls tend to have a higher prevalence of overweight and obesity than boys (Armstrong *et al.*, 2006; Cameron & Getz, 1997; Jinabhai *et al.*, 2003; Kruger *et al.*, 2006). Furthermore, overweight/obesity is often age-dependent, meaning that there is an increase in the prevalence of overweight/obesity with an increase in age. Children from rural areas are less exposed to an urban, westernised lifestyle which is linked to an increased prevalence of obesity (Kruger *et al.*, 2006; Puoane *et al.*, 2002). Smaller family size and increased household income have also been associated with higher body fat percentage in children (Kruger *et al.*, 2006; Mosuwan *et al.*, 2000; Ramachandran *et al.*, 2002). In addition to the characteristics of overweight/obesity listed above, the general African perception of overweight needs to be overcome (Puoane *et al.*, 2002). Different cultural and traditional perceptions with regard to overweight, especially in black women, are positive, and remain a challenge in addressing overnutrition (Mchiza *et al.*, 2011; Puoane *et al.*, 2010).

In conclusion, age, gender, ethnicity, cultural beliefs, family size and income are all determinants of overweight/obesity in South African children, and each of these factors deserves the necessary attention.

### **2.5.2.1 The link between overweight and obesity and the consumption of sugar-sweetened beverages**

Numerous studies have investigated the effects of the intake of sugar-sweetened beverages on weight status in children. In developed countries such as the United States, consumption of sugar-sweetened beverages has increased dramatically over past decades (Malik *et al.*, 2006). While most of the research investigating the possible contribution of sugar-sweetened beverages to obesity has been conducted in developed countries, the possible impact on children in South Africa, a country currently within the nutrition transition, cannot be overlooked.

Some evidence has shown that intake of sugar-sweetened beverages plays an independent role in promoting weight gain and obesity in children and adolescents (Cassady *et al.*, 2012; Malik *et al.*, 2006; Malik *et al.*, 2010). Sugar-sweetened beverages tend to be characterised by high calories and low satiety, leading to increased energy intake (DiMeglio & Mattes, 2000). However, the evidence seems to be equivocal. Bachman *et al.* (2006) investigated possible mechanisms to account for the relationship between sweetened beverage consumption and childhood obesity, and found that results were inconclusive. These mechanisms included: 1) the accumulation of total energy intake, which might include a sweetened beverage that could increase the risk of obesity; 2) the glycaemic load from a beverage that could increase insulin circulation; 3) the lower satiety levels of liquid intake, causing higher calorie intake; and 4) lower intake of milk and consequently of the obesity-lowering properties of calcium, as a result of the consumption of sweetened beverages. The authors also highlighted the difficulty of attributing the increase in body weight to one food group, because of the well-known fact that energy balance depends on energy consumption and energy expenditure. Similarly, it was reported that there is insufficient evidence to determine a causal relationship between sweetened beverage consumption and obesity. Furthermore, consumption of sweetened beverages does not contribute to overweight in a manner that is any different from that of other energy sources (Forshee *et al.*, 2007). Genetic factors that could predispose to obesity when sweetened beverage intakes are high have not been considered, but could provide new insight into this topic (Bachman *et al.*, 2006).

Two recent studies have been published on this controversial subject. De Ruyter *et al.* (2012) conducted a double-blind, randomised, controlled 18-month intervention trial in 641 Dutch children between the ages of four and eleven years, with normal body weight. Children received either 250 ml sugar-free beverage or a similar sugar-containing beverage that provided 104 ckal (436.8 kJ). Weight gain and body fat gain in healthy children were significantly

reduced by the masked replacement of a sugar-containing beverage with a sugar-free beverage.

While de Ruyter *et al.* (2012) looked at children of normal body weight, Ebbeling *et al.* (2012) investigated the effect that non-caloric beverages could have on the weight gain of 224 overweight and obese adolescents in Boston in the United States. The first year of intervention included home delivery of non-caloric beverages, motivational telephone calls with parents, and check-in visits. The second year of intervention served as a follow-up period. Results showed that with the provision of a non-caloric beverage the consumption of sugar-sweetened beverages was almost eliminated, and that this trend continued throughout the second year of the follow-up period. Significant group differences were reported for body weight after the one-year intervention, but these differences subsided during the follow-up year (Ebbeling *et al.*, 2012).

Section 2.6 provides general information on iron, vitamin A, zinc and iodine deficiencies and is followed by section 2.7, which highlights the relationship of these micronutrients with cognition.

## **2.6 Micronutrient deficiencies:**

For the purpose of this literature review, the focus will fall on vitamin A, zinc, iron and iodine deficiency. According to Black *et al.* (2008), the disease burden attributed to vitamin A and zinc deficiencies is still greater than that attributed to other micronutrients. While intervention programmes have reduced the disease burden of iron and iodine deficiencies, sustained effort is necessary for further reductions. As illustrated in the UNICEF framework (**Figure 2.3**), inadequate dietary intake is a major contributing factor to deficiencies in most micronutrients. While nobody is exempt from micronutrient deficiencies, children remain the most vulnerable, being the group most affected.

### **2.6.1 Vitamin A deficiency**

Worldwide, 33.3 percent of preschool-age children have serum retinol concentrations below 0.07  $\mu\text{mol/L}$ , thus being vitamin A deficient (WHO, 2009). This amounts to 190 million preschool-age children that are affected. After protein-energy malnutrition and iron deficiency, vitamin A deficiency is the most widespread nutritional disease found among children and remains the most common cause of preventable childhood blindness. Vitamin A deficiency can occur at any age, but it remains a serious public health problem for children under the age of six years (FAO & WHO, 2004). In South Africa, only one third of children between the ages of one

and nine years have been reported to have adequate vitamin A status (vitamin A concentration > 20 mg/dL) (Labadarios *et al.*, 2007).

Vitamin A status in a population can be assessed by measuring serum retinol concentrations. A cut-off concentration of 0.7 µmol/L indicates vitamin deficiency, below 0.35 µmol/L indicates severe vitamin A deficiency. Vitamin A is involved in many bodily processes and therefore the effect of vitamin A deficiency goes much further than child blindness alone. The main functions of vitamin A include vision (photopic and colour), cellular differentiation (gene transcription), immune response, haemopoiesis, growth and fertility (McLaren & Frigg, 2001).

Dietary vitamin A intake comes from a wide range of plant and animal sources and inadequate intake of these sources leads to vitamin A deficiency. Developed countries tend to obtain most of their vitamin A intake through animal sources of vitamin A, while developing countries rely on provitamin A carotenoids from plant sources (Ahmed & Darnton-Hill, 2004). Dark green leafy vegetables, tomato's, sweet potato, carrots, oils of palms and yellow fruits (mango, apricot and papaya) are the major sources of provitamin A (McLaren & Frigg, 2001). Plant sources are more affordable in developing countries but, unfortunately, the bio-availability of vitamin A from these sources is lower than it is from animal sources. The bio-availability of preformed vitamin A from animal sources can be from 70–90%, while the bio-availability of provitamin A carotenoids is much lower. Their bio-availability is influenced by the chemical nature, the amount ingested and the food preparation and cooking methods used for carotenoids (McLaren & Frigg, 2001).

In a rural village in South Africa, the production of yellow and dark-green leafy vegetables through a home gardening programme, together with nutrition education, significantly improved ( $p = 0.007$ ) serum retinol concentrations of two- to five-year-old children, whereas those in the control group showed a significant decrease in serum retinol concentrations ( $p = 0.01$ ) (Faber *et al.*, 2002). Therefore, even in communities where plant sources are more affordable than animal sources, the low bio-availability of plant sources should not prevent people from reaching adequate vitamin A intake.

### **2.6.2 Zinc deficiency**

Forty five percent of South African children aged 1–9 years were reported to be zinc deficient in 2005 (Dhansay *et al.*, 2007). Inadequate dietary intake of zinc, found naturally in foods such as meats, lentils (1.27 mg/100 g), rice (0.46 mg/100 g), potatoes (0.26 mg/100 g) and grains (South African fortified brown bread: 1.8 g/40 g slice) (Wolmarans *et al.*, 2010), together with

high intakes of phytates, is the most likely cause of zinc deficiency (Bhan *et al.*, 2001, Taras, 2005).

The International Zinc Nutrition Consultative Group (IZiNCG) describes zinc as being the most ubiquitous trace element that is involved in human metabolism. Together with vitamin A deficiency, it has the largest disease burden of all the micronutrients (Black *et al.*, 2008). Zinc takes part in all the major biochemical pathways and is involved in the perpetuation of genetic material, including deoxyribonucleic acid (DNA) transcription, ribonucleic acid (RNA) translation and cellular division (IZiNCG, 2004). Zinc deficiency can hinder several organ systems, and to a much greater extent during times of rapid growth and development, when nutritional demands are high (Black, 1998; Sandstead *et al.*, 2000).

In South Africa, Samuel *et al.* (2010) conducted a cross-sectional study in children 7 –11 years of age in a poor peri-urban settlement. Forty six percent of the children had zinc values lower than 70 µg/dL and it was suggested that this was a direct consequence of the poverty and food insecurity that contributes to low consumption of sources with high bio-availability of zinc (Samuel *et al.*, 2010). The diets of communities that are burdened by poverty tend to be low in animal products and high in phytate. Furthermore, a high prevalence of diarrhoea increases zinc deficiency because of intestinal losses (Bhan *et al.*, 2001).

Zinc deficiency results in symptoms such as stunted growth in infants, children and adolescents, decreased taste sensation and impaired immune function. Guatemalan children (81.5 ± 7 months, mean, SD) had the greatest response to zinc supplementation with regard to body composition. The effect was more significant in children with low initial hair zinc concentrations (Cavan *et al.*, 1993). Impaired immune function is usually accompanied by increased prevalence of childhood infections (diarrhoea and pneumonia) (IZiNCG, 2004). In cases of severe zinc deficiency dwarfism can occur (Samman, 2007). In addition, the far-reaching effect of zinc deficiency includes its possible effect on cognitive development (Black, 2003a).

### **2.6.3 Anemia**

In 2007, McLean *et al.* reported that more than half (56.3%) of the world's pre-school children were anaemic. More recently, the sixth report on the world nutrition situation noted that some 250 million children were anaemic (United Nations System & Standing Committee on Nutrition, 2010). Africa and Asia are the continents most affected by anaemia, with prevalences as high

as 64.6% and 47.7% respectively (McLean *et al.*, 2007). The African regional trends for anaemia in children under five years old can be viewed in **Table 2.3**. The small percentage of change that took place within a seven-year period is alarming. Given the implications of iron deficiency in children, urgent action to address anaemia is required.

**Table 2.3** Estimated prevalence of anaemia in children 0–5 years old, 2000–2007

Region	Prevalence (%)			Number	Rate (percentage
	2000	2005	2007	(thousand)	points per year)
<b>Year</b>	2000	2005	2007	2007	2000-2007
<b>East Africa</b>	70.7	68.8	66.7	34 524	-0.6
<b>Central Africa</b>	70.3	64.9	62.7	13 604	-1.1
<b>North Africa</b>	42.2	36.3	33.9	7 134	-1.2
<b>Southern Africa</b>	47.5	42.3	40.7	2 485	-1.0
<b>West Africa</b>	69.3	64.2	61.9	29 387	-1.1
<b>Region</b>	64.6	60.8	58.7	87 467	-0.8

Sixth report on the world nutrition situation, progress in nutrition (Adapted from United Nations system & Standing Committee on Nutrition, 2010)

Two South African national surveys reported on iron status. The first study was conducted in 1994 (SAVACG) on children 6–71 months of age while the second study, NFCS-FB, conducted in 2005, included children 1–9 years of age. Poor iron status has increased from a prevalence of 21.4% in 1994 among children 6–71 months of age, to 28.9% of children 1–6 years of age in 2005 (Labadarios & Louw, 2007). **Table 2.4** reflects the percentage of children with anaemia, iron depletion and iron deficiency in South Africa, as reported in the SAVACG-1994 and NFCS-FB-2005 surveys.

Anaemia is normally assessed by measuring haemoglobin levels (De Benoist *et al.*, 2008a). Clinical signs are easily overlooked and it is this less obvious clinical nature of anaemia that contributes to the lack of awareness of the problem (United Nations System & Standing Committee on Nutrition, 2010). Communities and populations all across the globe suffer the effects of poor iron status, making anaemia a worldwide public health problem. Inadequate iron intake, caused either by low dietary intake or by consumption of poorly bio-available iron, will force the body to use stored iron, which, when depleted, will result in iron deficiency (Gleason & Scrimshaw, 2007). The risk of developing IDA is furthermore enhanced in periods of rapid growth, pregnancy and menstruation (Falkingham *et al.*, 2010). Even when iron deficiency has

not yet resulted in anaemia, cognitive impairment, decreased physical capacity, and reduced immunity remain the negative effects found in children (Gleason & Scrimshaw, 2007). The serious implications of the effect of IDA on education are to be found across all cultures, in different settings and environments.

**Table 2.4** Proportion (%) of children with anaemia, iron depletion and iron deficiency in South Africa in 1994 and 2005

Indicator	SAVACG (1994)		NFCS-FB (2005)	
	age	%	age	%
<b>Anaemia</b>				
<b>Hb &lt;11g/dL; children ≤60 months</b>			1–5 years	28.9
<b>Hb &lt;11.5g/dL; children &gt; 60 months</b>	6 months–5 years	21.4	1–9 years	27.9
<b>Iron depletion</b>				
<b>Hb ≥11g/dL; children ≤60 months</b>			1–5 years	7.8
<b>or Hb ≥11.5 g/dL; children &gt;60 months and ferritin &lt;12µg/L</b>	6 months–5 years	4.8	1–9 years	5.7
<b>Iron deficiency anaemia</b>				
<b>Hb &lt;11g/dL; children ≤60 months</b>			1–5 years	11.3
<b>or Hb &lt;11.5g/dL; children &gt;60 months and ferritin &lt;12µg/L</b>	6 months–5 years	5.0	1–9 years	7.6

(Source: SAVACG, 1995 and Labadarios & Louw, 2007)

Hb, Haemoglobin, SAVACG, South African Vitamin A Consultative Group, NFCS-FB, National Food Consumption Survey-Fortification Baseline

#### 2.6.4 Iodine deficiency

Iodine deficiency is the world's most prevalent cause of brain damage, but is also easily preventable (WHO, 2012). For optimal brain development adequate levels of iodine are required. When iodine levels are too low *in utero*, the extreme result is cretinism. Because iodine deficiency impairs mental function in children, adolescents and adults, the potential of cognitive function in whole communities may be impaired (WHO, 2007). Thus, the greater public health consequence is the effect of iodine deficiency on entire populations when reduced cognitive capabilities occur (WHO, 2007).

Dietary iodine intake below recommended levels results in inadequate thyroid hormone production causing iodine deficiency, which results in iodine-deficiency disorders

(Zimmermann *et al.*, 2008). Goitre, the enlargement of the thyroid gland, is the most common clinical sign, and presents itself at all ages. With decreased iodine intake an increase in thyroid-stimulating hormone occurs, the uptake of available iodine is enhanced and the gland finally enlarges homogeneously and nodules develop (Zimmermann *et al.*, 2008).

In the past, goitre prevalence was used to determine iodine deficiency prevalence, and therefore iodine deficiency was regarded as having specific geographical characteristics. In contrast to this belief, the use of urinary iodine estimation to assess iodine deficiency showed that significant iodine deficiency may also occur where the prevalence of goitre is low, in coastal areas, in large cities, in highly developed countries, and where iodine deficiency has been considered to have been eliminated (WHO, 2007).

The WHO states that in 2012, the world is on the verge of eliminating iodine deficiency. This breakthrough is an excellent example to follow for remaining micronutrient deficiencies (WHO, 2012). Great progress has been made with the global decrease in iodine deficiency prevalence in primary school children (5%) but approximately 266 million children and 2 billion people are still at risk of iodine deficiency (de Benoist *et al.*, 2008b).

The most effective way to eliminate iodine deficiency in all regions is through universal salt iodisation, meaning the iodisation of all salt consumed by humans and livestock (WHO, 2007). The major obstacle to successful salt iodisation programmes remains the food industry, may be reluctant to comply. Salt intake per individual does not vary much throughout the year making salt ideal as an ideal iodine fortification vehicle. Furthermore, iodine does not change the appearance or the taste of salt (Zimmerman *et al.*, 2008).

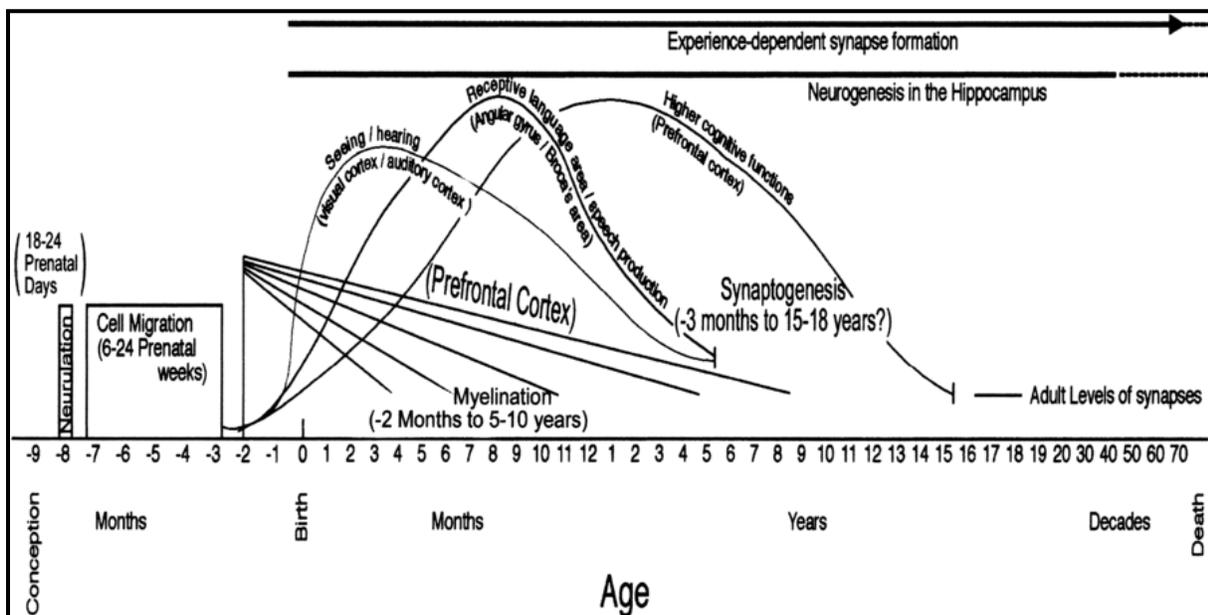
As for the worldwide success in addressing iodine deficiency, similar success stories can be told for South Africa. In 2008, Jooste and Zimmermann (2008) reported that South Africa stood on the verge of eliminating iodine deficiency. Dr Pieter Jooste from the Medical Research Council has passionately educated salt producers in the country over the past decade about iodisation and the effect of iodine deficiency on children's mental development (UNICEF South Africa & Department of Health, 2002). Because a turnover in the role-players involved will happen over time and is inevitable, it is important that education is continuous (Jooste & Zimmermann, 2008). An earlier part of this chapter dealt with the importance of education or the transfer of knowledge in bringing about change.

## 2.7 Nutrition and cognition

### 2.7.1 Nutrition and brain development

The cognitive function of a child is influenced through interaction between the brain and the environment. While genetic factors determine the physical characteristics of the foetal brain, an immediate influence on brain development is the environment (Isaacs & Oates, 2008). The brain development of two foetuses with identical genetic make-up but exposed to different nutritional regimes will ultimately differ. The variety of environmental influences will increase throughout life, but nutrition remains important for the period that brain development continues (Isaacs & Oates, 2008).

It is generally known that a child is most vulnerable during times of rapid growth spurts. Neurogenesis, axonal and dendritic growth, cell death, myelination, synaptic pruning and gliogenesis are all involved in the process of rapid brain development (Grantham-McGregor *et al.*, 2007). These processes occur at different times with overlaps of some of these processes (**Figure 2.4**). Therefore the stage of neural development and the effect that nutrition might have on brain functions are of major importance.



**Figure 2.4** Human brain development (Adapted from: Thompson & Nelson, 2001)

The first brain regions that mature are those involving visual control, balance and motor abilities. Through these a child can explore and interact with the environment. These are followed by structures such as the hippocampus, the left temporal lobes and the right hemisphere, which develops to enable language acquisition, memory, spatial ability and learning. Finally, the frontal lobes are the last brain areas to develop (Hughes & Bryan, 2003). These are responsible for higher-order cognitive activity. Since the different parts of the brain develop at different rates, it is clear that nutritional deficiencies are likely to affect the brain functions that are developing at the time, whether it is during gestation, infancy or childhood (Hughes & Bryan, 2003).

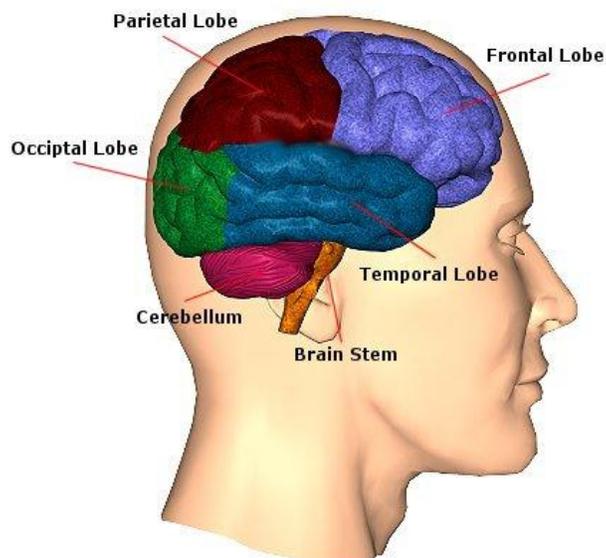
Because neural development can be described as a cascade of events, a small change in neural architecture early in the sequence could have a major impact on later development (Isaacs & Oates, 2008, Grantham-McGregor *et al.*, 2007). The effect of nutrition on brain development does not end when the growth spurts stop, and because brain development continues until adolescence, specific nutritional resources in adequate quantities are needed for the full developmental potential to be reached (Isaacs & Oates, 2008). Growth spurts in brain development may also occur from 2–4, 6–8, 10–12 and 14–16 years of age, and these may be the times recommended for nutrition interventions (Isaacs & Oates, 2008). The first 1000 days of pregnancy and infancy are particularly important to be targeted through nutrition interventions. It is expected that early interventions would reflect in intelligence quotient (IQ) scores, thus on an overall cognitive level, while later interventions could affect more specific domains such as attention and memory (Isaacs & Oates, 2008).

### **2.7.2 Basic brain functioning**

The human body is made of cells and the same is true for the brain. These cells are specialised cells called neurons. There are 180 billion neurons in the brain, of which 80 billion are involved in cognitive processes. The brain has different lobes and subcortical structures, all with specialised functions (**Figure 2.5**). The four lobes in the cerebral cortex can be described as shown in Box 2.1 (Goldstein, 2008):

**Box 2.1** The four brain lobes and their specialised functions

Temporal lobe:	important for language, memory, hearing and perceiving forms
Occipital lobe:	the first place in the cerebral cortex where visual information is received
Parietal lobe:	where signals are received from the touch system and which is also important for vision and attention
Frontal lobe:	higher functions such as language, thought, memory and motor functioning.

**Figure 2.5** The brain lobes

(Source: <http://www.neuroskills.com/brain-injury/brain-function.php>)

The neurons both generate and transmit electrical signals, and communicate with each other through neurotransmitters that are released at the synapse (Goldstein, 2008). Another important group of cells in the nervous system, called glial cells, make up approximately 60% of cellular brain mass (Fernstrom & Fernstrom, 2003). Glial cells provide insulation and physical support around neurons, provide the myelin sheaths that insulate axons and provide metabolic support for dendrites, axons, nerve terminals and synapses (Fernstrom & Fernstrom, 2003).

## 2.8 The role of glucose in brain development and cognitive function

Glucose is the primary energy source of the brain. From birth to four years, glucose utilization in the cerebral cortex rises. At the age of four, glucose usage is more than twice the amount used by adults. Over the next six years, till the age of ten, the high rates of glucose usage is maintained, where after glucose usage decline till it reach adult values at 16-18 years (Chugani, 1998). The brain of a child is more active (per unit of weight) than an adult brain and relatively bigger and therefore children may be particularly vulnerable to either a lack of glucose or glucose administration (Benton & Stevens, 2008). Bellisle (2004) suggested that even in children with maintained adequate nutrition status, the brain can still be sensitive to variation in glucose availability.

A number of studies have investigated the effect of breakfast on cognition. Most people would have fasted for about six to eight hours when they wake in the morning and it is possible that poorer cognitive performance is linked to low levels of energy and low blood glucose levels (Dye *et al.*, 2000).

The effect of fasting was investigated in children 9 – 11 years old, some who were well-nourished from middle-class income families and some children with or without nutrition risk from low-income families (Pollit *et al.*, 1998). Children either consumed a breakfast in the morning ( $\approx 2301\text{kJ}$ ) or not. Children who did not receive breakfast had slower stimulus discrimination, made more errors and had slower memory recall. It seems that by omitting breakfast the effect on cognition is more pronounce in children who are nutritionally at risk (Pollit *et al.*, 1998; Pollit & Mathews, 1998). Benton and Parker (1998) also reported that by eating breakfast, memory was improved when tested later in the morning. Furthermore it seems that by providing a source of glucose, the negative effects on memory by skipping breakfast may be reversed (Benton & Parker, 1998).

The effect of a glucose containing drink in school children has also been explored. As early as 1987, Benton *et al.* reported that a glucose drink administered to 6-7 year old children towards the end of the day improved attention. More recently, in a double blind, placebo controlled cross-over study, sixteen children aged 9-10 years were either given a glucose containing drink or a drink without glucose (Benton & Stevens, 2008). When memory was tested, increased blood glucose due to the glucose drink was associated with improved memory outcomes.

The vulnerability of the brain to fluctuations in glucose levels highlights the importance of the provision of food sources to children that will provide the brain with adequate fuel for optimal

brain functioning. It seems that memory in particular might be negatively influenced when inadequate glucose is provided to the brain as fuel.

## **2.9 Role of micronutrients in brain function**

The blood–brain barrier is located in the endothelial cells and forms the capillaries of the brain. The endothelial cells of the brain capillaries form tight junctions with each other so that no fluid or solutes can pass into or out of the brain without going through these cells. While the penetration of molecules into the brain is highly dependent on their lipid solubility, carriers offer a means of transporting nutrients such as vitamins and minerals into the brain. The access of any nutrient into the brain is dependent on certain properties of the blood–brain barrier and the transport systems that it offers for a specific nutrient (Fernstrom & Fernstrom, 2003).

Neurons and glia, like any other body cells, have demands for minerals. Furthermore, essential minerals are just as important for cellular function in the brain as in any other place in the body. The mechanisms by which vitamin A, zinc, iron and iodine in the brain may influence brain function and development are mostly unclear but existing knowledge will be discussed in the next section.

### **2.9.1 Vitamin A and cognition**

The specific role of vitamin A and its effect on brain function, behaviour and learning is still unclear and not well understood (Benton, 2008; Olson & Mello, 2010). In this relatively new research area, evidence suggests that vitamin A, through retinoic acid, plays an important role in brain physiology (Olson & Mello, 2010).

Like hormones, this fat-soluble vitamin has important physiological functions, usually in the derivative form of retinoic acid (Jiang *et al.*, 2012). Synaptic plasticity, learning and memory, sleep, schizophrenia, depression, Parkinson's disease and Alzheimer's disease might all be affected through retinoic signalling (Tafti & Ghyselinck, 2007). Most research concerning vitamin A and cognitive function has been conducted either in adults or in animal studies. Animal studies focus on vitamin A in learning and memory and are conducted mostly in vitamin A-deficient rodents and mice. Some research that addresses vocal and auditory learning has been done on songbird models (Denisenko-Nehrbass *et al.*, 2000; Scharff & Nottebohm, 1991).

The correct balance of retinoid acid influences behavioural plasticity, which, in turn, affects memory (Olson & Mello, 2010). Retinoid acid concentrations that are either too high or too low

result in negative effects on memory. Complex mechanisms maintain the correct levels of retinoic acid, but the precise pathways of retinoid acid signalling are still unclear.

Research by Etchamendy *et al.* (2003) indicates that spatial learning and memory of vitamin A-deficient adult mice were significantly impaired after 31 weeks of retinol acid treatment. Through acute administration of retinol acid, normal behaviour was partly restored but failed to restore cognitive deficits to the full (Etchamendy *et al.*, 2001; Etchamendy *et al.*, 2003). Adequate vitamin A concentrations in the brain are needed for the maintenance of mature brain functioning (Etchamendy *et al.*, 2003). Similar findings have been reported by Cocca *et al.* (2002), where rats were fed with a vitamin A-free diet for 12 weeks. Results indicated severe deficits in spatial learning and memory, and that cognitive impairment was fully restored when vitamin A was replaced.

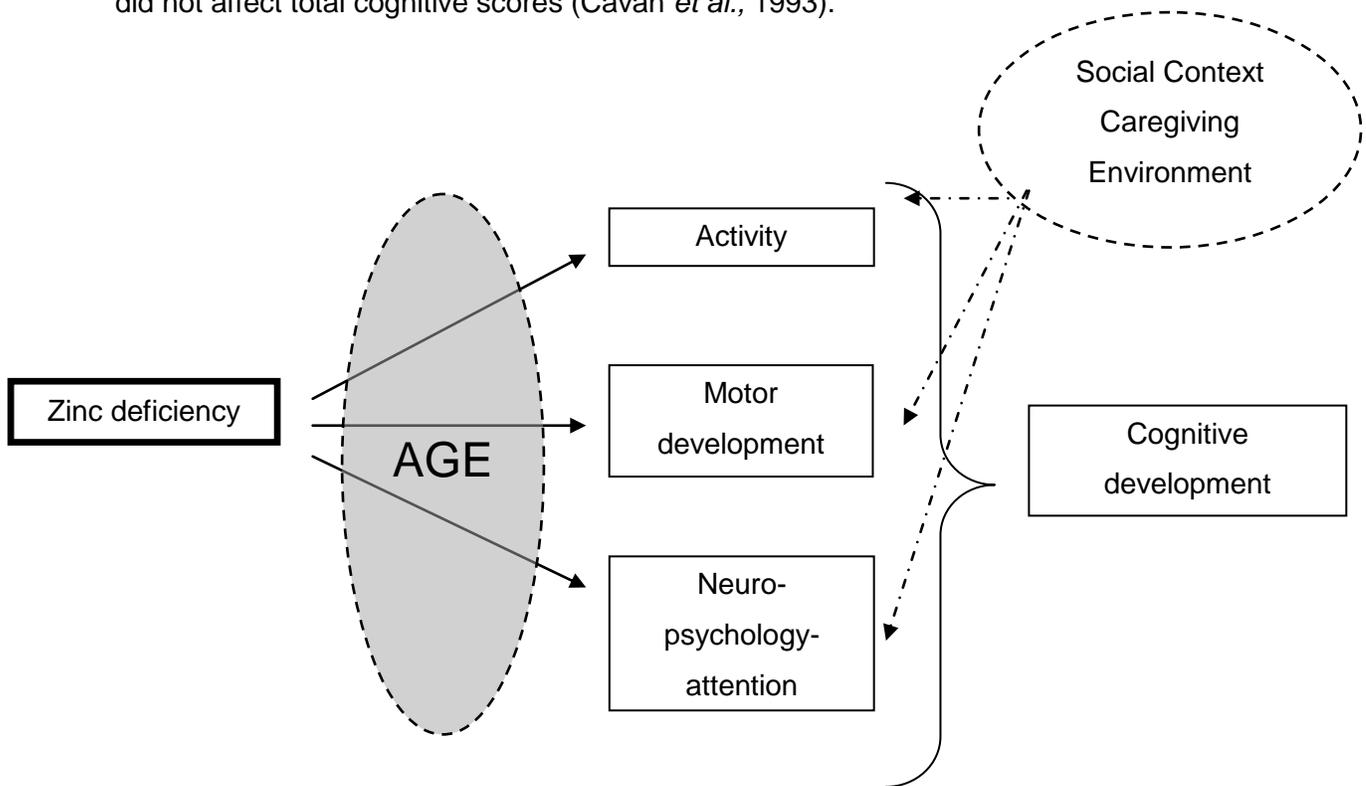
### 2.9.2 Zinc and cognition

While the mechanisms underlying zinc deficiency and cognitive development are unclear, it has been suggested that a child's neuropsychological functioning, activity and motor development are affected by zinc deficiency (Black, 2003b). Black (1998) proposed that zinc deficiency affects cognitive development through a change in attention, activity (neuropsychological behaviour features) and motor development. Five possible explanations have been listed and will be discussed briefly (**Figure 2.6**).

**Firstly**, zinc deficiency may affect a child's emotionality rather than cognitive performance *per se*, meaning that a child deficient in zinc would be especially affected by social context and environmental stress. This has been illustrated mostly in earlier animal studies such as a study by Sandstead *et al.* (1978), where zinc-deprived infant monkeys were reported to be more attached to their mothers and to explore the environment less than the control group.

**Secondly**, alterations in attention, activity and planning are all aspects of neuropsychological functions that might be affected. Randomised controlled trials have reported contrasting results. While there were some indications that zinc repletion might improve neuropsychological performance in children (aged 6 – 9 years) (Sandstead *et al.*, 1998), two earlier studies failed to report any beneficial effects (Cavan *et al.*, 1993; Gibson *et al.*, 1989). Sandstead *et al.* (1998) found improvement in performance only when combined with the supplementation of multiple nutrients and suggested that repletion of other limiting micronutrients is needed to fully observe the effect of zinc repletion (Sandstead *et al.*, 1998). An early study by Gibson *et al.* (1989) in Canada found no differences between children with and without zinc deficiency (serum

zinc < 1.68 $\mu$ mol/g) when children were tested with the Detroit Test of Learning Abilities. These results were similar to results found in Guatemalan children, where zinc supplementation alone did not affect total cognitive scores (Cavan *et al.*, 1993).



**Figure 2.6** A path model linking zinc deficiency in children to cognitive development.

(Adapted from Black, 1998)

**Thirdly**, zinc deficiency also decreases activity levels, which could inhibit cognitive development. Decreased activity has been reported in both animal and human studies (Bentley *et al.*, 1997; Sandstead *et al.*, 1978). Guatemalan infants (six–nine months of age) who were administered 10mg of oral zinc daily for seven months were significantly more frequently observed sitting up *versus* lying down, and playing when compared with the placebo group. They were also significantly less likely to be whining or crying (Bentley *et al.*, 1997). In Indian pre-school children similar results were reported (Sazawal *et al.*, 1996). Zinc supplementation of 10 mg/d for about three months was associated with significantly greater activity levels in children receiving the supplement versus the placebo group.

**Fourthly**, developmental stimulation and maternal responsibility could also contribute to the relationship between zinc and cognitive development (Black, 1998). **Lastly**, in order to fully

understand the association between zinc and cognitive development, it is important to consider the age of a child, for it is expected that the effect of zinc deficiency would be greater in times of rapid growth periods (Bhatnager & Taneja, 2001, Black, 1998; Sandstead *et al.*, 2000). Additional research is necessary to understand the threshold of severity of zinc deprivation in cognitive development, the critical periods sensitive to zinc deficiency or supplementation and the underlying biological mechanisms (Bhatnagar & Taneja, 2001).

### **2.9.3 Iron and cognition**

A great deal of research has been conducted in the field of iron and cognitive function and development, but it nevertheless remains a controversial topic. In this section attention will be given to mechanisms that have been postulated for the altering of cognition by iron deficiency as well as to studies conducted on iron deficiency and cognitive function.

#### **2.9.3.1 Mechanism by which iron deficiency may alter cognition**

Several mechanisms have been suggested by which iron deficiency may alter cognition. Most research in this field has been conducted on animals which suggest structural and functional changes of the central nervous system (Grantham-McGregor & Ani, 2001). While studies in children are limited, a study conducted in children with anaemia investigated auditory brain stem response (Roncagliolo *et al.*, 1998). The response provides a measure of the activation of the auditory pathway from the distal part of the acoustic nerve to the lateral lemniscus. Central conduction time is an indicator of development of the central nervous system. In anaemic infants of six months of age, the central conduction time was longer in comparison with non-anaemic children and did not improve with correction of anaemia but was even longer at 12 months (Roncagliolo *et al.*, 1998). It is possible that the longer central conduction time could be due to changes in myelination (Lozoff *et al.*, 2006), as has been previously reported by Yu *et al.* (1986). Research by Algarin *et al.* (2003) indicates that altered myelination during infancy could have a long-lasting effect on transmission through the auditory and visual systems.

A second hypothesis postulated by Grantham-McGregor and Ani (2001), was that functional isolation linked anaemia to poor development. Because anaemic children were less likely to explore their environment, their behaviour was less stimulating. However, iron deficiency is not the only factor influencing development and in Jamaica behaviour in undernourished children was changed through stimulation alone, without any change in the nutritional status of the children (Grantham-McGregor *et al.*, 1989). It is therefore important to control for background

factors and to take them into consideration when individual studies are reviewed (Lozoff *et al.*, 2006)

### **2.9.3.2 Studies conducted on the field of iron deficiency and cognitive function**

A longitudinal follow-up study of 191 children in Costa Rica aimed to determine the long-term effects of iron deficiency in infancy more than ten years after iron treatment (Lozoff *et al.*, 2000). Children with severe chronic iron deficiency in infancy had significantly lower scores on measures of mental and motor functioning, even after controlling for background differences. These iron-deficient children were, furthermore, more likely to have repeated grades and to have problematic behaviour such as social and attention problems (Lozoff *et al.*, 2000).

Metallinos-Katsaras *et al.* (2004) supplemented three- to four-year-old Greek pre-school children for a period of two months with 15 mg of iron, some of whom had good iron status and some who were iron deficient. In the study the effects of iron supplementation were examined based on the speed of information processing, accuracy of discrimination and conceptual learning. After iron treatment, the children with anaemia made significantly fewer errors of commission, had higher accuracy rates and were significantly more efficient than those receiving the placebo treatment. The authors suggested that advantages of using measures such as reaction time include that it is less sensitive to educational and cultural differences (Metallinos-Katsaras *et al.*, 2004). The reported effects were not found for children with good iron status. No effects were found on the learning test used. In contrast to the results of Metallinos-Katsaras *et al.* (2004), iron-folic acid supplements (20 mg elemental iron and 0.1 mg folic acid for 60 days) had a beneficial effect on cognitive performance in Indian children aged 5 – 8 years, as measured by Wechsler intelligence scale for children (WISC)-scores, in both anaemic and non-anaemic children.

In the United States a study conducted by Halterman *et al.* (2001) reported on the relationship between iron status and cognitive achievement by means of logistic regression analysis. This study was nationally representative of school-aged children and adolescents. The results indicated that lower standardised math scores were reported for iron-deficient school-aged children and adolescents, even in the absence of anaemia (Halterman *et al.*, 2001). Bruner *et al.* (1996) similarly reported in another study conducted in the United States in non-anaemic iron-deficient high school girls that even when anaemia was absent, certain aspects of cognitive function could be affected.

The few studies briefly discussed above direct one's attention to several points. It seems that although not all children respond equally to supplementation with regard to cognitive function and development, there is always a sub-set of children, characterised by diets low in micronutrients, that does respond. It is clear that the age of a child and the duration of iron deficiency have different effects on cognitive outcomes. Furthermore, it seems that it is not clear whether there is a threshold at which iron deficiency starts to impact on cognitive function. The final question should be to what extent supplementation can correct cognitive deficits that might occur due to iron deficiency. Since the year 2000, a number of reviews have been conducted in the field of poor iron status and the link with cognitive function in school children (Grantham-McGregor & Ani, 2001; Sachdev *et al.*, 2004; Falkingham *et al.*, 2010), as has been summarised in **Table 2.5**. Benton (2001) stated that overall, positive response to iron supplementation has been reported for non-verbal measures of intelligence, but not for verbal measures. While non-verbal tests look at biologically-based functioning, verbal tests reflect knowledge and vocabulary. Therefore non-verbal tests are expected to reflect dietary inadequacy while it is not expected that verbal tests would be improved with short-term micronutrient supplementation. In primary school children, it was suggested that iron therapy results in significant positive change in specific cognitive tests that include motor development and language tests (Beard & Connor, 2003)

A meta-analysis by Sachdev *et al.* (2004) reported modest improvement in mental development due to iron supplementation when IQ scores were examined. In results similar to those of Benton (2001), this improved effect was particularly apparent in initially anaemic or iron-deficient anaemic children, thus children with poor dietary intake of iron.

Recently, Falkingham *et al.* (2010), in a review on the effect on iron supplementation on cognition, concluded that well-powered, blinded, independently funded randomised controlled trials with a trial duration of at least a year were needed to confirm that iron supplementation improves attention, concentration and IQ. Particularly for outcomes such as scholastic achievement, duration is critically important. This is not to say that the duration needed for improved serum ferritin or haemoglobin concentrations is adequate time for performance to be affected. It might be that studies of up to 29 weeks' duration are not long enough to see effects on scholastic achievements, thus emphasising the importance of long-duration research in this area (Falkingham *et al.*, 2010). In order for children to catch up on school achievement, they have to learn material that they have missed throughout the years and in schools without the

necessary facilities to provide special attention, catch-up might not even occur (Grantham-McGregor & Ani, 2001).

**Table 2.5** A summary of reviews conducted on iron status, supplementation and cognition that includes data on school children since 2000

Author and type of study	Year of publication	Aim	Up and till the year	Number of articles reviewed	Age of subjects included	Conclusion
Grantham-McGregor & Ani (Review)	2001	To assess evidence of a causal relationship between iron status, cognition and behaviour.	2000	Longitudinal observation studies (n=10) Therapeutic treatment trials >2yrs (n=14)*	Longitudinal observation studies (birth to 14 years) Therapeutic treatment trials (2 to 24 years)	Longitudinal studies indicate that children who were anaemic in early childhood continued to be at a developmental disadvantage at one or more of the follow-up assessments. Therapeutic treatment trials indicate for children > 2 years that anaemic children usually had poorer cognition and school achievement than non-anaemic children. Anaemic children tend to catch up through treatment with regard to cognition but not in school achievement.
Sachdev et al. (Systematic review and meta-analysis)	2004	To evaluate the effect of iron supplementation on mental and motor development in children	1966-March 2003	Seventeen randomised controlled trials	Infants and toddlers (11 studies) Older children (6 studies)	Iron supplementation improves the mental development score of children marginally but significantly. The benefits were greater among initially anaemic or iron-deficient anaemic subjects. In children older than 7 years, iron administration resulted in a significant improvement in pooled intelligence quotient scores
Falkingham et al. (Systematic review and meta-analysis)	2010	To assess whether iron supplementation improves cognitive domains: concentration, intelligence, memory, psychomotor skills and scholastic achievement.	2008	Fourteen	Males and females > 6 years (children and adolescents: n=10, remaining subjects were females)	Regardless of baseline iron status, some evidence was found that attention and concentration in adolescents and women improved Iron supplementation also improved intelligence quotient in women and <b>children</b> who were anaemic at baseline, but had no effect on memory, psychomotor skills or scholastic achievement.

\*Studies for children younger than 2 years are not listed

Source: Grantham-McGregor & Ani, 2001; Sachdev *et al.*, 2004; Falkingham *et al.*, 2010

#### **2.9.4 Iodine and cognition**

While it is clear that *in utero* iodine deficiency impairs foetal growth and brain development, the postnatal effects on growth and brain development are less clear (Zimmermann *et al.*, 2008). In a meta-analysis conducted in populations with chronic iodine deficiency, a reduction in intelligence quotient of 5 to 13 points was reported (Qian *et al.*, 2005). Zimmermann *et al.* (2006) found that iodine supplementation (400 µg iodine as oral iodised oil) versus placebo improved cognition measured as information processing, fine motor skills and visual problem-solving in moderately iodine-deficient school children aged 10–12 years in Albania.

Similar results were reported in mildly iodine-deficient children in New Zealand. Iodine supplementation (150 µg/day for 28 weeks) improved perceptual reasoning (Gordon *et al.*, 2009). Gordon *et al.* (2009) aimed to answer the question whether or not supplementing iodine in mildly iodine-deficient children would improve cognition. The trial was conducted in 184 children 10 – 13 years of age. Except for the positive results on perceptual reasoning, the results showed that mild iodine deficiency in children could prevent children from reaching full intellectual potential.

In Malaysian children, no effect on mental performance was reported after 12 months of iodised oil supplementation, despite a significant increase in serum thyroxine hormone (T4) concentrations (Isa *et al.*, 2000). The authors concluded that environmental and genetic factors that could not be controlled for might have contributed to the results

It seems that for children born in areas with high risk for iodine deficiency, cognitive impairment could be partly reversible with iodine supplementation. If iodine deficiency is not corrected, even in cases of mild iodine deficiency children could fail to reach their full intellectual potential (Gordon *et al.*, 2009, Taras, 2005, Zimmermann *et al.*, 2008).

#### **2.9.5 Multiple-micronutrient supplementation and cognition at primary school level**

It is clear from the above section on single-micronutrient effect on cognition that extensive research has been conducted with contrasting results. Micronutrients however, do not function in isolation, and a diet that is deficient in one is likely to be deficient in other micronutrients too

(Benton, 2008). This forms important motivation to look at the effect of multiple-micronutrient supplementation on cognitive function/performance and development.

In Nigerian primary school children, a multi-micronutrient beverage enhanced vitamin A and zinc status over a period of six months but had no significant effect on haemoglobin or serum ferritin concentration (Aaron *et al.*, 2011). The authors suggested that one or more of the micronutrients in the beverage could have interfered with absorption and that subclinical inflammation was not controlled for in the analysis. While iron status was not significantly changed in the Nigerian school children (Aaron *et al.*, 2011) haemoglobin, serum ferritin and zinc protoporphyrin were significantly improved in Tanzanian school children who received a fortified beverage for six months (Ash *et al.*, 2003). Adolescent girls in Bangladesh also showed significantly improved concentrations of haemoglobin and serum ferritin after 12 months of administration of a multiple-micronutrient-fortified beverage (Hyder *et al.*, 2007). Additional intake of micronutrients is likely to result in improved micronutrient status; however, it is known that several factors, such as a diet high in phytate, might interfere with the absorption of micronutrients. In addition to the question on whether micronutrient status is improved through multiple-micronutrient supplementation, one might ask whether it is improved to the extent that cognitive performance is influenced.

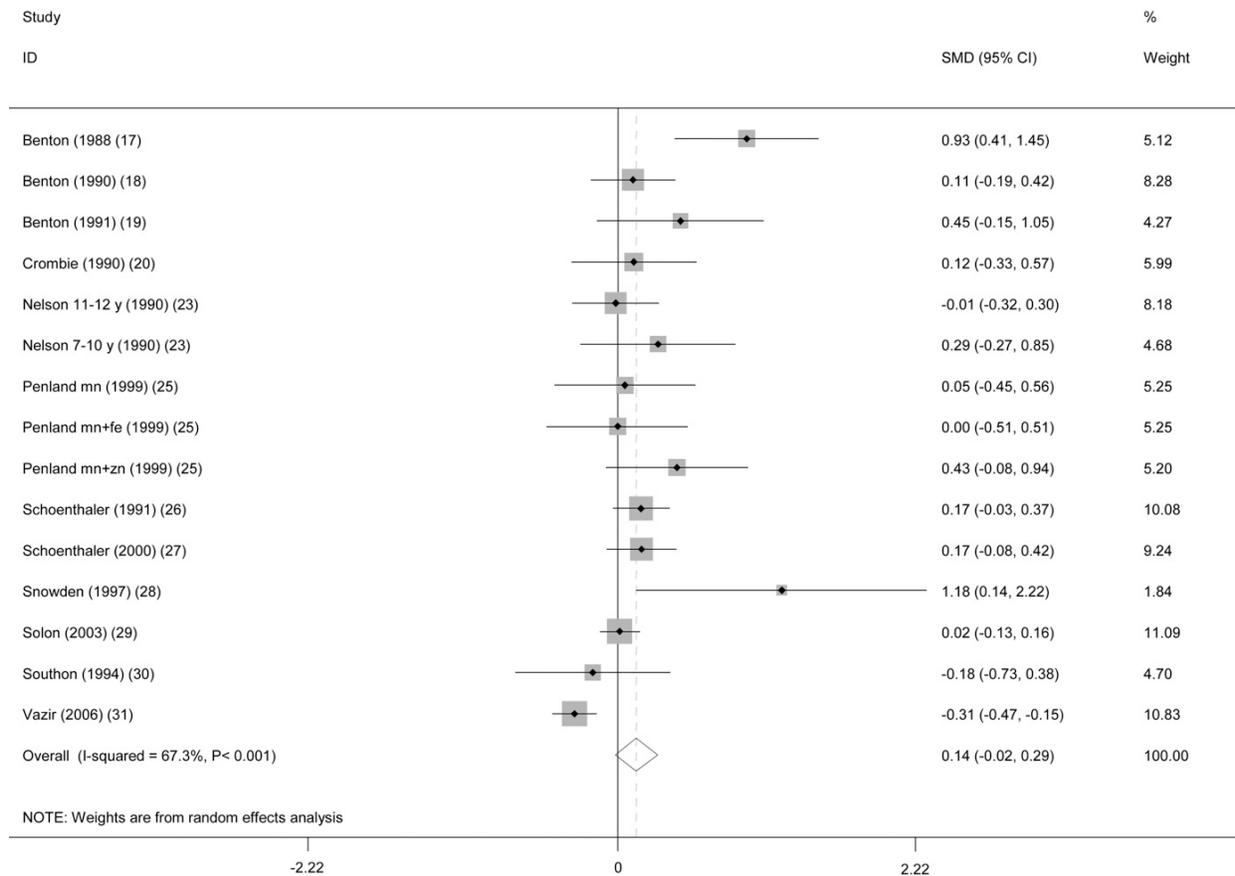
Vazir *et al.* (2006) reported significant improvement in attention-concentration in middle-income semi-urban Indian children when a multiple-micronutrient-fortified beverage was administered for 14 months. However, even though significant improvement in the micronutrient status of children in the micronutrient-supplemented group was reported, memory, school achievement and IQ were not influenced. Children included in the study had above-average IQ at baseline and therefore it is possible that the already relatively high IQ could explain the lack of effect being observed. Perlman *et al.* (2010) reported similar findings when school performance did not improve significantly when multi-micronutrient supplements were administered for almost nine months.

Two independent reviews have recently published results on the effects of multiple-micronutrient interventions, either *via* supplementation or fortification, and their effect on cognitive performance in children (Eilander *et al.*, 2010; Best *et al.*, 2011). In the review by Eilander *et al.* (2010), children of the ages of 0 – 18 years were included. Studies included in the review compared the effect of foods fortified with a minimum of three micronutrients with that of placebo/non-fortified foods. Twenty trials were identified, of which 18 were conducted in

children with a mean age between 6.5 and 14 years over a time period of twenty years, from 1988 to 2008. A key strength of this review was that it included a meta-analysis at the level of the different cognitive domains that has not been reported in previous literature (**Figure 2.7**). The meta-analysis suggested a small but non-significant positive effect of multiple-micronutrient supplementation on fluid intelligence (reasoning ability) (**Figure 2.7**). No effect was reported on crystallised intelligence (acquired knowledge) but academic performance was positively affected (limited number of four trials,  $p = 0.044$ ).

Crystallised intelligence is dependent on education (Kaufman *et al.*, 2005). It should not be associated with memory or knowledge but rather with the ability to use skills, knowledge and experience. Fluid intelligence includes non-verbal intelligence and is independent of previously attained knowledge. It refers to the ability to think logically and solve problems. Crystallised intelligence is resistant to age while fluid intelligence is more vulnerable to the effect of aging (Kaufman *et al.*, 2005).

Best *et al.* (2011) included only school children, with a minimum of 75% of the study population being in the range of 6–18 years. As was the case in the review of Eilander *et al.* (2010), food had to be fortified with a minimum of three micronutrients (micronutrients from the B-vitamin complex considered as one micronutrient) to be defined as multiple-micronutrient-fortified food. Twelve individual studies were included, ranging from 1999 to 2009. The authors indicated that although it seems clear that with multiple-micronutrient intervention, biochemical indicators of micronutrient status (with the exception of zinc) often result in positive effects in school children, it is unclear if this effect is such that a measurable impact can be found on functional health outcomes, including growth and cognitive development (Best *et al.*, 2011).



**Figure 2.7** Forest plot for fluid intelligence, SMD, standardised mean difference; mn, micronutrients (Taken from: Best *et al.*, 2011)

Best *et al.* (2011) reported that results of multiple-micronutrient-fortified foods on cognitive performance are inconsistent and differ by cognitive domain. **Table 2.6** gives an overview of the effect of multiple micronutrients that were provided in a food matrix on biochemical indicators and cognitive outcomes. It seems that working memory is mostly affected, and studies that do find a clear beneficial effect commonly have long trial durations (more than six months) and combine administration of iron, vitamin A, iodine and/or zinc (Van Stuijvenberg *et al.*, 1999; Vazir *et al.*, 2006). Several reasons for above findings have been hypothesised. It is possible that test kits are more sensitive to picking up changes in crystallised intelligence than in fluid intelligence. Furthermore, it could simply be that, as in reports on single-nutrient supplementation of iron, longer trial duration is needed to observe changes in cognitive function, and studies up to now have been too short to demonstrate effects.

There may also be differences found between developed and developing countries in terms of test kit requirements. It may be that the cognitive test kits that originate from developed

countries lose their sensitivity when adapted for developing countries. It is also likely that protein and energy malnutrition could occur simultaneously with micronutrient deficiencies and if this is uncorrected it may override the beneficial effect of micronutrient intervention on cognitive performance (Eilander *et al.*, 2010). As for all the micronutrients, the effect of environmental factors such as education, parenting styles, and socio-economic factors could play a more important role in crystallised intelligence than the correcting of micronutrient status (Eilander *et al.*, 2010).

As has been mentioned, different test kits are used to measure cognitive function and development. Cognitive tests are generally used for study outcomes but cognition is a relatively new field to nutritionists and is, to some extent, still an unfamiliar field. Assessment of cognitive abilities and test selection in children are discussed in the following section.

**Table 2.6** Overview of the results of studies assessing effect of multiple micronutrients provided in a food matrix on micronutrient status and cognitive outcomes in school-age children (Adapted from Best *et al.*, 2011)

	Micronutrient status					Cognitive performance			
	Iron	Hb/ Anaemia	Iodine	Vitamin A	Zinc	Memory	Fluid intelligence	School performance	Other cognitive outcomes
<b>Multiple micronutrient-fortified food versus unfortified food</b>									
Van Stuijvenberg <i>et al.</i> (1999)	✓	✓	✓	✓	-	✓	-	-	X <sup>1</sup>
Abrams <i>et al.</i> (2003)	✓	✓	-	X	-	-	-	-	-
Ash <i>et al.</i> (2003)	✓	✓	-	✓	-	-	-	-	-
Solon <i>et al.</i> (2003)	-	✓	✓	-	-	-	-	-	(✓) <sup>2</sup>
Sivakumar <i>et al.</i> (2006)	✓	(✓) <sup>3</sup>	✓	✓	X	X	X	X	X <sup>4</sup>
Hyder <i>et al.</i> (2007)	✓	✓	-	✓	X	-	-	-	-
Osendarp <i>et al.</i> (2007) (Australia)	✓	x	-	-	X	(✓) <sup>6</sup>	X	X <sup>5</sup>	X <sup>4</sup>
Osendarp <i>et al.</i> (2007) (Indonesia)	✓	✓	-	-	X	✓	X	X <sup>5</sup>	X <sup>4</sup>
Manger <i>et al.</i> (2008)	X	✓	✓	X	✓	✓	-	X	-
Lien do <i>et al.</i> (2009)	-	-	-	-	-	X	-	-	X <sup>7</sup>
Nga <i>et al.</i> (2009)	✓	✓	✓	✓	✓	-	-	-	-
<b>Multiple micronutrient-fortified food versus single-fortified food</b>									
Zimmerman <i>et al.</i> (2004)	✓	✓	-	✓	-	-	-	-	-

✓ Significant beneficial effect of MMN (comparing change from baseline between or within groups, or means between groups at follow-up)

(✓) Significant beneficial effect of MMN in subgroup only (comparing change from baseline between or within groups, or means between groups at follow-up)

-Parameter not assessed

X No significant effect

<sup>1</sup>Outcome measured: Speed performance

<sup>2</sup>Total cognitive score: effect only in severely-to-moderately anaemic children

<sup>3</sup>Effect only in deficient/anaemia/malnourished children

<sup>4</sup>Outcome measured: attention and concentration

<sup>5</sup>Results on academic performance were integrated in a cluster of other cognitive tests

<sup>6</sup>Effect on verbal learning and memory, only in girls

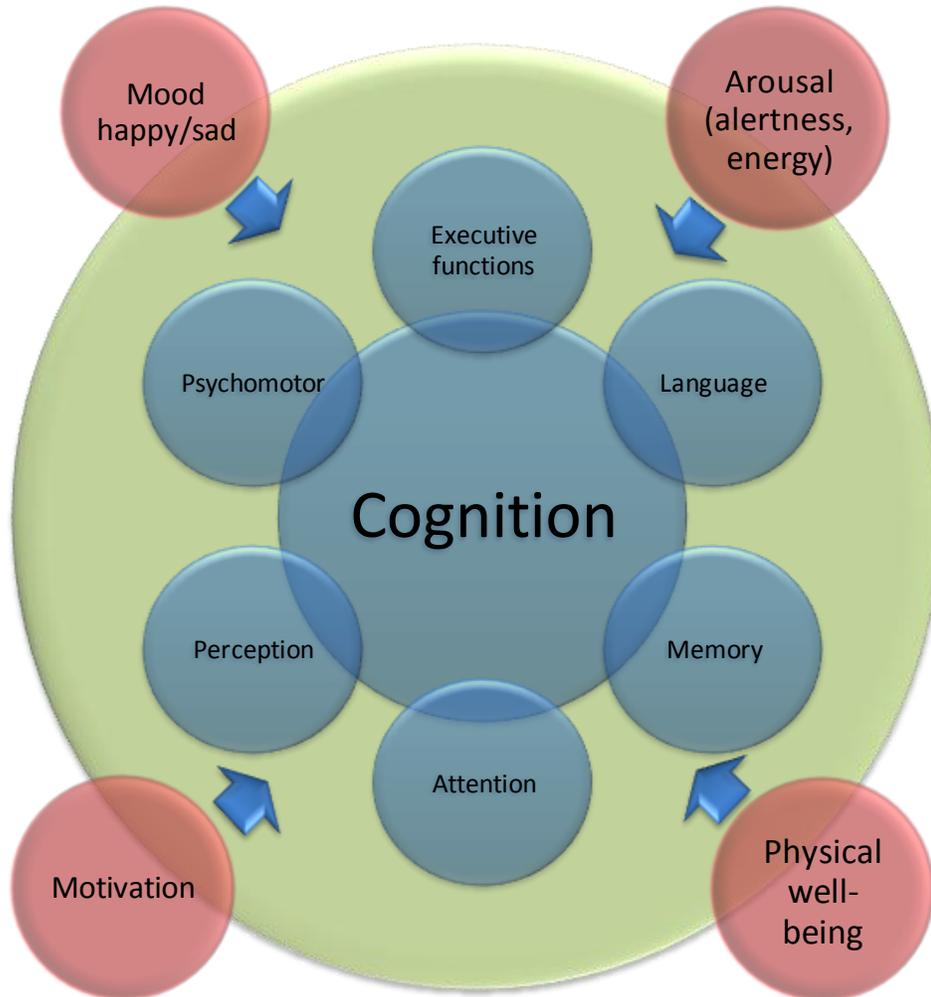
<sup>7</sup>Outcome measure: accuracy and efficiency of working

## 2.10 Assessment of cognitive abilities in children

The field of nutrition and cognitive function in children has caught the interest of many nutritionists. Though much research has been done on the underlying mechanisms by which nutrients might affect the brain, less research has been done on the correct cognitive test that should be selected (Hughes & Bryan, 2003). The growing awareness of the possible effects that nutrients might have on cognitive performance and the desire to measure these effects have forced food scientists, government, food industry representatives and nutritionists to recognise the importance of cognitive psychology (Schmitt *et al.*, 2005). Few nutritionists have been trained in the basic principles of assessing cognitive function in children (Isaacs & Oates, 2008). It is important, therefore, that nutritionists understand the basic principles of brain development, the relationship between cognition and nutrition and the cognitive functions that are tested.

When cognitive outcomes in children are assessed, behaviour (meaning performance rather than competence) is measured. A child is born with a certain intellectual potential that needs to be in a stimulating environment to reach the maximum potential. Nutrition is one of many contributing factors to the quality of the environment (Isaacs & Oates, 2008). However, nutrition provides building blocks for cellular growth and development. The expected effect of nutrition on cognitive abilities might be subtle but should not be underestimated (Hughes & Bryan, 2003).

**“Cognitive functions”** is a concept that can be explained as the process of taking information from the environment, processing this information internally and finally, responding to the information through specific behaviour (Isaacs & Oates, 2008). In other words, through these brain functions a person is able to perceive, evaluate, store, manipulate and use information obtained from internal (memory and thoughts) and external resources (environment) and act on it (Schmitt *et al.*, 2005). The six main domains of cognitive function include: language, executive functions, memory, attention, perception and psychomotor skills. Furthermore, these functions can be influenced by factors such as the mood, motivation, physical well-being and arousal (alertness) of the child (Schmitt *et al.*, 2005; Isaacs & Oates, 2008) (**Figure 2.8**).



**Figure 2.8** The interaction between the cognitive functions (in blue) and factors that may influence the efficiency of cognitive processing (in pink) [Adapted from Schmitt *et al.* (2005)]

The most general level of overall intellectual ability can be expressed as intelligence (often referred to as “g”). This “g” concept has led to what is now generally known as intelligence quotient (IQ). IQ is based on mental age, thus comparing the performance of an individual to the performance of a large group with similar chronological age. The next level refers to the six domains that were mentioned earlier (**Figure 2.8**). These cognitive domains are not mutually exclusive. This means that some of the domains may overlap, but because different tests are used to measure them it is more convenient to label them separately (Isaacs & Oates, 2008).

For the next level, each cognitive domain is broken down into sub-processes or components that are important for determining the level of function of each domain. In order to give a true

reflection of performance within a specific domain a series of tests should be administered and reporting only one test for a specific domain should be avoided.

### 2.10.1 Test choice

There are a number of tests that can be used to measure neuropsychological performance in an objective manner. Speed or accuracy is measured mostly as major test outcomes, but this may differ (Schmitt *et al.*, 2005). Because of the many factors influencing cognitive development, it is extremely difficult to single out the exact effect of nutrition, and therefore careful experimental study designs are extremely important (Isaacs & Oates, 2008).

It is important that researchers should be certain and specific about the exact cognitive functions that they wish to measure. The chosen tests should show adequate reliability and discrimination power and should be a valid measure of the function that they claim to assess. The ideal is that tests should be standardised for administration in the country and culture where they will be used, but this is not always possible as most test kits are developed in the United States and Europe (Hughes & Bryan, 2003). Where local tests are unavailable, existing tests are adapted for the unique cultural setting (Hughes & Bryan, 2003). Non-verbal performance tests are recommended in populations where it is likely that wide ranges of verbal abilities and cultural backgrounds may influence the outcomes.

While it has been common practice to make use of a global measure, such as IQ, it is now clear that a global measure without specific tests cannot adequately describe cognition. When the same tests are conducted at the beginning of an intervention and again at the end point, the initial score should be used as a covariate in the statistical data analysis. The “gold standard” for research in the field of nutrition remains the randomised controlled trial (Isaacs & Oates, 2008). Owing to the random nature of the design, many of the extraneous factors will be washed out. However, if there are certain socio-economic differences in groups then the factor needs to be controlled. This is done statistically either by proving that there was no difference between groups or by using it as a covariate in the analysis. When statistically significant differences are found the underlying effect sizes are of great importance and should be interpreted together (Isaacs & Oates, 2008).

The Kaufman assessment battery for children (KABC-II) is discussed in more detail. The KABC-II was chosen for the **B**everage **f**ortified with **m**icronutrient (BeForMi) study that was conducted in 2010 in the North-West province of South Africa and had been used in previous research trials in South Africa (Baumgartner *et al.*, 2012; Ogunlade *et al.*, 2010). The BeForMi

intervention forms part of the thesis. This KABC-II test kit is based on the theory of the Luria neuropsychological model and has been designed to minimise the influence of language and cultural knowledge on test outcomes (Kaufman *et al.*, 2005). The tests were devised in the United States and were intended to be used in multicultural populations in America. Some of the subtests were used in the BeForMi study to indicate group differences in particular aspects of cognitive processing in our population of school children in Africa.

### **2.10.2 Kaufman assessment battery for children (KABC)-II: Luria's Neuropsychological theory**

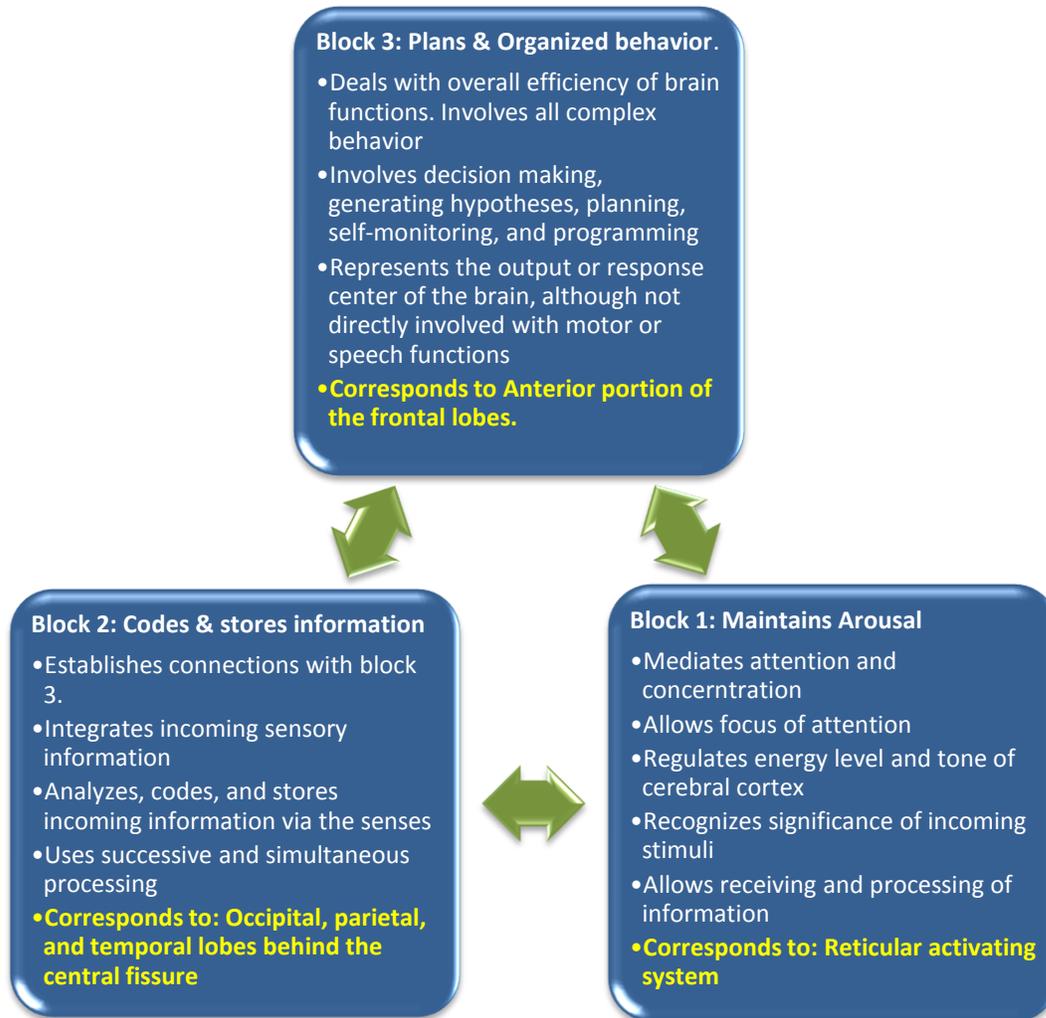
The theoretical foundation model, the Luria Theory, excludes measures of acquired knowledge. This model is preferred when a child is from a bilingual background and if his/her non-mainstream cultural background may have affected knowledge acquisition and verbal development. This global score is known as the Mental Processing Index (MPI).

According to the Luria Theory, the brain's basic functions can be divided into three main blocks or functional systems, which are interdependent (**Figure 2.9**). The KABC-II was designed to measure high-level, complex, intelligent behaviour, and the integration of the three Luria blocks fully represents this complexity (Kaufman & Kaufman, 2004; Kaufman *et al.*, 2005). The Luria theory emphasises the integration of incoming stimuli that are received, and block 2 (codes and stores information), which has the responsibility of connecting with block 3 (plans and organises behaviour). The KABC-II therefore includes subtests that focus on auditory and visual stimulation, and in order to capture the linkage between blocks 2 and 3, simultaneous processing is measured. These subtests not only need analysis, coding and storage of incoming stimuli but also require executive functioning with problem solving (Kaufman & Kaufman, 2004; Kaufman *et al.*, 2005). **Table 2.7** summarises the KABC-II scales and a gives a description of the subtests that can be used for each scale.

#### **Definitions for the Luria scale (Kaufman *et al.*, 2005):**

##### **Learning ability**

Learning ability reflects the integration of the processes associated with all three blocks. A premium is placed on the attention-concentration processes (Block 1) but coding processes from block 2 and strategy generation from block 3 are also required to learn and retain the new information.



**Figure 2.9** The three blocks of Luria's neuropsychological theory

[Adapted from Kaufman & Kaufman (2004) and Kaufman *et al.* (2005)]

### Sequential processing

Sequential processing measures successive coding function 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. Sequential processing is primarily associated with block 2.

### Simultaneous processing

Simultaneous processing measures the simultaneous coding function that is associated with block 2. For its tasks the input has to be integrated and synthesised simultaneously, usually spatially, to produce the appropriate solution. Block 2 and block 3 are blended to enhance the complexity of the simultaneous synthesis that is required.

### **Planning Ability**

Planning ability measures the high-level, decision-making executive processes associated with block 3. Because any cognitive task involves perception of sensory input and either a motor or a verbal response, the KABC-II measure of planning ability necessarily requires functions associated with the other two blocks as well.

**Table 2.7** Short summaries of the Kaufman Battery for Children subtests

<b>Scale/Subtest</b>	<b>Description</b>
<b>Sequential</b>	
Number recall	Child repeats a series of numbers in the same sequence as given by the examiner.
Word Order	The child touches a series of silhouettes of common objects in the same order as the examiner said the names of the objects.
Hand Movements	The child copies the examiner's precise sequence of taps on the table with the fist/palm/side of hand.
<b>Simultaneous</b>	
Block counting	The child counts the exact number of blocks in various pictures of stacks of blocks. The stacks are configured so that one or more blocks are hidden or partially hidden.
Conceptual thinking	The child views a set of four or five pictures and identifies the one picture that does not belong.
Face recognition	The child attends closely to photographs of one or two faces that are exposed briefly and then selects the correct face or faces, shown in a different pose, from a group photograph.
Pattern reasoning	Similar to planning
Rover	The child moves a toy dog to a bone on a checkerboard-like grid that contains obstacles and tries to find the "quickest" path - the one that takes the fewest moves.
Story completion	The child is given pictures depicting a story line, with some missing pictures. The child selects the picture that would complete the story correctly.
Triangles	The child assembles several identical foam triangles to match a picture of an abstract design. For easier items, the child assembles a set of colourful plastic shapes to match a model constructed by the examiner or shown on the easel.
Gestalt Closure	The child mentally "fills in the gaps" in a partially completed "inkblot" drawing and names the object or action depicted in the drawing.
<b>Planning</b>	
Pattern Reasoning	The child is shown a series of stimuli that form a logical, linear pattern, but one stimulus is missing. The child completes the pattern by selecting the correct stimulus from an array of four to six options at the bottom of the page.
Story completion	The child is shown a row of pictures that tell a story, but some of the pictures are missing. The child is given a set of pictures and places the missing pictures in their correct location.
<b>Learning</b>	
Atlantis	The examiner teaches the child the nonsense names for fanciful pictures of fish, plants, and shells. The child demonstrates learning by pointing to each picture when it is named.
Rebus	The examiner teaches the child the word or concept associated with each particular drawing (rebus) and the child reads aloud phrases and sentences composed from these rebuses.
Atlantis Delayed	The child demonstrates delayed recall of paired associations learned about 15–25 minutes earlier during Atlantis by pointing to the picture of the fish, plant, or shell that is named by the examiner.
Rebus Delayed	The child demonstrates delayed recall of paired associations learned about 15–25 minutes earlier during Rebus.

[Adapted from Kaufman &amp; Kaufman, (2004)]

### **2.11 Concluding remarks:**

There is still an unacceptably high prevalence of micronutrient malnutrition in South Africa. The magnitude of the nutrition problem and its far-reaching affects has been emphasised in recent years by the report from the Word Bank in 2006, the Lancet series in 2008 and the Copenhagen Consensus in 2012.

The mechanisms through which micronutrient malnutrition might affect cognition have been investigated, but most of these mechanisms still remain unclear. Vitamin A, iodine, zinc and iron have been investigated as independent vitamins and minerals but because micronutrients do not function in isolation, attention should be paid to the possible effect of multi-micronutrient fortification on improving micronutrient status and its effect on cognitive function. Nutrition intervention strategies that are known to work to reduce malnutrition should be utilised according to the needs and requirements of each country. One of these strategies is fortification. The aim of this thesis is, therefore, to determine the effect of a micronutrient-fortified beverage on cognition and on the nutritional status of primary school children.

## CHAPTER 3: ARTICLE 1



This article is based on the BeForMi intervention that was conducted. The aim of the study was to investigate the effects of micronutrients and sugar, alone and in combination, in a beverage, on growth and cognition in South African children. The article has been submitted for publication to the British Journal of Nutrition (BJN-2012-019248).

**Effects of a multi-micronutrient-fortified beverage, with and without sugar, on growth and cognition in South African schoolchildren: a randomised, double-blind, controlled intervention.**

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**Shortened title:** Fortification positively affects cognition

**Key words:** micronutrient status, cognitive function, anthropometric status, multi-micronutrient fortified beverage

**ABSTRACT**

Little is known about the effects of combined consumption of micronutrients and sugar on growth and cognitive function. We investigated the effects of micronutrients and sugar, alone and in combination in a beverage, on growth and cognitive function in schoolchildren. The children selected for the study were those with the poorest iron status. In a 2-by-2 factorial design, children (n = 408, aged 6–11 years) were randomly allocated to receive a beverage containing 1) micronutrients with sugar, 2) micronutrients with non-nutritive sweetener, 3) no micronutrients with sugar or 4) no micronutrients with non-nutritive sweetener, for 8.5 months. Growth was assessed by determining weight-for-age, height-for-age and body-mass-index-for-age z-scores. Cognition was tested by using sub-scales of the Kaufman Assessment Battery for Children. Micronutrients decreased the odds ratio (OR) for being iron-deficient at endpoint (OR 0.15, 95% CI: 0.03, 0.78). Micronutrients (intervention effect: 0.76; 95% CI: 0.10, 1.42) and sugar (0.71; 95% CI: 0.05, 1.37) increased Atlantis test scores, and sugar increased Rover test scores (0.72; 96% CI: 0.08, 1.35). Significant micronutrient x sugar interactions were found on Atlantis, Number Recall and Rover test scores, indicating that micronutrients and sugar in combination attenuated the beneficial effects of micronutrients or sugar alone. Micronutrients or sugar given alone lowered weight-for-age z-scores (WAZ), but when given in combination the lowering effect was attenuated. The provision of a beverage fortified with micronutrients or added sugar had a beneficial effect on cognitive function and a lowering effect on WAZ in school children. These effects were attenuated when micronutrients and sugar were provided in combination.

## INTRODUCTION

The most recent South African National Food Consumption Survey (NFCS) in 2005 reported that almost 14% of South African children aged 1–5 years were vitamin A deficient (Serum Retinol (SR) < 10 µg/dl) and 27.9% were anaemic (Hb < 11g/dl for children)<sup>(1)</sup>. The reported prevalences were reason for concern, mostly because it seemed that no improvement in vitamin A and iron status had been achieved since the national survey of 1994, where 3.3% of children were vitamin A deficient and 21.4% anaemic<sup>(2)</sup>.

A number of studies have reported on the adverse effects of missing breakfast on memory<sup>(3-5)</sup>. Furthermore, increased blood glucose concentrations due to a glucose-containing beverage have been reported to improve memory<sup>(6)</sup>. Glucose is the major fuel of the brain and is continuously maintained at appropriate levels through several mechanisms. The cognitive function of children, particularly malnourished children<sup>(7)</sup> has been reported to improve on consumption of a glucose-containing drink<sup>(8)</sup>.

In the past attention was drawn, with good reason, to the negative consequences of inadequate intake of protein and energy, but attention is now shifting towards the negative effect that micronutrient deficiencies could have on the cognitive development of children<sup>(9)</sup>. Research has shown that micronutrients such as iron, zinc, iodine and vitamin A<sup>(9-11)</sup> play an important role in cognitive function. Because brain development, especially with regard to the frontal lobe, continues throughout childhood, deficiencies of micronutrients and macronutrients during childhood are likely to influence cognitive function<sup>(12)</sup>. Nutrients do not function in isolation, and a diet that is deficient in one micronutrient is likely also to be deficient in others<sup>(13)</sup>. This underlines the importance of investigating the effects of multi-micronutrient supplementation on cognitive development, particularly in populations that are likely to suffer from multiple deficiencies.

Fortification of food with micronutrients is increasingly considered as an alternative strategy to micronutrient supplementation. A micronutrient-fortified beverage that forms part of a comprehensive integrated nutritional fortification programme has been reported to be of possible benefit in populations at risk of micronutrient deficiencies<sup>(14)</sup>. In 1994, the National School Nutrition Programme (NSNP) was introduced in South Africa<sup>(15)</sup> without targeting micronutrient deficiencies per se. The NSNP forms a highly suitable intervention channel for providing a large

number of children with micronutrient-fortified foods. At the present, because of mandatory fortification of maize meal and bread flour, school children do benefit from the fortification programme that is in place. Guidelines do state that maize meal, bread or flour and flour products to be used in the NSNP should have the logo indicating that they have been fortified.<sup>(16)</sup>The Beverage Fortified with Micronutrients (BeForMi) study was conducted in view of the possible adverse effect of poor micronutrient status on cognitive function, and the possible beneficial effect of micronutrient fortification on cognition. Furthermore, inadequate energy intake has also been reported to affect cognition adversely, and both micronutrients and sugar may influence growth. Therefore, the BeForMi study was a randomised, controlled intervention with the aim of investigating the effects of a multi-micronutrient-fortified beverage, with or without sugar, on growth and cognitive function in South African primary school children.

## **EXPERIMENTAL METHODS**

### **Study population**

The BeForMi study was conducted in primary school children between the ages of 6 and 11 years in a peri-urban settlement in the North West province in South Africa. The study was conducted in three pre-selected primary schools chosen by the Department of Education. The learners at all three schools were provided a single daily meal, sponsored by the National School Nutrition Programme. The study started in January 2010 and ended in November 2010. The inclusion criteria were as follows: 1) no health condition that would make cognitive testing impractical (e.g. dyslexia, hearing difficulties), 2) 6–10 years old by January 2010, 3) no use of medication or supplements that could affect nutritional status. Written informed consent was obtained for each child from a parent or guardian. Children had to be willing to have a blood sample taken. None of the children was diagnosed with haemoglobin concentrations below 7 g/dL at baseline and therefore none was excluded based on classification of severe anaemia.

In December 2009, study information and information on the screening process was provided to parents of learners in grades 1 to 3. Consent forms were compiled in the most commonly spoken languages of the catchment area of the schools. Written consent was obtained from the parents and oral assent from the children. This study was conducted according to the guidelines laid down in the Declaration of Helsinki. Ethical approval for the BeForMi study was granted by the

Research and Ethics Committee of the North-West University (NWU-00065-09-A1). The study was registered with the North West Department of Health (NWEP 04/2010) and permission was granted by the Department of Education as well as by the School Governing Body of each school.

Power calculations were based on a pilot study involving similar cognitive tests and pre-school children in a similar socioeconomic setting<sup>(17)</sup>. Point-of-use fortification of food with micronutrients in the pilot study resulted in a decrease of three mental processing index scores of the Kaufman Assessment Battery for Children II (KABC-II) of medium effect size ( $d = 0.4$ ). The power calculations indicated that 100 learners per treatment group would be sufficient to provide 80% power at a 0.05 significance level, and allowing for a 10% dropout rate. In total, 556 children participated in the baseline screening. After screening, the 414 children with the highest serum transferrin receptor (TfR) and thereafter the highest zinc protoporphyrin (ZnPP) values were enrolled into the study in order to include those children with the poorest iron status (**Figure 1**).

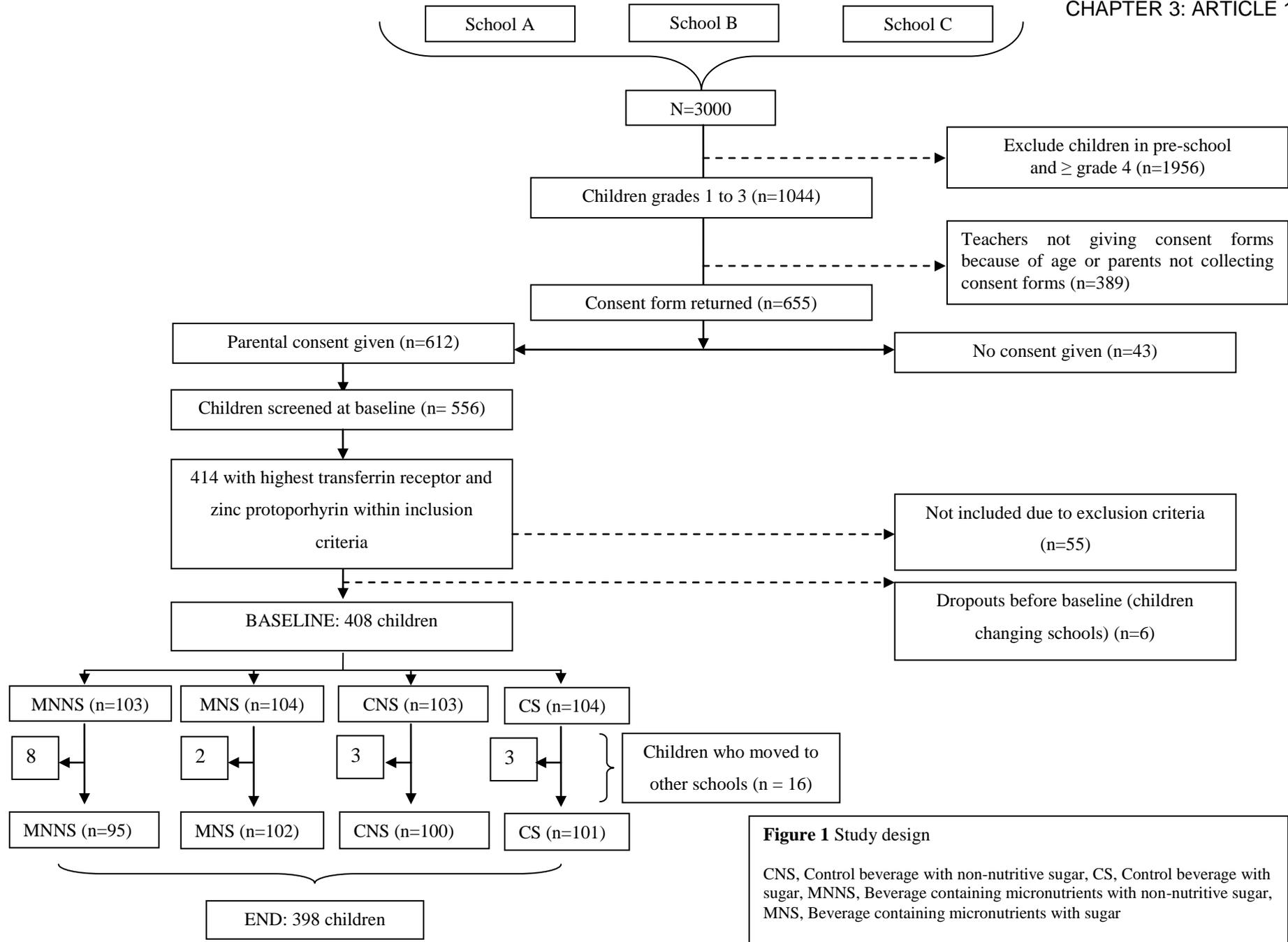
### **Study design**

In this double-blind, randomised, controlled intervention study, the 414 pre-selected children were randomly allocated to one of four treatment groups. The four different formulations of the beverage were: 1) micronutrients with sugar (MNS), 2) no micronutrients (control beverage) with sugar (CS), 3) micronutrients with non-nutritive sweetener (MNNS) and 4) no micronutrients (control beverage) with non-nutritive sweetener (CNS). Participants, investigators and school assistants were blinded to treatment assignments. Randomisation of the four beverage formulations was done within schools, classrooms and gender.

Baseline biochemistry and anthropometric data were collected within a month prior to the time that beverages were distributed. Beverages were administered for two weeks prior to taking cognitive baseline test. Distribution of the beverages started in March 2010 and continued until 30 November 2010. Children were de-wormed at baseline with 200 mg (100 mg twice daily) of Mebendazole for three consecutive days.

**Intervention**

The respective BeForMi formulations can be viewed in **Table 1**. Beverages (200 ml per child per day) were consumed during school hours, before the school meal, from Monday to Friday. The total amount of beverages needed for the day was mixed according to calculations, based on the number of children present each day, (1 sachet for 10 litres of beverage). Enough beverages were mixed to also provide to the rest of the school, but the rest of the school was not monitored. Each day the different beverages were freshly prepared and no beverages were kept for following days.



**Figure 1** Study design  
 CNS, Control beverage with non-nutritive sugar, CS, Control beverage with sugar, MNNS, Beverage containing micronutrients with non-nutritive sugar, MNS, Beverage containing micronutrients with sugar

**TABLE 1** Nutritional composition of the intervention products per 200 ml serving

Ingredients	MNS	CS	MNNS	CNS	% RDA for beverages containing micronutrients <sup>1</sup>
Energy (KJ) <sup>1</sup>	340	340	<14	<14	
Protein (g)	0	0	0	0	
Carbohydrate from sugar (sucrose) (g)	20.6	20.6	0	0	
Fat	Trace	Trace	Trace	Trace	
Dietary fibre	0	0	0	0	
Sodium	Trace	Trace	Trace	Trace	
Vitamin A (µgRE) (Beta - carotene) <sup>2</sup>	400		400		40
Vitamin E (mg)	7.5		7.5		75
Vitamin C (mg)	60		60		100
Vitamin B2 (mg)	0.4		0.4		25
Nicotinamide (mg)	2.7		2.7		15
Vitamin B6 (mg)	0.5		0.5		25
Folic acid (µg)	140		140		70
Vitamin B12 (µg)	1.0		1.0		100
Calcium (mg)	120		120		15
Iron (mg) (Ferrous chloride glycine)	7.0		7.0		50
Zinc (mg)	3.75		3.75		25
Iodine (µg)	60		60		40

MNS, beverage containing micronutrients with sugar; CS, control beverage with sugar; MNNS, beverage containing micronutrients with non-nutritive sweetener; CNS, control beverage with non-nutritive sweetener; RDA, Recommended dietary allowance.

<sup>1</sup>Non-nutritive sweetener (sucralose) used for CNS and MNNS groups

<sup>2</sup>Name of the colorant used: "yellow sunset E110"

The different beverage formulations were colour-coded, and children who participated in the study received identity cards with their respective colour codes. A school assistant was appointed to take care of a specific colour-coded beverage at each of the three schools for the duration of the study. The responsible field assistant served his/her learners with the correct BeForMi beverage in colour-coded cups. Each child had to finish his/her beverage in front of the school assistant. None of the children had problems to finish the beverage and drank it quickly. Adherence was monitored by the use of illness and adherence forms that were filled in on a daily basis.

When a child was absent, the school assistant followed up on the child the next day and the reason for absence was recorded. Visits by the BeForMi study team to the schools were made at least twice weekly, and the illness and adherence forms were collected at the end of each month. These forms were screened monthly to ensure that all the information needed was provided. The school assistants were instructed to report problems immediately, so that they could be addressed effectively.

The intervention started in March 2010 and continued until November 2010. As previously mentioned, children did not receive beverages over weekends and school holidays. The schools closed for public holidays in March/April for two weeks, in June/July for five weeks and in September for one week. In addition to the public holidays, 14 school days were lost when teachers participated in a national strike action. The BeForMi beverage was provided for 141 days over a period of eight and a half months.

### **Biochemical indicators**

A venous blood sample of 10 ml was collected at baseline and endpoint from each child. This included a 6 ml sample in a trace-element-free tube and a 4 ml sample in an EDTA-coated tube. Blood was transported on ice to the laboratory at the Centre of Excellence for Nutrition at the North-West University directly after blood was drawn. Haemoglobin (Hb) was determined from whole blood and ZnPP from washed red blood cells. Transferrin receptor (TfR), ferritin (SF), and C-reactive protein (CRP) were determined from serum. All these serum samples were stored at -80 °C until analysis and analysed after the intervention.

Hb concentrations were measured with an AcT 5Diff Cap Pierce Hematology Analyzer (Beckman Coulter, Miami, Florida, USA) together with 3-level controls provided by the manufacturer. Hb and ZnPP measurements were done on the same day the blood samples were collected. ZnPP was measured on washed red blood cells with a hematofluorometer (Aviv Biomedical, Lakewood, NJ, USA) with 3-level controls provided by the manufacturer. SF was measured using the WHO international standard (NIBSC code: 94/572). TfR and SF were measured using enzyme-linked immunosorbent assays (ELISA) (Ramco Laboratories Inc., Statford, TX, USA). CRP was measured using an immunoturbidimetric test (Human Biochemical and Diagnostic Laboratories, South Africa).

Serum zinc was determined by Flame Atomic Absorption Spectrometry (Thermo Elemental, S2 AAS SOLAAR AA series, Thermo Fisher Scientific Inc., Thermo Fisher Scientific, Cambridge UK) on an atomic absorption spectrometer (AA240FS, Varian Inc, Australia). Standard addition techniques were used to minimise matrix effects and commercial aqueous standards (Titrisol, Merck, Germany) were used for external calibration. Serum retinol was determined by a reversed phase High-Performance Liquid Chromatography (HPLC) method which is based on the method described by Catignani and Bieri<sup>(18)</sup>, using tocopherol acetate as internal standard and controls from the National Institute of Standards and Technology (Gaithersburg, MD, USA, SRM 986c).

Iron deficiency (ID) was defined according to: ZnPP  $\leq$  70 $\mu$ mol/mol heme (Aviv Biomedical, Lakewood, NJ, USA), TfR  $>$  8.3 mg/L (Ramco Laboratories Inc.) or SF  $<$  15  $\mu$ g/L (3<sup>rd</sup> WHO International Standard, 1996, 94/572). Anaemia was defined as Hb concentrations below 11.5 g/dL<sup>(18)</sup>. C-reactive protein concentration  $>$  5 mg/L indicated low grade inflammation<sup>(20)</sup>. SF values of children with CRP concentrations  $>$  5g/L were excluded from statistical analysis because of the confounding effect that inflammation has on SF.

Serum retinol concentration  $<$  20  $\mu$ g/dL (0.7  $\mu$ mol/L) was indicative of low vitamin A status<sup>(21)</sup> and serum zinc concentration of  $<$  65  $\mu$ g/dL (9.9  $\mu$ mol/L) indicated inadequate zinc status<sup>(22)</sup>.

### **Cognitive test**

Cognitive performance was assessed at baseline and endpoint using the Kaufman Assessment Battery for Children II (KABC-II)<sup>(23)</sup>. The tests included in this battery were devised in the United States and are based on the neuropsychological theories of Luria. These tests are further recommended for children from bilingual backgrounds, where cultural background could affect knowledge acquisition and verbal development. For the BeForMi study, sub-tests of the KABC-II were chosen to detect individual differences in African children, in particular in aspects of cognitive processing. Selected cognitive tests were used to assess different cognitive functions. “Story completion” and “Rover” were used to measure planning ability. Rover also measures simultaneous processing, along with “Triangles”. “Hand movements”, “Word order” and “Number recall” were used to measure sequential processing and “Atlantis” to measure learning ability<sup>(24)</sup>.

A team of eight locally trained cognitive assessors conducted the testing. All the cognitive assessors were blinded to treatment group allocation. The cognitive assessors underwent intensive training by a trained experienced assessor and a psychologist oversaw the process (JK). The week before baseline and endpoint cognitive measurements were taken, training was repeated. Children were assessed during morning school hours on the school premises in semi-private areas. The tests were administered to children using the language medium of education of the particular school.

The scores from the KABC-II tests were adjusted for age by using the KABC-II Assist software package. Inter-tester reliability was determined by using a test-retest process. This process included children who used the same language medium of education from two schools that did not form part of the study. In addition, adapted versions of the Cronbach alpha equation according to Foxcroft and Roodt<sup>(25)</sup> were used to determine intra-tester reliability coefficients based on the first eight children assessed by each assessor.

### **Anthropometry**

All anthropometric measurements were taken according to the International Standards for Anthropometric Assessment of the International Society for the Advancement of Kinanthropometry (ISAK) (ISAK, 2001).<sup>(26)</sup> Body weight of children wearing minimum clothing without shoes was measured on the SECA Robusta 813 digital scale (Hamburg, Germany). Body weight was recorded to the nearest 100 g. For height measurements, children stood upright without shoes with the head in the Frankfort plane. Height measurements were taken with the use of a calibrated stadiometer, measured to the nearest 0.1 cm. All the height and weight measurements were taken in duplicate and the average of the two values were used.

The 2007 WHO references (WHO AnthroPlus, version 1.0.2 software) were used to calculate age- and sex-specific weight-for-age (WAZ), BMI-for-age (BAZ) and height-for-age z-scores (HAZ). WAZ were only available for children younger than 11 years. A z-score below -2 indicated underweight ( $WAZ < -2$ ), wasting ( $BAZ < -2$ ) and stunting ( $HAZ < -2$ ).

### **Dietary vitamin A, zinc, iron and energy intake and socio-demographic questionnaire**

Three 24-hour recalls were conducted by trained school assistants at least one week apart on different days of the week during the study duration. Two recalls per child were conducted for

two different weekdays and one over the weekend, in all cases the caregiver together with the child was interviewed. Photo books, which are being used in the PURE study and were validated by Venter et al.<sup>(27)</sup> were used to estimate portion sizes. Dietary data were analysed with the Food Finder computer program (MRC, 2003). An existing socio-demographic questionnaire was adapted for this study and was administered to the parents/caregivers in their language of choice.

### **Statistical analysis**

Statistical analyses were performed using Statistica (StatSoft Inc, 2011) and IBM SPSS Statistics (version 19; IBM Co). Descriptive statistics were calculated to present means, standard deviation (SD) and frequencies of variables. Differences between treatment groups at baseline were investigated using ANOVA. Estimated intervention effects of micronutrient fortification (micronutrients vs control) and of sugar (sugar vs non-nutritive sweetener) and the interaction between micronutrients and sugar were analysed by using 2-factor ANCOVA on endpoint measurements, controlling for respective baseline values, gender and age. Only those children who completed the study were included in statistical analysis.

In addition, for cognitive tests, the years a child attended a crèche, the income of the head of the household and the education level of the mother were added as covariates. If one of these socio-demographic factors was a significant predictor of endpoint cognitive test scores, it was added as a covariate in the specific test, or removed from the analysis if not significant.

Odds ratios (OR) for being deficient at endpoint (anaemia, iron-, zinc-deficient and low vitamin A status) with treatment (micronutrients and/or sugar) were examined by using binary logistic regression analyses, adjusting for age, gender, respective baseline deficiency prevalence and adding a micronutrient x sugar interaction term. McNemar's test was used to determine whether the deficiency prevalence changed significantly from baseline to endpoint within groups. For dietary intake of micronutrients and energy, dependent T-tests were used to compare means before and after consumption of the beverage within each group. Independent T-tests were used to compare means between groups. Statistically significant results were those with *P*-values < 0.05.

## RESULTS

### Subjects

In the 556 children screened at baseline, 6.9% were anaemic (Hb < 11.5 g/dL), 23.0% were ID based on SF (< 15 µg/L) and 2.9% had iron-deficiency anaemia (IDA) (Hb < 11.5 g/dL and SF <15 µg/L). None of the children had Hb concentration below 8 g/dL and therefore none of the children were excluded based on the severity of anaemia. A total of 414 children was included in the study (52.2% boys), 408 of whom participated in the baseline assessments of cognition and anthropometry. Of the 414 children enrolled, 398 completed the study (**Figure 1**). All the children who dropped out from the study changed schools (n = 16). The majority (37.7%, n = 156) of children came from households with four to six persons living in same the household. More than 50% of the heads of the households had an income of less than or equal to R2000 (± 240 USD) per month (58.5%, n = 242). Fifteen percent (n = 61) of the mothers of the children had primary school education and 43% (n = 177) secondary school education. Fifty-eight percent (n = 240) of children attended crèche before primary school.

**Table 2** presents the baseline characteristics of the children by treatment group. There were no relevant differences between treatment groups in any baseline characteristics.

### Intervention adherence

Adherence of children to beverage intake was high. Based on the 141 days that the beverage was provided, a 95% adherence rate was achieved. Therefore, none of the children who completed the study was excluded from statistical analysis because of poor adherence.

### Dietary vitamin A, zinc, iron and energy intake

Complete dietary data of three 24-hour questionnaires per study child were obtained from 90% of the study children. The dietary vitamin A, zinc and iron intakes, with and without the beverage, as calculated by the 24-hour recalls are reported in **Table 3**. The drinks with micronutrients were coded in Food Finder (MRC, 2003) to include the levels of micronutrients in a 200ml sample. The ones without micronutrients were coded to have none. The calculations is not based on the 24 hour recall, but based on the coded information in Food Finder. As expected, the children who received the micronutrient-fortified beverages (MNS + MNNS) had a

significantly higher mean iron, vitamin A and zinc intake (intervention beverage included) than the children receiving the beverages without added micronutrients (CS + CNS). Furthermore, the micronutrient-fortified beverages significantly increased the daily mean dietary intake of vitamin A, zinc and iron. Mean dietary intakes of iron, vitamin A and zinc from food alone were above the estimated average requirements (EAR) in all treatment groups without the additional micronutrients provided by the BeForMi beverage.

**TABLE 2** Characteristics of the children at baseline by treatment group

	TREATMENT GROUPS			
	Control beverages		Beverages with micronutrients	
	CNS (n = 102)	CS (n = 104)	MNNS (n = 100)	MNS (n = 102)
Boys (%)	48.0	50.0	50.0	57.8
Age (Years)	8.2 ± 0.8 <sup>2</sup>	8.1 ± 0.9	7.9 ± 1.4	8.3 ± 0.9
<b>Anthropometric indexes (%)</b>				
Stunted (HAZ < -2)	12.0	14.7	8.0	14.7
Wasted(BAZ < -2)	7.1	10.8	6.0	10.9
Underweight (WAZ < -2)	13.4	13.4	14.4	16.5
<b>Deficiencies (%)</b>				
Anaemia (Haemoglobin < 11.5g/dL)	8.8	5.8	8.1	5.8
Low iron stores (Serum ferritin < 15 µg/dL) <sup>3</sup>	19.2	29.5	31.6	21.1
Iron deficiency based on transferrin receptors (Transferrin receptor > 8.3 mg/L)	7.8	4.9	10.0	7.8
Iron deficiency based on zinc protoporphyrin (Zinc protoporphyrin > 70 µmol/mol heme)	24.5	18.4	29.0	18.6
Zinc deficiency(Serum zinc < 65 µg/dL)	9.8	19.8	8.0	10.7
Low vitamin A status (Serum retinol < 20 dL)	6.9	6.0	3.0	8.8
Vitamin A deficiency (Serum retinol < 10 dL)	0	0	0	0

<sup>1</sup>CNS, Control beverage with non-nutritive sweetener; CS, Control beverage with sugar; MNNS, Beverage containing micronutrients with non-nutritive sweetener; MNS, Beverage containing micronutrients with sugar; HAZ, Height-for-age z-score; BAZ, Body mass index-for-age z-score; WAZ, Weight-for-age z-score.

<sup>2</sup>mean ± SD (all such values)

<sup>3</sup>Serum ferritin values of all children with a C-reactive protein concentration >5mg/L were excluded (baseline MNS, n = 31; MNNS, n = 24; CS, n = 21 CNS, n = 26)

Based on the results from the 24-hour recall questionnaires, the energy intake in the children consuming a beverage with sugar (CS + MNS) was significantly higher than in the children consuming a beverage with a non-nutritive sweetener (CNS + MNNS) (7014 ± 1860 vs 6594 ± 2199 kJ). Irrespective of the beverage formulation (with and without sugar), the mean energy

intake in the study children was below the estimated energy requirements (EER) (Institute of Medicine, 2002) of 9572 kJ for boys and 8698 kJ for girls.

**TABLE 3** Vitamin A, zinc and iron intake of BeForMi study children, with and without the beverage, compared within and between treatment groups

Dietary micronutrient intake	Unit	RDA <sup>1</sup> /EAR <sup>2</sup>	Micronutrient group <sup>3</sup>			Control group <sup>4</sup>			p-value <sup>5</sup>
			n	Mean	SD	n	Mean	SD	
Dietary vitamin A intake: food plus beverage consumption		500 (4-6 years)	189	1224	869	186	811	811	<0.001
Dietary vitamin A intake: food consumption alone	RE(µg)	445 RAE (M)	189	860	970	186	811	811	0.6
p-value (difference between intake from food consumption alone and from food and beverage) <sup>6</sup>		700 (7-10 years)		<0.001			1		
Dietary zinc intake: food plus beverage consumption		4 mg/d (4-8 years)	189	14.3	3.6	185	11.3	3.8	<0.001
Dietary zinc intake: food consumption alone	mg	7 mg/d (9-13 years)	189	10.5	3.6	185	11.3	3.8	0.1
p-value (difference between intake from food consumption alone and from food and beverage) <sup>6</sup>				<0.001			1		
Dietary iron intake: food plus beverage consumption		4.1 mg/d (4-8 years)	187	21.3	6.0	186	15.2	5.4	<0.001
Dietary iron intake: food consumption alone		5.9 mg/d (M)	187	14.3	3.0	186	15.2	5.4	0.2
p-value(difference between intake from food consumption alone and from food and beverage) <sup>6</sup>	mg	5.7 mg (F) (9-13 years)		<0.01			1		

<sup>1</sup>RDA (recommended dietary allowances, National Academy of Sciences, 1989)

<sup>2</sup>EAR (Estimated average requirements, Institute of Medicine, 2001); M, male; F, female. <sup>3</sup>Beverages with micronutrients with non-nutritive sweetener and with sugar

<sup>4</sup>Beverages without micronutrients but with non-nutritive sweetener and with sugar

<sup>5</sup>P value for comparison of groups consuming micronutrient containing beverage and combined control groups (T-test independent by group)

<sup>6</sup>P value for comparison between intake from food consumption alone and from food and beverage consumption (T-test dependent samples)

### Biochemical indicators

Baseline and endpoint concentrations of serum vitamin A, serum zinc and iron status indicators are presented in **Table 4**. There were no relevant differences at baseline for any of the reported variables. In order to determine treatment effects of micronutrient fortification (micronutrient vs control) and of sugar (sugar vs non-nutritive sweetener) and potential interaction effects of micronutrient x sugar on endpoint biochemical indicators, 2-factor ANCOVA was conducted, controlling for age, gender and respective baseline measurements. There were significant micronutrient effects on the iron status indicators SF ( $P = 0.008$ ) and ZnPP ( $P = 0.03$ ) for better iron status at endpoint. Furthermore, there was a significant effect of micronutrient fortification on higher Hb concentrations at endpoint ( $P = 0.007$ ). However, there was no significant improvement in anaemia prevalence from baseline to endpoint.

In binary logistic regression, controlling for age, gender and respective baseline ID prevalence, micronutrient fortification significantly decreased the OR for being ID (based on SF) at endpoint (OR = 0.15, 95% CI: 0.03, 0.78). The ID prevalence significantly decreased from 26.5% to 3.0% in children receiving a micronutrient-fortified beverage, while it remained unchanged (24.5% at baseline to 17.3% at endpoint) in children receiving a beverage without micronutrients. Micronutrient fortification did not significantly lower the risk for being ID at endpoint based on ZnPP and TfR ( $P > 0.05$ ).

The prevalence of zinc deficiency (serum zinc  $< 65 \mu\text{g/dL}$ ) significantly improved from baseline to endpoint in both the children receiving a beverage fortified with micronutrients (9.4% to 2.0%) ( $P = 0.007$ ) and the children who received a beverage without micronutrients (14.4% to 2.6%) ( $P < 0.001$ ). There was no significant effect of micronutrient fortification on vitamin A status.

**Table 4** Effect of a beverage with and without micronutrients by treatment groups on indicators of iron, zinc and vitamin A status

	Treatment groups								Estimated intervention effect				
	MNS (n = 101)		MNNS (n = 95)		CS (n = 98)		CNS (n = 92)		Micronutrient <sup>2</sup>		Sugar <sup>2</sup>		Micronutrient X Sugar <sup>2</sup>
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	B value	95% CI	B value	95% CI	p-value
<b>Blood haemoglobin (g/dL)</b>													
Baseline	12.7	0.9	12.5	1.1	12.7	0.8	12.7	1.0	0.31	(0.09, 0.53) <sup>4</sup>	0.04	(-0.19, 0.26)	0.42
End	13.0	0.9	12.9	1.0	12.7	0.9	12.7	1.1					
<b>Serum ferritin (µg/L)<sup>3</sup></b>													
Baseline	29.9	19.0	27.4	18.9	29.8	22.5	30.4	19.0	8.67	(2.32, 15.02) <sup>5</sup>	1.40	(-5.01, 7.80)	0.62
End	48.6	25.7	41.6	25.4	32.8	17.4	31.4	20.3					
<b>Zinc protoporphyrin (µmol/mol heme)</b>													
Baseline	59.4	23.9	63.4	26.1	57.6	16.8	61.4	22.5	-4.10	(-7.89, -0.32) <sup>6</sup>	2.25	(-1.52, 6.02)	0.54
End	65.8	24.6	65.5	21.8	66.6	20.2	68.5	26.8					
<b>Serum transferrin receptor (µg/L)</b>													
Baseline	6.0	1.3	6.3	1.9	6.0	1.1	6.3	2.1	-0.27	(-0.64, 0.10)	0.03	(-0.34, 0.40)	0.84
End	6.7	1.2	6.8	1.5	7.0	1.4	7.1	2.1					
<b>Serum zinc (µg/L)</b>													
Baseline	80.5	13.8	80.4	12.3	77.7	15.1	81.6	12.9	-1.41	(-5.25, 2.44)	-2.80	(-6.64, 1.04)	0.18
End	87.5	13.0	88.3	12.3	85.0	12.0	86.5	12.4					
<b>Serum retinol (µg/L)</b>													
Baseline	28.5	6.6	28.8	5.8	27.3	5.1	28.6	6.2	0.54	(-1.08, 2.15)	-0.46	(-2.09, 1.17)	0.60
End	32.4	8.3	32.3	7.5	30.3	6.1	31.5	6.3					

<sup>1</sup>MNS, Beverage containing micronutrients with sugar; MNNS, Beverage containing micronutrients with non-nutritive sweetener; CS, Control beverage with sugar; CNS, Control beverage with non-nutritive sweetener.

<sup>2</sup>Micronutrient and sugar group intervention effects as well as micronutrient X sugar interaction were estimated using two-way analysis of covariance (ANCOVA) comparing endpoints, adjusted for respective baseline values, age and gender

<sup>3</sup>Serum ferritin values of all children with C-reactive protein >5mg/L were excluded (endpoint MNS, n = 36; MNNS, n = 30; CS, n = 35 CNS, n = 36)

<sup>4</sup>P = 0.007

<sup>5</sup>P = 0.008

<sup>6</sup>P = 0.03

### **Cognitive performance**

Cronbach alpha for inter-tester reliability for cognitive assessors was 0.76 (95% CI, 0.61; 0.87). The intra-tester reliabilities for the eight assessors ranged from 0.67 to 0.91 (average = 0.80).

Intervention effects of micronutrient fortification and sugar, as well as possible micronutrient x sugar interactions were analysed by 2-way ANCOVA, adding age, gender and respective baseline cognitive test score as covariates (**Table 5**). The number of years a child spent in crèche, the education level of the mother, and the income of the head of the household were initially included as covariates. However, of these socio-demographic variables, the number of years a child spent in crèche was the only variable that significantly affected scores in the Triangles and Story completion tests, and thus was added as a covariate. A significant effect of micronutrient fortification ( $P = 0.02$ ) and of sugar ( $P = 0.03$ ) for higher endpoint scores was found in the Atlantis test (**Figure 2A**). There was also a significant micronutrient x sugar interaction, indicating that micronutrients or sugar provided alone had a beneficial effect on Atlantis scores, but when given in combination, this beneficial effect was attenuated. In the Rover test, there was a significant effect of sugar on higher endpoint scores ( $P = 0.03$ ), as well as a significant micronutrient x sugar interaction (**Figure 2B**), indicating again that sugar alone had a beneficial effect, but when given in combination this effect was attenuated. A significant attenuating micronutrient x sugar interaction was also found on the Number Recall test (**Figure 2C**). Even though not significant, a similar interaction pattern was also observed in the other tests.

### **Growth**

Baseline, endpoint and change in WAZ, HAZ and BAZ are reported in **Table 6**. WAZ improved in all four groups from baseline to endpoint. However, there were significant lowering effects of micronutrient fortification and of sugar on endpoint WAZ scores (micronutrient  $P = 0.03$ ; sugar  $P = 0.04$ ). Furthermore, there was a significant micronutrient x sugar interaction, indicating that these lowering effects of micronutrients or sugar alone on endpoint WAZ scores were attenuated when micronutrients and sugar were provided in combination (**Figure 3**).

**Table 5** Effect of a beverage with and without micronutrients by treatment groups on cognitive test scores

	Treatment groups								Estimated intervention effect				
	MNS (n = 101)		MNNS (n = 99)		CS (n = 99)		CNS (n = 100)		Micronutrient <sup>2</sup>		Sugar <sup>2</sup>		Micronutrient X sugar <sup>2</sup>
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	B value	95% CI	B value	95% CI	p-value
<b>Atlantis</b>													
Baseline	3.9	1.9	4.2	2.4	4.0	2.4	3.8	1.8	0.76	(0.10, 1.42) <sup>3</sup>	0.71	(0.05, 1.37) <sup>4</sup>	0.01
End	5.4	2.5	5.9	2.9	5.8	2.9	5.0	2.4					
<b>Story completion</b>													
Baseline	3.9	1.8	3.9	2.1	3.8	1.8	3.8	2.0	-0.02	(-0.57, 0.53)	0.11	(-0.42, 0.67)	0.36 <sup>6</sup>
End	3.5	1.9	3.9	2.4	3.9	2.0	3.8	2.1					
<b>Number recall</b>													
Baseline	6.2	2.2	6.5	2.5	6.5	2.0	6.4	2.3	0.24	(-0.39, 0.87)	0.48	(-0.15, 1.11)	0.04
End	6.1	3.2	6.6	2.5	6.8	2.4	6.3	2.3					
<b>Rover</b>													
Baseline	7.1	2.4	7.2	2.2	7.4	2.3	7.3	2.1	0.39	(-0.25, 1.02)	0.72	(0.08, 1.35) <sup>5</sup>	0.05
End	7.5	2.4	7.8	2.5	8.1	2.3	7.3	2.4					
<b>Triangles</b>													
Baseline	5.2	2.1	5.5	2.0	5.0	1.9	5.0	1.9	0.26	(-0.27, 0.79)	0.21	(-0.33, 0.74)	0.17 <sup>6</sup>
End	5.0	1.9	5.5	2.2	5.4	2.2	5.1	1.9					
<b>Word order</b>													
Baseline	6.1	1.5	6.0	1.5	6.6	1.8	6.0	1.6	0.03	(-0.46, 0.51)	0.04	(-0.45, 0.53)	0.56
End	5.8	1.9	6.0	1.9	6.1	1.9	5.9	1.7					
<b>Hand movements</b>													
Baseline	6.6	2.4	6.8	2.6	6.8	2.5	6.5	2.4	-0.33	(-0.98, 0.33)	-0.34	(-0.99, 0.32)	0.35
End	6.5	2.4	6.5	2.4	6.5	2.8	6.7	2.8					

<sup>1</sup>MNS, Beverage containing micronutrients with sugar; MNNS, Beverage containing micronutrients with non-nutritive sweetener; CS, Control beverage with sugar; CNS, Control beverage with non-nutritive sweetener.

<sup>2</sup>Micronutrient and sugar group intervention effects as well as micronutrient X sugar interaction were estimated using two-way analysis of covariance (ANCOVA) comparing endpoints, adjusted for respective baseline values, age and gender

<sup>3</sup>P = 0.02      <sup>4</sup>P = 0.03      <sup>5</sup>P = 0.03

<sup>6</sup>Adjusted for socio-demographic variable; number of years a child attended crèche.

**Table 6:** Effect of a beverage with and without sugar by treatment groups on anthropometric z-scores

	Treatment groups								Estimated intervention effect				
	MNS (n = 102)		MNNS (n = 100)		CS (n = 102)		CNS (n = 100)		Micronutrients <sup>2</sup>		Sugar <sup>2</sup>		Micronutrient X sugar <sup>2</sup>
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	B value	95% CI	B value	95% CI	p-value
Height for age z-score													
Baseline	-0.83	1.0	-0.88	0.98	-1.0	0.98	-0.78	1.01	-0.01	(-0.05,0.03)	-0.003	(-0.04, 0.03)	0.18
End	-0.73	1.0	-0.82	0.98	-0.94	0.95	-0.73	1.00					
Weight for age z-score <sup>3</sup>													
Baseline	-0.86	1.16	-0.86	1.04	-0.95	1.18	-0.92	1.07	-0.08	(-0.15, -0.01) <sup>4</sup>	-0.07	(-0.14, -0.002) <sup>5</sup>	0.03
End	-0.66	1.19	-0.82	1.06	-0.86	1.12	-0.83	1.08					
Body mass index for age z-score.													
Baseline	-0.58	1.13	-0.50	0.97	-0.54	1.11	-0.69	0.95	-0.06	(-0.16, 0.03)	-0.07	(-0.16, 0.03)	0.19
End	-0.55	1.14	-0.52	0.97	-0.58	1.09	-0.65	0.98					

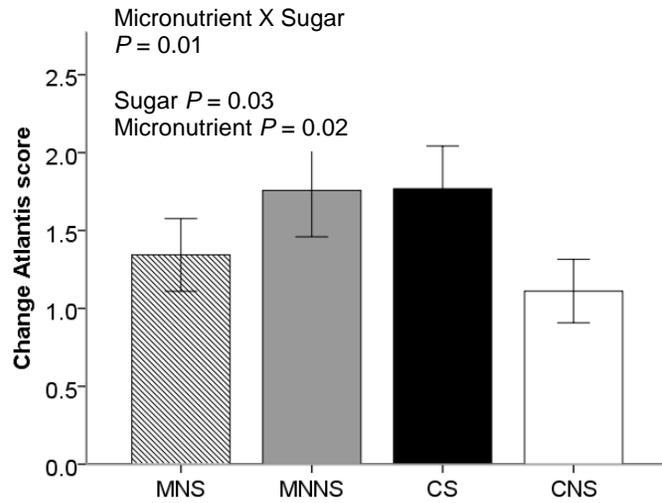
MNS Beverage containing micronutrients with sugar; MNNS, Beverage containing micronutrients with non-nutritive sweetener; CS, Control beverage with sugar; CNS, Control beverage with non-nutritive sweetener.

<sup>2</sup>Micronutrient and sugar group intervention effects as well as micronutrient X sugar interaction were estimated using two-way analysis of covariance (ANCOVA) comparing endpoints, adjusted for respective baseline values, age and gender

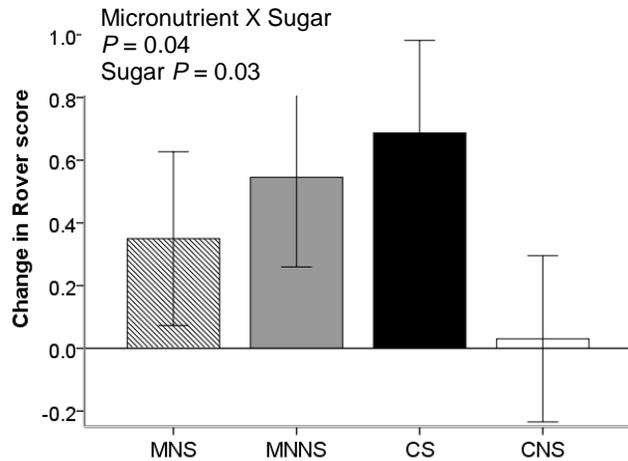
<sup>3</sup> Weight for age z-scores calculated for children <10 years (endpoint MNS, n = 88; MNNS, n = 91; CS, n = 91 CNS, n = 89)

<sup>4</sup>P = 0.04

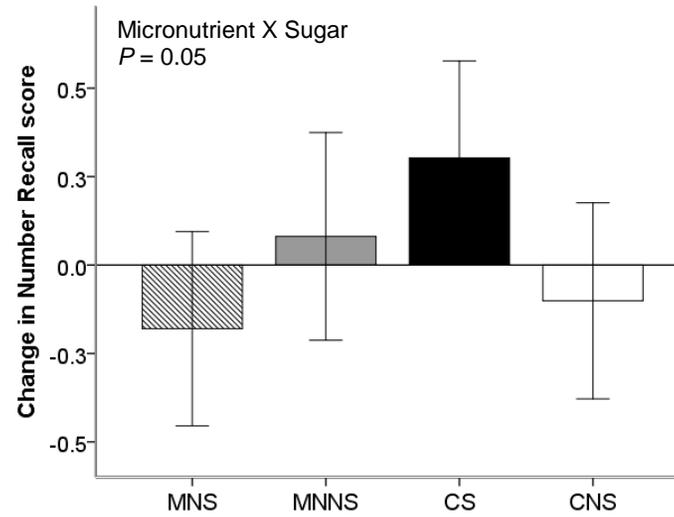
<sup>5</sup>P = 0.02



**Figure 2A**

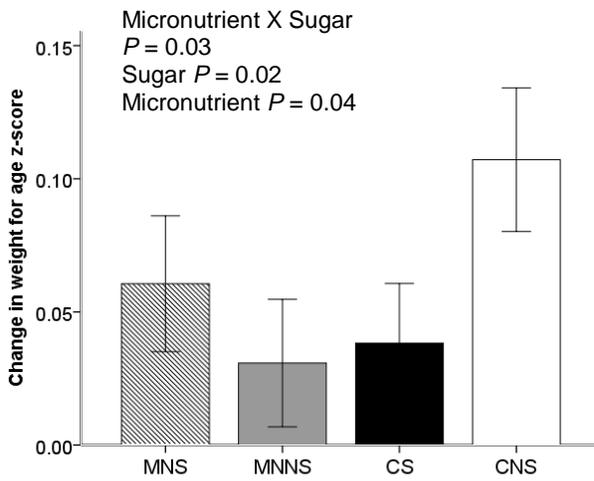


**Figure 2C**



**Figure 2B**

**Figure 2 A, B and C:** Mean (+SEM) change from baseline to endpoint in Atlantis (A), Number Recall (B) and Rover scores (C) by treatment group. CNS, Control beverage with non-nutritive sweetener; CS, Control beverage with sugar, MNNS; beverage containing micronutrients with non-nutritive sweetener, MNS; beverage containing micronutrients with sugar. A 2-factor ANCOVA was used to estimate the intervention effects on the endpoint measurements, adjusting for respective baseline measures, age and gender.



**Figure 3:** Mean (+SEM) change from baseline to end in weight-for-age z-score by treatment group. Intervention effects were estimated by using 2-factor ANCOVA on the endpoint measurement with adjusting for age, gender and respective baseline WAZ measurements.

## DISCUSSION

To our knowledge, this was the first randomised, controlled trial to investigate the effects of a beverage fortified with micronutrients, with and without sugar, on micronutrient status, growth and cognitive function using a 2-by-2 factorial design.

The prevalence of anaemia, ID, IDA, vitamin A and zinc deficiency in the study children was lower than expected. Compared with the South African NFCS-FB-2005, the prevalence of anaemia in children aged 7–9 years for the same province was three times higher (27%)<sup>(28)</sup> than what was reported in the current study. The dietary assessment done in our study showed that the children had an adequate intake of the micronutrients vitamin A, iron and zinc. In contrast with the adequate intakes of these micronutrients, the mean energy intake of the children did not meet the estimated energy requirements<sup>(29)</sup>. Further analysis of the dietary data would be useful to investigate which food items contributed the most to selected micronutrient intakes.

As expected, the results from this study showed that dietary vitamin A, iron and zinc intakes were significantly higher in the children consuming a micronutrient-fortified beverage than in the children consuming a beverage without micronutrients. Furthermore, the beverage with sugar contributed significantly to the energy intake of the children, but not to the extent that the EER was reached. While it is to be expected that micronutrient and energy intake would be higher in the intervention groups, the dietary intake (including macro and micronutrients) of each individual child and family differs, and it is not certain whether this contribution will be significant.

The micronutrient-fortified beverages significantly increased iron stores (based on SF concentrations) at endpoint and accordingly lowered the risk of a child being ID (SF < 15 µg/L) at endpoint. Even though micronutrient fortification significantly increased Hb concentration at endpoint, anaemia prevalence did not change from baseline to endpoint. Only 6.9% of the children were anaemic and even less than 3% of the children were anaemic owing to IDA. Given that the IDA prevalence in the study population was very low, a significant positive change in anaemia prevalence with micronutrient fortification was not expected. In agreement with our results, independent studies conducted in Botswana<sup>(14)</sup>, Bangladesh<sup>(30)</sup> and Tanzania<sup>(31)</sup> reported significantly higher levels of Hb and SF when experimental groups were compared with control groups after multi-micronutrient supplementation.

Micronutrient fortification had a beneficial effect on Atlantis test scores (Atlantis indicates learning ability and associative memory), and sugar in the form of sucrose positively influenced Atlantis and Rover test scores (Rover indicates simultaneous processing, visual memory). Since both micronutrients and sugar may have some beneficial effects on cognitive outcomes, it would be logical to expect that the combination of a micronutrient-fortified beverage with sugar would have an additive effect on cognitive test performance. However, we found significant attenuating interaction effects of micronutrients and sugar on Atlantis, Number recall and Rover test scores. The interaction effects consistently indicated that, while provision of micronutrients or sugar alone had a beneficial effect on cognitive test performance, when provided in combination, this beneficial effect was attenuated. Potential mechanisms that could explain these attenuating effects, and whether the main contributor was micronutrients or sugar, are at this point unclear.

In a previous South African study, provision of a multi-micronutrient-fortified biscuit resulted in improved short-term memory<sup>(32)</sup>. The authors reported, furthermore, that more significant intervention effects were obtained when only the children who were micronutrient-deficient at baseline were included. Similarly, only Filipino children who were anaemic at baseline (52% of study group) obtained significant cognitive benefits from a multiple-micronutrient-fortified fruit powder beverage<sup>(33)</sup>.

However, positive effects on cognition with multi-micronutrient fortification have also been reported in well-nourished children. The NEMO (Nutrition Enhancement for Mental Optimization) study group<sup>(34)</sup> reported that well-nourished school-aged children who received multi-micronutrient beverages for a year significantly improved verbal learning and memory. As in our study, the children included had a low prevalence of anaemia (< 14%) and IDA (< 6.1%). It is important then to acknowledge that in the current study, significant effects of micronutrient fortification on Atlantis was observed despite the low prevalence of vitamin A, iron and zinc deficiency and anaemia.

A number of studies have found that memory is positively affected by a glucose drink or breakfast consumption<sup>(3,6,8)</sup>. Bellisle (2004)<sup>(35)</sup> states that, even in well-nourished children, short-term variation of blood glucose levels might affect brain function. The sugar compound added to the beverage used in our study was sucrose, which is a disaccharide composed of the monosaccharides glucose and fructose, and which could explain the beneficial effects on

cognitive performance. The absence of breakfast may negatively affect memory through a number of mechanisms, of which one is decreased blood glucose levels<sup>(3)</sup>. Benton and Parker (1998)<sup>(3)</sup> reported that when a glucose beverage administered after breakfast was missed, some memory deficits were reversed.

Glucose utilisation by the brain across different age periods has been studied by using “positron emission tomography” (PET)<sup>(36)</sup>. The rates of glucose usage indicate that up until the age of 16 to 18 years the cerebral cortex is metabolically maturing<sup>(36)</sup>. From birth to 4 years, the usage of glucose by the human brain increases to the extent that the glucose usage is double the amount used by adults, and the high rate of glucose consumption continues from 4–10 years of age<sup>(36)</sup>. This emphasises the important role that glucose, as the metabolic fuel of the brain, plays in the brain function of children of primary school age. Therefore, children might be highly responsive to moment-to-moment fluctuation of glucose<sup>(35)</sup>.

Deficits in cognitive development cannot be explained merely by poor micronutrient status. It is well recognised that cognitive performance is influenced by a large number of factors, including socio-demographic factors such as income, crèche attendance and the education level of the mother. In the current study, all of these factors mentioned have been corrected for in statistical analyses, but it is impossible to correct for all factors contributing to cognitive function.

Micronutrient fortification and sugar significantly affected endpoint WAZ. WAZ improved in all four groups from baseline to endpoint, but this improvement in WAZ was lowered by the administration of micronutrients and sugar, particularly if micronutrients or sugar were provided alone. It can be speculated that micronutrients and sugar made the children more energetic and improved their well-being, thus making them more active and as a consequence lowering their weight gain. Future trials of micronutrient fortification and energy provision (in the form of sugar) should include measurements of physical activity levels too.

Interestingly, there was a significant micronutrient x sugar interaction, indicating that the decreasing effects of micronutrients and sugar on endpoint WAZ were attenuated when micronutrients and sugar were provided in combination. This interaction reflects the attenuating micronutrient x sugar interaction effect observed on cognitive outcomes and the underlying mechanisms need to be investigated in future research.

The main limitations of the study were the low prevalence of iron, vitamin A and zinc deficiency in the study population. It can be speculated that a higher prevalence of poor micronutrient status in the study population could have increased the likelihood of observing significant effects of micronutrient fortification on cognition. The results with regard to cognitive outcomes could be specific to our sample population owing to the lower-than-expected prevalence of micronutrient deficiency and adequate micronutrient intake in combination with inadequate energy intake. Even though the low prevalence of deficiencies is listed as a limitation, this emphasises that even in these children, cognition might still be positively influenced.

In conclusion, our data suggest that 1) both micronutrient fortification and sugar alone had a positive effect on cognitive test scores, but when these were given in combination, the positive effects were attenuated, 2) both micronutrient fortification and sugar had a lowering effect on WAZ, but when given in combination, the lowering effect was attenuated, 3) dietary micronutrient intake increased with the provision of a micronutrient-fortified beverage, 4) energy intake increased because of the sugar contained in the beverage and 5) micronutrient fortification positively influenced Hb, SF and ZnPP concentrations. Further research is needed to explain the mechanisms underlying the attenuating effect of micronutrient and sugar interactions on cognitive function and growth, and to confirm the current results.

#### **ACKNOWLEDGEMENTS**

The intervention study was supported by a research grant from Coca Cola South Africa (Pty). The company played no role in the decisions on study design or interpretation of the results. The authors had no conflict of interest. Authors' contributions were as follow: N Covic was the study director and J Jerling the project leader. All the authors provided scientific input into data interpretation and writing of the article. R van Reenen and J Baumgartner provided statistical guidance for data analysis. C Taljaard, the M.Sc and Ph.D student on the study, was involved in all aspects of the study and article writing. We thank Sr Chrissie Lessing and her team who conducted all the blood sampling, the logistics of the process, and assisted with baseline and end measurements. Furthermore, we thank Prof. S Ellis from the NWU statistical consultation services. Heartfelt thanks to the schools involved, the children, teachers and the school assistants for their hard work and contribution to the BeForMi intervention study.

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## CHAPTER 3: ARTICLE 2



This article aims to review evidence that has emerged on iron status and anaemia prevalence in South African children since the National Food Consumption Survey-Fortification Baseline-2005. The article was a logical progression based on the unexpectedly low baseline prevalence of micronutrient deficiencies for the BeForMi intervention. The article has been submitted for publication to the South African Journal of Clinical Nutrition.

## **Studies of South African schoolchildren since 2005 suggest lower anaemia prevalence in some regions**

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**Objectives:** To report on iron status of South African primary school children as observed by independent studies conducted since the last National Food Consumption Survey in 2005.

**Design:** Internet searches were conducted for cross-sectional and randomised controlled trials reporting on iron status of South African primary school children, published after the National Food Consumption Survey-Fortification Baseline (NFCS-FB-2005). Search engines used were Science Direct, Sabinet, PubMed, Ebsco Host (Academic Search Premier, Health Source, Medline) and Web of Knowledge. The search terms in different combinations were “South Africa”, “children”, “iron”, “anaemia”, “iron deficiency”, “micronutrient”, “malnutrition” and “nutritional status”. Secondary analysis was done on NFCS-FB-2005 data on children aged 7–9 years, at provincial level.

**Setting:** Different provincial locations in South Africa.

**Subjects:** South African primary school children.

**Outcome measures:** Haemoglobin, serum ferritin.

**Results:** The search identified four independent studies conducted in four different provinces: KwaZulu-Natal, North West, Western Cape and Northern Cape. All four studies were conducted in low socio-economic areas and selected children of poor iron status for intervention purposes. All studies reported anaemia prevalence lower than that of the NFCS-FB-2005 (KwaZulu-Natal: 11.5% vs 14.4%; North West: 6.9% vs 27%; Western Cape: 17.2% vs 18.8%; and Northern Cape: 5.4% vs 22.2%). Serum ferritin was more difficult to interpret due to different cut of points being used.

**Conclusion:** Anaemia prevalence based on haemoglobin concentration in primary school children might have improved in some regions since the NFCS-FB-2005. Regular national surveys are recommended to keep iron status of South African primary school children under surveillance.

## Introduction

Anaemia is a worldwide public health problem, with more than half of the world's children of preschool age being anaemic.<sup>1</sup> The wide-ranging impact on human health of iron deficiency (ID), with or without anaemia, includes increased fatigability and weakness, increased susceptibility to infection and delayed mental and physical development.<sup>2-3</sup>

In order to prevent the development of anaemia and its consequences it is important to monitor the iron status of individuals and populations. Worldwide, anaemia prevalence is determined by measuring blood haemoglobin (Hb) or haematocrit levels. Haemoglobin measurement is recommended where surveys related to public health are conducted, where resources are poor and where the prevalence of anaemia is high. Haemoglobin measurement is therefore the most popular choice for assessing iron status and has been used in the majority of South African surveys.<sup>4</sup> The limitation of Hb is that these measurements change only when ID is already severe; therefore, researchers turn to more sensitive tests such as serum ferritin (SF), transferrin receptor (TfR) and zinc protoporphyrin (ZnPP). Serum ferritin acts as a measure of the amount of iron in body stores if there is no current infection.<sup>2</sup> Transferrin receptor reflects the demand for iron and is less affected by infection than SF.<sup>2</sup> While SF as iron status indicator has commonly been used in South African studies, only a few studies have used TfR and ZnPP as iron status indicators in intervention studies.<sup>5-6</sup>

In 2005, almost 28% of South African children between the ages of one and nine years were anaemic<sup>7</sup>, classifying anaemia as a moderate public health problem.<sup>4</sup> In South Africa, three national nutritional surveys in children have been conducted since 1994: the South African Vitamin A Consultative Group (SAVACG-1994)<sup>8</sup>, National Food Consumption Survey (NFCS-1999)<sup>9</sup> and the National Food Consumption Survey-Fortification Baseline (NFCS-FB-2005).<sup>10</sup> In the South African field of nutrition, these studies are well known and in many cases used as reference material for the development of policies and programmes such as the vitamin A supplementation programme. National nutrition surveys include essential information with regard to the nutritional status of South African children, the foods that are purchased per household and what children consume.

Before 1994, the absence of a national nutritional surveillance programme was the main reason for the lack of data on the prevalence of malnutrition (overnutrition and undernutrition, including micronutrient malnutrition) on a national scale.<sup>8</sup> To address this problem, SAVACG was formed in 1993 with the aim of determining growth and micronutrient status in children younger than six years in order to guide the development of intervention programmes.<sup>8</sup> In 1999, the first NFCS was conducted, including children between the ages of one and nine years. In contrast with SAVACG-1994, the aim shifted to usual food consumption of children, nutrient intake and the factors influencing food consumption and nutrient intake. Furthermore, anthropometric status was determined but biochemical measurements were not taken. The results of the NFCS influenced food fortification strategies and nutrition education material.<sup>11</sup>

In 2003, the National Food Fortification Programme was implemented<sup>12</sup>, and the original intention was that the data collected by the second NFCS-FB-2005<sup>10</sup> would serve as baseline data. However, because the survey was conducted two years after fortification became mandatory, the data do not fully serve as baseline measurements for fortification but are nevertheless regarded as useful and valuable. The survey determined the anthropometric and micronutrient status of children between the ages of one and nine years. Because the NFCS survey in 1999 did not report on biochemical status, a ten-year gap exists between surveys reporting on iron status. Furthermore, SAVACG-1994 included only children younger than six years of age. This means that the only national data on biochemical micronutrient status of schoolchildren are based on the NFCS-FB-2005, and since then almost ten years of mandatory fortification have passed.

The scarcity of national data for primary school children forces researchers either to look at available data from smaller independent studies (referred to as independent studies) or to make use of older national data. While independent studies are not representative of the population, national data might be outdated. This review aims to report on iron status and anaemia prevalence in primary school children as observed in independent studies conducted since the last national study in 2005<sup>10</sup>, reporting any measure indicative of iron status (SF, TfR, ZnPP) and anaemia prevalence (Hb). Anthropometric status of the children was also reported as means of describing the characteristics of the population.

## Method

To retrieve all publications relating to iron status for South African primary school children, a search was conducted on published literature from January 2005 to April 2012. Computerised internet searches were conducted, using Science Direct, Sabinet, PubMed, Ebsco Host (Academic Search Premier, Health Source, Medline) and Web of Knowledge as search engines. Screening and selection of papers was conducted independently by an author, and an independent literature search was conducted by a librarian of the North-West University to ensure that all relevant articles were found.

Search strings included combinations of the terms “South Africa” “children” “iron”, “anaemia”, “iron deficiency”, “micronutrient”, “malnutrition” and “nutritional status” (Figure I). Reference lists of applicable articles were hand-searched for relevant articles, and researchers known to work in the field of iron status in South Africa were contacted with regard to any recent unpublished data.

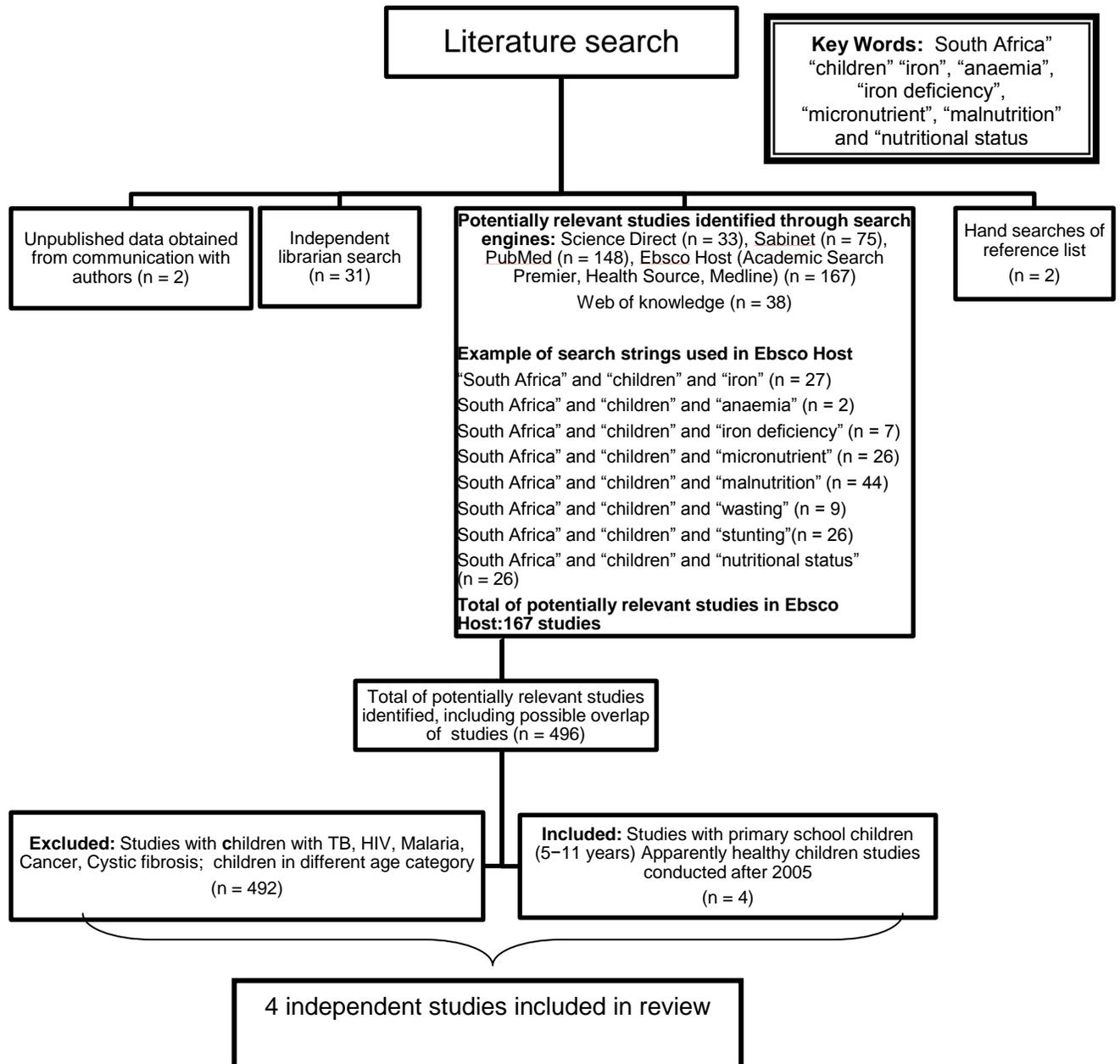
Papers were screened on the basis of title and abstract. Once potentially relevant literature was identified, full-text articles were retrieved and reviewed for inclusion on the basis of the predetermined inclusion criteria. To be included, publications (cross-sectional or randomised controlled intervention studies) needed to provide information on iron status or anaemia prevalence in primary school children aged 5 – 11 years. Only baseline results of randomised controlled intervention studies were used. Studies that were published after 2005 but were conducted prior to the NFCS-FB-2005 were excluded. Children had to be apparently healthy; studies including children with malaria, cystic fibrosis, tuberculosis or cancer, and human immunodeficiency virus infected children were excluded. Furthermore, the studies had to report on any iron status indicators, including (SF, TfR and ZnPP) or Hb, the indicator for anaemia. For the articles where anthropometric data were reported, this was also extracted to provide more information on the study population involved.

In three of the four studies, data on iron status and anaemia prevalence in a large number of children were available. Because these were intervention studies, only those children with the poorest iron status were selected from all the available children, to be included at baseline. For each study, power calculations were done by the respective authors to determine the required sample size. If the data before screening or after screening were not included in the original research article, the authors were contacted and requested to provide such data.

Data extraction was conducted independently by two of the authors (CT and JCJ). As mentioned above, in the case of any missing information with regard to data extraction, authors were directly contacted. A final number of four studies were included (Figure I).

The NFCS-FB-2005 reported iron status data by province for all children (age category 1–9 years). and nationally by age groups in the categories 1–3, 4–6, and 7–9 years.<sup>7</sup> In order to evaluate the data of the 7–9 year age groups at provincial level, secondary analysis of the data sets was done. Furthermore, the NFCS-FB-2005 reported z-scores according to the National Center of Health Statistics (NCHS) reference. The original

anthropometric data from the NFCS-FB-2005 were re-analysed by Kruger et al<sup>13</sup> using the reference values of the 2007 World Health Organization (WHO) (WHO AnthroPlus, version 1.0.2 software), and these results were used for the purpose of this review.



**Figure I** Flow chart of screening process for eligible articles

## Results:

Four studies conducted in four different provinces met the inclusion criteria. Of these, three were randomised controlled intervention studies<sup>6, 14-15</sup> and one a cross-sectional study that was the baseline data of a larger intervention study.<sup>5</sup> The four studies were reviewed, together with the re-analysed anthropometric data from the NFCS-FB-2005.<sup>12</sup>

All four of the independent studies selected children to be of poor iron status (Table I).<sup>5-6,14-15</sup> This is primarily because these were intervention studies designed to observe the greatest intervention effects according to their respective aims. Furthermore, in all four independent studies the children were de-wormed. Van Stuijvenberg et al<sup>14</sup> de-wormed four weeks prior to intervention, and Taljaard et al<sup>5</sup> de-wormed within one week prior to baseline measurements. The remaining two studies de-wormed just after baseline measurements.<sup>6,15</sup>

**Table I** Inclusion criteria based on iron status used by independent studies

	Haemoglobin (Hb)	Serum Ferritin (SF)	Transferrin Receptor (TfR)	Zinc Protoporphyrin (ZnPP)	Notes
<b>Van Stuijvenberg et al. (2008)</b>	≤12.5g/dL	-	-	-	Hb ≤ 7.2 g/dL excluded and referred to clinic
<b>Troesch et al.(2011)<sup>1</sup></b>	>9 g/dL	<20µg/L	>8.2 mg/L	-	-
<b>Taljaard (2011)</b>	-	-	414 children with highest TfR and then the highest ZnPP values were selected	-	-
<b>Baumgartner et al. (2012)<sup>2</sup></b>	-	<20µg/L	>8.3mg/L	>70 µmol/mol heme	Hb ≤ 8 g/dL excluded and referred to clinic

<sup>1</sup>Compliance with either Serum Ferritin OR Transferrin Receptor criteria

<sup>2</sup>Compliance with either Serum Ferritin OR Transferrin Receptor OR Zinc Protoporphyrin criteria

All four studies were conducted in low socio-economic areas. Study authors reported the demographic areas as follows: North West province (low socio-economic peri-urban area)<sup>5</sup>, KwaZulu-Natal (low income rural village)<sup>6</sup>, Western Cape (low socio-economic area)<sup>14</sup> and Northern Cape (low socio-economic area)<sup>15</sup>. The NFCS-FB-2005 included both rural and urban areas. General characteristics of the study population as well as anthropometric status are presented in Table II.

Anthropometric status was indicated by weight-for-age, height-for-age and BMI-for-age z-scores. According to the WHO classification normally used for children under 5 years of age, stunting prevalence reported by independent studies and the NFCS-FB (2005) was less than 15%.<sup>16</sup> The prevalence of underweight was less than 10% in all of the studies except for the study of Taljaard (14%).<sup>5</sup> Secondary analysis of the NFCS-FB-

2005 for children aged 7–9 years per province resulted in too small a number of participants per cell in some provinces (e.g.  $n = 9$  in the Northern Cape). The data of the independent studies were therefore compared with national data for children 7–9 years old ( $n = 462$ ) (Table II).

Anaemia prevalence ( $Hb < 11.5$  g/dl) varied from 5.4% in the Northern Cape to 11.5% in KwaZulu-Natal before screening was done (Table III).<sup>6,13</sup> After screening was done, the anaemia prevalence varied between 7.1% in the North West and 20.9% in KwaZulu Natal.<sup>5,6</sup> The NFCS-FB-2005 reported a higher prevalence of anaemia than did independent studies in all the provinces when compared with data before screening (Table III). Owing to the small sample sizes for the NFCS-FB-2005 provincial data, the national prevalence for children aged 7–9 years was also included to see if similar observations were found ( $n = 499$ ). In the studies of van Stuijvenberg et al<sup>14</sup> Taljaard<sup>5</sup>, and Baumgartner et al<sup>6</sup>, children with elevated CRP levels ( $> 10$  mg/L and  $> 5$  mg/L, respectively) were excluded from the SF analyses on the preselected children (Table III).

After personal communication with the authors, iron deficiency was re-calculated based on  $SF < 12$   $\mu$ g/L. The prevalence of iron deficiency before screening for the independent studies ranged from 3.3% in the Northern Cape<sup>15</sup> to 14.8% in the North West province.<sup>5</sup> The NFCS-FB-2005 reported ID based on the same cut-off as 4.4% for children 7–9 years (Table III).

Transferrin receptor as a measure of iron status was not measured in the NFCS-FB-2005. Taljaard<sup>5</sup> and Baumgartner et al<sup>6</sup> reported ID prevalence based on TfR values  $> 8.3$  mg/L. Iron deficiency prevalence was reported as 7.6% and 11.5% respectively. Troesch et al<sup>15</sup> and van Stuijvenberg et al<sup>14</sup> reported medians (95% confidence interval) and mean  $\pm$  SD of TfR concentrations respectively per treatment group, but not as ID prevalence.

Studies used either 10 mg/L or 5 mg/L as cut-off values for increased serum CRP concentrations as an indicator of low-grade inflammation. In all the independent studies low-grade inflammation was present in less than eight percent of the study samples.

**Table II** General and anthropometric characteristics of the children in the four independent studies and the NFCS-FB-2005

Author	Year and study design	n	Gender		Age yrs	Province	Data reported as:	Underweight WAZ<-2	Stunting HAZ<-2	Wasting BAZ<-2
			Boys	Girls						
<b>Van Stuijvenberg et al. (2008)<sup>1</sup></b>	2006 RCT	361	185	176	6-11	Western Cape	%	7.7	14.4	1.1
<b>Troesch et al. (2011)<sup>1</sup></b>	2009 RCT	200	111	86	5-11	Northern Cape	medians (95%CI)	Treatment group: WAZ=-1.5 (-1.9,-1.2) Control: WAZ=-1.4 (-1.6, -1.1)	Treatment group: HAZ=-1.6 (-2.0, -1.4) Control: HAZ=-1.4 (-1.6, -1.2)	NR
<b>Taljaard (2011)<sup>2</sup></b>	2010 Cross-sectional	407	211	196	6-11	North-West	%	14	12.8	8.4
<b>Baumgartner et al. (2012)<sup>2</sup></b>	2009-2010 RCT	321	163	158	6-11	Kwa-Zulu Natal	%	2.1	6.2	NR
<b>Kruger et al. (2011)<sup>2,3</sup></b>	2005 Cross-sectional	462	NR	NR	7-9	RSA	%	9.5 (6.8, 12.1)	14.3 (11.1, 17.5)	6.7 (4.3, 9.1)

<sup>1</sup>NCHS reference used<sup>2</sup>WHO reference used<sup>3</sup>Represents the reanalysed NFCS-FB-2005 data using the World Health Organization reference values

BAZ, BMI-for-age; HAZ, Height-for-age; NR, Not reported; RCT, Randomised controlled trial, RSA, Republic of South-Africa WAZ, Weight-for-age

**Table III** Iron status and anaemia prevalence indicated by haemoglobin and serum ferritin concentrations in the four independent studies and the NFCS-FB-2005<sup>1</sup>

Independent studies								NFCS-FB-2005 (by province)					
		Before screening <sup>2</sup>		After screening <sup>3</sup>									
Province	n	Anaemia prevalence <sup>4</sup>	ID prevalence <sup>5</sup>	SF levels per group	Hb per group	Anaemia prevalence	ID prevalence	n	Hb <sup>6</sup>	Anaemia prevalence	n	SF levels <sup>6</sup>	ID prevalence
(year)		%	%	µg/L	g/dL	%	%		g/dL	%		µg/L	%
Western Cape (2006)	361	Data not available	Data not available	<sup>7</sup> Control	<sup>8</sup> Control	17.2	23.1	32	12.4 (12.1-12.7)	18.8	24	46.4 (34.3-58.5)	4.2
				Tx 1	Tx1								
				Tx 2	Tx 2								
				Tx 3	Txt 3								
				20.1 (7.2, 44.9)	11.90 ± 4.4								
Northern Cape (2009)	200	5.4	3.3	<sup>9</sup> Tx	<sup>9</sup> Tx	7.3	Data not available	9	12.3 (10.9-13.6)	22.2	6	54.3 (31.6-77.0)	0
				Control	Control								
				19.4 (18.6-21.8)	12.6 (12.3-12.8)								
North West (2010)	407	6.9	14.8	30.56 ± 22.44	12.65 ± 0.96	7.1	15.7	37	12.1 (11.8-12.4)	27.0	35	33.9 (24.0-43.8)	2.9
KwaZulu Natal (2009)	321	11.5	7.3	<sup>10</sup> Tx 1	Tx 1	20.9	20.6	97	12.4 (12.1-12.7)	14.4	90	56.5 (48.3-64.8)	2.2
				Tx 2	Tx 2								
				Tx 3	Tx 3								
				20.0 (3.9-72.3)	12.16 ± 0.88								
				Control	Control								
19.46 (3.8-62.8)	12.06 ± 0.85												
								<b>NFCS-FB-2005 (national)</b>					
								499	12.3 (12.2-12.4)	18.6	430	46.3 (42.1-50.5)	4.4

ID, Iron deficiency; Tx, Treatment; NFCS-FB, National food consumption survey-fortification baseline

<sup>1</sup>Data presented for children 7-9 years

<sup>3</sup>Number of children selected according to power calculation

<sup>6</sup>mean (CI)

<sup>8</sup>Medians (95% CI)

<sup>2</sup>All available children at the study site

<sup>4</sup>Anaemia indicated as Hb < 11.5g/dL

<sup>7</sup>Median(10th, 90th percentile)

<sup>10</sup>Median, min, max

<sup>5</sup>Iron deficiency indicated as SF < 12µg/L

<sup>9</sup>Mean±SD (and all such values)

## Discussion

This review includes four independent studies that report on iron status and anaemia prevalence of primary school children in four different provinces of South Africa after the last NFCS-FB-2005. These studies had large study samples, were conducted on similar age groups and in low socio-economic areas. In order to evaluate the most comparable data from the NFCS-FB-2005, only children aged 7–9 years were included in this review. Further stratification of the anthropometric data from the national sample to provincial data yielded groups that were too small and therefore the national data of children aged 7–9 years were considered.

The prevalence for wasting, stunting and underweight in the independent studies was moderate or low and it seems that when compared with re-analysed NFCS-FB-2005 anthropometric data, the prevalence of wasting and stunting did not differ from the anthropometric status of children included in the NFCS-FB-2005.

The independent studies reported lower anaemia prevalence than the NFCS-FB-2005. Despite the children being preselected on the basis of having poor iron status in the respective studies, only one study reported higher anaemia prevalence (KwaZulu Natal)<sup>6</sup> than was reported in the NFCS-FB-2005. Anaemia prevalence data for the Western Cape<sup>14</sup> were available only after screening and the study reported prevalence similar to the NFCS-FB-2005 (17.2% vs 18.8%). The study was done only three years after the fortification programme commenced, while the remainder of the studies were conducted six to seven years after the fortification programme was initiated. However, when considering the final samples after screening in studies conducted in the North West and the Northern Cape, the NFCS-FB-2005 reported anaemia prevalence rates more than three times what was reported in the independent studies.<sup>6,15</sup>

Higher prevalence of ID based on SF concentrations was reported in most of the independent studies (before and after screening) compared with the NFCS-FB-2005. Comparisons between SF concentrations for children with CRP above 10 mg/L and below 10 mg/L in the NFCS-FB-2005 were mostly insignificant except for the rural ( $p = 0.05$ ) and tribal ( $p = 0.002$ ) areas. While the NFCS-FB-2005 did not correct for CRP, the independent studies corrected for CRP after screening. One would expect, therefore, that the independent studies would report higher prevalences of ID based on SF concentrations. For this reason it is more appropriate to make comparisons based on the pre-screening data.

In order to provide possible explanations for the differences observed in iron status and anaemia prevalence between the independent studies and the NFCS-FB-2005, the role of 1) infection/inflammation, 2) de-worming, 3) selection criteria, and 4) the NFFP on iron status in South Africa will be discussed.

### **1. The effect of low grade inflammation/infection on the iron status indicator, serum ferritin**

Acute and chronic infections lead to lower serum iron and higher SF concentrations. Research by Beard et al<sup>17</sup> suggested that with low prevalence of clinically defined inflammation (< 10%) there is little influence of inflammation on the distribution of iron biomarkers in large samples. It is still unclear at what point the prevalence of inflammation causes a shift in iron status indicators.<sup>17</sup> An increase in CRP concentration of 10–30 mg/L has been suggested as cut-off measure for SF to remain a valid diagnostic marker of iron status.<sup>18</sup> Furthermore, the duration of increase in CRP is generally shorter than the duration of increase in SF during the acute-phase response.<sup>18</sup> The concentration of serum iron decreases within several hours after the start of acute inflammation.<sup>19</sup> The decrease in serum iron is quickly followed by an increase in CRP. Serum ferritin reaches its maximum at approximately 48 hours after stimulation, while CRP concentrations start to decline 24–48 hours after the onset of inflammation.<sup>19</sup> Given the above information, for this review, estimations of iron status based on serum ferritin values are particularly difficult to compare because of the possible effect of inflammation. The influence of infection on SF is undeniable. Based on the reported number of children below and above elevated CRP concentrations (CRP > 10 mg/L) in the NFCS-FB-2005, approximately 12% of children had low grade inflammation nationally. This is above the <10% suggested by Beard et al<sup>17</sup> for minimal influence on the distribution of SF in a large population sample. Therefore, it is uncertain what the influence of low grade inflammation could have been on the prevalence of ID based on SF since a correction for CRP had not been made in the NFCS-FB-2005.

### **2. De-worming and the effect on iron status**

All the independent studies de-wormed the children prior to or shortly after starting the intervention. Children are de-wormed in intervention studies to avoid blood loss that is caused by intestinal worm infestation that negatively influences iron status.<sup>18</sup> The aim of the initial dose (usually albendazole or mebendazole) is to reduce the worm load by > 80%. Unfortunately, re-infection can occur directly after treatment. The likelihood of re-infection emphasises the importance of repeating treatment, especially in communities where sanitation circumstances do not improve.<sup>19-20</sup>

Stoltzfus et al<sup>21</sup> reported on 3595 schoolchildren from Zanzibar, 62.3% of whom were anaemic (82.7% associated with iron deficiency). Through multivariate analysis they determined that 73% of severe anaemia and 35% of iron deficiency anaemia could be explained by hookworm infestation. An increase in Hb-concentrations can be expected several weeks/months after the administration of de-worming medication. Furthermore, the lifespan of a red blood cell is 120 days<sup>22</sup> and it is therefore unlikely that de-worming could have had a significant effect on baseline Hb concentration reported in the independent studies.

### 3. Representativeness of national data *versus* data from independent studies

Although the national surveys of 1994, 1999 and 2005 have been widely used to describe the poor micronutrient status of South African children, the small studies such as the ones reported in this article has not been able to confirm this. Recent developments with regards to the South African National Health and Nutrition Survey (SANHANES) that was undertaken in 2012 may result in more up-to-date data if the survey was to include the micronutrient status of children. Data from independent studies cannot be extrapolated to larger population groups because of the manner in which the study samples were selected. In addition, extrapolation to what the situation might be at national level is not possible because of the unrepresentative nature of the samples. With regard to the inclusion criteria of the independent studies included in this review, children were selected on the basis of having poor iron status. It might therefore be expected that the independent studies included overestimated the prevalence of anaemia observed. This is further supported when taking into account that the prevalences reported before screening, as one would expect were lower than after screening.

### 4. The possible effect of the National Food Fortification Programme (NFFP) on iron status

The higher prevalence of poor iron status in 2005 than in 1994 (SAVACG, 1995) raised concern among nutritionists.<sup>7</sup> Despite national programmes in South Africa such as the Integrated Nutrition Programme, an increase in anaemia prevalence was found from 1994 to 2005. In 2003 mandatory food fortification legislation came into effect<sup>12</sup> and the question is: what has its impact been on micronutrient status and, more specifically, with regard to this article, on iron status?

According to the Foodstuffs, Cosmetics and Disinfectants Act of South Africa (54 of 1972), wheat flour and maize meal are fortified at a level of 35 mg/kg, wheat flour at 43 mg/kg, wheat bread (white) at 32 mg/kg and wheat bread (brown) at 34 mg/kg. Iron fortification has been found to be challenging because the compounds with the best bioavailability cause undesirable organoleptic changes in the fortification vehicles.<sup>23</sup> Furthermore, the correct foods should be fortified with adequate dosages in order for fortification to be effective in a population.<sup>18</sup> In a secondary analysis conducted by Steyn et al<sup>24</sup>, it appeared that fortifying the two most commonly consumed staple foods in South Africa, meant that micronutrient intake among children was likely to improve. However, for iron status per se, some randomised controlled intervention trials indicated that consumption of elemental iron at fortification levels of 35 mg/kg to 56 mg/kg for more than five months did not improve the iron status of primary schoolchildren.<sup>14,26</sup> In the above-mentioned studies, either bread or maize porridge was given as intervention, but not in combination. The quantity of micronutrients added in the study of van Stuijvenberg et al<sup>14</sup> was according to South African government regulations. Unfortunately, higher levels of elemental iron, as specified by the WHO/FAO<sup>23</sup>, were not included in the intervention groups of the studies conducted. It seems unlikely, then, that the current dosage of low bioavailable elemental iron now being used for fortification could be the reason for improved iron status, if indeed iron status is

improving. While micronutrient consultative meetings to address micronutrient deficiency are currently ongoing in South Africa, observations from independent studies conducted since fortification started are important in highlighting the need for a national study to inform policy in South Africa. Independent studies are certainly valuable in painting a picture of what the situation regarding iron status may be in some regions in the country.

## **Limitations**

The current data from independent studies are neither sufficient, nor sufficiently representative, for direct comparisons to be made with the NFCS-FB-2005. Nevertheless, such data, being the only data available, cannot be ignored. Data from the NFCS-FB-2005 that were re-analysed resulted in small sample sizes which further complicated observations for the same provinces. Data from the NFCS-FB-2005 include children from both rural and urban areas in order to provide a better picture of anaemia prevalence in the whole country.

## **Conclusion**

In four different independent studies, children were selected on the basis of having low iron status. These data suggest that the prevalence of anaemia is not as high as was measured in the NFCS-FB-2005. Observed SF concentrations are difficult to interpret owing to a correction for CRP that was not made in all the studies. Independent studies provide valuable information on iron status at a local level for a specific province.

The observations made in this review warrant a national survey to determine the current iron status of South African children. If iron status is indeed improving, questions, such as to what extent the National Food Fortification Programme has played a role, need to be answered. It seems unlikely that the poorly bioavailable iron that is currently used as fortificant would lead to improved iron status and, if this is the case, the question remains: which factors could be contributing to the improved iron status?

National data are needed to confirm the observations of the independent studies on iron status. A new national survey will be necessary to provide up-to-date data on the prevalence of anaemia. Updated information will guide nutritionists, dieticians and policy makers in focusing their attention on the correct age groups with the greatest need for nutrition interventions. In addition, updated national data are needed to evaluate the efficacy of current national programmes which aim to improve undernutrition in South Africa.

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## ACKNOWLEDGEMENTS

All the authors provided scientific input into data interpretation and writing of the article. C.Taljaard, the Ph.D student of the study, was involved in all aspects of the literature search, data analysis and article writing. Furthermore, thank you to Dr. L van Stuijvenberg, Dr. J. Baumgartner and Prof M. Smuts who provided addition information through personal communication. Heartfelt thanks to Hannelie Nel who did the secondary data analysis on the NFCS-FB-2005. Furthermore, we thank Anneke Coetzee from the North-West University library (Ferdinand Postma library) who helped with the literature search.

**CHAPTER 4:**

**GENERAL SUMMARY, CONCLUSION & RECOMMENDATIONS**



## 4.1 Introduction

The primary aim of this thesis was to investigate the effects of a beverage fortified with micronutrients, with or without sugar, on micronutrient status, growth and cognitive performance of South African primary school children between the ages of 6 and 11 years.

The secondary aim was to review the iron status of South African primary school children as observed by independent intervention and cross-sectional studies conducted in different parts of the country since the latest National Food Consumption Survey-Fortification Baseline (NFCS-FB, 2005) and to relate the findings reported by these local studies to the results of the NFCS-FB (2005).

The aim of this chapter is to summarise the main findings according to the hypotheses stated in chapter 1, to draw conclusions and to make recommendations for further research.

## 4.2 Main findings according to hypotheses given in Chapter 1

**Hypothesis 1: Administration of a micronutrient-fortified beverage for a period of 8.5 months in apparently healthy schoolchildren between the ages of 6 and 11 years,**

- a. improves dietary intake of iron, zinc and vitamin A, as measured by three repeated 24-hour recall questionnaires, to the extent that the estimated average requirements (EAR) are reached.

The micronutrient-fortified beverage significantly improved dietary micronutrient intake of iron, zinc and vitamin A. However, the children's dietary intake of iron, zinc and vitamin A already exceeded 100% of the EAR without beverage consumption.

- b. improves iron (based on the iron status indicators ZnPP, SF and TfR), zinc (based on SZn) and vitamin A (based on SR) status and anaemia prevalence (based on Hb).

The beverage fortified with micronutrients improved iron status based on ZnPP and SF concentrations, indicated by an increasing effect on endpoint SF and a decreasing effect on ZnPP concentrations. No significant intervention effects of micronutrient fortification were found on TfR. Furthermore, consuming the beverage fortified with micronutrients significantly reduced the odds ratio for a child to be ID at endpoint, based on SF concentrations. SZn and SR were not affected by micronutrient fortification. The beverages fortified with micronutrients did not

improve zinc status (based on SZn) in comparison with the control groups, or vitamin A (based on SR) status or anaemia prevalence based on Hb concentrations from baseline to end.

- c. improves cognitive performance, as assessed by selected Kaufman Assessment Battery for Children, Second Edition (KABC-II).

The micronutrient-fortified beverage improved cognitive performance as assessed by the sub-test Atlantis, indicative of associative memory. None of the other sub-tests (Story completion, Number recall, Rover, Triangles, Word order, Hand movements) was significantly improved at endpoint by the provision of a micronutrient-fortified beverage.

- d. improves growth, as indicated by increases in WAZ, HAZ and BAZ.

In contrast to our hypothesis, the micronutrient-fortified beverage had a significant lowering intervention effect on WAZ. Neither HAZ nor BAZ was affected by the micronutrient-fortified beverage.

**Hypothesis 2: Administration of a beverage with added sugar, for a period of 8.5 months in apparently healthy schoolchildren between the age of 6 and 11 years,**

- a. improves energy intake so that it reaches the estimated energy requirements (EER).

The sugar content of the beverage significantly increased mean energy intake. However, the energy intake did not increase to the extent that the estimated EER was reached by all children.

- b. improves cognitive performance, as assessed by selected KABC-II.

The sugar content of the beverage improved cognitive performance as assessed by the sub-tests of the KABC-II, Atlantis and Rover. These tests are indicative of associative memory and visual memory respectively. There was no intervention effect of sugar on any of the remaining sub-tests (Story completion, Number recall, Triangles, Word order and Hand movements).

- c. improves growth, as indicated by increases in WAZ, HAZ and BAZ

In contrast to our hypothesis, the addition of sugar content to the beverage had a significant lowering effect on WAZ. Neither HAZ nor BAZ was affected by the beverage with added sugar.

**Hypothesis 3: Administration of a beverage fortified with micronutrients and added sugar in combination,**

- a. improves cognitive performance assessed by the KABC-II to a greater extent than administration of micronutrients or sugar alone.

A beverage fortified with micronutrients and added sugar did not improve cognitive performance to a greater extent than administration of micronutrients or sugar alone. In contrast, when micronutrients and sugar were provided in combination, the positive intervention effects of micronutrients and sugar alone on Atlantis, Rover and Number recall were attenuated.

- b. improves growth, as indicated by increasing WAZ, HAZ and BAZ, to a greater extent than administration of micronutrients or sugar alone.

A beverage fortified with micronutrients and added sugar did not improve growth as indicated by increasing WAZ, HAZ and BAZ to a greater extent than administration of micronutrients or sugar alone. However, when micronutrients and sugar were provided in combination, the lowering effects of micronutrients and sugar alone on WAZ were attenuated.

**Hypothesis 4: South African independent studies report a lower prevalence of iron deficiency and anaemia compared with the NFCS-FB (2005).**

The four independent studies reported lower prevalence of anaemia compared with the NFCS-FB-2005. It is unclear whether iron deficiency, based on SF concentration, has changed. Interpretation of SF concentrations has been difficult because, unlike most of the independent studies, the NFCS-FB-2005 did not correct for elevated CRP (marker of acute inflammation).

### **4.3 Conclusions**

Multi-micronutrient fortification and glucose administration via a beverage has previously been reported to have a positive effect on certain cognitive outcomes in primary schoolchildren. The results of the BeForMi study indicated that both multi-micronutrient fortification and sugar positively affected cognitive function in primary schoolchildren. However, the attenuation of the positive effects of micronutrients and sugar alone that was observed when micronutrients and sugar were given in combination in a beverage was unexpected. Furthermore, the attenuating effect of micronutrients and sugar in combination on growth, as indicated by WAZ, was also unexpected. Since no other study has been conducted to investigate the interaction effects of

micronutrients and sugar, it is not clear whether the results are specific to this population sample.

Four independent studies conducted in KwaZulu Natal, NorthWest, Western Cape and the Northern Cape Province were identified by a thorough search strategy to provide data on iron status in primary school children after the National Food Consumption Survey-Fortification Baseline (2005). All four studies indicated a lower prevalence of anaemia (Hb<11.5g/dL) than did the NFCS-FB-2005. This was unexpected because the children in three of the four studies were selected on the basis of having poor iron status. The data from independent studies, therefore, suggest that the prevalence of anaemia is not as high as was measured in the NFCS-FB-2005. Reported SF concentrations in the different studies were difficult to interpret owing to the possible elevating effect of CRP on SF, which was not corrected for in all the studies.

The low prevalence of ID, IDA, anaemia, vitamin A and zinc deficiency, together with higher than expected dietary micronutrient intake but insufficient dietary energy intake, could have contributed to the findings of the BeForMi intervention study. The review article suggests that the low anaemia prevalence was not isolated to our study population but has also been observed in other study populations in different regions. Finally, the BeForMi intervention results emphasise the fact that, even in children with a low prevalence of micronutrient deficiency, cognitive function could still be influenced.

#### 4.4 Recommendations for further research

Both articles included in this thesis raise questions that can be answered only through new research initiatives. They also emphasise the remaining gaps, particularly in South African nutrition research on malnutrition in children.

- It is recommended that a large-scale high-quality national survey should be conducted to confirm possible improved anaemia prevalence observed in independent studies. This survey should include the distribution of micronutrient deficiencies in the different provinces of South Africa. This will provide up-dated information on the status of vitamin A and zinc, and on wasting, stunting, undernutrition and overnutrition in primary school children. It is important that the current magnitude of the problem of micronutrient deficiencies in children at a national level is determined so that it can be addressed accordingly.
- It is recommended that, at national level, the use of SF concentrations as a diagnostic criterion should be standardised in agreement with the current WHO recommendations in order to facilitate comparability between studies. Researchers could still include other ways of handling SF concentrations according to their own interest. Standardisation across different studies, as is done in correction for CRP/low-grade inflammation, would have similar benefits.
- To our knowledge, this was the first study that investigated the combined effects on cognitive function of micronutrients and sugar in a beverage. The results need to be verified. Our population had specific characteristics and it is possible that in settings where children have poorer micronutrient status the beverage might have an even greater positive effect on cognitive function.
- It is recommended that physical activity should be included in similar studies where the effects of sugar and micronutrients on growth are investigated.
- It is recommended that the mechanisms by which sugar and micronutrients may interact to influence cognition should be further investigated by using animal models.

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## **ADDENDA**

### **ADDENDUM A: 24-HOUR RECALL QUESTIONNAIRE**

### BeForMi 24-HOUR RECALL INTERVIEW FORM

Subject number: \_\_\_\_\_ Interviewer: \_\_\_\_\_

School: \_\_\_\_\_ Date: \_\_\_\_/\_\_\_\_/200\_\_\_\_

Tick what the day was yesterday:

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

Is this your first, second or third interview about what you ate

First	Second	Third
-------	--------	-------

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

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

I want to find out about everything you ate or drank yesterday, including water or food you pick from the veld. 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 school, or starting day's activities					
During the morning at school or at home					

Time (approximately)	Place (Home, school, etc)	Description of food and preparation method	Amount	Amount in g (office use only)	Code (office use only)
Middle of the day (Lunch time)					
During the afternoon					
At night (dinner time)					
After dinner, before going to sleep					
Do you take any vitamins (tablets or syrup)?			Yes	1	No
Give the brand name and dose of the vitamin/tonic:					

## **ADDENDUM B: SOCIO-DEMOGRAPHIC QUESTIONNAIRE**

### BeForMi Socio-demographic questionnaire

(All information in this questionnaire is confidential).

a. Interviewer Name: \_\_\_\_\_

b. Interview Date: \_\_\_\_\_

c. Subject number \_\_\_\_\_

d. Name of Child: \_\_\_\_\_

e. School: \_\_\_\_\_

f. Date of birth of child: Day ..... Month ..... Year .....

g. Gender	1		2											
	Male		Female											
h. Home language	1		2		3		4		5		6		7	
	Zulu		English		Sesotho		Setswana		Xhosa		Afrikaans		Other specify	
i. Type of dwelling: <i>(You can tick more than one block if necessary)</i>	1		2		3		4		5					
	Brick, Concrete		Traditional mud		Tin		Plank, Wood		Other, specify					
j. Number of people living in the your household <i>(Tick one)</i>	1		2		3		4		5					
	<4 persons		4-6 persons		7-8 persons		>8 persons		Don't know					
k. The people in your household who work <i>(Tick one)</i>	1		2		3		4		5		6 (specify)			
	Mother/mother figure to child		father/father figure to child		grandmother		grandfather		sibling		Other funds			
l. Where do you get drinking water most of the time <i>(Tick one)</i>	1		2		3		4		5					
	Own Tap		Public Tap		River, Dam		Borehole, Well		Other: Specify					
m. What type of toilet does your household have? <i>(Tick one)</i>	1		2		3		4		5					
	Flush		Pit		Bucket, Pot		Ventilated Improved Pit latrine (VIP)		Other (Specify)					

n. What fuel is used for cooking most of the time in your household? ( <i>You can tick more than one</i> )	1	2	3	4	5	6	7
	Electric	Gas	Paraffin	Wood/ coal	Sun	Open Fire	Don't know
o. Do you have access to electricity inside your house?	1	2					
	Yes	No					
p. Does your household have a working:	1	2	3	4	5		
1. Refrigerator / Freezer	Fridge	Freezer	Fridge/freezer combination	None	Don't know		
2. Stove ( <i>You can tick more than one</i> )	1	2	3	4	5	6	7
	Coal	Paraffin	Gas	Electric	With oven	Without oven	None
3. Washing machine	1	2					
	Yes	No					
4. Microwave oven	1	2					
	Yes	No					
5. Television	1	2					
	Yes	No					
6. Radio	1	2					
	Yes	No					

**q. Household Composition (Defined as people who regularly eat together)**

Name	Age (yrs)	Gender 1= Female 2= Male	Relationship to child 1=mother 2=father 3=sibling 4=aunt 5=uncle 6=grandmother 7=grandfather 8=other	Currently Schooling? 1 = Yes 2 = No	Head of Household (Mark X)	Marital status 1= Single 2= Married 3= Divorced	Current perceived health status 1= bedridden/disabled, 2= ill, 3 average health 4= good health 5= excellent	Educational level 1=no schooling 2=primary 3=secondary 4=tertiary	Monthly income 1= < 500 2=500-1000 3=1000-2000 4= 2000-2500 5=2500-3000 6=3000-3500 7=3500-4000 8= >4000

**Access to Food/Food Security:**

r. Do you grow any food or vegetables for self consumption? Tick the relevant one

Food		Vegetables	
1. Yes	2. No	3. Yes	4. No

s. How many days in the last month has your family run out of food completely? (Record the actual number of day.) \_\_\_\_ days (if none then record 0)

t. How many days in the last month has your family not had enough food to eat? (Record the actual number of day.) \_\_\_\_ days (if none then record 0)

u. How many days in the last month did you reduce the number of meals in a day because there was not enough food? (Record the actual number of day.) \_\_\_\_ days (if none then record 0)

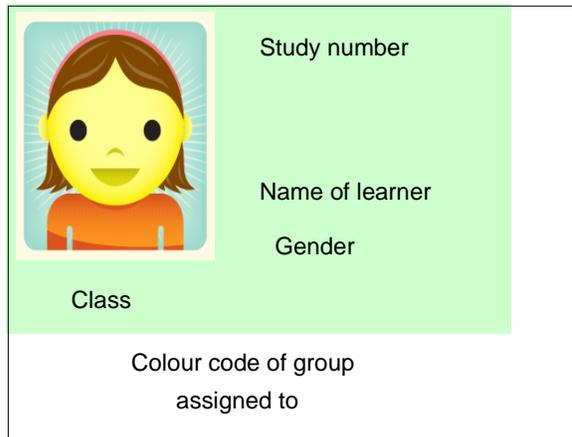
**Health Service Utilization**

Select one by marking the box with X:

v. What services does this household mostly utilize for their health needs?					
1. Public Hospital	2. Private Doctor	3. Traditional Healers	4. Others	5. Public Clinic	6. None

**ADDENDUM C: IDENTITY CARD**

### Sketch of learner identity card



**ADDENDUM D: INFORMED CONSENT FORM**

## BeForMi Informed Consent Form and Information



*Dear Parent / legal guardian*

### **Re: INFORMATION about the BeForMi study and request for informed consent**

The North West University wants to undertake a research study to determine the effect of a flavoured cold drink that contains vitamins and minerals (BeForMi) on the learning ability in primary school learners in grades 1 to 3 at the school where your child is enrolled. Research studies that have been done in the past have shown that additional vitamins and minerals may improve a child's ability to learn. We have designed 4 different cold drinks, some with vitamins and some without, to enable us to find out if these vitamins and minerals can improve your child's learning ability. We have planned a study in which the children will be given 200ml of BeForMi each day before break for the duration of the whole year. Your child may receive any one of the four. We are asking for your consent for your child to take part in this study. Participation is completely voluntarily and the child can drop out of the study at any time.

The study will involve the following:

1. We want to take a picture of each child to help the study assistants recognise the children in the study and that we are sure to compare measurements of the same child before and after the study.
2. Your child's height, weight, arm circumference and fat skin-folds will be measured. The skin-fold measurements are taken by using a machine that folds the skin in order to measure how thick it is.
3. To measure your child's ability to learn, your child will be asked questions in a personal interview by a trained adult assistant.
4. Your child will be asked to step on a machine which looks like a scale and some measurements will be taken. These measurements will be used to calculate how much fat and muscle the child has in the body.

5. For us to know how much nutrients the child has we will need to take a small blood sample (about two teaspoons). The blood samples will be taken by an experienced registered nurse who normally does this type of work. She will use sterile equipment (only used once and then discard it).
6. All the measurements will be taken once at the beginning of the study and again at the end of the school year.
7. During the study period you will be visited at your home by a study assistant. The assistant will interview you about the food you ate the previous day and also ask about your general living conditions.

***Benefits of the study to your child:***

- 1) Every day all children will receive a cold drink as part of their meal at school during the 2010 school year.
- 2) After the study has finished all learners in the school will be given the drink that has been shown to improve their learning ability to the greatest extent for the first half of the 2011 school year.
- 3) Children who are found to be severely anaemic when we test the blood will be identified confidentially and you will be referred to the clinics so that the child can receive treatment immediately.
- 4) The children will be given de-worming medication before the study. This will ensure that the children may benefit from the micronutrients they get.
- 5) All the utensils that will be used to serve the children with the drinks will be left at the school for the sole use of the school feeding programme.

If you have any questions please feel free to phone

Dr. Namukolo Covic                      018 299 4037 or 072 443 6895

Professor Johann Jerling              018 299 2481

Yours Sincerely,

Dr. Namukolo Covic



**INFORMED CONSENT FORM**

**Effect of long-term consumption of a beverage fortified with vitamins and minerals on the learning ability of primary school children aged 7-9 years in North-West Province of South Africa: the BeForMi study**

I have been informed about the purpose and nature of the study and that all information will be regarded as confidential.

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.

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.

Nutritional status will be assessed by means of the measurement of height, weight, skinfold measurements, body composition measurement and analysis of blood samples. A small blood sample of about two teaspoons will be taken from the child's arm by a nursing sister at the beginning of the year and end of the study at the end of the year.

Name of child ..... My child is allergic to: .....

Date of birth of child: Day ..... Month..... Year .....

The gender of the child Male  Female  *please tick one as appropriate*

Residential address.....  
.....  
.....

Name of Parent or Legal Guardian ..... Signature:.....

Signed this..... Day Of ..... 2010 at .....

Telephone number of Name of Parent or Legal Guardian

**ADDENDUM E: AUTHORS GUIDELINES FOR THE BRITISH  
JOURNAL OF NUTRITION**

## Directions to Contributors

*British Journal of Nutrition*

(Revised January 2012)

The *British Journal of Nutrition* is an international peer-reviewed journal that publishes original papers and review articles in all branches of nutritional science. The underlying aim of all work should be, as far as possible, to develop nutritional concepts. The *British Journal of Nutrition* encompasses the full spectrum of nutritional science including epidemiology, dietary surveys, nutritional requirements and behaviour, metabolic studies, body composition, energetics, appetite, obesity, ageing, endocrinology, immunology, neuroscience, microbiology, genetics and molecular and cell biology. The journal does not publish case studies; papers on food technology, food science or food chemistry; or papers of primarily local interest.

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Spelling should generally be that of the *Concise Oxford Dictionary* (1995), 9th ed. Oxford: Clarendon Press. Papers should normally be divided into the following parts:

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1. Setchell KD, Faughnan MS, Avades T *et al.* (2003) Comparing the pharmacokinetics of daidzein and genistein with the use of <sup>13</sup>C-labeled tracers in premenopausal women. *Am J Clin Nutr* 77, 411–419.
2. Barker DJ, Winter PD, Osmond C *et al.* (1989) Weight in infancy and death from ischaemic heart disease. *Lancet* ii, 577–580.
3. Forchielli ML & Walker WA (2005) The role of gut-associated lymphoid tissues and mucosal defence. *Br J Nutr* 93, Suppl. 1, S41–S48.
4. Bradbury J, Thomason JM, Jepson NJA *et al.* (2003) A nutrition education intervention to increase the fruit and vegetable intake of denture wearers. *Proc Nutr Soc* 62, 86A.
5. Frühbeck G, Gómez-Ambrosi J, Muruzabal FJ *et al.* (2001) The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation. *Am J Physiol Endocrinol Metab* 280, E827–E847.
6. Han KK, Soares JM Jr, Haidar MA *et al.* (2002) Benefits of soy isoflavone therapeutic regimen on menopausal symptoms. *Obst Gynecol* 99, 389–394.
7. Uhl M, Kassie F, Rabot S *et al.* (2004) Effect of common Brassica vegetables (Brussels sprouts and red cabbage) on the development of preneoplastic lesions induced by 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) in liver and colon of Fischer 344 rats. *J Chromatogr* 802B, 225–230.
8. Hall WL, Vafeiadou K, Hallund J *et al.* (2005) Soy isoflavone enriched foods and inflammatory biomarkers of cardiovascular risk in postmenopausal women: interactions with genotype and eouol production. *Am J Clin Nutr* (In the Press).
9. Skurk T, Herder C, Kraft I *et al.* (2004) Production and release of macrophage migration inhibitory factor from human adipocytes. *Endocrinology* (Epublication ahead of print version).
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11. Bradbury J (2002) Dietary intervention in edentulous patients. PhD Thesis, University of Newcastle.
12. Ailhaud G & Hauner H (2004) Development of white adipose tissue. In *Handbook of Obesity. Etiology and Pathophysiology*, 2nd ed., pp. 481–514 [GA Bray and C Bouchard, editors]. New York: Marcel Dekker.
13. Bruinsma J (editor) (2003) *World Agriculture towards 2015/2030: An FAO Perspective*. London: Earthscan Publications.
14. Griinari JM & Bauman DE (1999) Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In *Advances in Conjugated Linoleic Acid Research*, vol. 1, pp. 180–200 [MP Yurawecz, MM Mossoba, JKG Kramer, MW Pariza and GJ Nelson, editors]. Champaign, IL: AOCS Press.
15. Henderson L, Gregory J, Irving K *et al.* (2004) *National Diet and Nutrition Survey: Adults Aged 19 to 64 Years*. vol. 2: *Energy, Protein, Fat and Carbohydrate Intake*. London: The Stationery Office.
16. International Agency for Research on Cancer (2004) *Cruciferous Vegetables, Isothiocyanates and Indoles*. IARC Handbooks of Cancer Prevention no. 9 [H Vainio and F Bianchini, editors]. Lyon, France: IARC Press.
17. Linder MC (1996) Copper. In *Present Knowledge in Nutrition*, 7th ed., pp. 307–319 [EE Zeigler and LJ Filer Jr, editors]. Washington, DC: ILSI Press.
18. World Health Organization (2003) *Diet, Nutrition and the Prevention of Chronic Diseases*. Joint WHO/FAO Expert Consultation. WHO Technical Report Series no. 916. Geneva: WHO.
19. Keiding L (1997) *Astma, Allergi og Anden Overfølsomhed i Danmark – Og Udviklingen 1987–1991 (Asthma, Allergy and Other Hypersensitivities in Denmark, 1987–1991)*. Copenhagen, Denmark: Dansk Institut for Klinisk Epidemiologi.

References to material available on websites should include the full Internet address, and the date of the version cited. Thus:

20. Department of Health (1997) Committee on Toxicity of Chemicals in Food Consumer Products and the Environment. Statement on vitamin B<sub>6</sub> (pyridoxine) toxicity. <http://www.open.gov.uk/doh/hcf/B6.htm>
21. Kramer MS & Kakuma R (2002) *The Optimal Duration of Exclusive Breastfeeding: A Systematic Review*. Rome: WHO; available at [http://www.who.int/nut/documents/optimal\\_duration\\_of\\_exc\\_bfeeding\\_review\\_eng.pdf](http://www.who.int/nut/documents/optimal_duration_of_exc_bfeeding_review_eng.pdf)
22. Hooper L, Thompson RL, Harrison RA *et al.* (2004) Omega 3 fatty acids for prevention and treatment of cardiovascular disease. *Cochrane Database of Systematic Reviews*, issue 4, CD003177. <http://www.mrw.interscience.wiley.com/cochrane/clsysrev/articles/CD003177/frame.html>
23. Nationmaster (2005) HIV AIDS – Adult prevalence rate. [http://www.nationmaster.com/graph-T/hea\\_hiv\\_aid\\_adu\\_pre\\_rat](http://www.nationmaster.com/graph-T/hea_hiv_aid_adu_pre_rat) (accessed June 2005).

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**Mathematical modelling of nutritional processes.** Papers in which mathematical modelling of nutritional processes forms the principal element will be considered for publication provided: (a) they are based on sound biological and mathematical principles; (b) they advance nutritional concepts or identify new avenues likely to lead to such advances; (c) assumptions used in their construction are fully described and supported by appropriate argument; (d) they are described in such a way that the nutritional purpose is clearly apparent; (e) the contribution of the model to the design of future experimentation is clearly defined.

**Units.** Results should be presented in metric units according to the International System of Units (see *Quantities, Units and Symbols in Physical Chemistry*, 3rd ed. (2007) Cambridge: RSC Publishing), and *Metric Units, Conversion Factors and Nomenclature in Nutritional and Food Sciences* (1972) London: The Royal Society – as reproduced in *Proceedings of the Nutrition Society* (1972) **31**, 239–247). SI units should be used throughout the paper. The author will be asked to convert any values that are given in any other form. The only exception is where there is a unique way of expressing a particular variable that is in widespread use. Energy values must be given in Joules (MJ or kJ) using the conversion factor 1 kcal = 4.184 kJ. If required by the author, the value in kcal can be given afterwards in parentheses. Temperature is given in degrees Celsius (°C). Vitamins should be given as mg or µg, not as IU.

For substances of known molecular mass (Da) or relative molecular mass, e.g. glucose, urea, Ca, Na, Fe, K, P, values should be expressed as mol/l; for substances of indeterminate molecular mass (Da) or relative molecular mass, e.g. phospholipids, proteins, and for trace elements, e.g. Cu, Zn, then g/l should be used.

Time. The 24 h clock should be used, e.g. 15.00 hours.

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Comparisons between means can be made by using either confidence intervals (CI) or significance tests. The most appropriate of such measures is usually the standard error of a difference between means (SED), or the standard errors of the means (SE or SEM) when these vary between means. The standard deviation (SD) is more useful only when there is specific interest in the variability of individual values. The degrees of freedom (df) associated with SED, SEM or SD should also be stated. The number of decimal places quoted should be sufficient but not excessive. Note that pH is an exponential number, as are the log(10) values often quoted for microbial numbers. Statistics should be carried out on the scalar rather than the exponential values.

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Where the method of analysis is unusual, or if the experimental design is at all complex, further details (e.g. experimental plan, raw data, confirmation of assumptions, analysis of variance tables, etc.) should be included.

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In curves presenting experimental results the determined points should be clearly shown, the symbols used being, in order of preference, ○, ●, △, ▲, □, ■, ×, +. Curves and symbols should not extend beyond the experimental points. Scale-marks on the axes should be on the inner side of each axis and should extend beyond the last experimental point. Ensure that lines and symbols used in graphs and shading used in histograms are large enough to be easily identified when the figure is reduced to fit the printed page.

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Some image processing is acceptable (and may be unavoidable), but the final image must accurately represent the original data. Authors should provide sufficient detail of image-gathering procedures and process manipulation in the Methods sections to enable the accuracy of image presentation to be assessed. Grouping or cropping of images must be identified in the legend and indicated by clear demarcation. Adjustment of brightness, contrast or colour balance is acceptable if applied to the whole image and to controls and if data do not disappear as the result of the manipulation.

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For indicating statistical significance, superscript letters or symbols may be used. Superscript letters are useful where comparisons are within a row or column and the level of significance is uniform, e.g. <sup>a,b,c</sup>Mean values within a column with unlike superscript letters were significantly different ( $P < 0.05$ ). Symbols are useful for indicating significant differences between rows or columns, especially where different levels of significance are found, e.g. 'Mean values were significantly different from those of the control group: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ '. The symbols used for  $P$  values in the tables must be consistent.

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**Descriptions of solutions, compositions and concentrations.** Solutions of common acids, bases and salts should be defined in terms of molarity (M), e.g. 0.1 M- $\text{NaH}_2\text{PO}_4$ . Compositions expressed as mass per unit mass (w/w) should have values expressed as ng,  $\mu\text{g}$ , mg or g per kg; similarly for concentrations expressed as mass per unit volume (w/v), the denominator being the litre. If concentrations or compositions are expressed as a percentage, the basis for the composition should be specified (e.g. % (w/w) or % (w/v) etc.). The common measurements used in nutritional studies, e.g. digestibility, biological value and net protein utilization, should be expressed as decimals rather than as percentages, so that amounts of available nutrients can be obtained from analytical results by direct multiplication. See *Metric Units, Conversion Factors and Nomenclature in Nutritional and Food Sciences*. London: The Royal Society, 1972 (para. 8).

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**Gene nomenclature and symbols.** The use of symbols and nomenclature recommended by the HUGO Gene Nomenclature Committee (<http://www.genenames.org/>) is encouraged. Information on human genes is also available from Entrez Gene (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene>), on mouse genes from the Mouse Genome Database (<http://www.informatics.jax.org/>) and on rat genes from the Rat Genome Database (<http://rgd.mcw.edu/>).

**Nomenclature of vitamins.** Most of the names for vitamins and related compounds that are accepted by the Editors are those recommended by the IUNS Committee on Nomenclature. See *Nutrition Abstracts and Reviews* (1978) 48A, 831–835.

Acceptable name	Other names*
<i>Vitamin A</i>	
Retinol	Vitamin A <sub>1</sub>
Retinaldehyde, retinal	Retinene
Retinoic acid (all-trans or 13-cis)	Vitamin A <sub>1</sub> acid
3-Dehydroretinol	Vitamin A <sub>2</sub>
<i>Vitamin D</i>	
Ergocalciferol, ercalciol	Vitamin D <sub>2</sub> calciferol
Cholecalciferol, calciol	Vitamin D <sub>3</sub>
<i>Vitamin E</i>	
$\alpha$ -, $\beta$ - and $\gamma$ -tocopherols plus tocotrienols	
<i>Vitamin K</i>	
Phylloquinone	Vitamin K <sub>1</sub>
Menaquinone-n (MK-n) <sup>†</sup>	Vitamin K <sub>2</sub>
Menadione	Vitamin K <sub>3</sub>

	menaquinone, menaphthone
Vitamin B <sub>1</sub> Thiamin	Aneurin(e), thiamine
Vitamin B <sub>2</sub> Riboflavin	Vitamin G, riboflavine, lactoflavin
Niacin Nicotinamide Nicotinic acid	Vitamin PP
Folic Acid Pteroyl(mono)glutamic acid	Folacin, vitamin B <sub>c</sub> or M
Vitamin B <sub>6</sub> Pyridoxine Pyridoxal Pyridoxamine	Pyridoxol
Vitamin B <sub>12</sub> Cyanocobalamin Hydroxocobalamin Aquocobalamin Methylcobalamin Adenosylcobalamin	Vitamin B <sub>12a</sub> or B <sub>12b</sub>
Inositol Myo-inositol	Meso-inositol
Choline Pantothenic acid Biotin	Vitamin H
Vitamin C Ascorbic acid Dehydroascorbic acid	

\*Including some names that are still in use elsewhere, but are not used by the *British Journal of Nutrition*.

†Details of the nomenclature for these and other naturally-occurring quinones should follow the Tentative Rules of the IUPAC-IUB Commission on Biochemical Nomenclature (see *European Journal of Biochemistry* (1975) 53, 15–18)

*Generic descriptors.* The terms vitamin A, vitamin C and vitamin D may still be used where appropriate, for example in phrases such as 'vitamin A deficiency', 'vitamin D activity'.

**Vitamin E.** The term vitamin E should be used as the descriptor for all tocol and tocotrienol derivatives exhibiting qualitatively the biological activity of  $\alpha$ -tocopherol. The term tocopherols should be used as the generic descriptor for all methyl tocols. Thus, the term tocopherol is not synonymous with the term vitamin E.

**Vitamin K.** The term vitamin K should be used as the generic descriptor for 2-methyl-1,4-naphthoquinone (menaphthone) and all derivatives exhibiting qualitatively the biological activity of phyloquinone (phytylmenaquinone).

**Niacin.** The term niacin should be used as the generic descriptor for pyridine 3-carboxylic acid and derivatives exhibiting qualitatively the biological activity of nicotinamide.

**Vitamin B<sub>6</sub>.** The term vitamin B<sub>6</sub> should be used as the generic descriptor for all 2-methylpyridine derivatives exhibiting qualitatively the biological activity of pyridoxine.

**Folate.** Due to the wide range of C-substituted, unsubstituted, oxidized, reduced and mono- or polyglutamyl side-chain derivatives of pteroylmonoglutamic acid that exist in nature, it is not possible to provide a complete list. Authors are encouraged to use either the generic name or the correct scientific name(s) of the derivative(s), as appropriate for each circumstance.

**Vitamin B<sub>12</sub>.** The term vitamin B<sub>12</sub> should be used as the generic descriptor for all corrinoids exhibiting qualitatively the biological activity of cyanocobalamin. The term corrinoids should be used as the generic descriptor for all compounds containing the corrin nucleus and thus chemically related to cyanocobalamin. The term corrinoid is not synonymous with the term vitamin B<sub>12</sub>.

**Vitamin C.** The terms ascorbic acid and dehydroascorbic acid will normally be taken as referring to the naturally-occurring L-forms. If the subject matter includes other optical isomers, authors are encouraged to include the L- or D- prefixes, as appropriate. The same is true for all those vitamins which can exist in both natural and alternative isomeric forms.

*Amounts of vitamins and summation.* Weight units are acceptable for the amounts of vitamins in foods and diets. For concentrations in biological tissues, SI units should be used; however, the authors may, if they wish, also include other units, such as weights or international units, in parentheses.

See *Metric Units, Conversion Factors and Nomenclature in Nutritional and Food Sciences* (1972) paras 8 and 14–20. London: The Royal Society.

**Nomenclature of fatty acids and lipids.** In the description of results obtained for the analysis of fatty acids by conventional GLC, the shorthand designation proposed by Farquhar JW, Insull W, Rosen P, Stoffel W & Ahrens EH (*Nutrition Reviews* (1959), 17, Suppl.) for individual fatty acids should be used in the text, tables and figures. Thus, 18 : 1 should be used to represent a fatty acid with eighteen carbon atoms and one double bond; if the position and configuration of the double bond is unknown. The shorthand designation should also be used in the abstract. If the positions and configurations of the double bonds are known, and these are

important to the discussion, then a fatty acid such as linoleic acid may be referred to as *cis-9,cis-12-18 : 2* (positions of double bonds related to the carboxyl carbon atom 1). However, to illustrate the metabolic relationship between different unsaturated fatty acid families, it is sometimes more helpful to number the double bonds in relation to the terminal methyl carbon atom, *n*. The preferred nomenclature is then: 18 : 3*n*-3 and 18 : 3*n*-6 for  $\alpha$ -linolenic and  $\gamma$ -linolenic acids respectively; 18 : 2*n*-6 and 20 : 4*n*-6 for linoleic and arachidonic acids respectively and 18 : 1*n*-9 for oleic acid. Positional isomers such as  $\alpha$ - and  $\gamma$ -linolenic acid should always be clearly distinguished. It is assumed that the double bonds are methylene-interrupted and are of the *cis*-configuration (see Holman RT in *Progress in the Chemistry of Fats and Other Lipids* (1966) vol. 9, part 1, p. 3. Oxford: Pergamon Press). Groups of fatty acids that have a common chain length but vary in their double bond content or double bond position should be referred to, for example, as C<sub>20</sub> fatty acids or C<sub>20</sub> PUFA. The modern nomenclature for glycerol esters should be used, i.e. triacylglycerol, diacylglycerol, monoacylglycerol *not* triglyceride, diglyceride, monoglyceride. The form of fatty acids used in diets should be clearly stated, i.e. whether ethyl esters, natural or refined fats or oils. The composition of the fatty acids in the dietary fat and tissue fats should be stated clearly, expressed as mol/100 mol or g/100 g total fatty acids.

**Nomenclature of micro-organisms.** The correct name of the organism, conforming with international rules of nomenclature, should be used: if desired, synonyms may be added in parentheses when the name is first mentioned. Names of bacteria should conform to the current Bacteriological Code and the opinions issued by the International Committee on Systematic Bacteriology. Names of algae and fungi must conform to the current International Code of Botanical Nomenclature. Names of protozoa should conform to the current international Code of Zoological Nomenclature.

**Nomenclature of plants.** For plant species where a common name is used that may not be universally intelligible, the Latin name in italics should follow the first mention of the common name. The cultivar should be given where appropriate.

**Other nomenclature, symbols and abbreviations.** Authors should consult recent issues of the *British Journal of Nutrition* for guidance. The IUPAC rules on chemical nomenclature should be followed, and the recommendations of the Nomenclature Committee of IUBMB and the IUPAC-IUBMB Joint Commission on Biochemical Nomenclature and Nomenclature Commission of IUBMB in *Biochemical Nomenclature and Related Documents* (1992), 2nd ed., London: Portland Press (<http://www.chem.qmul.ac.uk/iupac/bibliog/white.html>). The symbols and abbreviations, other than units, are essentially those listed in *British Standard 5775* (1979–1982), *Specifications for Quantities, Units and Symbols*, parts 0–13. Day should be abbreviated to d, for example 7 d, except for 'each day', '7th day' and 'day 1'.

Elements and simple chemicals (e.g. Fe and CO<sub>2</sub>) can be referred to by their chemical symbol (with the exception of arsenic and iodine, which should be written in full) or formula from the first mention in the text; the title, text and table headings, and figure legends can be taken as exceptions. Well-known abbreviations for chemical substances may be used without explanation, thus: RNA for ribonucleic acid and DNA for deoxyribonucleic acid. Other substances that are mentioned frequently (five or more times) may also be abbreviated, the abbreviation being placed in parentheses at the first mention, thus: lipoprotein lipase (LPL), after that, LPL, and an alphabetical list of abbreviations used should be included. Only accepted abbreviations may be used in the title and text headings. If an author's initials are mentioned in the text, they should be distinguished from other abbreviations by the use of stops, e.g. 'one of us (P. J. H.)...'. For UK counties the official names given in the *Concise Oxford Dictionary* (1995) should be used and for states of the USA two-letter abbreviations should be used, e.g. MA (not Mass.) and IL (not Ill.). Terms such as 'bioavailability' or 'available' may be used providing that the use of the term is adequately defined.

Spectrophotometric terms and symbols are those proposed in *IUPAC Manual of Symbols and Terminology for Physicochemical Quantities and Units* (1979) London: Butterworths. The attention of authors is particularly drawn to the following symbols: m (milli, 10<sup>-3</sup>),  $\mu$  (micro, 10<sup>-6</sup>), n (nano, 10<sup>-9</sup>) and p (pico, 10<sup>-12</sup>). Note also that ml (millilitre) should be used instead of cc,  $\mu$ m (micrometre) instead of  $\mu$  (micron) and  $\mu$ g (microgram) instead of  $\gamma$ .

Numbers. Numerals should be used with units, for example, 10 g, 7 d, 4 years (except when beginning a sentence, thus: 'Four years ago...'); otherwise, words (except when 100 or more), thus: one man, ten ewes, ninety-nine flasks, three times (but with decimal, 2.5 times), 100 patients, 120 cows, 136 samples.

**Abbreviations.** The following abbreviations are accepted without definition by the *British Journal of Nutrition*:

ADP (GDP)	adenosine (guanosine) 5'-disphosphate
AIDS	acquired immune deficiency syndrome
AMP (GMP)	adenosine (guanosine) 5'-monophosphate
ANCOVA	analysis of covariance
ANOVA	analysis of variance
apo	apolipoprotein
ATP (GTP)	adenosine (guanosine) 5'-triphosphate
AUC	area under the curve
BMI	body mass index
BMR	basal metabolic rate
bp	base pair
BSE	bovine spongiform encephalopathy
CHD	coronary heart disease
CI	confidence interval
CJD	Creutzfeldt-Jacob disease
CoA and acyl-CoA	co-enzyme A and its acyl derivatives
CV	coefficient of variation

CVD	cardiovascular disease
Df	degrees of freedom
DHA	docosahexaenoic acid
DM	dry matter
DNA	deoxyribonucleic acid
dpm	disintegrations per minute
EDTA	ethylenediaminetetra-acetic acid
ELISA	enzyme-linked immunosorbent assay
EPA	eicosapentaenoic acid
Expt	experiment (for specified experiment, e.g. Expt 1)
FAD	flavin-adenine dinucleotide
FAO	Food and Agriculture Organization (except when used as an author)
FFQ	food-frequency questionnaire
FMN	flavin mononucleotide
GC	gas chromatography
GLC	gas-liquid chromatography
GLUT	glucose transporter
GM	genetically modified
Hb	haemoglobin
HDL	high-density lipoprotein
HEPES	4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography
Ig	immunoglobulin
IHD	ischaemic heart disease
IL	interleukin
IR	infra red
kb	kilobases
$K_m$	Michaelis constant
LDL	low-density lipoprotein
MHC	major histocompatibility complex
MRI	magnetic resonance imaging
MS	mass spectrometry
MUFA	monounsaturated fatty acids
NAD <sup>+</sup> , NADH	oxidized and reduced nicotinamide-adenine dinucleotide
NADP <sup>+</sup> , NADPH	oxidized and reduced nicotinamide-adenine dinucleotide phosphate
NEFA	non-esterified fatty acids
NF- $\kappa$ B	nuclear factor kappa B
NMR	nuclear magnetic resonance
NS	not significant
NSP	non-starch polysaccharide
OR	odds ratio
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PG	prostaglandin
PPAR	peroxisome proliferator-activated receptor
PUFA	polyunsaturated fatty acids
RDA	recommended dietary allowance
RER	respiratory exchange ratio
RIA	radioimmunoassay
RMR	resting metabolic rate
RNA, mRNA etc.	ribonucleic acid, messenger RNA etc.
rpm	revolutions per minute
RT	reverse transcriptase
SCFA	short-chain fatty acids
SDS	sodium dodecyl sulphate
SED	standard error of the difference between means
SFA	saturated fatty acids
SNP	single nucleotide polymorphism
TAG	triacylglycerol
TCA	trichloroacetic acid
TLC	thin-layer chromatography
TNF	tumour necrosis factor
UN	United Nations (except when used as an author)
UNICEF	United Nations International Children's Emergency Fund

UV	ultra violet
VLDL	very-low-density lipoprotein
V <sub>O2</sub>	O <sub>2</sub> consumption
V <sub>O2max</sub>	maximum O <sub>2</sub> consumption
WHO	World Health Organization (except when used as an author)

Use of three-letter versions of amino acids in tables: Leu, His, etc.  
CTP, UTP, GTP, ITP, as we already use ATP, AMP etc.

**Disallowed words and phrases.** The following are disallowed by the *British Journal of Nutrition*:

- deuterium or tritium (use <sup>2</sup>H and <sup>3</sup>H)
- c.a. or around (use approximately or about)
- canola (use rapeseed)
- ether (use diethyl ether)
- free fatty acids (use NEFA)
- isocaloric/calorie (use isoenergetic/energy)
- quantitate (use quantify)
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**ADDENDUM F: AUTHORS GUIDELINES FOR THE SOUTH AFRICAN  
JOURNAL OF CLINICAL NUTRITION**

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Identifying information should not be published in written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) gives informed written consent for publication. Informed consent for this purpose requires that the patient be shown the manuscript to be published. ([www.icmje.org](http://www.icmje.org))

### Ethnic classification

Work that is based on or contains reference to ethnic classification must indicate the rationale for this.

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Manuscript should not exceed 4000 words in total all contents inclusive.

**Original articles** of 4 000 words or less, with up to 6 tables or illustrations, should normally report observations or research of relevance to the field of nutrition. References should preferably be limited to no more than 25.

**Short reports or scientific letters**, which include case reports, side effects of nutrient supplements/drugs and brief or negative research findings should be 1000 words or less, with 1 table or illustration and no more than 6 references.

**Editorials, Opinions, Issues in the field of nutrition** should be about 1000 words and are welcome, but unless invited, will be subjected to the SAJCN peer review process.

**Review articles** are rarely accepted unless invited.

**Letters to the editor**, if intended for the correspondence column, should be marked 'for publication', signed by all authors and presented in triple spacing. Letters should be no longer than 400 words with only one illustration or table.

**Obituaries** should not exceed 400 words and may be accompanied by a photograph.

### Manuscript preparation

- Please submit your manuscript electronically at [www.sajcn.co.za](http://www.sajcn.co.za)
- Research articles should have a structured abstract not exceeding 250 words (50 for short reports) comprising: Objectives, Design, Setting, Subjects, Outcome measures, Results and Conclusions.
- Refer to articles in recent issues for guidance on the presentation of headings and subheadings.
- Abbreviations should be spelt out when first used in the text and thereafter used consistently.
- Scientific measurements should be expressed in SI units except: blood pressure should be given in mmHg and haemoglobin values in g/dl.

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1. Figures consist of all material that cannot be set in type, such as photographs and line drawings.
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5. If any tables or illustrations submitted have been published elsewhere, written consent to republication should be obtained by the author from the copyright holder and the author(s).
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A quote will be provided on request. Consider sponsorship.

### References

References should be inserted in the text as superior numbers and should be listed at the end of the article in numerical and not in alphabetical order.

Authors are responsible for verification of references from the original sources.

References should be set out in the Vancouver style and approved abbreviations of journal titles used; consult the List of Journals in Index Medicus for these details.

Names and initials of all authors should be given unless there are more than six, in which case the first three names should be given followed by et al. First and last page numbers should be given.

**Journal references** should appear thus:

1. Price NC .Importance of asking about glaucoma.BMJ 1983; 286: 349-350.

**Book references** should be set out as follows:

1. Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975: 96-101.

2. Weinstein L, Swartz MN. Pathogenic properties of invading microorganisms. In: Sodeman WA jun, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974: 457-472.

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***Unpublished observations and personal communications*** may be cited in the text, but not in the reference list.

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5. The text adheres to the stylistic and bibliographic requirements outlined in the [Author Guidelines](#), which is found in About the Journal.
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7. The manuscript has an abstract.

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BJN-2012-019248

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Yours sincerely,

Professor Philip Calder

Editor-in-Chief

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**ADDENDUM H: CONFIRMATION EMAIL FROM THE SOUTH  
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## **[SAJCN] Submission Acknowledgement**

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