Effect of a multiple micronutrient enriched maize-based liquid meal supplement on iron status of grade 3 and 4 learners attending Sunnyside primary school, Pretoria

by

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DECLARATION

I declare that the dissertation, which I hereby submit for the degree MSc nutrition at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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DATE: February 2015

ABSTRACT

Background: About one half of school-age children in developing countries are estimated to be affected by iron deficiency. Failure to treat micronutrient deficiencies can negatively affect health and economic development.

Objective: To determine the effect of multiple micronutrient maize-based liquid meal supplement on the iron status and the nutritional status of primary school children.

Design: A randomized double-blind placebo controlled trial was conducted.

Setting and subjects: The study took place at Sunnyside Primary School in Sunnyside, Pretoria, in the urban area of Tshwane (Gauteng Province, South Africa). Grades 3 and 4 male and female learners aged 8-12 years, enrolled in the 2010 academic year were recruited for the study.

Methods: Participants were dewormed, to eliminate parasitic infestaion at the beginning of the study. The experimental product was a maize-based liquid meal supplement enriched with macronutrients and micronutrients including chelated ferrous bisglycinate, while the control had the same macronutrient profile but no added micronutrients. The learners took the meal supplement every morning on school days for 14 weeks. Iron status was measured by Haemoglobin (Hb) levels and the nutritional status was measured by anthropometric measures at baseline and end. Groups were compared with respect to change in Hb and change in anthropometry using an analysis of covariance (ANCOVA) with baseline Hb values as covariate. Testing was done at the 0.05 level of significance.

Results: There was no significant difference in the Hb levels at baseline (12.6 ± 1.1 g/dL and 12.8 ± 1.1 g/dL) (P = 0.250) between the experimental and control groups respectively. The prevalence of mild anemia (Hb< 11g/dL) was low in both the experimental and control groups. Over the 14 weeks study period, consumption of experimental products was similar and there was no significant effect on Hb levels of the participants observed (P = 0.806) in the experimental and control groups. There was also no significant change observed in the anthropometry of the participants.

Conclusion: The maize-based liquid meal supplement enriched with multiple micronutrients did not have a significant effect on the iron status of the participants in this study, possibly owing to low prevalence of anemia, a low rate of consumption and therefore iron absorption.

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ABREVIATIONS

ADA	American Dietetic Association
AGP	alpha-1 glycoprotein
AIDS	Acquired Immunodeficiency Syndrome
ALA	Eicosapentaenoic acid
ALC	Active learning capacity
ANCOVA	Analysis of Covariance
BAZ	Body Mass Index for age z-score
BMI	Body Mass Index
CI	Confidence Interval
CRP	C- reactive protein
DCYTB	duodenal cytochrome
DHA	Docosahexaenoic acid
DMT1	Divalent metal ion transporter 1
DOH	Department of Health
FAO	Food and Agriculture Organization
FBDG	Food based dietary guidelines
Fe^{2+}	Ferrous ion
Fe^{3+}	Ferric reductase
H^{+}	Hydrogen
HAZ	Height for age z- score
Hb	Haemoglobin
HCP1	Heme carrier protein 1
HIV	Human Immuno-deficiency Virus
ICSH	International Committee for Standardization
IDA	Iron deficiency anaemia
MVC	Mean corpuscular volume
n	number

Na^+	Sodium
NaFe EDTA	Sodium Ferredetate ethylenediamine tetracetic acid
NFCS	National Food Consumption Survey
NSNP	National Schools Nutrition Program
PSNP	Primary School Nutrition Program
RDA	Recommended dietary allowance
RDI	Recommended dietary intake
SANHNES	South African National Health and Nutrition Examination Survey
sd	standard deviation
sTfR	soluble transferrin receptor
UNICEF	United Nations Children's Fund
UNU	United Nations University
WMD	Weight mean difference
WHO	World Health Organization
WHZ	Weight for height z- score
ZnPP	Zinc protoporphyrin

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

Micronutrient malnutrition is considered as a public health problem affecting more than 2 billion people worldwide.[1] In developing countries the magnitude is much greater because malnutrition, infection and poverty are most common, and often interlinked.[1,2] Failure to treat micronutrient deficiencies can negatively affect health and economic development.[1] Iron, vitamin A and iodine deficiencies are the major micronutrient deficiencies affecting children including school children in developing countries. In addition deficiencies of vitamin C, zinc and B vitamins often occur concurrently with the 3 major micronutrient deficiencies. About one half of school age children in developing countries are estimated to be affected by iron deficiency. [3]

The school age years are therefore an opportune time for addressing iron deficiency because of the following reasons: iron deficiency impairs fitness and work capacity thus interventions to improve iron status may enhance fitness and work capacity of children.[4] Improving iron status may enhance learning potential of children.[5] Improving iron status of girls may help prevent anemia in their reproductive years. Most importantly, the school offers an ideal distribution system for several types of public health interventions.[6] Micronutrient deficiencies are also a risk factor for frequent and severe infections. These infections in turn may have adverse effects on nutritional status. [7]

School children are significantly disadvantaged in terms of nutrition interventions and/or programs and in urgent need of additional attention, if they are to reach their full developmental potential.[8] The full genetic potential of the child for physical growth and mental development may be compromised due to deficiency (even subclinical) of micronutrients. Children and adolescents with poor nutritional status are exposed to alterations of physical, mental and behavioral functions that can be corrected to certain extend by dietary measures.[9]

Therefore, in trying to alleviate micronutrient malnutrition, the South African Government designed a 3-way food-based approach which includes mandatory food fortification. The other

two approaches include a micronutrient supplementation program for women and children, and an educational program to promote better dietary habits, including breast-feeding initiatives, school feeding program and campaigns to encourage people to grow their own vegetables and fruits to improve household food security as well as increasing intakes of micronutrient-rich foods. The approaches are known as the Integrated Nutrition Program (INP).[10]

School feeding has the potential to contribute toward alleviating both short-term hunger and hidden hunger (micronutrient deficiencies) for school children to reach their full mental and physical potential and perform optimally in school.[8] Therefore, to ensure good nutritional status and improvement of the general health as well as learning capacity, a comprehensive Primary School Nutrition Program (PSNP) was introduced twenty years back in South Africa. In its first ten years of implementation PSNP was coordinated by the Department of Health, in 2004 it was relocated to the Department of Education. The decision was based on consideration that school feeding had important educational outcomes which are the functional responsibility of the Department of Education.[11]It was then renamed the National Schools Nutrition Program (NSNP). The primary aim of the program was to improve the educational experience of the disadvantaged primary school learners through promoting punctuality, alleviating short term hunger, improving concentration and contributing to general health development.[12]

In 1996, an evaluation on the PSNP showed a high prevalence of malnutrition especially amongst the black and colored primary school children, and reports have shown poor and inconsistent coverage of the program in several parts of the country. Numerous challenges were encountered in the program, such as inappropriate feeding times and food of a sub-standard quality and quantity.[13] Furthermore, several schools were found to have poor infrastructure to be able to adequately support the implementation of NSNP effectively. Lack of proper kitchen infrastructure, cooking equipment and storage facilities such as refrigerators for storing perishables, have been a drawback in the preparation of school meals. As a result, the complete advantage of school feeding has not been realized. Therefore, micronutrient

deficiencies (including iron deficiency) are still highly prevalent in South African school children despite the existence of a national school-feeding program. School-feeding program often focus on relieving short-term hunger, and do not always concentrate on alleviating or preventing hidden hunger.[8]

To alleviate short term and hidden hunger, deworming, nutrition education and micronutrient supplementation are recognized as more cost-effective interventions. However, there has been a lack of systematic implementation of these interventions as part of the NSNP.[13] Controversy surrounding the use of supplementation products as part of NSNP has been another issue. The concern has been that: use of commercial supplements may defeat the aim of nutrition education and may not be in line with local eating habits in addition, enriched commercial foods tend to be more expensive and do not contribute to "community involvement". Unfortunately, the chance of local food based meal to provide the same micronutrient contribution as a meal that includes a fortified product is very slim, unless it contains fortified ingredients. In addition, food sources of iron are relatively expensive. Alternative sources of iron which are cheaper include spinach and legumes, but unfortunately their iron content is smaller and less bioavailable, presenting yet another challenge.[13]

This study therefore sought to provide solid scientific evidence describing the magnitude of impact to be expected from enriched maize-based meal supplements on iron status in primary school children. The results from this project are meant to assist in making informed choices about the importance and potential impact of multiple micronutrient interventions in primary school children. The study sought to provide input for the Integrated Nutrition Program of South Africa, especially the NSNP on the use of maize-based supplementation in school feeding schemes to reach the most vulnerable groups. The study investigated the effect of a ready to use multiple micronutrient enriched maize-based liquid meal supplement on iron status of primary school children aged 8 to 12 years.

1.2 RESEARCH HYPOTHESIS

1. Consumption of a multiple micronutrient enriched maize-based liquid meal supplement 5 days a week for 14 weeks, will improve iron status as measured by hemoglobin, in primary school children (8 – 12 years old). Iron therapy is expected to increase Hb values.[14] The supplement contains Ferrous bisglycinate chelate which has a higher bioavailability (3.4 -4 times higher) than ferrous sulpphate.[15]

2. Consumption of a multiple micronutrient enriched maize-based liquid meal supplement, will improve nutritional status. Multiple micronutrient interventions containing iron improve nutritional status compared to placebo or single nutrient interventions.[16]

1.3 OBJECTIVES

1. To determine the effect of multiple micronutrient enriched maize - based liquid meal supplement on the iron status of primary school children using hemoglobin as a biomarker.

2. To determine the effect of multiple micronutrient enriched, maize-based liquid meal supplement on the nutritional status of primary school children using anthropometry.

1.4 CONCEPTUALIZATION

The conceptual framework used in this study shows that nutrition status results from several interrelated causes. Anemia, which is one indication of poor nutrition status, is an outcome of poor diet, increased iron demand, infection and sometimes inherited conditions. Anemia can be assessed by measuring hemoglobin concentration, and can be classified as mild, moderate or severe. To correct anemia, a multiple micronutrient dietary supplement which contains chelated iron can be useful, as this type of iron is more bioavailable, and the presence of the other nutrients can work synergistically with the iron to correct anemia. If anemia is not corrected it may result to poor health, low activity, defects in growth, affect cognition, in severe cases it may lead to death (Figure 1.1)

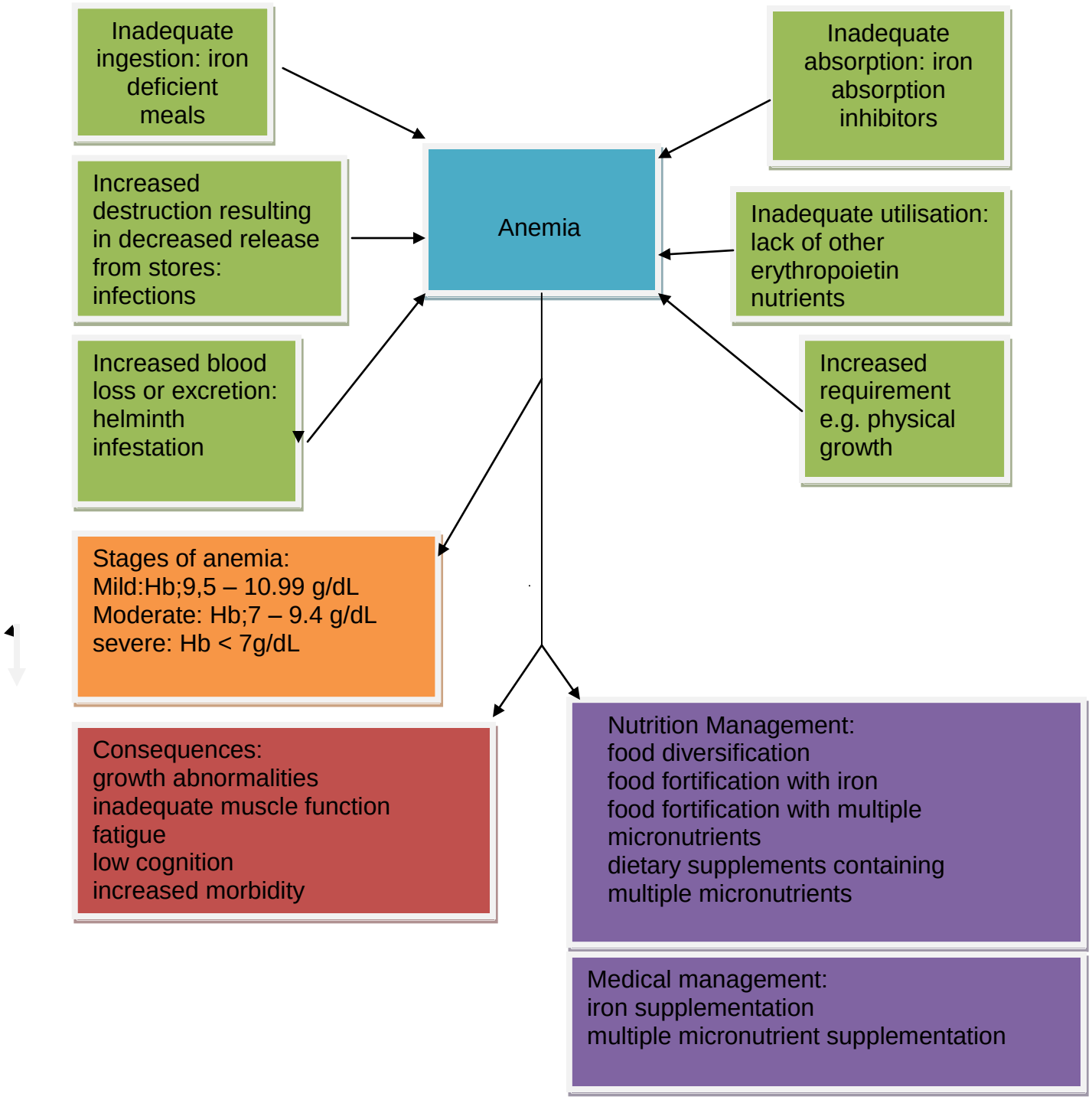

Figure 1. Conceptual framework[17]

Table 1.1: Conceptual definitions and operationalization

Terminology	Conceptual definition	Operationalization
Anemia	Condition indicating a deficiency of the size or number of red blood cells or the amount of haemoglobin they contain.[17]	Indicated by decrease in the quantity of hemoglobin i.e. < 7g/dL indicate severe anemia, 7 – 9.4 g/dL indicate moderate anemia and 9.5 – 10.99 g/dL indicate mild anemia[18]
Grade 3 and 4 learners	Primary school pupils in Grade 3 and 4 classes.	Sunnyside Primary school boys and girls (8 -12years old)
Multiple micronutrient enriched maize-based meal supplement	A 250ml ready to drink, made from maize meal and enriched with multiple nutrients including chelated iron.	Experimental product
Chelated iron	Two molecules of amino acid bound with a covalent bond to an iron molecule.[19]	Ferrous bisglycinate chelate (Ferrochel®)
Hb: Hemoglobin	A conjugated protein containing four heme groups and globin; it is the oxygen carrying pigment of erythrocytes. [17]	Cut off points indicate iron status Biomarker for Iron status
Iron status	Can range from overload to deficiency and anemia	Iron status has a variety of indicators. Haemoglobin was used in this study.
Mild anemia	Low Hb, but not severe.	Hb: (9.5g/dL to ≤10.99)[18]

1.5 STRUCTURE OF THIS DISSERTATION

A chapter format has been used in presentation of this dissertation.

Chapter 1 is an introductory chapter, followed by Chapter 2, which is a review of the literature. This review covers the nutrition status of school children including their iron status, the role of iron in growth and development, iron metabolism, the epidemiology of iron deficiency anemia. The etiology of iron deficiency anemia (IDA) and the involvement of other micronutrients in the cause of IDA are also dealt with in this review. Also forming part of the review are the consequences of IDA, methods of diagnosis as well as the strategies for fighting anemia. A Review on randomized controlled trial studies, on dietary supplements involving primary school children are also laid out in Chapter 2.

Chapter 3 shows Methodology including: ethical approval of the study, description of the study design, the recruitment of the subjects, inclusion and exclusion criteria, screening, randomisation, blinding, anthropometric and hemoglobin assessment methods as well as the data collection, capturing and analysis methods used in this study.

Presentation of the results and its discussion is in Chapter 4. In this chapter the description (demographic information) of the participants is presented. Baseline and end assessment data are reported in this chapter. In the discussion the results are compared to available literature and possible interpretation for results is given. Limitations of this study are reviewed in this chapter.

Chapter 5 gives a conclusion based on all assessments. Recommendations for future research are given in this chapter.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

School age children suffer from multiple micronutrient deficiencies like most people in developing countries.[20] It is estimated that 13 – 27% pre – school children have two or more micronutrient deficiencies, indicating that 100millions of these children are affected.[21] This indicates that if these children's condition is not corrected they will move on to primary school with the same or even worse nutritional status. Reports of impairment in growth, immune function and cognitive performance have been made, concerning school age children who are deficient in iron, zinc, vitamin A and iodine.[22] Reduction in both productivity and cognitive performance in adult hood can occur due to the health consequences of micronutrient deficiencies. Therefore, reducing the prevalence of micronutrient deficiencies is of importance to several policy makers in developing countries.[23]

Malnutrition has a negative impact on morbidity, mortality, educability and productivity. In South Africa, the nutritional status of the population has not improved over the last fourteen years except for the folate and iodine status. The prevalence of micronutrient deficiencies (i.e. vitamin A and iron) has increased the double burden of disease in the population.[24] Micronutrient deficiencies usually occur concurrently, they tend to interact and coexist. For example, iron deficiency and vitamin A deficiency usually occur concurrently in the same group of people. Thus, providing vitamin A supplements for example, can have a positive outcome on vitamin A status and can improve iron metabolism in affected groups.[20]

2.1.1 Focus of literature review

The literature review focuses on the role of iron in growth and development, iron metabolism and homeostasis, etiology of iron deficiency anaemia, diagnosis of iron deficiency anaemia, epidemiology of iron deficiency and anaemia, iron status and strategies to address iron deficiency and anaemia.

2.2 THE ROLE OF IRON IN GROWTH AND DEVELOPMENT

Iron is a component of every living cell primarily involved in transport and storage of oxygen,

oxidative metabolism and several physiological processes. It is necessary for cellular growth and functioning.[25] Together with other micronutrients, iron is necessary for promotion of physical growth, sexual maturity and neuromotor development. A number of vitamins and trace minerals including iron play an important role in boosting both cell-mediated and humoral immune body defenses. Production of various enzymes, hormones and biochemical mediators for controlling biological processes and energy production, are shared function of iron with vitamins and other trace minerals.[26] Iron plays a very crucial role in the functioning of the neurotransmission system through production of dopamine and serotonin.[26] The content of iron in the brain is lowest at birth and increases with age, and reaches adult concentration after puberty.[27] Iron requirements are most likely to exceed intake at 6 – 8months after birth and during adolescence (for girls).[25]

2.2.1 Iron metabolism and homeostasis

Iron is a main component of hemoglobin (Hb), needed for basic cellular function in all human tissues, especially the muscles, brain and blood cells.[28] Human beings cannot actively excrete iron, therefore iron concentration is controlled in the proximal small intestine, at the site of iron absorption (Figure 2).[29] The haem and non haem iron from the diet have specific transporters. Iron deficiency and hypoxia up regulate heme carrier protein1 (HCP1), a putative haem transporter.[30,31] The divalent metal ion transporter 1 (DMT1), mediates the transport of non haem iron from the intestinal lumen to the enterocytes.[32] DMT1 can only transport ferrous iron and yet most of the iron enters the duodenum in a ferric form. It is therefore necessary that it must first be reduced to ferrous iron, ferric reductase, duodenal cytochrome b (DCYTB),[33] or possibly by other reducing agents, such as vitamin C.

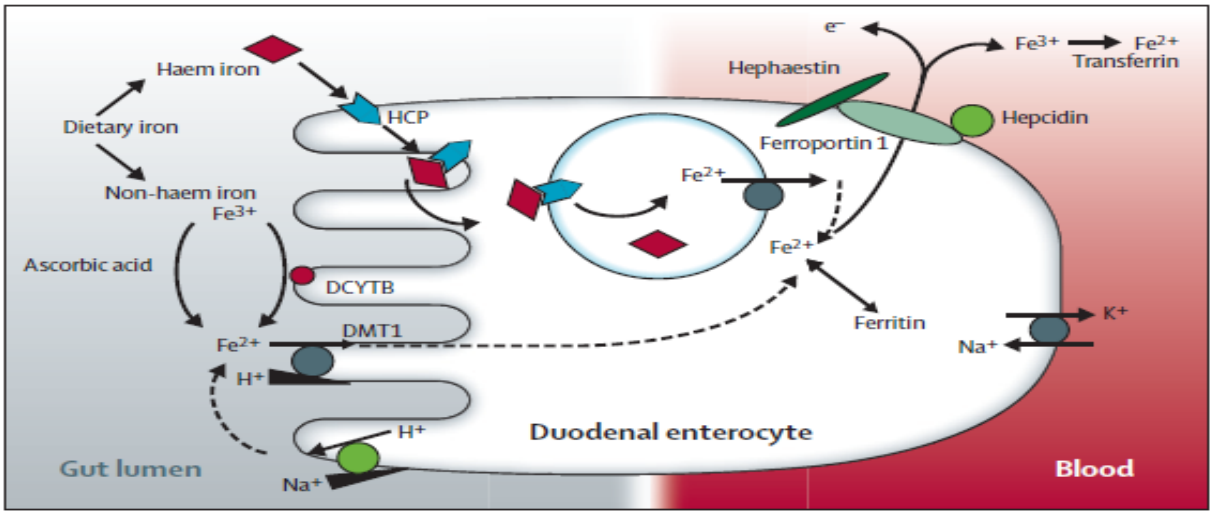

Figure 2. Regulation of intestinal iron uptake[29]

HCP: Heme carrier protein; DCYTB: duodenal cytochrome b; DMT1: The divalent metal ion transporter 1.

Fe^{2+}: ferrous iron: Fe^{3+}: ferric reductase: H+: Hydrogen: Na+: Sodium

Iron that is not transferred to the circulation is stored as ferritin and, is finally lost when the cell sloughs off at the villus tip. Movement of iron across the basolateral membrane is controlled by ferroportin 1 and the iron oxidase, hephaestin. The transport protein ferroportin also mediates iron movement form other cells, including macrophages. Hypoxia and iron deficiency encourages DMT1, DCYTB and ferroportin stimulation thus increasing iron uptake, iron deficiency increases iron uptake.[33]

Hepcidin, a hormone produced by the liver inhibits both absorption and release of iron from macrophages and other cell types. Therefore, during iron deficiency hepcidin secretion from the liver is decreased to enable maximum iron absorption.[34,35] In the erythroid iron cycle, old red cells are broken down in the spleen by macrophages, secreted iron returns to the circulation and binds to transferrin receptors (TfRs) in the bone marrow on erythroid precursors, and completion of the cycle occurs on entrance of the erythrocytes into the circulation in the 7-10 days that follows. Iron deficiency encourages maximum iron transfer through the cycle by increasing expression of ferroportin on macrophages hepatic synthesis,[29]

and TfR1 expression in the bone marrow and other tissues.[36] Although the body's homeostatic mechanisms are efficient in iron conservation, deficiency of iron can still occur, particularly when physiologic needs exceed intake or in the case of depletion of iron stores.[14]

2.2.2 Etiology of anemia

A number of factors may contribute to the development of iron deficiency; low intake and poor absorption of iron from the diet due to the presence of iron absorption inhibitors such as polyphenols and phytates, or lack of absorption enhancers such as poor ascorbic acid and meat intake. High physiological demands of iron during menstruation, pregnancy, and growth may also contribute to iron deficiency.[37,38] Other risk factors include heavy menstrual blood loss, parasitic infection, acute infection, other micronutrient deficiencies, haemoglobinopathies,[50] Human Immune Deficiency Virus (HIV), and other chronic diseases.[40,41]

2.2.2.1 Iron content in the diet

There are two forms of iron that can be taken up from the diet, haem and non haem iron. Haem iron is usually obtained from the hemoglobin and myoglobin in animal foods, whereas, non haem iron comes from cereals, pulses, fruits, and vegetables.[42] A majority of diets in developing countries comprise mainly of cereals and pulses, which means that the form of iron such a population is likely to get from its diet is non haem iron. Non haem iron, however, has a low bioavailability.[43]

2.2.2.2 Bioavailability of iron

Iron bioavailability is defined as the amount of ingested iron which is absorbed and used for metabolic functions.[43] Meals can be categorized into three broad categories in terms of their iron bioavailability; low, intermediate and high bioavailability.[42] The low bioavailability diet consist of cereals, roots and/tubers and a negligible amount of meat, fish or ascorbic acid. Such meals have absorption of approximately 5%. Intermediate bioavailability diets have absorption of approximately 10% and usually consist of cereals, roots and/tubers and negligible food of animal origin and/vitamin C. The high bioavailability diet is usually composed of generous quantities of meat, poultry fish, and/foods containing high quantities of ascorbic acid. The iron absorption of such meals is approximately 15%. The regulation of iron absorption is usually

regulated by the iron status; people with normal or high iron stores have a low iron absorption.[46] Sustained negative iron balance can lead to anemia.[44] Excessive iron absorption can have negative effects on the body; it can cause diseases such as hepatic cirrhosis and diabetes mellitus.[47]

2.2.2.3 Parasitic infestation

Approximately 35% (320 million) of school age children have round worm infestation; 25% (233 million) are infested with whipworm, and 26% (239 million) have hookworm infestation.[48,49,50] Intestinal worms alone account for 11 % and 12% of the total disease burden in 4 -14 years old boys and girls (from low income countries) respectively.[51] It is possible for children to be concurrently affected by a number of parasitic species.[52] Worm infestation may build up over time and may cause chronic and long lasting health problems. Worms can contribute to malnutrition through causing lack of appetite, malabsorption and anemia may occur due to blood loss.[52] Parasitic infestation is the most significant infection causing blood loss at the site of feeding, it also exacerbates bleeding by the secretion of anticoagulants and interferes with iron uptake in the duodenum as it impairs appetite (with moderate to heavy infection).[41] A relationship between infection intensity and hemoglobin levels has been shown in several studies and increase in worm infestation results in decreased hemoglobin levels.[53,54,55]

A study done in East Africa showed that the degree of iron deficiency anemia due to hook worm infestation was dependant on the intensity and duration of the infection, the iron stores of the host as well as the species of hookworm. Therefore, *Ancylostoma duodenale* was more significant in the prevalence of IDA compared to *Necator Americanus.*[56]

Malaria is another parasitic infection which occurs mostly in tropical and sub tropical countries. Malaria can contribute to iron deficiency and anemia.[24] In a cross sectional study from Nigeria, school children were evaluated to determine the effect of low level Plasmodial infection. The results indicated that even low level plasmodial infection contributes to anemia.[57]

2.2.2.4 Other micronutrients in the etiology of anemia

Populations in developing countries are usually affected by concurrent micronutrient

deficiencies.[29] African school children are vulnerable to coexisting deficiencies of vitamin A and iron.[58,59] A survey carried out in Sri Lanka adolescents reported that 54% males and 55% females had folate and zinc deficiencies. Moreover, 30% males and 48% females were iron deficient. The odds ratio of having at least two deficiencies at a time among the iron deficient children were; 1.6 (95%CI: 0.6 - 4.2) in boys and 0.8 (95%CI: 0.5 - 1.5) in girls. One micronutrient deficiency could negatively affect the absorption, metabolism and/excretion of another micronutrient, hence the coexistence. For example, iodine deficiency goiter may be aggravated by iron deficiency anemia.[59]

Other micronutrient deficiencies such as riboflavin, folate, vitamin C, A, and B12 may affect hemoglobin synthesis either by weakening erythropoiesis or indirectly by affecting uptake or mobilization of iron. Nutrient deficiencies also weaken immune response against helminthic infections.[60]

Vitamin A

Vitamin A deficiency and anemia have long been recognized to be linked. Positive correlation between serum retinol and hemoglobin concentrations have been reported in surveys carried out in developing countries. Populations with low vitamin A showed a stronger association between serum retinol and hemoglobin concentrations.[61,62]

Vitamin A status has an effect on mobilization of iron stores.[29] In a study where children were given soup fortified with iron and vitamin C, an increase in serum iron levels and transferrin were reported when serum retinol levels were > 40ug/dL than when they were < 20ug/ dL.[63] Hemoglobin increases in response to improved vitamin A status in pre-school and primary school children.[63,64]

Anemic school children in Tanzania were given on daily basis a placebo, vitamin A (1.5 mg RE), iron (40mg), or iron plus vitamin A. An increase in hemoglobin was reported in the following fashion; 3.6g/L, 13g/L, 17.5g/L, and 22.1g/L in the placebo; vitamin A (1.5 mg RE); iron (40mg), and the iron plus vitamin A groups respectively.[64] Anemic and vitamin A deficient pregnant

women in Indonesia received a placebo; vitamin A (2.4 mg RE/d), iron (60mg/d) or iron plus vitamin A 60 mg iron/d, 2.4 mg RE/d.[65] Biochemical measures after 8 weeks showed that 16%, 35%, 68% and 97% of pregnant women respectively were no longer anemic. The suggestion is that dual fortification or supplementation with iron and vitamin A is more effective in controlling iron or vitamin A deficiency, compared to individual micronutrient fortification or supplementation.[29]

Vitamin A status affects anemia in many ways[66]as follows: vitamin A deficiency results in decreased resistance to infection, therefore, it gives rise to anemia of infection. Vitamin A affects iron absorption and/or metabolism, and it is directly involved in the modulation of erythropoiesis. Given the high incidence of infectious diseases in developing countries, vitamin A deficiency may aggravate infection, thereby perpetuating anemia of infection.[67]

Riboflavin

Low intakes of meat and dairy products increase the risk of riboflavin deficiency. In both developing and developed countries, school children are highly vulnerable to riboflavin deficiency[68] which may affect erythropoiesis thus contributing to the aetiology of anemia (Hb < 11g/dL).[69,70] The mechanism by which this impairment occurs is through reduced mobility of stored iron,[71,72] diminished iron absorption and increased iron losses.[73,74]

A more effective way of improving iron status in adult males and children is to give riboflavin and iron supplements together.[75,76] Three trials carried out with children as well as pregnant women compared the efficacy of iron supplementation given alone to iron supplementation together with riboflavin. It was found that dual supplementation enhanced hemoglobin production. However, the folic acid given with iron may have compromised the accuracy of the results obtained with the former.[77,78] In another trial, riboflavin and iron supplementation produced no better results than iron supplementation alone.[79] Likewise, a trial with Croatian children showed no added benefit of riboflavin supplementation in school children with adequate hemoglobin levels.[80] The results of these studies suggest that the effect of riboflavin on hemoglobin status varies and can be affected by a number of factors.[29]

Zinc

Factors that affect iron bioavailability, such as low meat, high phytate and polyphenol intake, are the same as those affecting zinc absorption.[81] Although the data do not suggest that zinc deficiency plays a role in anemia, iron and zinc deficiencies often coexist and supplements containing these elements may therefore prove helpful in vulnerable populations.[29] However, numerous studies have reported reduced iron efficacy when zinc and iron are taken concurrently, possibly due to impairment of iron absorption. An increased intake of non-heme lowers the biovailability of zinc.[82,83] On the contrary, a high dietary zinc to iron ratio can inhibit iron absorption.[104,105] This effect was demonstrated in a study where adults received micronutrients in water solution,[104,05]but not when micronutrients were added as dietary supplements for infant formulations or maize meal preparations.[83,85,86] The exact nature of the mechanism involved in this instance is not clear, but it is likely to be due to competition for uptake in the eneterocyte. Both iron and zinc ions depend on DMT1 for transportation.[32] It is therefore possible that high zinc concentrations lower iron uptake by the intestines even though this effect has not been demonstrated in mammalian systems.[29]

In a randomised controlled supplementation trial in Vietnam, infants were given a daily dosage respectively alternated as (placebo, 10 mg iron, 10mg zinc or 10mg iron plus 10mg zinc). It was reported that the zinc and iron supplements were as effective as the iron supplementation alone in combating iron deficiency and anemia.[87] Similar effects were reported in a study carried out in Mexican children.[88,89] Contrasting results were reported in an Indonesian study conducted with children who received a similar dose for the same amount of time as the Vietnamese children.[87] In the Indonesian study, iron supplementation alone had a better effect on iron status than combined iron and zinc supplementation, which suggests that the efficacy of iron absorption is reduced when zinc and iron supplementation coincide.[90] The difference in results of these two studies is attributable in principle to the baseline iron status of the Vietnamese children,[85] who had severe anemia compared to their Indonesian counterparts.[90]

According to a review by Fischer Walker *et al.* 2005,[91] iron status is not affected when zinc supplements are given alone. The same review also suggests, however, that iron status is not

improved beyond the effect of iron supplementation on its own when zinc and iron supplements are given concurrently. More studies are therefore needed to investigate the interaction between the two nutrients.

Folate and vitamin B12

Macrocytic anemia, a type of anemia where the red cells were found to be larger than normal was discovered by the end of the nineteenth century. Poor intake of folate from the diet and insufficient folate absorption and utilisation, contributes to suppression of bone marrow proliferation as part of macrocytic anemia.[92] Vitamin B_{12} deficiency can also contribute to macrocytic anemia. This anemia is characterised by abnormal red cell precursors in the bone marrow called megaloblasts. Iron deficiency anemia can occur concurrently with folate and vitamin B_{12} deficiency anemia, which results in normocytic anemia. As a result, it may be difficult to diagnose iron deficiency anemia.[93]

2.2.3 Diagnosis of iron deficiency and anemia

Iron deficiency can be diagnosed by using a number of indicators such as clinical indicators where chronic fatigue is usually important. However, clinical indicators are usually not specific symptoms.[14] Dietary evaluation can also be done to assess how much haem and non haem iron is taken in the diet. The dietary method might also be helpful but better diagnosis relies on biochemical indicators, particularly for the early stages of deficiency.[94]

The three stages of iron deficiency are characterised respectively by depletion of iron stores, followed by iron deficiency eryhropoiesis and iron deficiency, in that order. The first one involves the depletion of iron stores, the second one is iron deficiency erythropoiesis, and the third one is iron deficiency anemia. All these stages can be analysed biochemically (Table 2.1).[94] Iron deficiency (usually defined as ferritin level < 12 ug/L) is the most prevalent nutritional deficiency.[15] Iron deficiency anemia occurs when there is severe iron deficiency that causes reduced erythropoiesis, thus reducing the red blood count, which leads to anemia (Hb level < 11.5g/dL).[95]

It has been agreed that iron status is best determined with the aid of measurements of hemoglobin, ferritin, soluble transferrin receptor (sTfR), as well as chronic infections serving as

indices. However, this procedure is usually expensive and difficult.[94] Hemoglobin therefore has been successfully used in situations where there were financial constraints and field work in remote areas.[96]

Table 2.1: Influence of iron status on various indicators in absence of other diseases[94]

	Hb	**Ferritin (ug/L)**	**STfR**
Iron overload	Above cut off	> 300	Low
Normal	Above cut off	100+/-60	Normal
Depleted iron status	Above cut off	<20	Normal
Iron deficient erythropoiesis	Above cut off	<12	High
Iron deficiency anemia	Below cut off	<10	High

2.2.3.1 Iron status indicators

A number of factors can affect an individual's iron status, including limited food choice due to poverty, micronutrient deficiencies, or interaction between nutrients and helminth infestation.[131] Parasitic infestation can affect iron status due to loss of blood, reduced appetite and lowered rate of absorption.[132]

A national food consumption survey conducted in 1999 showed that for South African children as a whole, the intake of calcium, iron, zinc, selenium, vitamin A, D, C and E, riboflavin, niacin, vitamin B6 and folic acid were below two-thirds of the Recommended Dietary Allowance. [109]Children living in urban areas, however, had a significantly higher iron intake ($p < 0.05 – 0.001$) than those living in rural areas.[109] A more recent food consumption survey found that the prevalence of poor iron and vitamin A status in children in the country appears to have increased compared with previous national data. In addition, 45.3% of children nationally were found to have an inadequate zinc status and to be at risk of zinc deficiency.[133]

A study by Keskin *et al.* (2005) showed that the prevalence of iron deficiency was relatively high among school boys of low socio-economic status (SES). Higher tea intake and lower intake of citrus fruits, red meat and fish among the low SES group, were cited as the major reason for

the results obtained in the study.[134]Biochemical indicators that can be used as indicators for iron status, include hemoglobin, ferritin and sTfR. Other parameters include, hematocrIt, iron saturation of plasma transferrin, and zinc protoporphyrin (ZnPP).[94]

Hemoglobin (Hb)

Anemia can be diagnosed by administering Hb tests. This is an inexpensive and common measurement. However, hemoglobin concentration, can be affected by a variety of conditions and diseases, and in any case only becomes noticeable in the third stage of iron deficiency. It may therefore be necessary to use very specific and sensitive indices to determine whether iron deficiency is the specific cause of anemia.[94] However, hemoglobin measurement alone can be used to assess prevalence and etiology of anemia when it is not feasible to use multiple biochemical tests for iron status due to cost or other operational limitations.[96] Anemia is graded variously as mild, moderate or severe anemia (Table 2.2).[97]

Table 2.2: Stages of anemia and values used in demographic and health surveys [97]

	Anemia measured by hemoglobin (g/dL)			
	Anemia	**Mild**	**Moderate**	**Severe**
Children 6-59 months	<11.0	10-10.9	7.0-9.9	<7.0
Children 5-11 years	<11.5	10-11.4	7.0-9.9	<7.0
Children 12-14 years	<12.0	10-11.9	7.0-9.9	<7.0
Non-pregnant women above 15 years	<12.0	10-11.9	7.0-9.9	<7.0
Men (above 15 years)	<13.0	12-12.9	9.0-11.9	< 9.0

Note: Hemoglobin values change with altitude.

Hemoglobin levels depend on factors such as age, sex, biological variation, race, pregnancy, altitude (Table2.3), iron deficiency anemia, other micronutrient deficiencies, parasitic infection, certain disease state as well as cigarette smoking.[18] Table 2.4 illustrates the adjustments that need to be made to Hb cutoffs for altitude and ethnicity.[18]

Table 2.3: Cut-off values for anemia at sea level and above sea level using hemoglobin concentration [98,99]

Target	Age	Hb at sea level (g/dL)	Hb above sea level > 1.500m (g/dL)	Hb above sea level > 2.700m (g/dL)
Infants [a]	6 -11 months	< 11.0	< 12.0	< 13.0
Children	1 – 4 years	< 11.0	< 12.0	< 13.0
School age	5 – 11 year	< 11.5	< 12.5	< 13.5
School age	12 – 13 years	< 12.0	< 13.0	< 14.0
Pregnant women		< 11.0	< 13.0	< 14.0
Non pregnant women		< 12.0	< 12.0	< 13.0
Men		< 13.0	< 14.0	<1 5.0

Iron deficiency is rare among infants of an age below six months, unless the birth weight is low. Hemoglobin is best determined using venous blood anticoagulated with EDTA. Blood from the heel, ear or finger pricks collected in heparinised capillary tubes can be used as an alternative.[100] A cyanmethemoglobin method is most reliable, provided the blood specimens are correctly diluted. This method is also recommended by the International Committee for Standardization in Hematology (ICSH).[101]

Table 2.4*:* Adjustments to hemoglobin cutoffs and individual values for altitude and ethnicity[18]

	Adjustment to hemoglobin cut-off value (g/dL)
Altitude (m) $\geq$ 1250, < 1750	+0.5
Ethnicity: African extraction	-1.0

The method involves converting all the encountered form of hemoglobin into cyanmethemoglobin, which is then analysed with a spectrophotometer.[101] Hemoglobin levels can also be determined from field-collected blood spots.[102]Alternatively a portable hemoglobin photometer can be used in remote field settings. The HemoCue is a battery-operated device that uses a dry reagent (sodium azide) in a microcuvette for direct blood collection and measurement. The accuracy and precision of hemoglobin values based on the HemoCue are

comparable to those obtained by following standardised cyanmethemoglobin-based procedures and methods.[103]

Ferritin

Ferritin is currently the most useful indicator of iron status. It is the most sensitive parameter in detecting the first stage of iron deficiency. Plasma content correlates well with iron stores, hence a lowered, ferritin concentration may indicate depletion of iron stores. However, ferritin can also be increased by other factors such as infection and inflammation, which means high ferritin level may not always be an indication that the iron status is within acceptable limits. To minimize this problem, therefore, chronic and acute infection parameters must also be measured to determine whether a raised ferritin level is attributable to infection.[94] C-reactive protein (CRP) is currently used to detect the presence of acute infection, while alpha-1 glycoprotein (AGP) is used for chronic infections. A ferritin value below 10ug/L shows definite iron deficiency despite unclear cutoff values. Another indicator such as sTfR may be used as it is not likely to be influenced by infection.[94]

Soluble transferrin receptor

Iron status can be reliably determined with the aid of sTfR where infection is a factor. Iron requirement has an effect on the release of sTfR from the cells into the blood stream. In the second stage of iron deficiency sTfR concentration is increased if the Hb concentration remains above cutoff level after the iron stores are exhausted. Therefore, sTfR is less sensitive than ferritin but more sensitive than Hb.[94] Bone marrow staining is by far the gold standard in defining iron deficiency.[94]

Other iron status indicators

(i) HematocrIt: This parameter usually correlates with hemoglobin, but is relatively insensitive compared to Hb. It is therefore, not a good diagnostic nutritional anemia indicator[94]

(ii) Iron saturation of plasma transferrin (ratio of plasma iron to total iron binding capacity) and mean corpuscular volume (MCV): These two indicators are well established and inexpensive to measure when hematology analysers are available. Iron deficiency is marked by low saturation of transferrin with iron and decreased size of erythrocytes. Specificity of these indicators is low, due to the large number of clinical disorders that may affect transferrin saturation.[104] Plasma

has a diurnal variation and MVC can therefore only indicate the late stage of iron deficiency. It may be difficult to take these measurements accurately without analysers, as measurements may be difficult and likely to have errors.[96] Ferritin or sTfR are important alternatives in such situations.[94]

(iii) Zinc protoporphyrin (ZnPP)

Iron in protoporphyrin is replaced by zinc in cases of iron deficiency and can be measured with the aid of haematoflourometry,[96]at the second stage of iron deficiency before Hb levels decline below cutoff, thus making ZnPP a more sensitive indicator than Hb. However, note that ZnPP can be influenced (increased) by lead levels.[104]

2.2.4 Consequences of anemia

As noted, the final stage of iron deficiency is iron deficiency anemia, which is characterised by low hemoglobin levels[105]and has been reliably found to retard physical development, undermine the immune function, inhibit growth and advance onset of fatigue. Cognitive function and school achievement can also be affected by iron deficiency anemia.[106]According to the World Health Organization (WHO), 8000 000 deaths each year are attributable to iron deficiency anemia. With regard to loss of healthy life, iron deficiency anemia accounts for 25 million disability-adjusted life years.[107]

2.2.4.1 Effect of on anthropometry

In reality, the double burden of disease has become more severe with the increased prevalence of micronutrient deficiencies (vitamin A and iron) together with high levels of overweight and obesity.[24] Adding micronutrients to children's supplementary feeds and fortification of food have frequently proved to alleviate micronutrient deficiencies and thus helpful in improving the population's well being.[109] A study conducted in India showed an increase in the mean height-for-age z-score (HAZ) among school children after they had taken a multiple micronutrient fortified drink for 14 months.[110]

According to a review of studies including infants, pre-school and school children, there is a positive correlation between iron supplementation and linear growth of anemic children.[111] In a

study by Chwang *et al* (1988),[112]an increase in height, weight, and arm circumference (compared to a control group) was observed in anemic school children who were given iron supplements for 12weeks. In a study on anthelmintic treatment and iron fortification conducted with iron deficient primary school children in South Africa, the height-for-age and weight-for-height z-scores of the subjects was found to have improved significantly.[113] Conversely, however, in some studies conducted with iron-replete children it was found that iron supplementation had proved counterproductive, while in others a similar group of subjects had proved unaffected by supplementation.[113,115] The inconsistency in the results of these studies could be due to coinciding multiple deficiencies, variation in the duration of studies and the iron dosages used, different age groups and different degrees of iron deficiency.[116]

2.2.4.2 Effect on immunity

In cases of infection occurring in the presence of iron deficiency with or without anemia, normal resistance mechanisms including functioning of phagocytic, T- and B- cells, may be compromised while the infection lasts, because large doses of iron given to such children may aggravate the infection. This is because the infectious organism also gets supplied with the iron resulting in its replication before the immune system of the host has had time to recover.[118] Thus deficient as well as excessively high iron levels could compromise the immune function, which suggests that an iron status within normal parameters should be sought that would ensure a complete phagocytic and immune response to pathogens.[118]

Untargeted supplementation in tropical countries where malaria transmission is high, was found to be associated with an increased risk of severe infection.[119,120] Hence the WHO has suggested (in light of the potential adverse effects of supplementation on malaria infected individuals) that, iron and folic acid supplementation should be given to anemic children who are at risk of iron deficiency, and that in such instances concurrent protection against malaria (such as treated bed nets and anti-malarial drugs) and other infectious diseases should be in place.

2.2.4.3 Effect on cognition and school performance

Results from several randomised trials have shown a causal relationship between iron deficiency and deficient cognitive function, also suggesting that short-term iron supplementation can reverse some aspects of impaired cognition. Children suffering from anemia have demonstrated poor physical and cognitive development. Anemia results in severe lethargy and low physical capacity for activity, which negatively affect the time spent by children playing and exploring.[122]

A study carried out in Malawi demonstrated a significant increase in fluid intelligence in school children supplemented with iron for 10 months.[123] A study carried out in Thailand reported a great difference between the scores obtained respectively by anemic iron-deficient and iron-replete children in a Thai language test, as well as in a test gauging general reasoning ability. The same difference was not evident in arithmetic scores obtained by the same group, however.[124] On the other hand differences between scores of Indonesian school children who were iron deficient and iron-replete, respectively, were not markedly different for a number of exams, as well as a test for concentration. However, scores across the board were improved for the same exams as well as the concentration test as a result of iron supplementation.[125]

Amplified vulnerability to infections as a result of iron shortage in school children could lead to lowered school attendance, which could therefore compromise performance.[105] Fewer school days were missed by children fed with biscuits fortified with multiple micronutrients than by a control group because the intervention had caused a decline in respiratory and diarrhea related illness.[105] A review by Taras (2005) demonstrated an association between iron deficiency and poor academic performance.[126] However, academic performance (at school) improved as result of iron supplementation administered to normalize depleted iron stores.[126]

2.2.5. Epidemiology of iron deficiency and anemia

Iron deficiency (ID) and iron deficiency anemia (IDA) are prevalent in women and young children. More people in the world are affected by iron deficiency than any other type of malnutrition.[95] It is estimated that more than 2 billion people are affected by iron deficiency,

and 1.2 billion of these suffer from iron deficiency anemia.[127] Anemia is most prevalent in developing countries, thus 39% of children < 5 years old, 48% of children 5-14 years old, 42% of all women and 52% of expectant women are suffering from anemia. About 50% of anemia is due to iron deficiency.[97] Estimates show that 53% or 210 million school age children suffer from IDA.[44,45] A recent South African national health and nutrition examination survey showed that, provincially, the prevalence of iron depletion was the highest in women from Gauteng (11.2%) and lowest in Eastern Cape women (0.7%).[128] Prevalence among younger South African women was higher (10.5%) than among older women (8.5%). Reports suggest that Asia has the highest IDA prevalence (58.4%), followed by Africa (49.8%).[129] Several studies have been done to try and capture the IDA prevalence in school children.

A survey of nearly 14000 rural school children in Africa and Asia, showed that IDA prevalence was more than 40% among children aged 7-11 years old in five African countries (Mali, Tanzania, Mozambique, Ghana and Malawi).[130] IDA prevalence in Asian children aged (7- 11 years) in Vietnam and Indonesia was low (12 and 28 % respectively). Prevalence was found to be higher among the older than the younger group. Boys had a higher hemoglobin concentration than girls. However, the IDA prevalence was higher in boys than in girls.[130] Results could have been attributable to a higher incidence of parasitic infections among boys, a higher growth rate (e.g. onset of a more pronounced “growth spurt” than among girls), or other confounding factors. Certainly a variety of causes are possible.

2.3 STRATEGIES TO ADDRESS IRON DEFICIENCY AND ANEMIA

There are three main strategies for correcting iron deficiency in populations, and they can be used alone or in combination.[125]These strategies are: education combined with dietary modification or diversification, or both to improve iron intake and bioavailability; iron supplementation and iron fortification of foods. A new approach is biofortification via plant breeding or genetic engineering. Dietary modification and diversification are the most sustainable approaches. However, it may be difficult to change dietary practices and preferences. Moreover, good sources of highly bioavailable iron are expensive.[125]

2.3.1 Food fortification

Even though iron is the most difficult mineral to add in food and ensure adequate absorption, iron fortification of foods is still the most practical, sustainable and cost-effective long-term solution to combating iron deficiency.[100,101,102] Fortification of staple foods is even more important as a long-term strategy for addressing micronutrient deficiencies, including iron deficiency.[43] Different foods can be used as vehicles for several iron fortificants (Table 2.5). In South Africa the fortification of bread, flour and maize meal was legislated in 2003.[108] Maize and wheat flour are currently fortified to provide a person of 10 years or older with electrolytic iron (25% from unsifted maize and 50% from maize meal) of the recommended dietary allowance.[108]

The most bioavailable iron compounds often lead to the development of unacceptable sensory changes, such as off flavours and colour change.[103] Therefore less soluble forms of iron in low doses are usually used to avoid organoleptic changes.[29] Fortification may be the safest intervention as low doses similar to the physiological environment are used.[101,121] Analysis of studies where infants received iron fortified foods showed no adverse effects and demonstrated a significant protection effect against development of respiratory tract infections.[119]

Most staple foods contain some iron, however, the quantities differ with the different cultivars.[135] This suggests that selective breeding (biofortification) might increase the iron content of staple foods.[29] But then the high phytate content of most staple foods could still pose a challenge when it comes to bioavailability. Therefore breeding should also be aimed at producing cultivars low in iron absorption inhibitors.[29] A study in which the aim was to lower the phytic acid content of rice was done by Lucca *et al.* (2001), it involved introducing phytase from *Aspergillus fumigates.*[136] The results indicated a seven fold increase in phytase activity.[136] Some studies showed that iron uptake from the soil could be increased by introducing a ferric reductase gene into the plant root systems.[137] Breeding or genetic engineering can be useful in increasing iron content in staple foods.[29]

Table 2.5: Suggested iron fortification compounds for different food vehicles[138]

Food vehicle	Iron Fortificant
Low extraction (white) wheat flour or degermed corn flour	Dry ferrous sulfate Ferrous fumarate Electrolytic iron (2x amount) Encapsulated ferrous sulfate Encapsulated ferrous fumarate
High extraction wheat flour, corn flour, corn masa flour	NaFeEDTA Ferrous fumarate (2x amount) Encapsulated ferrous sulfate (2x amount) Encapsulated ferrous fumarate (2x amount)
Pasta	Dry ferrous sulfate
Rice	Ferric pyrophosphate (2x amount)
Dry milk	Ferrous sulfate plus ascorbic acid
Fluid milk	Ferric ammonium citrate Ferrous bisglycinate Micronized dispersible ferric pyrophosphate
Cocoa products	Ferrous fumarate plus ascorbic acid Ferric pyrophosphate (2x amount) plus ascorbic acid
Salt	Encapsulated ferrous sulfate Ferric pyrophosphate (2x amount)
Sugar	NaFeEDTA
Soy sauce, fish sauce	NaFeEDTA Ferrous sulfate plus citric acid
Juice, soft drink	Ferrous bisglycinate, ferrous lactate Micronized dispersible ferric pyrophosphate
Bouillon cubes	Micronized dispersible ferric pyrophosphate
Cereal based complementary foods	Ferrous sulfate Encapsulated ferrous sulfate Ferrous fumarate Electrolytic iron (2x amount) All with ascorbic acid (2:1 molar ratio of ascorbic acid: iron)
Breakfast cereals	Electrolytic iron (2x amount)

Ferrous bisglycinate

Ferrous bisglycinate (used in as a fortificant in the experimental product) is a chelated form of an iron fortificant. The chelation occurs when amino acids are attached to a mineral. In the case of ferrous bisglycinate two molecules of amino acid are bound with a covalent bond to an iron

molecule. Absorption of this type of iron in the small intestine is similar to that of amino acids: no irritation or constipation or any other side effects are experienced as with other forms of iron supplementations. In addition, the mechanism by which this type of iron is absorbed seems to be determined by blood hemoglobin levels. This is important in preventing toxic levels of iron in the body.[19] Furthermore, it has been reported that losses of vitamins in multivitamin mixtures caused by amino acid chelates are lower than those caused by ferrous sulphate.[139] Ferrous bisglycinate is usually recommended for liquid milk and other beverages: it is classified under the rubric Generally Recognized as Safe (GRAS).[140]

The relative bioavailability of iron compounds is articulated by comparing their bioavailability with ferrous sulphate (relative bioavailability of ferrous sulphate = 100%). Compared to ferrous sulphate, iron from ferrous bisglycinate chelate (Ferrochel®) has been found to have a 3.4 – 4 times higher relative absorption rate in infants with iron-deficiency anemia,[141] iron-sufficient men[142] and anemic adolescents.[143] A study by Layrisse *et al.* (2000),[144]showed that even in the presence of iron absorption inhibitors (phytates and polyphenols), the relative bioavailability of iron from Ferrochel® in non-anemic adults is twice as high as that achieved with ferrous sulphate. Despite contradictory reports concerning ferrous bisglycinate efficacy[145,146] in the prevention and control of iron deficiency and iron deficiency anemia it has been proved conclusively in several supplementation trials with infants, preschool children and adolescents that ferrous bisglycinate can improve the iron status of children.[15,143,144]

Ferrous bisglycinate appears to be a good fortificant because of its high bioavailabilty and relatively low reactivity, particularly in milk products.[139] Its efficacy in fortified liquid milk, sweetened bread rolls and whey-based beverage was reported satisfactory in three studies carried out in Brazil,[147,148,149] and also in an iron fortified milk drink trial in Saudi Arabia.[150]A South African study on the efficacy of bread made from high-extraction flour fortified with ferrous bisglycinate, reported a small but significant increase in both hemoglobin and ferritin in school children.[151]

2.3.2 Education combined with dietary diversification

Dietary diversification which involves nutrition education is a long term strategy for controlling any micronutrient deficiency. Nutrition education helps to create awareness which has to be converted into action.[105] International strategies customized to South African context such as Food based dietary guidelines (FBDGs) are part of the nutrition education strategy.[24] Dietary diversification aiming at improving iron status should focus on increasing bioavailability of iron in the diet through high intakes of enhancers and reduced intake of inhibitors.[105]However, in light of challenges that might confront prospective behavior change the purpose in view may be equally served by employing other strategies such as fortification and supplementation besides food diversification.[105]

2.3.3 Supplementation

According to the ADA Report (2005), a supplement is a product (excluding tobacco) intended to complement the diet that contains a few, or most, or a combination, of the following the dietary ingredients: a vitamin or mineral; a herb or other botanical; an amino acid; a dietary substance for human use to supplement the diet by increasing the total dietary intake; or a concentrate metabolite, constituent or extract.[152] It can also be described as a product to be taken orally in a tablet, gel cap, or liquid form; and as a product that is not meant for use as a conventional food or a sole item of a meal forming part of a dietary regimen.[152] Supplementation should therefore proceed with due consideration of the fact that a healthy diet should comprise a balanced diversity of foods.[152] By the same token, however, it should be noted that the view commonly held within the ambit of nutritional science to the effect that a balanced diet can meet all nutritional requirements has been challenged.

For example the Nutrition United Nations Sub Committee on Nutrition has declared that dietary sources alone cannot provide 100% RDA of micronutrients.[153] It is justified to aver, therefore, that nutritional supplements can play a crucial role in improving physical growth, mental development, and the prevention of common infections.[26]

Supplementation can be cost-effective when given to targeted high-risk groups.[100]Quality

control during manufacture and correct dosing are important. Overages are usually included in the formulation of vitamin supplements during manufacture, to ensure that a certain dosage is still available by the end of the shelf life. A high- dose Vitamin A supplementation programme in South Africa is being followed since 2001.[24]Supplementation seeks to control existing imbalances which may have pathogenic consequences, such as severe iron deficiency. The purpose of iron therapy is to increase hemoglobin values. Restoration of iron stores may take about 4 months because of the lifespan of the red blood cells, which is approximately 120 days.[14]

2.3.3.1 Iron supplementation

Women of reproductive age and young children have been the main focus for IDA reduction programmes. However, the recent increase in studies reporting on IDA in school- children has resulted in a programmatic response for the relevant age group.[20] A summary of studies on the effect of iron supplementation on the iron status of school children is given in Table 2.6. Iron supplementation has been found to have a positive effect on the Hb concentration in treatment groups, with more significant changes observed in subjects who were anemic at baseline. This indicates that iron-replete groups were unable to absorb much iron. Deworming of subjects at the start of the interventions boosted Hb concentration even in the placebo group, which explains the importance of eradicating parasitic worms in order to improve iron status.[154]

Table2.6: Overview of iron supplementation trials on iron status of school age children.

Reference	Country	Initial sample size	Type of study	Age group	Baseline Hb concentration (g/dL)	Duration/supplement	Hb outcome (g/dL)
Seomantri (1989)[155]	Indonesia	130 ANPL:24 NAPL:35 ANFe:34 NAFe:37	Double blind randomised clinical trial	8.1 – 11.6 yrs	ANPL:9.6 NAPL:13.2 ANFe:9.7 NAFe:13.3	3 mo Iron sulphate 10 $mg.kg^{-1}.d^{-1}$	After treat. 3mo.later ANPL:9.5 ANPL 9.6 NAPL:13.3 NAPL 13.4 ANFe13.0 Ante 13.0 NAFe:13.6 NAFe 13.8 Treatment effect observed in anemic but not non anemic children. Not indication whether outcome was significant or not.
Sungthon et al.(2004)[156]	Thailand	(397) Daily;140 Weekly; 134 Placebo;123	Double blind, randomised placebo controlled trial	Grade 1 to 6	Daily;12.1 Weekly; 12.2 Placebo;12.1	16 wks Ferrous sulphate 30.0mg	Daily;12.8 Weekly; 12.7 Placebo;12.5 A positive treatment effect was observed
Rochnick et al.(2004)[157]	Phillipines	1510 Interv: 708 Con: 802	Randomised controlled trial	7 – 12 yrs	Intervention:12.4 Control:12.6	17 wk Ferrous sulphate 32.5mg	Intervention:12.4 Control:12.2 Hb concentration of children in intervention group did not change significantly. Hb for untreated group fell.

Hb: hemoglobin; ;ANPL:anemic placebo treated group ; NAPL:non anemic placebo treated group; ANFe:anemic iron supplementation treated group ; NAFe:non anemic iron supplementation treated group.

Best absorption of iron supplements is achieved on an empty stomach. However, gastrointestinal side effects such as nausea, epigastric discomfort and distention, heartburn, diarrhea or constipation may reduce tolerance and compliance. Oral iron supplements such as ferrous iron salts (ferrous sulphate and gluconate) are usually preferred because of their low cost and high bioavailability. Other supplements include: amino acid chelated ferrous bisglycinate, synthetic chelated NAFerredetate, and EDTA (ethylenediaminetetraacetic acid). However, the efficacy of ferrous bisglycinate has proved superior to that of its rivals and less prone to produce undesirable side effects.[19]

The studies show that iron supplementation on its own has a positive effect on the iron status of school age children. Significant changes were particularly noticeable in anemic children.

2.3.3.2 Multiple micronutrient supplementation or fortification

Micronutrient deficiencies have been observed to overlap and occur simultaneously in the same group of people.[20] Provision of multiple micronutrient supplementation or fortified foods to the affected or vulnerable groups may therefore be cost-effective in addressing nutrient deficiencies.[20]

A summary of studies on the effect of micronutrient supplementation on the iron status of school children is provided in Tables 2.7 and 2.8. The latter gives detailed information on the nutritional content of the supplements used in the studies summarised in Table 2.7. The intervention/treatment groups involved in these randomised controlled trials were given foods, beverages, seasoning, biscuits, bread or tablets enriched with multiple micronutrients, whereas the controls received placebo and/or iron supplements alone. The treatment groups showed an increase in Hb concentration at varying quantities. The conclusion favouring iron supplementation alone in some instances, especially in virtue of apparent lack of increase in Hb after the multiple micronutrient intervention, was most probably drawn as a result of measurement error.[160] It is also important to note the possible effect due to the variability in the duration of the intervention studies, some had a duration as short as 8 wks[158] while some went on for as long as 8 to 12months[161,163,65,166].

A systematic review of randomized studies on the placebo effect on Hb response, compared to that of combining multiple micronutrient with iron supplementation, showed a significant increase in Hb concentration in children's weight mean difference (WMD) = 0.65 g/dL, 95% CI 0.50, 0.80, P < 0.001). An initial greater rise was seen in anemic children and in children in the lower ranges of height-for-age z-scores.[159]

A pooled analysis of studies comparing combined Fe and micronutrient supplementation with Fe supplementation alone showed that the addition of multiple micronutrients to Fe resulted in a small but significant increase in Hb (WMD = 0.14 g/dL, 95% CI 0.00, 0.28, P = 0.04) over Fe supplementation alone.[159] According to expectation, therefore, synthesized evidence alone shows that instead of impairing the Hb response to iron supplementation children, a judicious addition of multiple micronutrients may have marginal benefits compared to iron supplementation alone.[159] However, given the mixed results of previous studies, interaction may be more likely with high dosages of micronutrient supplementation and shifting from a single to multiple micronutrient supplementation may therefore still have to overcome challenges such as deficient programme efficacy.[159]

A food based strategy involving enriched food products, for example, nevertheless remains a promising nutritional intervention. However, more evidence on the efficacy and effectiveness of this type of intervention is needed for policy and programme planners to have it implemented.[62]

Table 2.7: Overview of multiple micronutrient supplementation/ fortification trials on mean Hb concentration in school age children

Reference	Country	Sample size	Type of study	Age group	Baseline Hb concentration (g/dL)	Duration	Intervention	Hb outcome (g/dL)and comments
Ayoya *et al* (2009)[162]	Mali	847	Randomised controlled trial	7 – 12 yrs	10.37 (P) 11.42 (P+Fe) 10.57 (P+MM) 10.59 (P+Fe+MM)	12wk	Praziquantel (P) Praziquantel + Iron (P+Fe) Praziquantel+multiple micronutrient supplementation(P+MM) Praziquantel +iron +multiple micronutrient supplementation (P+Fe+MM)	11.54(P+Fe) 10.81 (P) 11.28(P+MM) 11.35 (P+Fe+MM) Possible explanation for low effects of MM on Hb, could be negative interactions among nutrients that interfered with the use of iron or other erythropoietin nutrients
Zimmerman *et al.* (2004)[58]	Morocco	157 goitrous school children with vitamin A and iron deficiency	Randomsed double blind trial	10-13yrs	Iodized salt(IS): 11.6 Triple fortified salt(TFS): 11.4	10 mo	Triple fortified salt	I S: 11.5 TFS: 12.9 Triple fortification of salt effective in increasing hemoglobin levels, possibly because most of the children were taking three main meals plus to snacks per day, all of which had some salt. Thus iron absorption was enhanced by repeated delivery of small doses throughout the day.
vanStuivernberg *et al.* (2006)[151]	South Africa	160	Randomised controlled trial	6-11yrs school children	Control:12.7 Electrolytic iron:12.6 Ferrous bisglycinate:12.7	7.5 months	Fortified bread	Control:12.8 Electrolytic iron:12.7 Ferrous bisglycinate:12.9

Hb : Haemoglobin , Praziquantel (P) , Praziquantel + Iron (P+Fe) , Praziquantel+multiple micronutrient supplementation(P+MM) , Praziquantel +iron +multiple micronutrient supplementation (P+Fe+MM), Iodized salt(IS), Triple fortified salt(TFS).

Table 2.7:(cont.) Overview of multiple micronutrient supplementation/ fortification trials on mean Hb concentration in school age children

Reference	Country	Sample size	Type of study	Age group	Baseline Hb concentration (g/dL)	Duration	Intervention	Hb outcome (g/dL)and comments
Osei *et al*. (2010)[163]	India	499	Randomised control trial	1-8 yrs	Micronutrient premix fortified: 12.2 Non fortified: 12.17	8 mo	Micronutrient premix added in lunch meals Nonfortified lunch meals	Micronutrient premix fortified: 12.32 Nonfortified: 12.25 Slight increase in Hb of boh groups
Jinabhai *et al*. (2001) [164]	South Africa	579	Double blind randomised placebo controlled trial	8 – 10 yrs	Vit A +iron grp;12.8. Vit A group;12.7. Non fortified group;12.8	16 wks	Fortified biscuit	Vit A +iron grp;12.9. Vit A group;12.8. Non fortified group; 12.9. No treatment effect, may have been due to low prevalence of anemia at baseline.
vanStuijvernberg *et al.* (1999) [167]	South Africa	Experimental; 115 Control;113	Randomized controlled trial	6 -11 yrs	Intervention grp; 12.5 Control grp;12.6	3 wks over a 12 mo period	Fortified biscuit and cold drink	Intervention grp; 6 mo; 12.4 12 mo; 12.9 Control grp; 6 mo; 12.4 and 12 mo; 12.7

Hb: hemoglobin, Vit A: Vitamin A, mo: month, grp: group

Table 2.7 (cont.) Overview of multiple micronutrient supplementation/ fortification trials on mean Hb concentration in school age children

Reference	Country	Sample size	Type of study	Age group	Baseline Hb Conc (g/dL)	Duration	Intervention	Hb outcome (g/dL)and comments
Abrams (2003) [158]	Botswana	311 Exp:164 Con:147	Non random clinical trial	6 -11yrs	Exp: 12.9 Con: 12.9	8wk	Fruit flavoured fortified beverage	Exp: 12.6 Con: 12.2 Changes in Hb significantly different between experimental and control group. Reduction in Hb level may have been due to change in measuring equipment
Taljaarrd *et al*.(2013)[168]	South Africa	CNS;103 CS;104 MNNS;103 MNS;104	Randomised double - blind, controlled intervention	6 -11yrs	CNS; 12.7 CS;12.7 MNNS;12.5 MNS;12.7	8.5 mo	A beverage with and without micrnutrients	CNS; 12.7 CS;12.7 MNNS;12.9 MNS;13.0
Ash *et al.* (2003)[132]	Tanzania	841	Randomized double blind placebo controlled	6 -11 yrs	11.9 (MM fort. bev) 11.9 (non fort bev)	6 mo	Multiple micronutrient fortified beverage	11.6 (MM fort. bev) 11.2 (non fort bev) Hb decrease in both groups due to seasonal influence on dietary quality and morbidity pattern with regards to malaria.

Hb: hemoglobin, Ex: experimental group; Con : control proup; CNS : no micronutrients (control beverage) with non nutritive sweetener , CS : no micronutrients(control beverage) with sugar, MNNS : micronutrients with a non nutritive sweetener, MNS: micronutrients with sugar, mo: months, MM fort bev: multiple micronutrient fortified beverage, non fort bev: non fortified beverage.

Table2.8: Nutritional content of the supplements /fortified foods used in the multiple micronutrient studies

Nutrients	158	132	162	58	163	164	167	168	151
Vitamin: A (ug) (RE)	B carotene 2400	Retinyl palmitate 1750 IU	Vitamin acetate 1030	60	30	350	B carotene 2.0		257.2
Thiamin(mg			1.5		0.25				0.28
Riboflavin (mg)	0.4	0.6	1.7						0.26
Niacin (mg)	2.7		20						3.41
Pyridoxine (mg)	0.5	0.7	400						0.38
Folic acid (ug)	14	0.14	10					1: 0.1 2: 0.2	206
B12 (ug)		3							
Biotin (ug)			30						
Ascorbic acid (mg)	60	72	120				110		
Pantothenat			10						
Cynocobalamin (ug)	1.0								
Tocopherol (mg)	7.5	10.5	23						
Calcium (mg)			250						
Iron (mg)	Ferrous bisglycinate chelate 7.0	Ferrochel 5.4	Ferrous fumerate 18	Fe PP 2	Ferrous sulphate 3mg of elemental iron.kg body weight^{-1}.d^{-1}	FeEDTA 5	Fe fumerate 5.9	**1**.EleFe 20 **2.**eleFe 40 **3**. Fe sul30 **4**.eleFe 60	**Elect: 5.04** **Ferro bisgly:5.04** **Contr;±1.8**
Magnesium (mg)			100						

Nutrients	158	132	162	58	163	164	167	168	151
Iodine (ug)	60	45	150				95.4		
Phosphorus (mg)			77						
Zinc (mg)	3.75	5.25	15			2.5			2.16
Selenium (ug)			25						
Potassium (mg)			40						
Molybdenum (ug)			25						
Boron (mg)			150						
Chloride (mg)			36						
Nickel (ug)			5						
Copper (mg)			2						
Chromium (mg)			120						

2.4. LITERATURE REVIEW SUMMARY

Anemia is most prevalent in developing countries (i.e. 39% of children aged < 5years, 48% of children aged 5-14 years, 42% of all women and 52% of expectant women). Iron deficiency accounts for about 50% of anemia.[97]Iron deficiency anemia is the final stage of iron deficiency and is characterised by low hemoglobin levels.[105] Factors that may contribute to the development of iron deficiency include low intake and poor absorption of iron from the diet due to the presence of iron absorption inhibitors or lack of absorption enhancers; and a greatly heightened physiological need for iron during menstruation, pregnancy and growth.[37,38] It is common cause that measurement of hemoglobin, ferritin and sTfR in conjunction with chronic infections as further indices, produces the best results when assessing iron status. However, this procedure is usually expensive and difficult.[94] Hemoglobin measurement has therefore been successfully substituted as an alternative method in situations challenged by financial constraints and in remote field works.[96]

There is good evidence that Iron deficiency anemia can cause retarded physical development, low cognitive function, weak immune function, growth decline, and accelerated fatigue.[106] Micronutrient deficiencies have been observed to overlap and occur simultaneously in the same group of people.[20] Provision of multiple micronutrient supplementation or fortified foods to the affected or vulnerable groups may therefore be cost effective in addressing nutrient deficiencies.[20]

CHAPTER 3: METHODOLOGY

3.1 ETHICAL CONSIDERATIONS

Ethical approval to undertake this study was granted by the Research Ethics Committee of the Faculty of Health Sciences (University of Pretoria) (Addendum 1). Permission to conduct the study was given by the Gauteng Department of Education and the Sunnyside Primary School. Informed consent was also sought and granted by the parents or legal guardians of the learners. All aspects of the protocol were explained to the learners in their classrooms. Learners with moderate to severe anemia (Hb < 9.5) were not included in the study were referred for treatment.

3.2 RESEARCH DESIGN

An experimental study design in the quantitative domain was used. The study was a randomised double blind placebo controlled trial (Figure3).

3.3 STUDY SETTING

Sunnyside Primary School in Sunnyside, Pretoria, situated within the municipal confines of Tshwane Metro Council (Gauteng Province, South Africa).

3.4 STUDY POPULATION

The study population consisted of male and female learners aged 8 – 12 years enrolled for Grades 3 and 4 the academic year 2010.

3.4.1. Recruitment and screening

Parents or guardians of learners enrolled for Grade 3 and 4 at Sunnyside Primary School were informed during a scheduled general parents meeting about the study and its purpose, and were given an opportunity to ask questions. Children whose parents/guardians had signed consent forms for participation in the study were eligible for participation in the study and had to sign assent forms (Addendum 2). Screening was done and children who met the inclusion criteria were enrolled in the study.

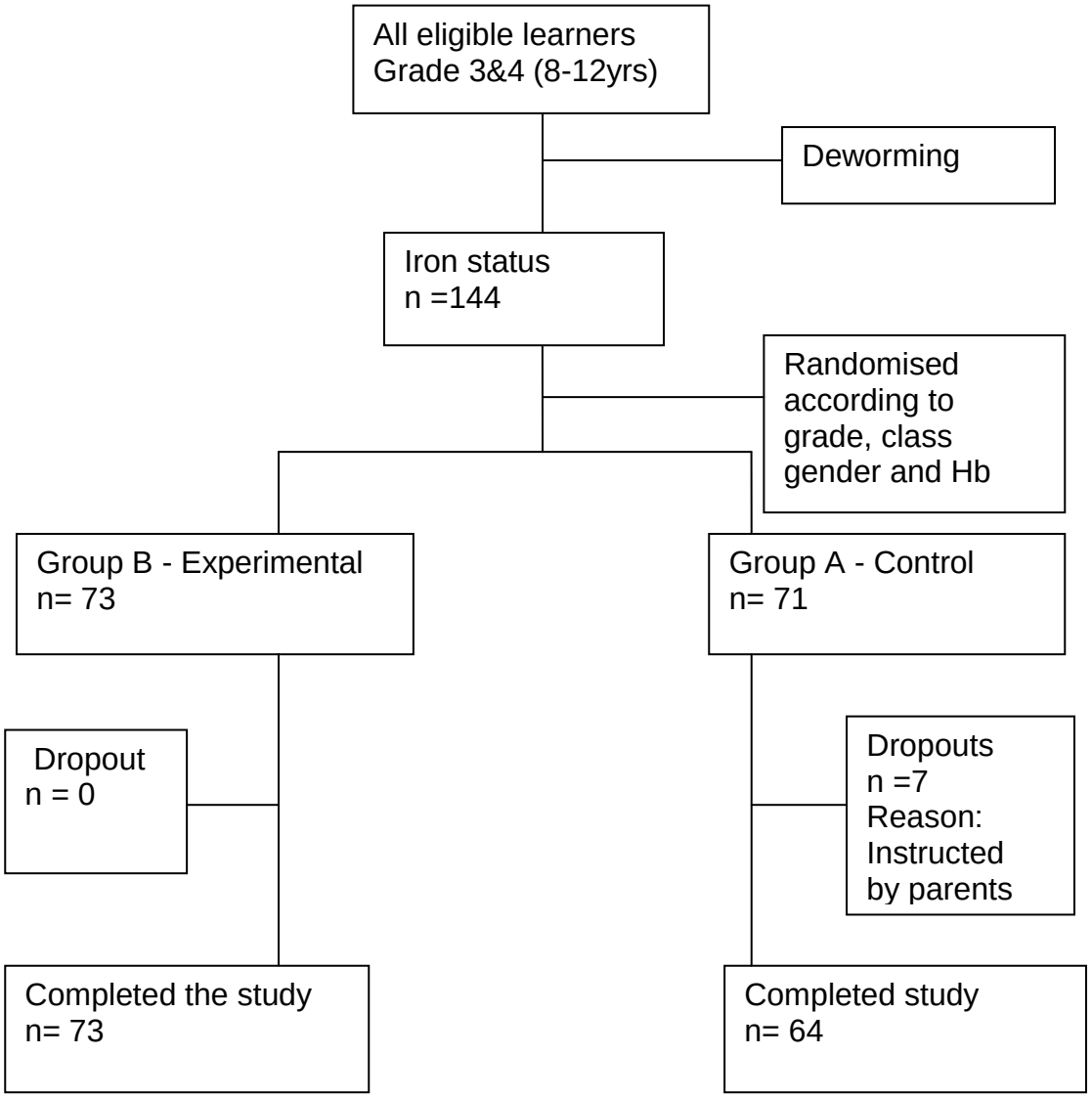

Figure 3. Trial profile of the 14 weeks intervention study.

Inclusion criteria

_ Boys and girls in Grades 3 and 4

_ English literate

_ Children with normal iron status (Hb $\geq$ 11 g/dL) and mild anemia (Hb $\geq$ 9.5 g/dL)

Exclusion criteria:

_Children with moderate to severe anemia (Hb<9.5 g/dL)

3.5 SAMPLING METHOD

A convenient purposive sampling method was used for this research study. A primary school with a need for school feeding and with an existing school feeding program in place was chosen. Convenient sampling was used for logistical ease.

3.5.1 Sample size

Sample size was calculated based on the comparison of the two groups with respect to their change in Hb following 14 weeks of intervention treatment. This was done with either micronutrient enriched or none micronutrient enriched maize based liquid meal supplement. A difference in change from baseline of 0.8 g/dL between the groups was regarded as clinically significant and a standard deviation of 1.5g/dL was assumed (range/4 = (15.5 – 9.5)/4 =1.5g/dL). For a one sided test at the 0.05 level of significance a sample of 61 subjects per group had a 90% power to detect the clinically significant difference of 0.8g/dL. To account for a dropout rate of 30%, a sample size of 80 subjects per group was aimed for, however, only 71 and 73 learners were included, allowing for a dropout rate of about 20%.[158]

3.6. INTERVENTION

3.6.1 De-worming

All participating children were dewormed before the start of the intervention to eliminate parasitic infestation which results in chronic intestinal blood loss due to the release of anticlotting agents.[56] A single dose of mebendazole (Vermox 500 mg tablet) was administered.

3.6.2 Experimental product

3.6.2.1 Description

The meal supplement used in the study was produced and provided by a South African based company supplying locally manufactured maize-based ready-to-use “TetraPak” packed meal supplements. Unfortified maize as the staple food of the target population was used as the foundation of the products.The meal supplement was a lactose free, gluten free enteral feed,

providing 1 kcal/ml and 9 g protein per 250 ml Tetra-Pak portion. The meal supplement was fortified with micronutrients and had the following nutritional specifications:

- High in energy (>250 kJ per 100 ml)
- High in vitamins (A, D, E, C, B_1, B_2, Niacin, B_6, B_{12}, Biotin & Pantothenic acid)
- High in minerals: calcium, phosphorus, iron, magnesium, zinc & iodine
- Source of protein (>2.5 g per 100 ml and >2.5 g per 418 kJ)
- High in carbohydrates (>6.5 g per 100 ml)

The control meal supplement had a similar macronutrient profile but without any added micronutrients (Table 3.1).

Table 3.1: Nutritional composition of experimental and control product for boys and girls

Nutrient	Experimental product	%RDA(9yrs)	%RDA(12yrs)	Control product	% RDA
Energy	1050 KJ	11		1050 KJ	11
Protein (g)	9	26	16	9	26
Carbohydrate (g)	32.5			32.5	
Fat (g)	10			10	
Fibre-inulin FOS(g)	3			3	
Sodium (mg)	275			275	
Potassium (mg)	375			375	
Chloride (mg)	355			355	
Vit A (ug RE)	452.5	65	45		
Vit D (ug)	2.5	25	50		
Vit E (mg)	9.5	136	95		
Vit C (mg)	45	100	75		
Vit B1(mg)	0.8	67	57		
Vit B2 (mg)	1	71	63		
Niacin (mg)	15	94	83		
Vit B6 (mg)	1.5	94	75		
Folic Acid (ug)	50	17	25		
Vit B12 (ug)	0.75	25	75		
Biotin (ug)	50	42	50		
Pantothenic acid (mg)	4.88	98	81		
Calcium (mg)	325	41	41		
Phosphurus (mg)	312.5	39	39		
Iron (mg)	5	63	63		
Magnesium (mg)	105	42	35		
Zinc (mg)	5	50	33		
Iodine (ug)	100	83	67		
Selenium (mg)	0.13	65	-		

Other characteristics of the product include: Gluten and lactose free; prebiotics (inulin), all essential amino acids and non-essential amino acids. The product incorporated bioavailable amino acid chelated minerals including ferrous bisglycinate chelate (Ferrochel®) whose bioavailability was found to be far superior to the iron from ferrous sulphate.

The maize-based liquid meal supplement was a product used for the following conditions:

- Full fluid liquid diet
- Remedial treatment for malnutrition, underweight and micronutrient deficiencies
- Immuno-compromised conditions, e.g. HIV & AIDS
- Tuberculosis
- Gastro-intestinal disease, e.g. diarrhea, Irritable bowel syndrome
- Supplementation in addition to meals
- Poor appetite
- Cerebral palsy
- Mental health conditions
- All other clients as identified by a health professional

The product came in three different flavours (vanilla, chocolate, banana). The vanilla-flavoured product was used in the study under review.

3.7 RANDOMISATION AND BLINDING

Learners in each class were stratified according to gender and baseline haemoglobin levels then randomly assigned to two groups (Group A and Group B). A double blind study design was used for this study. To prevent bias neither the subjects nor the investigator assessing the response were told of the treatment the subjects were receiving.

3.7.1 Preparing and administering of experimental product

As a "ready to drink" product the maize-based liquid meal supplement did not require any further preparation before uses. The pre-portioned meal supplements were numbered before being given to the learners. Supplements intended for Groups A and B were marked with numbers to be administered accordingly to specific subjects. Children were given supplements each morning before 10 am. Each learner produced an identity tag before she/he could be

given the drink allocated to him/her by number as indicated. The said numbering by subject was employed as a safeguard against dispensing product twice to a specific subject.

3.7.2 Subject compliance and monitoring

Each child was given an identity tag with the subject number, name, and class (reflecting the relevant school grade) on it. These identity tags were yellow for Group A and blue for Group B (Addendum 3). The researcher and assistant monitored compliance by observing the children as they imbibed their drinks, making sure they did not share. Empties or leftovers were collected and records of attendance and amounts left over were weighed and recorded in grams on compliance sheets (Addendum 4) and filed. Compliance or consumption was expressed as portion percentage (%).

3.7.3 Packaging and blinding of experimental products

The packaging and labeling of the two products were identical. The expiry date printed in lower cases on the upper surface of the packaging had different dates for each product, and was the only mean of differentiating between the two products.

3.8 DATA COLLECTION

3.8.1 Screening

Screening of the learners for eligibility to participate in the study was done in July 2010. Standardised procedure was employed to measure Hb with the aid of a portable HemoCue photometer (HemoCue Hb 201^{+}Analyser, Angelhom, Sweden).[123] Adjustment to hemoglobin cutoffs was carried out according to altitude and ethnicity (Table 2.5).[18] The adjustment led to a Hb cutoff of 11.0 g/dL for the school children serving as subjects. Learners whose Hb was above 9.5 g/dL were included/invited to participate in the study.

3.8.2 Schedule of measurements

Weight, height, and Hb measurements were taken at the beginning of the intervention (July 2010), and again at the end of the intervention (November 2010).

3.8.3 Variables

3.8.3.1 Iron status

The assessment of iron status was achieved by using hemoglobin as the biomarker. No other parameters were used due to financial and ethical constraints. Baseline and end assessments were carried out by qualified dietitians and trained dietetic students. Hemoglobin concentrations were determined with the aid of a portable HemoCue photometer (HemoCue Hb 201^{+}Analyser, Angelhom, Sweden). The accuracy and precision of hemoglobin values measured with the aid of the HemoCue photometer are comparable to those obtained with the cyanmethemoglobin method (the most reliable method recommended by the International Committee for Standard Hematology). Finger pricks were done by using a single-use lancing device (Accu-Check® Safe-T-Pro Uno). This lancing device is safety engineered with safety wings that break during the use to prevent re-use, avoid accidental finger pricks, and eliminate cross-contamination.[103]

3.8.3.2 Anthropometric measurements

Anthropometric measurements were recorded at two different time-points (baseline and end) according to standard techniques by trained dietitians and dietetics students.[96] Measures included height and weight. Body mass index (BMI) for age and height for age were expressed as z- scores.[169]

Height

Height was measured with a portable stadiometer (the Leicester height measure, England max height 2.10 m). In measuring height, clothes were minimal so that posture could be clearly seen and shoes were taken off. Height measurement was taken using standard techniques to the nearest 0.1 cm.[96]

Weight

Weight was measured with the aid of a digital personal scale (Body – Check Analysis- Seca sense 804, Germany). The scale was placed on a hard flat surface (not carpet), checked and adjusted for zero balance before each measurement. Body weight was measured according to standard techniques and recorded to the nearest 0.1 kg.[96]

Z -scores

Z-scores for height-for-age (HAZ) and BMI-for-age (BAZ) were determined with the aid of the WHO Anthro Plus Software.[169] A HAZ (z-score < -2SD) was indicative of moderate stunting and HAZ < -1SD was indicative of mild stunting. On the other hand a BAZ < -3 SD reflects severe thinness, BAZ <-2SD indicated thinness, and BAZ > +1SD indicated overweight and BAZ >+2 SD reflected obesity.

3.8.3.3 Socio-demographic information

Socio-demographic information was solicited from participants in a standard, made-to-measure questionnaire (Addendum 5). Parents/guardians were asked to fill in the questionnaires and return them through their children.

3.8.3.4 Sickness diary

Children were given a sickness diary, to be filled in by their parents whenever they suffered a bout of illness during the period of study (Addendum 6). The diaries were collected at the end of the study.

3.9 STATISTICAL ANALYSIS

This randomised, controlled trial was conducted to compare the Hb levels of children aged, 8 – 12 years in experimental and control groups. Only subjects with both baseline and end data were included in the statistical analysis.

The statistical software STATA Release 11 was used for statistical analyses.

Descriptive statistics were used for all measurements. Groups were compared with respect to change in Hb based on an analysis of covariance (ANCOVA) with baseline Hb values as covariate. Testing was done at the 0.05 level of significance.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 CHARACTERISTICS

4.1.1 Socio-demographic data

Of the 144 learners who started as participants in the study 137 (95%) remained in it for the duration of 14 weeks. Seventy three (73) were in Group B (experimental group) and 64 in Group A (control group) (Table4.1).

Table 4.1: Characteristics of learners who participated in the 14 week study

	Experimental group (n= 73)	**Control group** (n=64)
Male: n (%)	31 (42)	28 (44)
Female: n (%)	42 (58)	36 (56)
Mean age (years)	9 (0.8) [a]	9 (0.9) [a]

[a] standard deviation

Data affecting socio-demographic characteristics (Table 4.2) were obtained from 49% of the participants who remained in the study for the duration, while 51% did not return the questionnaires. As noted by Keskin *et al*.(2005), a high prevalence of iron deficiency anemia correlates closely with low socio-economic status (SES).[134] It was noted that, the consumption of tea was high in the low socio-economic groups, whereas the consumption of red meat, fish and citrus fruits tended to be lower by comparison in that group.[134]

Children whose parent/guardian (head of household) had no education, a low income and no car, were considered to be of low socio-economic status in Keskin *et al.*'s study.[134] However, the findings (Table4.2) in this study were to the positive, meaning the children were of high SES. Therefore, it can be assumed that the low prevalence of anemia among the children in this study was attributed to their high SES.

Table 4.2: The socio-demographic characteristics of the study population (n = 67)

Characteristic	N	%
Size of family		
2	6	9
3	9	3
4	19	28
5	16	24
6	6	9
7	7	10
8	4	6
Mother's marital status		
Unmarried	14	21
Married	41	62
Divorced	3	5
Separated	3	5
Widowed	3	5
Traditional marriage	2	3
Who cooks food?		
Father	2	3
Mother	54	81
Sibling	5	7
Grandmother	2	3
Aunt	3	4
Other	1	1
Who buys food?		
Father	1	1
Mother	59	88
Sibling	3	4
Grandmother	2	3
Aunt	2	3
Head of household		
Father	34	51
Mother	27	40
Grandmother	2	3
Aunt	2	3
Friend	1	1
Other	1	1
Who decides how much money is spent on food?		
Father	13	20
Mother	50	76
Grandmother	2	3
Aunt	1	2
Mom's level of education		
None	2	3
Primary school	4	6
Std 6-8	1	2
Std 9-10	18	3
Tertiary education	37	57
Not applicable	3	5
Household income		
None	3	4
R100-500	2	3
R500-1000	5	7
R1000-3000	12	18
Over 5000	15	22

4.1.2. Anthropometric characteristics

Anthropometry determines either the cross-sectional nutritional status of a population, or the nutritional status of an individual.[109] The anthropometric characteristics of participants in the study under review are presented in Table 4.3. There was no significant difference in the weight of the experimental (33.6 ± 9.6kg) and control (35.3 ± 9.0kg) (P= 0.281) groups at baseline. Both groups gained some weight, though not significantly, during the period of the study. There was also no significant difference in the height of the groups at baseline. The participants were not stunted. The mean BMI z-scores indicated that the learners were not obese. These findings indicate that there was no significant difference in the nutritional status of the participants at baseline.

Genetics and the environment play a major role in influencing physical growth. The environment, and the quality and quantity of food available are important determinants of growth rate.[170,171] The NFCS showed that one in ten of all children aged 1 – 9 years were underweight, more than one in five were stunted and 6% of children were overweight. The same survey found that the prevalence of stunting declined while that of excess body weight rose as maternal education levels increased.[109] Although the average age of children involved in the study was slightly higher than that of their counterparts in the national survey, their average age (9.8 years) nevertheless fell within the same age bracket as the said counterparts, therefore comparison of the survey results with those of the present study is justifiable. Thus, as in the national survey, few participants (< 10%) were underweight.[109,172] By contrast, however, the incidence of stunting and overweight recorded for the survey was different from that recorded in the study at issue.

In particular, stunting was lower among participants in the present study (< 10%) compared to the moderate prevalence recorded in the NFCS (20 – 29.9%). Contrariwise, the prevalence of overweight participants was higher (> 20%) in the present study than that reported by the NFCS (6%).[109] The results emanating from this study were comparable to those of the South African National Health and Nutrition Examination Survey (SANHENS), which showed that the prevalence of undernutrition had declined among children of all age groups in South Africa.[128]

Table 4.3: Anthropometric characteristics of the study population

Variable	**Experimental group** (n= 73)	**Control group** (n=64)	**p- value**1
	Mean (sd)	**Mean (sd)**	
Weight (kg)			
Baseline	33.6 (9.6)	35.3 (9.0)	0.281
End	34.6 (10.1)	36.5 (10.1)	0.276
Height (cm)			
Baseline	136.2 (7.8)	137.9 (9.3)	0.255
End	137.7 (0.9)	139.6 (1.3)	0.219
HAZ (stunting)			
Baseline	0.009 (1.08)	0.265 (1.20)	0.153
End	0.037 (1.095)	0.292 (1.249)	0.152
Change from baseline to end	0.028 (1.082)	0.027 (1.198)	0.237
BAZ (wasting)			
Baseline	0.442 (1.18)	0.650 (1.18)	0.309
End	0.321(1.352)	0.537(1.302)	0.367
Change from baseline to end	0.121 (1.181)	0.113 (1.177)	0.704

HAZ: Height for age z-scores; **BAZ:** Body mass index for age z- scores
Stunted (HAZ < -2); **Wasted** (BAZ < -2); [1](ANCOVA, P = 0.05)

At the end of the study no significant changes had become evident in the anthropometry of the participants (Table 4.3). Other studies undertaken with school children showed significant increases in growth as well as height-for-age and weight-for-age z- scores.[112,113] The positive results recorded in these studies were attributed to participant's low iron stores at baseline. Results that seemed to vary erratically in the instance of studies conducted with iron-replete children could have been attributable to variations in administered iron dosages, coinciding

deficiencies, studies running over varying length of time or combinations of these factors.[116]

4.1.3 Consumption

The maize-based liquid meal supplement enriched with multiple micronutrients as used in the study under review was provided for a total of 69 school days over a period of 14 weeks (shortened due to strike action). Mean consumption (defined as the actual amount of drink consumed during the study, expressed as a percentage of the total amount provided over the trial period) was 49.34 % (SD: 27.43) and 53.71% (SD: 31.94) in the experimental and control groups respectively. The difference in consumption of the drink between the two groups was insignificant (P = 0.394)

The experimental product provided 5 mg iron per day providing 63% DRI of iron intake when consumption is complete. However, since the consumption was 49.34% the average daily intake per individual can be assumed to have been 2.47 mg, thus amounting to 30.9% of DRI per day. The school children took the maize-based liquid meal supplement in the early hours of the day, which probably meant that they had taken the supplement shortly after consuming a breakfast meal, which would therefore not have been fully digested by the time they ingested the supplement, thus possibly accounting for the low consumption recorded in the course of the study. Other possible reasons for the low consumption levels may have been absenteeism, unavailability of child during allocated scheduled handout of supplements, and the observed relative distaste for the experimental products. Consumption overall as observed in the study under review is lower than that found in comparable studies.

A consumer sensory evaluation (data not included) of the same product showed that the learners preferred (not markedly though) the chocolate flavoured maize-based liquid meal supplement enriched with multiple micronutrients to the other two flavours (vanilla and banana).[173] This preference could be the reason why consumption as observed in this study was low.

4.1.4 Iron status

The prevalence of anemia at baseline in the experimental and control groups is shown in Table 4.4. In the experimental group six (6) cases of mild anemia were diagnosed at baseline (Hb < 11g/dL) while only one (1) participant in the control group was diagnosed as mildly anemic at that stage. By the end of the study, however, only one (1) participant in the experimental group had mild anemia, while the control group still had one (1) mildly anemic participant.

This was not unexpected as the learners in the control group were not getting any micronutrient from their product. Table 4.5 shows the mean Hb levels of the experimental and control groups. There was no significant difference between the experimental and control groups in the Hb levels (12.6 ±1.1 g/dL in the experimental group and 12.8±1.1 g/dL in the control group) (P = 0.250) at baseline (Table 4.5). It should be noted however that Hb was used in the study as an inexpensive and common measurement, otherwise when used alone Hb is not a very specific and sensitive indice to determine whether iron deficiency is the specific cause of anemia.[96]

Table 4.4: Anemia prevalence in the experimental and control groups at baseline and at end

Mild anemia (Hb 9.5 to ≤ 10.99g/dL)	**Experimental n(%)**	**Control n(%)**
Baseline	6 (8)	1(2)
End	1(1)	1(2)

The intervention had no significant effect on participants' Hb levels over the intervention period of 14 weeks. However, at the end of the trial a slight increase in Hb levels (0.08 ± 1.210g/dL) was found in the experimental group, while a decline (-0.249 ± 1.191g/dL) was observed in the control group (Table 4.5); moreover the prevalence of mild anemia (Hb< 11g/dL)) in the experimental group had decreased from 8% at baseline to 1%.

Table 4.5: Iron status of participants at baseline, end and change from baseline to end

	Experimental **Mean ± sd**	**Control** **Mean ± sd**	**p-value**[1]
Baseline Hb (g/dL)	12.6 ± 1.1	12.8 ± 1.1	0.250
End Hb (g/dL)	12.7 ± 0.12	12.6 ± 0.11	0.806
Change from baseline to end	0.087 ± 1.210	-0.249 ± 1.191	0.477

Hb: Hemoglobin; **sd:** standard deviation; [1](ANCOVA, P = 0.05)

The prevalence of anemia declined in the course of this study from a level regarded as a mild public health problem (10%)[128] to an almost negligible level (2%) despite the low mean consumption observed (50%). An improvement was mainly observed in the experimental group. Table 4.6 shows that a negative change irrespective of rate of intake of the product was observed in the iron status of learners in the control group (-0.241 ± 1.258 in members whose consumption was < 50% and -0.241 ± 1.191 in members whose consumption was ≥ 50%). On the other hand a positive change (0.008 ± 1.097) was evident in members of the experimental group whose consumption was < 50% and 0.123 ± 1.301 in members whose consumption was ≥ 50%. On balance, though, the changes were negligible.

Assimilation of iron is low when iron status is at repletion levels.[174] If a high bioavailability diet is followed absorption averages at 15% in non-anemic individuals but climbs to 50% in anemic subjects .[174] The fact that 90% of the children in this study had normal Hb levels (Hb > 11g/dL) could be the main reason why the experimental product used in this study, despite its high highly bioavailable iron content, made no appreciable difference to subjects' iron status. Iron absorption improves when taken on an empty stomach or between meals. The liquid meal supplement used in the instance under review was taken early in the day when subjects' breakfast probably had not had time to digest, with the result that intake of the supplement as well as absorption of its iron content were low.

Results were comparable with those obtained in other studies conducted with subjects whose

iron status was at repletion levels. For instance, no observable result materialized from administering fortified biscuits during an intervention conducted over a period of 16 weeks in iron replete (12.8 g/dL) school children aged 8 – 10 years old.[164] Supplementation also had no appreciable effect in the instance of another intervention study carried out in Tanzania[132] where participants' baseline iron status had been normal (11.9 g/dL for both the experimental and control groups). Seasonal influence in dietary quality and the malaria-related morbidity pattern were given as reasons for the insignificant results achieved by conducting the intervention. This shows that iron repletion may not be the only reason for insignificant results obtained with supplementation venture.

The mean age of participants in the study under review was 9 years, but their age range was 8 – 12 years, which means that some were pre-adolescents or even adolescents who might have started menstruating, in which case the iron requirements of the menstruating female participants would have been higher than those of other participants and might not have been met in full by the supplementation.(not assessed).

Since the experimental product utilized in the study under review contained other chelated micronutrients besides chelated ferrous bisglycinate (Table 3.1), it follows that some of these additional nutrients may have interacted biologically with iron supplement because they (the ferrous and other nutrients) have chemically similar absorption and transport mechanisms (e.g. calcium).[175] A number of interactions between micronutrients could take place when a high dose of a single nutrient is given or when the supply of an individual micronutrient is inadequate.[175] In such instances iron indicators do not improve as greatly as when iron is given alone.[175] Such interaction could have served to suppress the efficacy of supplementation and could therefore have been partly responsible for the observed insignificant effect of supplementation over the study period. Moreover, no indication was found in the observed outcome of supplementation that vitamin C had enhanced iron intake as might be expected.

Similar results were observed in a study by Ayoya[162] where a multiple micronutrient

supplement administered to school children aged 7- 12 years old schoolchildren (baseline Hb: 10.42 g/dL; 10.57 g/dL; 10.59 g/dL for the Fe, MM, and Fe+MM groups respectively), barely lifted Hb values over a study period of 12 weeks, as opposed to when iron supplementation was taken alone. Negative interactions among nutrients were reported to have interfered with the use of iron or other erythropoietin nutrients. In another study conducted with Botswana school children aged 6 – 11 years over a period of 8 weeks (baseline Hb:12.9 g/dL for both the experimental and control groups), it was found that administering a fortified, fruit-flavoured drink over the study period had resulted in lowered Hb levels[158] possibly because in this instance too, negative interaction had occurred between nutrients. By contrast, however, other studies involving multiple micronutrient supplementation have reported a positive effect on the anemia status of the population concerned.

A positive effect for instance, was observed in a study done in Morocco by Zimmerman[58] where it was found that the Hb levels of school children aged 10 – 13 years had been raised by administering triple fortified salt (baseline Hb: 11.4g/dL and 11.6 g/dL for the experimental and control groups respectively). The likely reason for these results may have been the fact that the children were taking three meals per day plus snacks, all of which contained triple fortified salt. Iron absorption was therefore enhanced by repeated delivery of small doses throughout the day,[58] unlike Zimmerman's study, the experimental product in this study was only given once a day, therefore there was no repeated delivery of the micronutrients.

A significant increase in Hb levels as well as high consumption levels (>90%) were reported by Van Stuijvernberg's study in which South African school children aged 6 -11years old (baseline Hb: 11.5g/dL and 11.3 g/dL for the experimental and control groups respectively) were given biscuits to eat that had been fortified with multiple micronutrients.[167] The high consumption meant that, unlike the results obtained with the low consumption of participants in the present study, the children absorbed useful amounts of the nutrients as result of the continuity.

Positive results on the bioavailability of ferrous bisglycinate have been shown in several studies.

For example, in a study conducted in Valencia (Carabobo State, Venezuela) on men aged (15 – 50 yrs old) and women, of whom 40 were menopausal, showed that Ferrochel could partially prevent the inhibitory effects of phytates, mainly because it is highly soluble even at pH 6, highly assimilable and not inclined to interact with food.[144] The same effect was expected in the present study as the basis of the liquid meal supplement was maize which contains some phytates

It is understandable therefore, that the children did not absorb useful amounts of the nutrients, either because they were iron replete, or because they did not consume sufficient amounts of the supplement. This is why the intervention did not show a significant impact on participants' Hb levels.

4.2 LIMITATIONS

The intervention only took place during school days but not on school holidays, weekends or public holidays. The study was also limited to 14 weeks instead of the envisaged 16 weeks because of a strike action mounted by teachers in the public schools. The unforeseen break may have been partly responsible for the insignificant effect of the intervention on learner's Hb levels. Research ethics imposed another limiting factor in that its disapproval of participation by children with a very low iron status naturally precluded participation by moderately and severely anemic children. It is understandable, therefore, that absorption in children whose iron status was replete was less pronounced than it could have been with moderately or severely anemic children. The lack of dietary data was also a limitation as it would have helped in the interpretation of the intervention results,

A further limitation that may have affected the outcome of the study was the fact that the product was not subjected to a consumer sensory evaluation test before it was administered, with the result that no data were obtained about its acceptability prior to the intervention. It stands to reason, therefore, that a responsive flavour adjustment to appease participants' preference might have proved instrumental in increasing consumption, which could have led to different results.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

It can be concluded from measurements of Hb levels that the maize-based liquid meal supplement enriched with multiple micronutrients administered to participants in the study at issue did not have a significant effect on their iron status, possibly owing to a low prevalence of anemia and, for that reason, a low rate of consumption and therefore of iron absorption among members of the participating group.

It can also be concluded from anthropometric data that the liquid meal supplement did not have a significant effect on the nutritional status of participants.

RECOMMENDATIONS

- It is recommended that the intervention be taken to a primary school where the children are really needy and anemic to see if a significant effect will be forthcoming from the intervention.
- Since the poor consumption observed in the study under review could have been a result of aversion to the taste (vanilla flavor) of the maize-based liquid meal supplement enriched with multiple micronutrients, it is recommended that the chocolate flavoured product be substituted, since it proved to be the most liked in the consumer sensory evaluation which was carried out later.
- To increase consumption children should be given the supplement between meals when the digestive process is sufficiently advanced to prevent interference with absorption of the iron supplement.
- It is also recommended that the female participants be questioned in retrospect to discover whether any of them had begun to menstruate at the time of experimental supplementation, and that a statistical analysis be done accordingly to accommodate the outcome of what the recommended questioning reveals.

REFERENCES

1. UNICEF/Micronutrient Initiative. Vitamin and mineral deficiency. Global progress report. Ottawa: Micronutrient Initiative; 2004.
2. Kurz KM. Adolescent nutritional status in developing countries. Proc Nutr Soc. 1996;55:321–31.
3. Hyder SMZ, Haseen F, Khan M, et al. A multiple micronutrient fortified beverage affects hemoglobin, iron and vitamin A status and growth in adolescent girls in rural Bangladesh. J Nutr. 2007;137:2147-2153.
4. Rees JM, Neumark-Sztainer D, Kohn M, et al. Improving the nutritional health of adolescents - Position statement - Society of Adolescent Medicine. J Adolesc Health. 1999;24:461–2.
5. Brabin L, Brabin BJ. The cost of successful adolescent growth and development in girls in relation to iron and vitamin A status. Am J Clin Nutr. 1992;55:955–8.
6. Standing Committee on Nutrition. Adolescence: a pivotal stage of the life cycle. SCN News. 2005–2006;31:6–13.
7. Allen LH, Peerson JM, Onlney DK. Provision of multiple rather than two or fewer micronutrient more effectively improves growth and other outcomes in micronutrient deficient children and adults. J Nutr. 2009;139:1022–1030.
8. van Stuijvenberg ME. Using the school feeding system as a vehicle for micronutrient fortification: Experience from South Africa. Food Nutr Bull. 2005;26(2):S213-S219.
9. Bourre JM. Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirements for brain. Part 1: Micronutrients. J Nutr Health and Ageing. 2006;10(5):377-385.
10. Kloka D. Integrated Nutrition Programme. 2003.
11. Ministry of Education–UNICEF Provincial Report. Evaluation of the school nutrition programme Gauteng Province. 2008.
12. DOH. The Integrated Nutrition Programme. 2002. Issue 3.
13. Health Systems Trust. An Evaluation of South Africa's Primary School Nutrition

Programme. 1997.http://www.healthlink.org.za

14. Clark SF. Iron deficiency anemia. Nutr Clin Pract. 2008;23:128-141.
15. Pineda O, Ashmead HD. Iron-deficiency anemia in infants and young children with ferrous bisglycinate chelate. Nutr J. 2001;17(5):381-384.
16. Ramakrishan U, Goldenberg T, Allen LH. Do multiple micronutrient interventions improve child health, growth, and development? J Nutr. 2011;141:2066–2075.
17. Mahan LK, Escott-Stump S, Raymond JL. Krause's food and nutrition therapy. Edition 13. St Louis Missouri. 2012.
18. Sullivan KM, Mei Z, Grummer-Strawn L, et al. Hemoglobin adjustments to define anemia. Trop Med Intern Health. 2008;13(10):1267–1271.
19. Pendleton J. Ferrous bisglycinate. A gentle form of iron that is easier to absorb. 2008. http://vitamins-minerals.suite1oi.com/article.cfm/ferrousbisglycinate.
20. Partnership for child development (The World Bank, UNFPA, WFP,Unicef). School age children; their nutrition and health. 2002; www.schoolsandhealth.org.www.child-development.org.www.imperial.ac.uk/ (accessed on 12 April 2010).
21. Mason JB. The micronutrient report: Current progress and trends in the control of vitamin A, Iodine and Iron deficiencies. The micronutrient Initiative: Ottawa, Canada. 2001.
22. Viteri FE, Gonzalez H. Adverse outcomes of poor micronutrient status in childhood and adolescence. Nutr Rev. 2002;60 (Suppl):577–83.
23. Manger MS, Mc Kenzie JE, Winichagoon P, et al. A micronutrient fortified seasoning powder reduces morbidity and improves short term cognitive function, but has no effect on anthropometric measures in primary school children in North East Thailand: a randomized controlled trial. Am J Clin Nutr. 2008;87:1715.
24. Swart R, Sanders D, Mc Lachlan M. Nutrition: A primary health care perspective, 2002.
25. Lynch S. Iron metabolism in Nutritional anemia. Edited by Kraemer K and Zimmermann MB, Sight and Life Press, California. 2007.
26. Singh N. Role of micronutrients for physical growth and mental development. Indian J Pediatr. 2004;71 (1):59-62.

27. Aggett J, Agstonic C, Axelsson I, et al. Iron metabolism and requirement in early childhood: Do we know enough? ESPGHA Committee on nutrition. J Pediatr Gastro and Nutr. 20002;34:337-345.

28. WHO. World Health Report. Reducing risk, promoting healthy life. Geneva: WHO. 2002.

29. Zimmermann MB. Interactions in the etiology of anemia. Nutritional anemia. Sight and Life. 2007;119–213.

30. Shayeghi M, Latunde- Dada GO, Oakhil JS, et al. Identification of an intestinal heme transporter. Cell. 2005;122:789-801.

31. Qui A, Jansen M, Sakaris A, et al. Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption. Cell. 2006;127:917-28.

32. Gunshin H, Mackenzie B, Berger UV, GunshinY, et al. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature. 1997;388:482–8.

33. McKie AT, Barrow D, Latunde-Dada GO, et al. An iron-regulated ferric reductase associated with the absorption of dietary iron. Science. 2001;291:1755-59.

34. Nicolas G, Chauvet C, Viatte I. et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. J Clin Inves. 2002;110:1037-44.

35. Nemeth E, Valore EV, Territo M, et al. Hepcidin a putative mediator of anemia of inflammation is a type 11 acute phase protein. Blood. 2003;101:2461-63.

36. Skikne BS, Flowers CH, Cook JD. Serum transferring receptor: a quantitative measure of tissue iron deficiency. Blood. 1990;75:1870-76.

37. Stoltzufus R, Chwaya HM, et al. Epidemiology of iron deficiency and anaemia in Zanzibar school children: The importance of hookworms. Am J Clin Nutr. 1997;65:153 -9.

38. Falkingham M, Abdelhamid A, Curtis P, et al. The effects of oral iron supplementation on cognition in older children and adults: a systematic review and meta analysis. Nutr J. 2010;9:4

39. Nestel P, Bouis HE, Meenakshi JV, et al. Biofortification of staple food crops. J Nutr. 2006;136:1064–67.

40. Labadarios D. Estimating the burden of disease attributable to iron deficiency anemia in

South Africa in 2000. S Afr Med J. 2007;97.8:741–6.

41. Lynch SR. The impact of iron fortification on nutritional anemia. Best Pract Res Clin Haematol. 2005;18:333–46.

42. FAO/WHO. Requirements of vitamin A, iron, folate and B12. Report on joint FAO/WHO expects consultation. FAO Food Nutr Series no. 23 Rome FAO. 1988.

43. Hurrell RF. Preventing iron deficiency through fortification. Nutr Rev. 1997;55:210-222.

44. Partnership for Child Development. An association between chronic under nutrition and educational test score in Vietnamese children. Eur J Clin Nutr. 2001;55:801-804.

45. Partnership for Child Development. Short stature and the age of enrollment in primary school: Studies in two African countries. Social Science and Medicine. 1999;48:675–682.

46. Hallberg L, Hultén L, Gramatkovski E. Iron absorption from the whole diet in men: how effective is the regulation of iron absorption? Am J Clin Nutr. 1997;66:347–56.

47. Beutler E. How little we know about the absorption of iron. Am J Clin Nutr. 1997;66:419–20.

48. Partnership for Child Development. This wormy world: Fifty years on. Parasitology Today. 1997 (poster).

49. Chan MS. The global burden of intestinal nematode infections – fifty years on. Parasitology Today. 1997;13(11):438–443.

50. Bundy DAP. This wormy world – then and now. Parasitology Today. 1997;13(11):519–528.

51. World Bank. World development Report Investing in Health. Oxford: Oxford University Press. 1993.

52. Drake IJ, Bundy DAP. Multiple helminth infections in children: impact and control. Parasitology. 2001;122:S73–S81.

53. Brock C, Cammish N. Factors affecting female participation in seven countries. DFID. 1997.

54. Lwambo NJS, Bundy DAP, Medley GFH. A new approach to morbidity risk assessment in hook worm endemic communities. Epidemiol infection. 1992;108:469.

55. Beasley NMR, et al. The impact of population level deworming on the hemoglobin

levels of school children in Tang Tanzania. Tropical Medicine and International Health. 1999;4:744–750.

56. Albonico M, Stoltzfus RJ, Savioli L, et al. Epidemiological evidence for differential effect of hookworm species, Ancyclostoma duodenale or Necator amaricans, J Epidemiol. 1998;27:S30-S37.

57. May J, et al. Impact of sub patent multispecies and multiclonal plasmodial infections on anemia in children from Nigeria. Transactions of the Royal Society of Trop Med Hygiene. 2000;94:399-403.

58. Zimmermann MB, Wegmüller R, Zeder C, et al. Triple fortification of salt with microcapsules of iodine, iron and vitamin A. Am J Clin Nutr. 2004;80:1283–90.

59. Zimmermann MB, Adou P, Zeder C, et al. Persistence of goiter despite oral iodine supplementation in goitrous children with iron deficiency anemia in the Côte d'Ivoire. Am J Nutr. 2000;71:88–93.

60. Nga T, Winichagoon P, Dijkhuizen MA, et al. Multi-micronutrient–fortified biscuits decreased prevalence of anemia and improved micronutrient status and effectiveness of deworming in rural Vietnamese school children. J. Nutr. 2009;139:1013–1021.

61. Hinderaker SG, Olsen BE, Lie RT, et al. Anemia in pregnancy in rural Tanzania: associations with micronutrients status and infections. Eur J Clin Nutr. 2002;56:192–9.

62. Dijkhuizen MA, Wieringa FT, West CE, et al. Concurrent micronutrient deficiencies in lactating mothers and their infants in Indonesia. Am J Clin Nutr. 2001;73:786–91.

63. Muhilal, Permeisih D, Idjradinata YR, et al. Vitamin A-fortified monosodium glutamate and health, growth, and survival of children: a controlled field trial. Am J Clin Nutr. 1988;48:1271–6.

64. Mwanri L, Worsley A, Ryan P, et al. Supplemental vitamin A improves anemia and Interactions in the etiology of anemia growth in anemic school children in Tanzania. J Nutr. 2000;130:2691–6.

65. Suharno D, West CE, Muhilal, et al.Supplementation with vitamin A and iron for nutritional anemia in pregnant women in West Java, Indonesia. Lancet. 1993;342:1325-8.

66. Semba RD, Bloem MW. The anemia of vitamin A deficiency: epidemiology and pathogenesis. Eur J Clin Nutr. 2002;56:271–81.

67. Means RT, Jr. The anemia of infection. Baillieres Best Pract Res Clin Haematol. 2000;13: 151–162. Medicine. J Adolesc Health. 1999;24:461–2.

68. Powers HJ. Riboflavin (vitamin B-2) and health. Am J Clin Nutr. 2003;77:1352–60.

69. Foy H, Kondi A. A case of true red cell aplastic anemia successfully treated with riboflavin. J Pathol Bacteriol. 1953;65:559–64.

70. Foy H, Kondi A, Mbaya V. Effect of riboflavin deficiency on bone marrow function and protein metabolism in baboons. Preliminary Report. Br J Nutr 1964;18:307–18.

71. Sirivech S, Frieden E, Osaki S. The release of iron from horse spleen ferritin by reduced flavins. J Biochem. 1974;143:311–5.

72. Powers HJ. Riboflavin-iron interactions with particular emphasis on the gastrointestinal tract. Proc Nutr Soc. 1995;54:509–17.

73. Adelekan DA, Thurnham DI. The influence of riboflavin deficiency on absorption and liver storage of iron in the growing rat. Br J Nutr. 1986;56:171–9.

74. Butler BF, Topham RW. Comparison of changes in the uptake and mucosal processing of iron in riboflavin-deficient rats. Biochem Mol Biol Int. 1993;30:53–61.

75. Buzina R, Jusic M, Milanovic N, et al. The effects of riboflavin administration on iron metabolism parameters in a school going population. Int J Vitam Nutr Res. 1979;49:136–43.

76. Ajayi OA, Okike OC, Yusuf Y. Haematological response to supplements of riboflavin and ascorbic acid in Nigerian young adults. Eur J Haematol. 1990;44:209–12.

77. Charoenlarp P, Pholpothi T, Chatpunyaporn P, et al. The effect of riboflavin on the hematologic changes in iron supplementation of schoolchildren. Southeast Asian J Trop Med Public Health. 1980;11:97–103.

78. Suprapto B, Widardo, Suhanantyo. Effect of low-dosage vitamin A and riboflavin on iron-folate supplementation in anemic pregnant women. Asia Pac J Clin Nutr. 2002;11:263–7.

79. Powers HJ, Bates CJ. Micronutrient deficiencies in the aetiology of anemia in a rural area in the Gambia. Trans R Soc Trop Med Hyg. 1987;81:421–5.

80. Suboticanec K, Stavljenic A, Schalch W, et al. Effects of pyridoxine and riboflavin supplementation on physical fitness in young adolescents. Int J Vitam Nutr Res. 1990;60:81–8.

81. Lonnerdal B. Dietary factors influencing zinc absorption. J Nutr. 2000;130(5S):1378S–83S.

82. Solomons NW, Jacob RA. Studies on the bioavailability of zinc in humans: effects of heme and non heme iron on the absorption of zinc. Am JClin Nutr. 1981;34:475–82.

83. Sandstrom B, Davidsson L, Cederblad A, et al. Oral iron, dietary ligands and zinc absorption. J Nutr. 1985;115:411–4.

84. Crofton RW, Gvozdanovic D, Gvozdanovic S, et al. Inorganic zinc and the intestinal absorption of ferrous iron. Am J Clin Nutr. 1989;50:141–4.

85. Rossander-Hulten L, Brune M, Sandstrom B, et al. Competitive inhibition of iron absorption by manganese and zinc in humans. Am J Clin Nutr. 1991;54:152–6.

86. Fairweather-Tait SJ, Wharf SG, Fox TE. Zinc absorption in infants fed iron-fortified weaning food. Am J Clin Nutr. 1995;62:785–9.

87. Berger J, Ninh NX, Khan NC, et al. Efficacy of combined iron and zinc supplementation on micronutrient status and growth in Vietnamese infants. Eur J Clin Nutr. 2006;60(4):443–54.

88. Rosado JL, Lopez P, Munoz E, et al. Zinc supplementation reduced morbidity, but neither zinc nor iron supplementation affected growth or body composition of Mexican preschoolers. Am J Clin Nutr. 1997;65:13–9.

89. Munoz EC, Rosado JL, Lopez P, et al. Iron and zinc supplementation improves indicators of vitamin A status of Mexican preschoolers. Am J Clin Nutr. 2000;71:789–94.

90. Lind T, Lonnerdal B, Sherlund H, et al. A community based randomized controlled trial of iron and zinc supplementation in Indonesian infants: interactions between iron and zinc. Am J Clin Nutr. 2003;77:883–90.

91. Fischer Walker C, Kordas K, Stoltzfus RJ, et al. Interactive effects of iron and zinc on biochemical and functional outcomes in supplementation trials. Am J Clin Nutr. 2005;82:5–12.

92. Chanarin I. The megaloblastic anaemias. 2nd ed. Oxford: Blackwell Scientific Publications. 1979:1–3.

93. Scott JM. Nutritional anemia: B-vitamins: Nutritional anemia. 2007;111–131

94. Bresalki HK, Erhardt JG. Diagnosis of nutritional anemia- Laboratory assessment of iron status (Nutritional Anaemia), 2007.

95. Stoltzufus RJ. Update on issues related to iron deficiency and anemia control In: Labadarios D. Estimating the burden of disease attributable to iron deficiency anemia in South Africa in 2000. S Afr Med J. 2007;97.8:741–6.

96. Gibson RS. Principles of Nutritional Assessment. 2nd ed. New York: Oxford University Press, 2005.

97. WHO/UNICEF/UNU. Iron deficiency anemia assessment, prevention, and control. Geneva: World Health Organization, 2001.

98. Stoltzfus RJ, ML Dreyfuss. Guidelines for the Use of Iron Supplements to Prevent and Treat Iron Deficiency Anemia. International Nutritional Anemia Consultative Group. Washington, DC: International Life Sciences Institute Press, 1998.

99. United States Institute of Medicine. Nutrition During Pregnancy and Lactation. An Implementation Guide. Washington, DC: National Academy Press, 1992.

100. Baltussen R, Knai C, Sharan M. Iron fortification and iron supplementation are cost-effective interventions to reduce iron deficiency in four sub regions of the world. J Nutr. 2004;134:2678–84.

101. WHO/FAO. Guidelines on Food Fortification with Micronutrients. Eds. Allen L, de Benoist B, Dary O, Hurrell R. Geneva, WHO, 2006.

102. Laxminarayan R, Mills AJ, Breman JG, et al. Advancement of global health: key messages from the Disease Control Priorities Project. Lancet. 2006;367:1193–208.

103. Burger SE, Pierre-Louis JN. How to assess iron deficiency anemia and use of hemocue. Helen Keller International. 2002;17.

104. Cook JD. Diagnosis and management of iron deficiency anemia. Best Pract Res Clin Haematol. 2005;18(2):319–32.

105. Van Stuijvernberg ME. A biscuit fortified with iron, iodine and B-carotene as a strategy

to address micronutrient deficiencies in primary school children. Thesis; department of paediatircs: University of CapeTown, 2001.

106. Grantham-Mcgregor S, Ani C. Undernutrition and mental development. Nutrition and brain, Nestle Nutrition Worksop Series Clinical and Performance Programme. 2001;5:1–18.

107. Arcanjo FPN, Amanco OMS, Braga JAP etc. Randomized controlled trial of iron-fortified drinking water in Pre-school children. J Am Coll Nutr. 2010;29(2):122-29.

108. Government Gazette Republic of South Africa Regulation Gazette. Foodstuffs, cosmetics and disinfectants act 1972 Act No. 54 of 1972. Regulation relating to the fortification of certain food stuffs. 2003;No. 7634:vol 454, Pretoria 7.

109. Labadarios D, Steyn NP, Maunder E, et al. The National Food Consumption Survey (NFCS): Children aged 1- 9 in South Africa. 2000.

110. Sarma KVR, Udayakuma P, Balakrishna N et al. Effect of micronutrient supplementation on health and nutrition status of school children : growth and morbidity. Nutrition. 2006;22(suppl):8S–14S.

111. Bhandari N, Bahl R, Taneja S. Effect of micronutrient supplementation on linear growth of children. Br J Nutr. 2001;85:S131-S137.

112. Chwang L, Soemantri AG, Pollit E. Iron supplementation and physical growth of rural Indonesian children. Am J Clin Nutr. 1988;47:496–501.

113. Kruger M, Badenhorst CJ, Mansvelt EPG, et al. Effects of iron fortification in a school feeding scheme and anthelmintic therapy on iron status and growth of six to eight year old children. Food Nutr Bull. 1996;17:11–21.

114. Idjradinata P, Watkins WE, Pollit E. Adverse effect of iron supplementation on weight gain of iron–replete young children. Lancet. 1994;343:1252–1254.

115. Aguayo VM. School administered weekly iron supplementation – effect on growth and hemoglobin status of non –anemic Bolivian school- age children. A randomized placebo controlled trial. Euro J Nutr. 2000;39:263–269.

116. Allen LH, Ahluwalia N. Nutritional influences on linear growth. Euro J Clin Nutr. 1994; 48:S75–S79.

117. Kibel MA, Wagstaff LA. Child health for all: A manual for Southern Africa 2nd edition. Oxford University Press Southern Africa, Cape Town. 1995.

118. Hershko C. Iron and infection. In van Stuijverngerg ME. A biscuit fortified with iron, iodine and B-carotene as a strategy to address micronutrient deficiencies in primary school children. Thesis ; department of pediatrics: University of Cape Town. 2001;17–18.

119. Gera T, Sachdev HP. Effect of iron supplementation on incidence of infectious illness in children: systematic review. BMJ. 2002;325:1142.

120. Sazawal S, Black RE, Ramsan M, et al. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomized, placebo-controlled trial. Lancet. 2006;367:133–43.

121. WHO. WHO Statement: Iron supplementation of young children in regions where malaria transmission is intense and infectious disease highly prevalent. http://www.who.int/child-adolescent health/New_Publications/CHILD_HEALTH/WHO_statement_iron.pdf. 2007 (accessed April 8, 2010).

122. Solon FS, Sarol JN, Bernado Jr ABI, et al. Effect of a multiple-micronutrient fortified fruit powder beverage on the nutrition status, physical fitness and cognitive performance of school children in the Philipines. Food and Nutr Bull. 2003;24,4 (Suppl):S129–S140.

123. Shrestha RM. Effects of iodine and iron supplementation on physical, psychomotor and mental development in primary school children in Malawi. PhD thesis. Wageningen Agricultural University, Wageningen, Netherlands. 1994.

124. Pollit E, Hathirat P, Kotchabhakdi NJ, et al. Iron deficiency and educational achievement in Thailand. Am J Clin Nutr. 1989;50(3 Suppl):687–696.

125. Soemantri AG, Pollit E, Kim I. Iron deficiency anemia and educational achievement. Am J Clin Nutr. 1985;42(6):1221–1228.

126. Taras H. Nutrition and student performance at school. J School Health. 2005;75(6):199–13.

127. Gillespie S. Major issues in the control of iron deficiencies. The micronutrient initiative.

In: Partnership for child development (The world bank,UNFPA,WFP,Unicef). School age children; their nutrition and health.;www.schoolsandhealth.org.www.child-development.org.www.imperial.ac.uk/ 2002. (accessed on 12 April 2010).

128. Shisana O, Labadarios D, Rehle T, et al. South African National Health and Nutrition Examination Survey (SANHNES-1). 2013;Cape Town:HSRC Press.

129. ACC/SCN. Nutrition of the school age child. SCN News.1998;16:3–25.

130. World Bank. Focusing resources on effective school health: a FRESH start to improving the quality of education (http://wbln0018.worldbank.org/HDnet/Fresh/).2000.

131. Ramakrishnam U. Prevalence of micronutrient malnutrition worldwide. Nutr Rev. 2002;60:546-52.

132. Ash DM, Tatala SR, Frongillo EA, et al. Randomized efficacy trial of a micronutrient fortified beverage in primary school children in Tanzania. Am J Clin Nutr. 2003;77: 891–8.

133. Labadarios D, Swart R, Maunder EMW, et al. The National Food Consumption Survey-Fortification Baseline
(NFCS-FB-I): South Africa, 2005. S Afr J Clin Nutr. 2008;21(3)(Suppl 2):245-300.

134. Keskin Y, moschonis G, Dimitriou M, et al. Prevalence of iron deficiency among schoolchildren of different scio-economic status in urban Turkey. Eur J Clin Nutr. 2005;59:64-71.

135. Nestel P, Bouis HE, Meenakshi JV, et al. Biofortification of staple food crops. J Nutr. 2006;136:1064–67.

136. Lucca P, Hurrell R, Potrykus I. Genetic engineering approaches to improve the bioavailability and the level of iron in rice grains. Theor Appl Genet. 2001;102:392–97.

137. Samuelsen AI, Martin RC, Mok DW, et al. Expression of the yeast FRE genes in transgenic tobacco. J Plant Physiol. 1998;118:51–58.

138. World Health Organization. Guidelines on food fortification with micronutrients. Geneva: World Health Organization, 2006.

139. Marchetti M. Ashmead H, Tossani N et al. Comparison of the rates of vitamin degradation when mixed with metal sulphates or metal amino acid chelates. J Food

Compst Anal. 2000;13:875–884.

140. Hurrell R, Egli I. Optimizing bioavailability of iron compounds for food fortification in Nutritional anemia. Ed. Kraemer K and Zimmermann MB, Sight and Life Press, California. 2007.

141. Pineda O, Ashmead HD. Iron-deficiency anemia in infants and young children with ferrous bisglycinate chelate. Nutrition, 2001;17(5):381-384.

142. Bovell-Benjamin AC et al. Iron absorption from ferrous bisglycinate and ferric trisglycinate in whole maize is regulated by iron status. AJCN, 2000;71(6):1563-1569.

143. Pineda O et al.. Effectiveness of iron amino acid chelate on the treatment of iron deficiency anemia in adolescents. J Appl Nutr. 1994;46:1 & 2.

144. Layrisse M, Garcia-Casal MN, Solano L, et al. Iron bioavailability in humans from breakfasts enriched with iron bis-glycine chelate, phytates and polyphenols. J Nutr. 2000;130(9):2195-2199.

145. Hallberg L, Hulthen L. No advantage in using ferrous bisglycinate as an iron fortificant. Am J Clin Nutr. 2000;72:1583–93. (letter).

146. Pineda O. Heme and Fe bisglycinate share similar absorption properties. Am J Clin Nutr. 2003;78:493-7. (letter).

147. Lost C, Name JJ, Jeppsen RB, et al. Repleting hemoglobin in iron deficiency anemia in young children through liquid milk fortification with bioavailable iron amino acid chelate. J Am Coll Nutr. 1998;17:187–94.

148. Giorgini E, Fisberg M, De Paula RAC, et al. The use of sweet rolls fortified with iron bis-glycinate chelate in the prevention of iron deficiency anemia in preschool children. Arch Latinoam Nutr. 2001;51:48–53.

149. Miglioranza LH, Matsuo T, Caballero-Cordoba GM, et al. Effect of long-term fortification of whey drink with ferrous bisglycinate on anemia prevalence in children and adolescents from deprived areas in Londrina, Parana, Brazil. Nutrition. 2003;19:419–21.

150. Osman AK, Al-Othaimeen A. Experience with ferrous bis-glycine chelate as an iron fortificant in milk. Int J Vitam Nutr Res. 2002;72:257–63.

151. van Stuijvenberg ME, Smuts CM, Wolmarans P, et al. The efficacy of ferrous bisglycinate and electrolytic iron as fortificants in bread in iron-deficient schoolchildren. Brit J Nutr. 2006; 95:532–8.

152. ADA Reports. Position of the American Dietetic Association: Fortification and nutrition supplements. J Am Dietetic Association. 2005;105:1300–1311.

153. Allen L, Gillespie S. What works? A review of the efficacy and effectiveness of nutrition interventions. United Nations Administrative Committee on Nutrition, Asian Development Bank, September 2001, pp23-41.

154. Winichagon P, McKenzie JE, Chavasit V, et al. A multifortified seasoning powder enhances the hemoglobin, zinc and iodine status of primary school children in North East Thailand: A randomized controlled trial of efficacy. J Nutr. 2006;136:1617–23.

155. Soemantri AG. Preliminary findings on iron supplementation and learning achievement of rural Indonesian children. Am J Clin Nutr. 1989;50:698–702.

156. Sungthon R, Mo-suwan L, Cngsuvivatwong V, et al. Once weekly and 5 Days a week iron supplementation differentially affect cognitive function but not school performance in Thai children. J Nutr. 2004;134:2349–2354.

157. Roschinick N, Parawan A, Baylon MAB, et al. Weekly iron supplementation given by teachers sustain the hemoglobin concentration of school children in Philipines. Trop Med and International Health 2004;9(8):904-909.

158. Abrams SA, Mushi A, Hilmers DC, et al. A micronutrient fortified beverage enhances the nutritional status of children in Botswana. J Nutr. 2003; 133:1834–1840.

159. Gera T, Sachdev HP, Nestel P. Effect of combining multiple micronutrients with an iron supplementation on Hb response in children: Systematic review of randomized controlled trials. Public Health Nutr. 2008;12(6):756-773.

160. Pineda O. Heme and Fe bisglycinate share similar absorption properties. Am J Clin Nutr. 2003;78:493-7. (letter).

161. Friis H, Mwaniki D, Omondi B, et al. Effects on hemoglobin of multi –micronutrient supplementation and multi – helminth chemotherapy: a randomized controlled trial in Kenyan school children. Eur J of Clin Nutr. 2003;57:573–579.

162. Ayoya AM, Spiekermann-Brouwer GM, Traoere-A, et al. Multiple micronutrients including iron are not more effective than iron alone for improving hemoglobin and iron status of Malian school children. J Nutr. 2009;139:19721979.

163. Osei AK, Rosenberg IH, Huser RF, et al. Community-level micronutrient fortification of school lunch meals improved Vitamin A, Folate, and Iron status of schoolchildren in Himalayan villages of India. J of Nutr. 2010;140:1146-1154.

164. Jinabhai CC, Taylor M, Coutsoudis A, et al. A randomized controlled trial of the effect of antihelminthic treatment and micronutrient fortification on health status and school performance of rural primary school children. Annals of Tropical Paediatr. 2001;21:319–333.

165. Muthayya S, Eilander A, Transler C, et al. Effect of fortification with multiple micronutrient and n-3 fatty acids on growth and cognitive performance in Indian school children; the CHAMPION (Children's Health and Mental Performance Influenced by Optimal Nutrition) Study. Am J Clin Nutr. 2009;89:1766-75.

166. Kumar M, Rajagoplan S. Impact of a multiple – micronutrient food supplement on the nutritional status of school children. Food and Nutr Bull. 2006;27(3):203–211.

167. van Stuijvernberg ME, Kvalsvig JD, Faber M, et al. Effect of iron, iodine and B carotene fortified biscuits on the micronutrient status of primary school children: A randomized control trial. Am J Clin Nutr. 1999;69:497–503.

168. Taljaard C, Covic NM, van Graan AE, et al. Effects of multi-micronutrient-fortified beverage, with and without sugar, on growth and cognition in South African schoolchildren: a randomized, double blind, controlled intervention. Brit J Nutr. 2013; May:1-4.

169. WHO Anthro Plus software for the global application of the WHO References for 5 – 19 years to monitor growth of school age children and adolescents; 2007. http://www.who.int/growthref.

170. Bellany C. The state of the world's children-UNICEF. 2000 pp71 and Tables 1 and 2.

171. Scrimshaw NS. The challenge of global malnutrition to the food industry. Food Technol. February 1993;60–71.

172. Dean AG, Dean JA, Coulombier D, et al. Epi Info Version 6 : a word processing database, and statistics program for epidemiology on microcomputers. Centre for Disease Control and Prevention. ATLANTA, Georgia.USA. 1994.

173. Khumalo S. Mineral solubility and consumer acceptability testing of three flavoured enriched liquid maize based meal supplement. Hons dissertation, University of Pretoria, 2011.

174. FAO/WHO. Requirements of vitamin A, iron, folate and vitamin B12. Report of a joint consultation FAO Food Nutr Series No 23. Rome: FAO, 1988.

175. Sandstrom B. Micronutrient interactions; effect on absorption and bioavailability. Brit J Nutr. 2000;85,suppl2:S181–S185.

ADDENDUM 1: ETHICAL APPROVAL

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

* FWA 00002567, Approved dd 22 May 2002 and Expires 13 Jan 2012.
* IRB 0000 2235 IORG0001762 Approved dd Jan 2006 and Expires 13 Aug 2011.

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UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee
Fakulteit Gesondheidswetenskappe Navorsingsetiekkomitee

DATE: 20/11/2009

PROTOCOL NO.	**213/2009**
PROTOCOL TITLE	Comparison of the effect of three meal supplements on the cognitive performance of primary school children
INVESTIGATOR	**Principal Investigator:** Dr Z White
SUBINVESTIGATOR	Ms GJ Gericke
DEPARTMENT	**Dept:** Human Nutrition, Steve Biko Academic Hospital
	Phone:0123541993 **Fax:**0123541232 **Mobile:**0827382916 **E-Mail:** zelda.white@up.ac.za
SPONSOR	RESAF International Pty Ltd
VAT NO.	Not Applicable
POSTAL ADDRESS	P O Box 1946, Umhlanga Rocks, 4320, KZN, RSA
MEETING DATE	18 November 2009

This **Protocol** and **Informed Consent Document** were considered by the Faculty of Health Sciences Research Ethics Committee, University of Pretoria and approved by a quorum of committee members on 18/11/2009.

Members of the Research Ethics Committee:

Prof VOL Karusseit	MBChB; MFGP(SA); MMed(Chir); FCS(SA) - Surgeon
Prof JA Ker	MBChB; MMed(Int); MD – Vice-Dean (ex officio)
Dr NK Likibi	MBBCh – Representing Gauteng Department of Health)
Prof TS Marcus	(female) BSc(LSE), PhD (University of Lodz, Poland) – Social scientist
Dr MP Mathebula	(female) Deputy CEO: Steve Biko Academic Hospital
Prof A Nienaber	(female) BA(Hons)(Wits); LLB; LLM(UP); PhD; Dipl.Datametrics(UNISA) – Legal advisor
Mrs MC Nzeku	(female) BSc(NUL); MSc(Biochem)(UCL, UK) – Community representative
Snr Sr J Phatoli	(female) BCur(Eet.A); BTec(Oncology Nursing Science) – Nursing representative
Dr L Schoeman	(female) B.Pharm, BA(Hons)(Psych), PhD – Chairperson: Subcommittee for students' research
Mr Y Sikweyiya	MPH; SARETI Fellowship in Research Ethics; SARETI ERCTP; BSc(Health Promotion) Postgraduate Dip (Health Promotion) – Community representative
Dr R Sommers	(female) MBChB; MMed(Int); MPharmMed – **Deputy Chairperson**
Prof TJP Swart	BChD, MSc (Odont), MChD (Oral Path), PGCHE – School of Dentistry representative
Prof C W van Staden	MBChB; MMed (Psych); MD; FCPsych; FTCL; UPLM - **Chairperson**

DR R SOMMERS; MBChB; MMed(Int); MPharmMed.
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ADDENDUM 2: ASSENT FORM FOR 7-8 YEARS FOR CLINICAL TRIAL/INTERVENTION RESEARCH

ASSENT FORM FOR PROTOCOL TITLE: Effect of enriched maize-based liquid meal supplement on the cognitive performance of primary school children.

We wish to know if you would like to volunteer to be part of a research study in which you will consume a maize – based- ready- to- use meal supplement (let's call it a maize sip for short). We are asking you to help us to gather information on the effect of this on learning and concentration abilities (cognitive performance) and the amount of iron in the bodies of children. It will also tell us if it improves nutritional status in children like you.

About 160 children are going to take part in this study, and the study will last for 16 weeks over the first two school terms of the year. During that time you will have to consume one of two maize sips 5 days a week for the 16 week study period at a specific location at the school. The sip you will receive will be determined by chance like flipping a coin.

During the study you will undergo different kinds of procedures and tests. We will measure your weight and height and assess your learning and concentration abilities (cognitive performance). This will be done before and after the study period. At the beginning and end of the study, they will also take a tiny amount of blood (5 drops) from your finger. This may hurt, but will only take a minute. These tests will take about 3 hours in total but will only take place two times during the whole study.

You will receive deworming medication, 1 tablet, once off before the start of the study. The unpleasant effects that can occur after taking the medication may include: temporary stomach pain, diarrhea and vomiting, breaking out with a rash and hives, headaches and agranulocytosis (sudden fever, shaking and sore throat).We will give you a sickness diary in which you or your parents must record every time you are ill. It is very important that you tell your doctor, nurse or your parents if you don't feel well at anytime during the study.

If you do not want to take part anymore you may decide at anytime during the study to stop participating, no one will force you to carry on. No one will be cross or upset with you if you don't want to. You don't have to give us your answer now, take your time and read through the form again before you decide. If you sign at the bottom it will mean that you have read this paper and that you would like to be in this study.

	Your name	Person obtaining consent	Parent /Guardian as witness
Name			
Signature			
Date	15 July 2010		

ADDENDUM 3:

NAME BADGES FOR CHILDREN

(yellow)

NAME: _____

SUBJECT # :_____

(blue)

NAME: _____

SUBJECT # :_____

ADDENDUM 4: COMPLIANCE SHEET

COMPLIANCE			**JULY – NOV 2010**			**NAME:……** **……** **SUBJECT #:** **GRADE:……..**
	Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**COMMENTS**
WK1	**July 19**	**20**	**21**	**22**	**23**	
						……
WK2	**26**	**27**	**28**	**29**	**30**	**……**
						……
WK3	**2**	**3**	**4**	**5**	**6**	**……**
						……
WK4	**9**	**10**	**11**	**12**	**13**	**……**
	Public holiday					**……**
						……
WK5	**16**	**17**	**18**	**19**	**20**	**……**
						……
WK6	**23**	**24**	**25**	**26**	**27**	**……**
						……
WK7	**30**	**31**	**Sept. 1**	**2**	**3**	**……**
						……
WK8	**6**	**7**	**8**	**9**	**10**	**……**
						……
WK9	**13**	**14**	**15**	**16**	**17**	**……**
						……
WK10	**20**	**21**	**22**	**23**	**24**	**……**
					School holiday	**……**
	27	**28**	**29**	**30**	**Oct. 1**	**……**
	SCHOOL HOLIDAY					**……**
WK11	**4**	**5**	**6**	**7**	**8**	**……**
						……
WK12	**11**	**12**	**13**	**14**	**15**	**……**
						……
WK13	**18**	**19**	**20**	**21**	**22**	**……**
						……
WK14	**25**	**26**	**27**	**28**	**29**	**……**
						……
WK15	**Nov. 1**	**2**	**3**	**4**	**5**	**……**
						……
WK16	**8**	**9**	**10**	**11**	**12**	**……**
						……
						……

ADDENDUM 5:
SOCIO-DEMOGRAPHIC QUESTIONNAIRE

Subject no. ☐☐☐☐☐☐
Birth date: ☐☐☐☐☐☐
Interview date: ☐☐☐☐☐☐

Child's name……Gender: M ☐ F ☐
Address ……Religion…… ☐ ……. ☐ ……
……Mother's language……
Tel :(H)……(W)……
1. Relationship to child: Mother ☐ Father ☐ Grandparent ☐ Sibling ☐ Aunt/Uncle ☐ Other ☐
2.Household composition

Name of household members	Age (yrs)	Gender		Family Relationship to the child		Does this person eat and sleep at home at least 4 days a week?	
		M	F	Relationship	Code	Yes	No
Relationship (use as child reference): Father (1), Mother (2),Sibling (3),Grandmother (4), Grandfather(5), Aunt (6). Uncle (7), Cousin (8), Friend (9), Other (10)							

3. Marital status of mother (tick one)

1	2	3	4	5	6	7	8
Unmarried	Married	Divorced	Separated	Widowed	Living together	Traditional marriage	Other please specify

Tick one block only for every question	father	Mother	Sibling	grandma	Grandpa	uncle	Aunt	cousin	friend	Other
4. Who is mainly responsible for food preparation in the house	1	2	3	4	5	6	7	8	9	10
5. Who decides on what type of foods are bought for the household?	1	2	3	4	5	6	7	8	9	10
6. Who is mainly responsible for serving or feeding the child	1	2	3	4	5	6	7	8	9	10
7. Who is the head of this household?	1	2	3	4	5	6	7	8	9	10
8. Who decides how much is	1	2	3	4	5	6	7	8	9	10

spent on food?										

Now look at this child and tick one block of every question

9. Would you consider this to be a healthy child?	1 ……	2 ……	If no, specify
10. Is this child disabled?	1 ……	2 ……	If yes, specify

Now decide on the following (considering where the child lives)

11. Type of dwelling: **You can tick more than one block if necessary**	1 Brick concrete	2 Traditional Mud	3 Tin	4 Plank, wood	5 Other Specify	
12. number of people sleeping in the house for at least 4 nights per week?						
13. Number of rooms in the house (excluding bathroom, toilet and kitchen, if separate):						
14. Number of people per dwelling, living/sleeping **(tick one)**	1 0-2 persons	2 3-4 persons	3 More than 4 persons			
15. Where do you get drinking water from? **(tick one)**	1 own tap	2 Communal tap	3 River, dam	4 Borehole Well	5 Other specify	
16. What type of toilet does this household have? **(tick one)**	1 Flush	2 pit	3 bucket/ Pot	4 VIP	5 Other specify	
17. What fuel is used for cooking most of the time? **(you can tick more than one)**	1 electric	2 gas	3 paraffin	4 wood	5 sun	6 Open fire

Tick one box only:

18. Does the child's home have a working (i) Refrigerator/freezer	1 Fridge	2 Freezer	3 Both	4 None
(ii) Stove	1 Yes	2 No	If yes choose one …… Gas, coal, electric	If yes choose one …… With oven without oven
(iii) Primus or paraffin stove	1 Yes	2 No		
(iv) Microwave	1 Yes	2 No		
(v) Hot plate	1 Yes	2 No		
(vi) Radio or Television	1 Radio	2 TV	3 Both	4 None

Now ask questions about:

19. Education level of mother **(tick only one)**	1 None	2 Primary school	3 Std 6 -8	4 Std 9 – 10	5 Tertiary educatio n	6 Don't know			
20. Mother's employment status **(choose one)**	1 House wife by choice	2 Unempl oyed	3 Self-employed	4 Wage earner	5 Other specify	6 Don't know			
21. Education level of caregiver **(Tick only one)**	1 None	2 Primary school	3 Std 6 -8	4 Std 9 – 10	5 Tertiary educatio n	6 Not applicabl e			
22. Father's employment status **(can tick more than one)**	1 Unemployed	2 self employ ed	3 wage earner	4 retired by choice	5 other specify	6 not applicabl e e.g. deceased			
23. How many people contribute to the total income **(Tick one only)**	1 1 person	2 2 persons	3 3-4 persons	4 5-6 persons	5 Over 6 persons				
24. Household income per month (including wages ,rent, sales of veg. etc, state grants **(Tick only one)**	1 None	2 R100-R500	3 R500-R1000	4 R1000-R3000	5 R3000-R5000	6 Over R5000	7 Don't know		
25. Is this the usual income of the household? **(Tick one box only)**	1	2	If NO what other income is available? Specify						
26. Is this more or less the income you had over the past six months**? (Tick one only)**	1 Yes	2 No							
27. How much money is spent on food weekly? **(Tick one only)**	1 R0-R50	2 R50-R100	3 R100-R150	4 R150-R200	5 R200-R250	6 R250-R300	7 R300 R350	8 R350-R400	9 Over R400	10 Don't know

ADDENDUM 6

SICKNESS DIARY

NAME: _____ _____ SUBJECT #:_____

Condition			Medication			
	Start date	End date	Name	Dosage & frequency	Start date	End date
E.g. Cough	6 Feb 2010	8 Feb 2010	BRONCLEER	5ml 3 times a day	6 Feb 2010	8 Feb 2010