A BISCUIT FORTIFIED WITH IRON, IODINE AND ß-CAROTENE AS A STRATEGY TO ADDRESS MICRONUTRIENT DEFICIENCIES IN PRIMARY SCHOOL CHILDREN

MARTHA ELIZABETH VAN STUIJVENBERG

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DECLARATION

This thesis is presented in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) in the Department of Paediatrics, Faculty of Health Sciences, University of Cape Town. The work on which this thesis is based is the author's original research except as stated in the acknowledgements, and has not, in whole or in part, been submitted for another degree at this or any other university. The university is empowered to reproduce all or part of the contents for the purpose of research.

Signed by candidate

15 August 2001

Martha Elizabeth van Stuijvenberg

Date

To my husband

" of the making of many books there is no end,
and much study wearies the body "

Ecclesiates 12:12

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ABSTRACT

Deficiencies of vitamin A, iron, and iodine continue to be prevalent in developing countries worldwide and can, in addition to the classic consequences such as nutritional anaemia, goitre, cretinism, xerophthalmia and blindness caused by severe deficiencies, also affect the growth, development and immunity of young children. The various internationally acknowledged strategies for combating micronutrient deficiencies include high-dose supplementation, food fortification, dietary diversification and nutrition education. The aim of this research was to evaluate a micronutrient-fortified biscuit as a strategy to address micronutrient deficiencies in primary school children from a poor rural community.

The research comprised three phases. During the first phase the effect of a biscuit fortified with iron, iodine, and ß-carotene on the vitamin A, iron and iodine status of 115 children was evaluated and compared with 113 controls, in a randomised placebo-controlled trial. To enhance the absorption of iron a vitamin C-fortified cold drink was given together with the biscuit. Anthropometric status, cognitive function and morbidity were assessed as secondary outcomes. The 12-month intervention resulted in a significant improvement in serum retinol, serum ferritin, transferrin saturation, haemoglobin and urinary iodine excretion. Morbidity and cognitive function, particularly the cognitive function in the children presenting with low iron status and with goitre at baseline, were also favourably affected. Linear growth was positively affected only in the children with marginal iron stores at baseline.

During the second phase of this study the long-term effectiveness of the biscuit programme, in terms of elimination of micronutrient deficiencies, compliance, acceptability and sustainability, was evaluated in a longitudinal study over a period of 30 months. In addition, cross-sectional data on vitamin A and iron status from subsequent studies conducted in the same school at 33, 42 and 45 months after the start of the original biscuit intervention, during which time the fortified biscuit continued to be distributed at the school, are reported. Although micronutrient status improved significantly during the 12 months of the first study, all variables (except urinary

iodine) returned to pre-intervention levels when the schools reopened after the summer holiday. Serum retinol increased again during the next nine months, but was significantly lower in a subsequent survey, carried out directly after the summer holiday; this pattern was repeated in two further cross-sectional surveys. Iron status showed no recovery during a subsequent intervention period when the vitamin C-fortified cold drink was supplied on a less frequent basis, or during the period that ferrous bisglycinate was used as iron fortificant. Because of the compulsory iodisation of salt, that came into effect halfway through the first phase of the study, improved iodine status, as measured by urinary iodine excretion, was maintained.

In the third phase of the research, red palm oil, a rich natural source of ß-carotene, was examined as an alternative vitamin A fortificant in the biscuit. This study contained elements of both a randomised placebo-controlled trial and an equivalence trial. The biscuit with a red palm oil-based shortening was shown to be as effective as the biscuit with ß-carotene from a synthetic source in improving the vitamin A status of these children.

In conclusion, the results of the studies described in this thesis showed that a micronutient-fortified biscuit is a feasible, practical and effective way of improving the micronutrient status of primary school children from a poor rural community. Long-term evaluation of this programme, however, showed that improved micronutrient status is not sustained during the long summer school holidays, and it is suggested that the biscuit programme is supplemented with other strategies, such as local food production programmes and nutrition education. Red palm oil, with all of its additional qualities (i.e. no *trans* fatty acids; rich source of antioxidants), appears to be an attractive alternative for use as a vitamin A fortificant. The choice of the iron compound to be used in the biscuit, however, needs further investigation.

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ABBREVIATIONS

CRP	C-reactive protein
epg	eggs per gram
EDTA	sodium iron ethylenediamine tetra acetic acid
FAO	Food and Agriculture Organization
HPLC	high performance liquid chromatography
ICCIDD	International Council for Control of Iodine Deficiency Disorders
IDD	iodine deficiency disorders
MI	Micronutrient Initiative
MRDR	modified relative dose response
NCHS	National Center for Health Statistics
RCT	randomised controlled trial
RDA	Recommended Dietary Allowance
RDR	relative dose response
TIBC	total iron binding capacity
TS	transferrin saturation
UNICEF	United Nations Children's Fund
UNU	United Nations University
WHO	World Health Organization

Chapter 1

GENERAL INTRODUCTION

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GENERAL INTRODUCTION

1.1 INTRODUCTORY COMMENTS

Micronutrient deficiencies, particularly of iron, iodine and vitamin A, continue to be prevalent in developing countries worldwide and, apart from the classic consequences such as nutritional anaemia, goitre, cretinism, xerophthalmia and blindness, caused by severe deficiencies, they can also affect the growth, development and immunity of young children.

In 1990 world leaders gathered at the World Summit for Children in New York (United Nations, 1990). The meeting issued a declaration on the survival, protection and development of children, and a plan of action for the 1990s. The plan included seven major goals and 26 supporting sectoral goals, of which three relate specifically to micronutrient malnutrition. They were: (i) the virtual elimination of vitamin A deficiency; (ii) the virtual elimination of iodine deficiency; and (iii) the reduction of iron deficiency anaemia in women by one-third. These goals were subsequently endorsed at the "Ending Hidden Hunger" Policy Conference on Micronutrient Malnutrition held in 1991 in Montréal (WHO/UNICEF, 1991), and also at the 1992 International Conference on Nutrition in Rome (FAO/WHO, 1992).

Deficiencies of vitamin A, iron and iodine are also prevalent in South Africa, and exist in both preschool (South African Vitamin A Consultative Group, 1996) and school-aged children (Lamparelli *et al.*, 1988; Benadé *et al.*, 1997; Sickle *et al.*, 1998; Oelofse *et al.*, 1999) at levels that require intervention.

There are several internationally acknowledged strategies for addressing micronutrient deficiencies. These strategies include micronutrient supplementation, food fortification, dietary diversification and nutrition education (Ramalingaswami, 1992). Although nutrition education and dietary diversification offer long-term solutions and are the most sustainable, they may take decades to show an effect. Micronutrient supplementation, on the other hand, is an immediate

solution to acute nutritional deficiencies. Food fortification offers a solution in both the medium and long term and can be aimed at a population in general or targeted at specific segments of a population. All strategies therefore have a role to play, and should complement each other, rather than stand on their own. For fortification to be successful, it is important that a suitable vehicle is identified; it is also desirable that the feasibility and efficacy of a planned fortification strategy is evaluated in a scientific trial.

1.2 AIM AND OBJECTIVES

The aim of the research described in this thesis was to determine the feasibility and impact of a micronutrient-fortified biscuit as a strategy to address deficiencies of vitamin A, iron and iodine in primary school children from a poor rural community.

Specific objectives were:

(i) To determine the efficacy of a biscuit fortified with iron, iodine and ß-carotene in improving the iron, iodine and vitamin A status of the study population;

(ii) To determine the effect of the fortified biscuit on the cognitive function of the study population;

(iii) To determine the effect of the fortified biscuit on the anthropometric status of the study population;

(iv) To determine the effect of the fortified biscuit on morbidity patterns of the study population;

(v) To evaluate the long-term effectiveness of the fortified biscuit intervention over a period of 3.75 years;

(vi) To examine the use of red palm oil as an alternative source of ß-carotene in the biscuit.

1.3 THESIS OUTLINE

An overview of the literature on the state of knowledge with regard to micronutrient deficiencies and the available strategies to overcome these deficiencies, with special emphasis on fortification, is given in Chapter 2. The general methods employed in the execution of the various studies described in this thesis are set out in Chapter 3. Chapter 4 describes a randomised placebo-

controlled trial which evaluated the effect of a biscuit, fortified with iron, iodine and ß-carotene, on the micronutrient status, cognitive function, morbidity and anthropometric status of primary school children. The long-term effect of this biscuit, over a period of approximately four years, with regard to maintaining improved micronutrient status, compliance, acceptability and sustainability, is examined in Chapter 5. In Chapter 6 the use of red palm oil as an alternative source of ß-carotene in the biscuit is examined in a randomised controlled trial, and also compared with ß-carotene from a synthetic source. A general discussion of the results, as well as the conclusions, is presented in Chapter 7.

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Chapter 2

REVIEW OF THE LITERATURE

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REVIEW OF THE LITERATURE

The micronutrients discussed in this review will be restricted to the three that were included in the end-decade goals of the World Summit for Children (United Nations, 1990) and in the interventions described in this thesis, i.e. vitamin A, iron and iodine. First, the magnitude, causes and consequences of the deficiencies of each of these three micronutrients will be discussed individually. This will then be followed by a brief overview of the various strategies available for overcoming these deficiencies, whereafter an indepth overview of food fortification as a strategy to combat micronutrient deficiencies will be given.

2.1 VITAMIN A DEFICIENCY

2.1.1 Magnitude

It is estimated that 2.8 and 250 million preschool-age children worldwide suffer from clinical and subclinical vitamin A deficiency, respectively (WHO/UNICEF,1995). In South Africa, according to the national survey of the South African Vitamin A Consultative Group (1996), 33% of children aged 6-71 months are subclinically deficient in vitamin A (serum retinol < 20 μg/dL), with the prevalence being higher in the rural than in urban areas (38% *vs* 25%, respectively). Bitot's spots were found in 0.8% of the children, and 3.3% had serum retinol concentrations below10 μg/dL. Although there are no data at national level on the vitamin A status of South African school-aged children, there are indications that the problem also exists in this age category (Sickle *et al.*, 1998; Oelofse *et al.*, 1999).

2.1.2 Causes of vitamin A deficiency

(i) Insufficient intake and low bioavailability

Insufficient intake of vitamin A is the primary cause of vitamin A deficiency. In the industrialised world vitamin A is mostly derived from animal products as preformed vitamin A. In developing countries, however, where the intake of animal products is limited, individuals largely rely on plant sources for their intake of vitamin A (FAO/WHO, 1988). Diets in these countries are often

monotonous, consisting mainly of traditional staples such as rice, maize, cassava, potatoes, millet or sorghum, all of which are relatively devoid of provitamin A activity (Solomons, 1995). Up until recently it was assumed that 6 µg ß-carotene from a mixed diet was necessary to provide 1µg of retinol (FAO/WHO, 1988). The bioavailability of ß-carotene from vegetable sources has, however, in recent years been challenged. A study by De Pee *et al.* (1995) showed a significant increase in serum retinol concentrations in lactating women given a wafer fortified with ß-carotene, but no increase in the group that received a portion of dark-green leafy vegetables, providing the same amount of ß-carotene. A subsequent study (De Pee *et al.*, 1998) showed the apparent effectiveness of yellow fruit and dark-green leafy vegetables in improving vitamin A status to be respectively only 50% and 23% of the value that was previously assumed, suggesting bioconversion factors of 12:1 and 26:1 for yellow fruit and dark-green leafy vegetables, respectively. West (2000) suggests a bioconversion factor of 21:1, assuming that the ratio of provitamin A intake from dark-green leafy vegetables to that from yellow/orange fruits is 4:1. It is thought that the ß-carotene from dark-green leafy vegetables is poorly absorbed, because it is trapped in a complex matrix within the chloroplasts of the plant cell; the ß-carotene in orange fruit, on the other hand, may be more accessible, because it is found in the lipid droplets and chromoplasts (De Pee *et al.*, 1995).

Apart from the food matrix, ß-carotene absorption is also influenced by other factors, such as the amount of ß-carotene present in a meal, the concurrent intake of fat (a minimum amount of 3-5g dietary fat is needed in a meal to ensure intestinal carotene uptake), genetic factors, and the presence of gastrointestinal infections and parasites causing malabsorption (De Pee *et al.*, 1996; Roodenburg *et al.*, 2000).

(ii) Infection burden

Another factor contributing to vitamin A deficiency is the burden of repeated infections. Serum retinol has been shown to be depressed during infections (Buyukgebiz *et al.*, 1990; Filteau *et al.*, 1993). This may in part be attributed to a decrease in the carrier protein, retinol binding protein, which acts like a negative acute-phase reactant, and thereby causes a transient shift in retinol from

the circulation to tissues (Mitra *et al.*, 1998a). However, loss of vitamin A from the body also occurs during states of infection. Campos *et al.* (1987) reported a deterioration, not only in serum retinol, but also in liver stores, as measured by the relative dose response test, in children during an acute outbreak of chickenpox. An increase in urinary retinol excretion has been observed during acute episodes of infection accompanied by fever (Alvarez *et al.*, 1995; Mitra *et al.*, 1998b), and repeated infections may therefore be an important contributor to vitamin A depletion.

(iii) Parasitic infestation

Evidence from several studies suggest that infestation with *Ascaris lumbricoides* and *Giardia lamblia* interferes with the uptake of vitamin A (Mahalanabis *et al.*, 1979; Marinho *et al.*, 1991). A recent study by Jalal *et al.* (1998) reported improved vitamin A status in children receiving anthelmintic therapy, but only in those with a high initial load of *Ascaris* infestation (egg counts > 3 200 epg). *Ascaris* infection is thought to alter the structure of the intestinal mucosa and also reduces the absorption of dietary fat, which is a necessary factor in the absorption of vitamin A (Jalal *et al.*, 1998).

2.1.3 Consequences of vitamin A deficiency

(i) Xerophthalmia

The clinical manifestation of vitamin A deficiency is xerophthalmia, a collective term for a series of ocular changes associated with deteriorating vitamin A status. These abnormalities range from night blindness in its mildest form through to corneal ulceration and in its severest form to permanent blindness. It is estimated that 350 000 children worldwide are blinded every year as a result of vitamin A deficiency (Whitcher et al, 2001).

(ii) Effect on mortality and morbidity

The effect of vitamin A deficiency on infectious morbidity and mortality, even at subclinical level, is well documented. Beaton *et al.* (1993) conducted a meta-analysis on the results of eight community trials, evaluating the effect of vitamin A supplementation on morbidity and mortality, and found the overall reduction in all-cause mortality to be 23%; results from a meta-analysis by

Glasziou & Mackerras (1993) suggest a reduction of 30% in all-cause mortality. Morbidity conditions affected by vitamin A deficiency are particularly that of diarrhoea, respiratory disease and measles (Hussey & Klein, 1990; Sommer & West, 1996). Ross (1996) has suggested two mechanisms to explain the protective action of vitamin A against infection. The first is the *epithelial barrier hypothesis* where vitamin A is necessary for the structural integrity of the epithelial membrane, thus protecting the body against invasion by pathogens; intervention with vitamin A therefore will result in a decreased *incidence* of infection in deficient children. The second is the *immunologic response hypothesis* where vitamin A deficiency decreases the body's defence against invading pathogens at immunological level. It has been shown for example that children with xerophthalmia have underlying abnormalities in T-cell subsets, which are reversed when they are supplemented with vitamin A (Semba *et al.*,1993). The immunologic response mechanism is involved once invasion by pathogens has occurred, and intervention with vitamin A is expected to result in a decrease in the *duration* and/or *severity* of the infection.

(iii) Effect on growth

Animal studies from as far back as the early 1900s have shown that vitamin A is a necessary factor for growth (McCollum & Davis, 1913). It is, however, difficult to demonstrate the impact of vitamin A on growth in human beings, because it would not be ethical to experimentally induce a state of deficiency in children. Vitamin A deficiency also often co-exists with deficiencies of other growth-limiting nutrients, and is also frequently accompanied by infections, which in turn may influence vitamin A status. Results on the effect of vitamin A deficiency on growth in humans are therefore often conflicting (Ramakrishnan *et al.*, 1995). However, data from recent trials involving vitamin A supplementation suggest that vitamin A deficiency does impair growth, but only at severe levels of deficiency, i.e. where xerophthalmia is present (West *et al.*, 1997), or in children with serum retinol concentrations below 10 µg/dL (Hadi *et al.*, 2000).

(iv) Effect on iron metabolism

The role of vitamin A in iron metabolism has received increased attention following the classic study by Hodges *et al.* in 1978. In this study vitamin A deficiency was experimentally induced

in eight healthy male volunteers. Despite an adequate intake of iron, five of the subjects developed a mild anaemia, which did not respond to iron therapy, but improved only when the vitamin A deficiency was corrected. Several subsequent studies have demonstrated either an association between vitamin A and iron status (Mejía *et al.*, 1977; Bloem *et al.*, 1989), or an improvement in iron status following vitamin A supplementation (Mejía & Arroyave, 1982; Bloem *et al.*, 1990). In two randomised controlled trials, anaemic children (Mejía & Chew, 1988) and anaemic pregnant women (Suharno *et al.*, 1993) were supplemented with either vitamin A alone, iron alone, a combination of vitamin A and iron, or a placebo for a period of two months. In both studies the iron and haematological indicators responded to all three non-placebo treatments, but the best response was obtained when vitamin A and iron were given simultaneously. Although the mechanism involved is not completely understood, it is thought that vitamin A may be necessary for the mobilisation of iron from storage depots, resulting in an increase in serum iron, and thereby favouring haematopoiesis (Bloem, 1995). There are indications that vitamin A may also influence the absorption of iron. Recent studies suggest that vitamin A and ß-carotene promote iron absorption by preventing the inhibitory effect of phytates and polyphenols (Gracía-Casal *et al.*, 1998; Layrisse *et al.*, 2000). Regardless of the mechanism, however, this interaction between vitamin A and iron is an important factor that should also be considered when iron intervention strategies are planned.

(v) Effect on cognitive performance

Although vitamin A deficiency may not affect cognition and learning ability of the schoolchild directly, it may do so indirectly via its impact on resistance to infections, which in turn may have an effect on school attendance and consequently performance. Vitamin A deficiency may also affect cognition and performance via its effect on iron metabolism (Sommer & West, 1996).

2.2 IRON DEFICIENCY

2.2.1 Magnitude

Iron deficiency is the most common nutritional deficiency in both the industrialised and developing world and affects more than 3.5 billion people around the world

(UNICEF/UNU/WHO/MI, 1999), with approximately 1 billion being anaemic. The most affected groups are women of child-bearing age, especially pregnant women, as well as infants and school-aged children. The prevalence in developing countries is three to four times higher than in industrialised countries, with the prevalence of anaemia in African preschool children ranging from 42% to 53% (WHO, 1998). In South Africa, according to a national survey, 21% of children between the age of 6 and 71 months are anaemic, using haemoglobin < 11 g/dL as a cut-off (South African Vitamin A Consultative Group, 1996). There are no national data on the prevalence of iron deficiency in primary school-aged children, but several studies suggest that the problem does exist (Lamparelli *et al.*, 1988; Kruger *et al.*, 1996; Oelofse *et al.*,1999).

2.2.2 Causes of iron deficiency

(i) Iron content of the diet

Iron can be taken in in the form of either haem or non-haem iron. Haem iron, which comprises 10-15% of food iron consumed in industrialised countries, is contained in the haemoglobin and myoglobin in animal foods. Non-haem iron, contained in cereals, pulses, fruits and vegetables comprises the major and often only source of dietary iron in developing countries (FAO/WHO, 1988).

(ii) Low bioavailability

Adequate iron nutriture, however, depends not only on the iron *content* of the diet, but also, and to a much greater extent, on the *bioavailability* of the iron from the diet. Iron bioavailability is defined as the amount of ingested iron which is absorbed and utilised for metabolic functions (Hurrell, 1997a). On average, only 5-15% of the iron consumed is absorbed by the body. Haem iron is more bioavailable than non-haem iron, and absorption of iron from diets containing large quantities of meat, fish or poultry may be as high as 20-30%; iron absorption from diets consisting entirely of cereals can be as low as 1-2% (FAO/WHO, 1988).

The bioavailability of iron in the diet depends on the balance between factors that stimulate and inhibit the absorption of iron, especially that of non-haem iron (Hallberg, 1981). Absorption of

iron is also inversely related to the size of body iron stores (Baynes & Bothwell, 1990). Inhibitors of iron absorption bind iron in insoluble complexes which make it unavailable for absorption, while enhancers of iron absorption reduce iron from insoluble ferric iron (Fe^{3+}) to its more soluble ferrous form (Fe^{2+}), and may also bind the iron in soluble complexes, making it available for absorption (Hurrell, 1997a). The major inhibitors and enhancers of iron absorption are discussed below.

Inhibitors of iron absorption

Phytic acid: Phytic acid (inositol hexaphosphate) is widely present in cereals, nuts, seeds and legume seeds, and has a dose-dependent inhibitory effect on iron absorption (Hallberg *et al.*, 1989). Phytates accumulate under the bran of cereal grains and can be reduced during milling, fermentation, germination or baking (Allen & Ahluwalia, 1997). It has been shown that removal of phytates in bran by endogenous phytase can increase iron absorption ~3.5 times (Hallberg *et al.*, 1987). In legumes, such as soy, phytic acid is found in the body of the endosperm, and a four- to five-fold increase in iron absorption has been demonstrated when phytic acid was enzymatically removed from a soy protein isolate (Hurrel *et al.*, 1992).

***Polyphenols*:** Polyphenols are particularly high in beverages such as tea, coffee, herb teas, cocoa and red wine, with the tannins in black tea being the most potent inhibitor. Disler *et al.* (1975) showed the mean absorption of iron from a meal of rice with potato and onion soup to be 2.5% when taken with tea, compared to 10.8% when the meal was taken with water. One cup of coffee reduces iron absorption from a hamburger meal by 39% compared to a 64% reduction in absorption with tea (Morck *et al.*, 1983). Cook *et al.* (1995) showed that iron absorption from white wine, containing a small quantity of polyphenols, was two to three times higher than the absorption from red wine, which contains 10 times as much polyphenols.

Calcium: Calcium can inhibit iron absorption when fed as an organic calcium compound or when consumed in dairy products such as milk or cheese. The inhibitory effect of calcium added to wheat roles was found to be dose-related over the range 40 to 300 mg, with the absorption of

iron being reduced by 60% when 300 mg calcium was added, but with no further increase in inhibition at higher levels of calcium (Hallberg *et al.*, 1991). Giving 165 mg calcium as milk or cheese with a wheat roll reduced iron absorption by 57% and 46%, respectively (Hallberg *et al.*, 1991). The level of inhibition also depends on the size and composition of a meal, and is more pronounced in small simple meals. In complex meals, the combined effect of all the enhancers and inhibitors present may mask the potential inhibitory effect of calcium (Hurrell, 1997a). Haem iron absorption also appears to be inhibited by calcium. Hallberg *et al.* (1993) showed that calcium reduced the absorption of haem and non-haem iron to the same extent, and suggests that the mechanism is calcium interfering with the transport of iron through the mucosal cell.

Protein: During digestion, food proteins are transformed into peptides which can bind iron in the intestinal lumen and influence its absorption. Peptides can both inhibit or enhance iron absorption depending on their nature (Hurrel, 1997a). Soy protein, egg albumin and milk casein are important inhibitors of iron absorption. The inhibitory effect of soy protein has been observed, even after removal of virtually all the phytic acid (Hurrel *et al.*, 1992).

Enhancers of iron absorption

Ascorbic acid: Ascorbic acid is the most potent enhancer of iron absorption, both in its natural form in fruit and vegetables (Ballot *et al.*, 1987), and when added as a free compound. Cook & Monsen (1977) have shown that iron absorption from a semisynthetic meal, containing 4.1 mg non-haem iron, was directly proportional to the amount of ascorbic acid added to the meal; over a range of 25 to 1000 mg of ascorbic acid added, iron absorption progressively increased from 0.8% to 7.1%. Ascorbic acid, at high enough concentrations, may also overcome the inhibitory effect of phytic acid in cereals (Hallberg *et al.*, 1989; Siegenberg *et al.*, 1991) and in soy formula (Davidson *et al.*, 1994a), and can partially overcome the effect of polyphenols from tea (Disler *et al.*, 1975; Siegenberg *et al.*, 1991). The higher the content of inhibitors in a meal, the more pronounced the effects of ascorbic acid on iron absorption. The bioavailability of iron fortification compounds is also increased by ascorbic acid (Hurrell, 1992).

Ascorbic acid is thought to enhance the absorption of iron by promoting acid conditions in the stomach so that dietary iron is efficiently solubilised, by reducing ferric iron to ferrous iron, by forming chelates with iron in the stomach, and by maintaining the solubility of non-haem iron when the food enters the alakaline environment of the small intestine (Allen & Ahluwalia, 1997). Ascorbic acid is, however, easily oxidised when exposed to air, heat and humidity, and when stored under suboptimal conditions, especially if packaging materials are not air-tight; these factors may limit its use as an enhancer of iron absorption in food fortification (Davidsson *et al.*, 2001).

Recent studies suggest that the effect of vitamin C on iron absorption is exaggerated when iron absorption is measured from single meals compared to complete diets (Hunt *et al.*, 1994; Cook & Reddy, 2001). The reason for this is not clear, but may in part be due to the effect of dietary inhibitors also concurrently present in the diet (Cook & Reddy, 2001).

Muscle tissue: Beef, veal, lamb, chicken, fish, pork and liver have an enhancing effect on iron absorption which appears to be related to the high level of cysteine-containing proteins in these tissues. The enhancing effect of cysteine-containing peptides on iron absorption could be explained by their potential to chelate iron, and to reduce ferric iron to the more soluble ferrous iron (Hurrell, 1997a). Muscle tissue also appears to enhance the absorption of haem iron. The bioavailability of haem iron in foods prepared from blood, but given without meat, was found to be much lower than if given together with meat (10% *vs* 25%; Hallberg, 1981).

Other organic acids: Organic acids, such as citric acid, malic acid, tartaric acid, and lactic acid also have an enhancing effect on iron absorption. Ballot *et al.* (1987) demonstrated a significant correlation between iron absorption from a rice meal and the citric acid content of various fruits added to the meal; the enhancing effect of citric acid was additive to that of ascorbic acid.

Prediction of iron bioavailability

Iron bioavailability is determined by the net effect of all the inhibitors and enhancers of iron

absorption in the diet. Over time several attempts have been made to develop models to estimate the bioavailabilty of the dietary iron content in meals (Monsen *et al.*, 1978; Murphy *et al.*, 1992). The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have separated typical meals in various regions of the world into three broad categories (i.e low, intermediate and high bioavailability) and assigned an average percentage of iron absorption to each category (FAO/WHO, 1988):

- The *low bioavailability diet* has an iron absorption of approximately 5%, and is a simple, monotonous diet containing cereals, roots and/or tubers and negligible quantities of meat, fish or ascorbic acid-rich foods. This diet contains a preponderance of foods that inhibit iron absorption (maize, beans, wholewheat flour, sorghum, etc.) and is dominant in many developing countries, particularly among lower socio-economic groups.

- The *intermediate bioavailability diet* has an iron absorption of approximately 10%, and consists mainly of cereals, roots and/or tubers and negligible quantities of food of animal origin and/or ascorbic acid. A low bioavailability diet can be converted to this level by increasing the intake of foods which enhance iron absorption, such as ascorbic acid-rich foods, meat and fish. Similarly, a high bioavailability diet can be reduced to this intermediate level by the regular consumption of meals containing higher amounts of inhibitors of iron absorption, such as tea or coffee.

- The *high bioavailability diet* has an iron absorption of appoximately15%, and is a diversified diet containing generous quantities of meat, poultry, fish, and/or foods containing high amounts of ascorbic acid, and is typical for most segments of the populations in industrialised countries.

The 5%, 10% and 15% absorption values refer to non-anaemic individuals with no iron stores. If anaemia is present, absorption would be increased by 50%, i.e. to 7.5%, 15% and 22.5% absorption for the *low*, *intermediate*, and *high bioavailability* categories, respectively.

A recent model developed by Hallberg & Hulthén (2000) suggests an algorithm for calculating the absorption and bioavailability of dietary iron for each meal, taking into account the quantitive effects of eight dietary factors known to inhibit or enhance iron absorption (i.e. a phytate factor, an ascorbic acid factor, a polyphenol factor, a calcium factor, a meat factor, a soy protein factor, an egg factor, and an alcohol factor). For each factor an equation describing the dose-effect relation was derived; interactions between individual factors were also considered. The amount of iron absorbed from the whole diet was obtained by summing the amounts of iron absorbed from all the single meals for a certain period of time. The authors found that iron absorption from the complete diet, estimated by using this algorithm, agreed well with measured iron absorption using radioiron tracers.

(iii) Parasitic infestations

Iron status is affected by infestation of both hookworm (*Ancylostoma duodenale* and *Necator americanus*) and *Trichuris trichiura*. Hookworms cause chronic intestinal blood loss by attaching themselves to the mucosa of the upper small intestine, ingesting blood and tissue, and changing their feeding site every 4-6 hours. Blood loss occurs both from ingestion by the worm and through bleeding from the damaged mucosa; a moderate hookworm infestation can approximately double the iron requirement of a non-pregnant women (Stoltzfus *et al.*, 1997a). Highly significant relationships between haemoglobin levels and intensity of hookworm infestation has been documented (Layrisse & Roche, 1964; Stoltzfus *et al.*, 1997b; Brooker *et al.*, 1999); however, a parasite egg load below 2 000 eggs per gram of stool does not appear to affect haemoglobin status (Stoltzfus *et al.*, 1997a). Iron loss from *Trichuris trichiura* infestation is approximately one-tenth of the iron loss caused by hookworm infestation (Stoltzfus *et al.*, 1997a), and an association between *Trichuris* infestation and anaemia is found only at infestation loads of more than 10 000 eggs per gram of stool (Ramdath *et al.*, 1995). *Ascaris lumbricoides* infestation does not appear to have an effect on iron absorption (İşlek *et al.*, 1993), but in some studies has been associated with slightly reduced haemoglobin levels (Stoltzfus *et al.*, 1997b). Several studies have demonstrated a positive impact of anthelmintic treatment on haemoglobin concentrations (Beasley *et al.*, 1999; Stephenson *et al.*, 1985). The greatest effects of deworming on iron status

are, however, seen in trials where anthelmintic therapy is combined with increased dietary iron (Kruger *et al.*, 1996; Gilgen & Mascie-Taylor, 2001).

2.2.3 Consequences of iron deficiency

The final stage of iron deficiency is iron deficiency anaemia, a condition where there are not enough red blood cells to transport oxygen to the tissues, and which is characterised by low haemoglobin concentrations. In its severest form, iron deficiency anaemia can cause increased risk of child (Brabin *et al.*, 2001a) and maternal mortality (Brabin *et al.*, 2001b). Iron deficiency, however, also adversely affects other metabolic processes, such as electron transport, catecholamine metabolism, DNA synthesis, and several enzyme systems (Baynes & Bothwell, 1990), which may lead to deficits in functional outcomes such as work performance and child development. The impact of iron deficiency on child development, particularly the schoolchild, is discussed below.

(i) Effect on cognitive development and function

An association between haemoglobin concentrations and measures of cognitive development, motor development or school achievement has been found in several correlational and case-control studies (Grantham-McGregor & Ani, 2001). Longitudinal studies also consistently demonstrate that children who were anaemic in early childhood continue to have poorer cognition and school achievement in later childhood (Cantwell, 1974; Lozoff *et al.*, 1991; Hurtado *et al.*, 1999.) Lozoff *et al.* (2000) re-evaluated children treated for iron deficiency during infancy in a longitudinal follow-up study after a period of more than 10 years. Although the children were free of iron deficiency and growing normally at that stage, those who had chronic and severe anaemia in infancy scored lower on tests measuring mental and motor functioning, performed worse at school, and tended to experience more anxiety/depression, social problems, and attention problems. Both anaemia and poor cognitive development are, however, associated with low socioeconomic status, and the possibility that the latter may confound results prevents causal relationships from being concluded from longitudinal studies (Grantham-McGregor & Ani, 2001).

A causal relationship between iron deficiency and cognitive development has, however, been suggested by the results of several randomised controlled trials, which also suggest that there are some aspects of impaired cognition that can be reversed by short-term iron treatment. A significant improvement in achievement test scores in iron deficient, anaemic schoolchildren given an iron supplement was shown in a three-month trial carried out in Indonesia by Soemantri *et al.* (1985). Groner *et al.* (1986) showed a significant improvement in short-term memory in young pregnant women (aged 14-24 years) receiving a vitamin supplement plus iron for one month, compared with a group receiving vitamins only. Seshadri & Gopaldes (1989) described four iron supplementation studies carried out in Indian schoolchildren and found a suggestion of treatment effect in all four studies. Shrestha (1994) showed a significant increase in fluid intelligence in a group of 6-8-year-old Malawian schoolchildren supplemented with iron for a period of 10 months. Even in non-anaemic, iron deficient children iron supplementation appears to have a positive effect on cognitive function. Bruner *et al.* (1996) conducted an eight-week randomised controlled trial on non-anaemic, iron deficient adolescent girls, and found a significant improvement in verbal learning and memory. Not all studies, however, showed positive results. Pollitt *et al.* (1989) failed to show an improvement in educational achievement in Thai schoolchildren receiving iron supplementation for a 16-week period; an improvement in haemoglobin was, however, also observed in the placebo group (probably secondary to deworming) and might have confounded their results. In other randomised controlled trials that failed to demonstrate significant treatment effects, the sample sizes were small, i.e. < 30 subjects per treatment group (Pollitt *et al.*, 1985; Soewondo *et al.*, 1989).

(ii) Effect on immune function

The role of iron deficiency in immune function is controversial. On the one hand, iron is required for microbial growth. Several studies have shown increased morbidity from malaria and from other infections with oral administration of therapeutic doses of iron (Oppenheimer, 2001). During infection there is an increased rate of ferritin synthesis and a reduction in serum iron levels. This may be a defence mechanism of the body to prevent microbial access to iron during infections (Hershko, 1992). Iron deficiency, on the other hand, is also associated with an

increased incidence of infections (Oppenheimer, 2001). Abnormalities in cell-mediated immunity, in neutrophil function, and in the secretory response of macrophages during iron deficiency have also been described (Baynes & Bothwell, 1990). The role of iron in infection is thus far from clear. Hershko (1992), however, suggests that both iron deficiency and iron excess may result in impaired immunological function, and that the optimum condition probably is a normal iron status which will allow a full phagocytic and immune response to pathogens.

Implication for the schoolchild:

Increased susceptibility to infections as a result of iron deficiency in the schoolchild may lead to lower school attendance rates, and consequently compromised performance.

(iii) Effect on growth

The results of various studies suggest that iron may have an effect on the growth of young children (Judisch *et al.*, 1966; Aukett *et al.*, 1986; Angeles *et al.*, 1993). The beneficial effect of iron supplementation on growth has also been demonstrated in the school-aged child. Chwang *et al.* (1988) observed a significant increase, compared with an anaemic control group, in weight, height and arm circumference in 8-13-year-old anaemic children treated with iron for a period of 12 weeks. Lantham *et al.* (1990) also showed a significant improvement in weight gain in Kenyan schoolchildren receiving iron for 15 weeks, though observed no effect on linear growth. A South African study by Kruger *et al.* (1996) showed that iron fortification combined with anthelmintic therapy in primary school children had a significantly greater effect on both the height-for-age and weight-for-age Z-scores in a subgroup of children with low iron stores at baseline.

Adverse effects of iron supplementation have, however, been reported in children who are iron-replete. Idjradinata *et al.* (1994) studied the effect of iron supplementation (3 mg/kg/day) on the growth rate of iron-sufficient children, aged 12-18 months, over a period of four months, and found the rate of weight gain to be significantly lower than in the placebo group, receiving no iron. In the study by Kruger *et al.* (1996), however, no such effect was observed in 6-8-year-old iron-replete children, receiving 20 mg of iron per day. Aguayo (2000) also observed no negative

effect on growth of weekly iron supplementation in non-anaemic school-age children. Lack of consistency among studies, according to Allen (1994), may be due to the co-existence of deficiencies of other growth-limiting nutrients in the same children, difference in study duration periods, different doses of iron, different age groups, and varying degrees of iron deficiency.

2.3 IODINE DEFICIENCY

2.3.1 Magnitude

The term iodine deficiency disorders (IDD) is used to collectively describe the whole spectrum of conditions resulting from iodine deficiency. They can range from cretinism and severe mental retardation at one end of the scale to only mild forms of motor and cognitive deficits without any clinical signs at the other end (Hetzel, 1983). Globally 740 million people are affected, and more than 2 billion are estimated to be at risk of IDD (WHO/UNICEF/ICCIDD, 1999). The majority of countries in Africa has IDD, with the central parts of the continent being affected most (Lamberg, 1993). In South Africa, goitre, the visible sign of iodine deficiency, has been reported in certain geographical "pockets" for many years (Steyn *et al.*, 1955; Benadé *et al.*, 1997; Jooste *et al.*, 1997). Before 1995, salt in South Africa was iodised on a voluntary basis (less than one-third of table salt was iodised), and many segments of the population were not reached (Jooste *et al.*, 1995). Mandatory iodisation of salt came into effect in December 1995, and the effectiveness of this programme is currently being monitored. Jooste *et al.* (2000) reported significantly improved urinary iodine levels in a group of schoolchildren from a previously endemically goitrous area, but no reduction in the prevalence of goitre after one year of mandatory salt iodisation. The situation continues to be monitored.

2.3.2 Causes of iodine deficiency

(i) Lack of iodine in the environment

Inadequate intake of iodine is the main cause of iodine deficiency. Iodine does not occur naturally in specific foods; it is present in the environment, and the iodine content of foods depends on the iodine content of the soil in which they are grown. In areas where the soil is lacking in iodine, especially mountainous areas and areas subject to high rainfall and frequent flooding, locally

produced foods will not provide enough iodine, and populations living in such environments are likely to be iodine deficient. Sea water, on the other hand, contains adequate amounts of iodine, and people living near the sea and those eating sea fish and other sea products are less likely to be iodine deficient (Mannar & Dunn, 1995; WHO, 1996). Because there are no specific foods, other than sea products, that contain iodine, iodine deficiency cannot be corrected by changing dietary habits; iodine has to be supplied from an external source, either via supplementation with iodised oil, or by fortifying food items or condiments that are regularly consumed (Mannar & Dunn, 1995).

(ii) Intake of goitrogenic substances

Goitre sometimes occurs in the presence of adequate iodine intake, and can be caused by the excessive intake of goitrogenic substances. Goitrogens act by blocking the uptake of iodine by the thyroid gland, and occur naturally in foods such as cabbage, turnips, brussels sprouts, sweet potatoes, maize, lima beans, bamboo shoots, millet and cassava, and also in the drinking water in some areas. They are present in small quantities and do not normally pose a major risk. The exception, however, is when goitrogenic foods are consumed as staples, like for example, cassava which is a staple food in many regions in Africa (Gaitan, 1990; Lamberg, 1993; WHO, 1996).

2.3.3 Consequences of iodine deficiency

Iodine deficiency is the leading preventable cause of intellectual and neurological impairment in the world today (Ramalingaswami,1992). Iodine is required for the synthesis of thyroid hormones, which are involved in regulating metabolic activities of all cells throughout the life cycle. With inadequate intake of iodine the secretion of thyroid stimulating hormone is increased, resulting in overstimulation and hyperplasia of the thyroid, which visibly manifests as goitre. Iodine also plays a key role in cell replication, including the replication of brain cells, which multiply mainly *in utero* and during the first two years of life. Adequate iodine status is therefore of crucial importance during this stage, and inadequate intake may result in irreversible damage. In its severest form iodine deficiency leads to cretinism, which is characterised by severe mental retardation, deaf mutism, spastic diplegia, and dwarfism. It can also lead to increased rates of

abortion, stillbirths, congenital abnormalities, and neonatal and infant mortality (Hetzel, 1983; Delange, 1994; WHO, 1996; Cobra *et al.*, 1997). The implications of milder forms of iodine deficiency, particularly for the schoolchild, are discussed below.

(i) Impaired mental and psychomotor development

Iodine deficiency does not have to be severe to affect mental ability; less severe iodine deficiency can also lead to impaired mental and psychomotor development, with the level of impairment depending on the degree of deficiency. Boyages *et al.* (1989) demonstrated that even mild iodine deficiency without any clinical signs can reduce IQ scores in schoolchildren by 10-15%. Tiwari *et al.* (1996) studied the effect of prolonged iodine deficiency on learning and motivation in schoolchildren and found the severely iodine-deficient children to be slower learners and less motivated to achieve than mildly iodine deficient children. A study in a South African rural community from an endemically goitrous area showed that the schoolchildren with goitre scored consistently worse in their Zulu (the local language) examination papers than those with no goitre (Benadé *et al.*, 1997). The long-term consequences of a low iodine status during foetal development was illustrated by Pharoah *et al.* (1984), who showed that cognitive and motor performance was depressed in children aged 10-12 years who were born to women known to have been iodine deficient during pregnancy.

(ii) Reversible consequences

Although damage due to iodine deficiency early in life is usually irreversible, there are studies that suggest that iodine supplementation in later childhood may still have beneficial effects on mental and psychomotor development. A study by Bautista *et al.* (1982) in 5-12-year-old children from a goitrous area, showed no significant difference in IQ scores between those children receiving iodised oil and those that did not; this was probably because, for some unknown reason, iodine status also improved in the control group. However, the authors did find a significant correlation between reduction in goitre size and improvement in IQ score for the group as a whole, which suggests that correction of iodine deficiency may have an effect on mental performance in school-aged children. A double-blind placebo-controlled trial in Malawi showed

a significant improvement in various aspects of mental and psychomotor development in 6-8-year-old primary school children supplemented with iodised oil (Shrestha, 1994). Van den Briel *et al.* (2000) showed a significant association between improvement in iodine status and mental performance in 7-11-year-old schoolchildren from Benin supplemented with iodised oil. The above studies suggest that improved mental performance as a result of iodine supplementation is to some extent still possible in children of school-going age. Whether there is an age threshold beyond which restoration of mental performance is no longer possible is not known. The effect of iodine supplementation does, however, appear to be less pronounced in older children (Van den Briel *et al.*, 2000).

2.4 STRATEGIES FOR ADDRESSING MICRONUTRIENT DEFICIENCIES

The different internationally acknowledged strategies for combating micronutrient deficiencies, i.e. micronutrient supplementation, food fortification, and dietary diversification, are schematically presented in **Figure 2.1**. All three strategies have an important role to play. They should not stand on their own, but should rather be seen to complement each other. Indirect strategies to address micronutrient deficiencies include improved public health measures, such as improved sanitation, infection control, parasite control and immunisation coverage (Ramalingaswami, 1992; Stoltzfus *et al.*, 1997a).

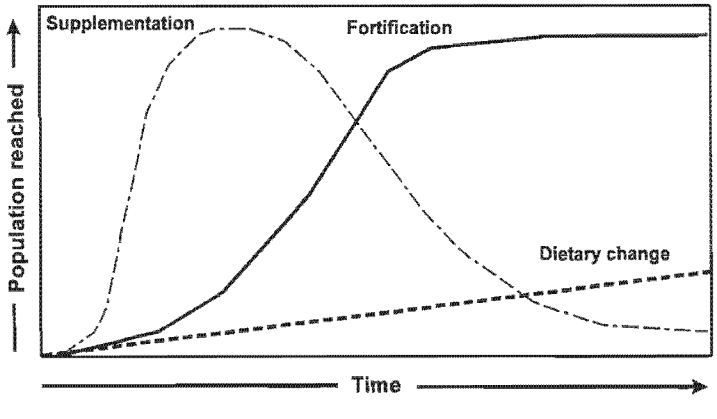

Figure 2.1: Strategies for the eradication of micronutrient deficiencies other than iodine (Adapted from "Forum on Food Fortification, Ottawa, Canada, 1995")

2.4.1 Supplementation

Supplementation is a solution to acute nutritional deficiencies, and can produce immediate results while longer-term strategies are being developed. An example is the periodic distribution (every 4-6 months) of high-dose vitamin A capsules (50 000 - 200 000 IU) to children below the age of six years, as well the distribution of a single dose (200 000 IU) to mothers within eight weeks of delivery. Capsule distribution is usually linked to immunisation programmes, but can also be delivered through community-based outreach programmes. High dose vitamin A supplementation can reduce the incidence of both corneal and non-corneal xerophthalmia by 90%. Vitamin A supplementation programmes are, however, only effective if they are sustained and if the coverage rate exceeds 85%, and it is often the children with the highest risk that have the lowest coverage (Ramalingaswami, 1992; Sommer and West, 1996).

Iron supplementation has been the key strategy for short-term control of iron deficiency and is primarily targeted at pregnant women attending antenatal clinics, but is also advocated for young children (Viteri, 1997). Unfortunately, iron supplementation programmes are seldom effective. The main reasons for this are insufficient supply of iron tablets, low coverage of the target population, and poor compliance with tablet intake. Schultink *et al.* (1993) for example, showed compliance with tablet intake in pregnant Indonesian women to be only 36%. The main reason for poor compliance usually is the undesirable gastrointestinal side-effects that are experienced. An alternative approach that has emerged in the last few years is that of weekly iron supplementation instead of a daily dose. Weekly supplementation was shown to be as effective as daily supplementation in improving iron status (Ridwan *et al.*, 1996; Muslimatun *et al.*, 2001), and fewer side-effects compared to daily supplementation have also been reported (Angeles-Agdeppa *et al.*, 1997). Weekly supplementation is less expensive and may therefore be easier to sustain (Gross *et al.*, 1997). However, whether weekly supplementation is effective in terms of coverage and long-term compliance at programmatic level remains to be demonstrated (Yip,1996). A meta-analysis on the subject by Beaton & McCabe (1999) concludes that, although both daily and weekly iron supplementation appear to be efficacious, there is no reason to expect that weekly supplementation would be more effective than daily supplementation in programme

settings; the authors recommend that, until the latter has been proven, pregnant women continue to be supplemented on a daily basis.

Supplementation with iodine is usually in the form of an iodised oil, given either orally or by intramuscular injection. The effect of a single injection lasts three to five years, and the effect of the oil taken orally is approximately two years. Iodised oil is used as an interim measure in at-risk populations that do not have access to iodised salt, and also as an emergency measure in populations where severe iodine deficiency and cretinism are present. Target groups are infants and women of child-bearing age (WHO, 1996).

2.4.2 Food fortification

Food fortification has been successfully used to control micronutrient malnutrition in industrialised countries for over 50 years, and has also become a feasible option for developing countries. It offers a direct, effective, inexpensive and sustainable way to control micronutrient deficiencies in both the medium and long term. A main advantage of food fortification is that it does not require the active co-operation of the individual. Large segments of a population can be reached, including those not reached by supplementation programmes e.g., school-aged children and non-pregnant women of child-bearing age. Fortification can, however, also be targeted at sections of a population that are most at risk (Cook & Reusser, 1983; Hurrell, 1997b). Food fortification will be discussed in more detail in **Section 2.5**.

2.4.3 Dietary diversification

Dietary diversification is a long-term strategy and should be the ultimate goal of any initiative to control micronutrient deficiencies. Nutrition education is an essential component of this strategy and must go beyond simply creating an awareness; the awareness must also be converted into action. However, because change in dietary behaviour takes time, this strategy should be complemented with other approaches that give more immediate results, such as supplementation or food fortification. The dietary diversification strategy is particularly relevant to vitamin A and iron, and should focus on the production and consumption of vitamin A-rich foods, and on

increasing the bioavailability of iron in the diet by increasing the intake of enhancers and limiting the intake of inhibitors in meals. Promotion of breastfeeding and improvement of maternal nutrition are also of critical importance (Ramalingaswami, 1992).

An example of a successfully applied dietary diversification strategy is the Bangladesh homestead gardening programme, which increased the availability and consumption of vitamin A-rich foods by promoting gardening throughout the year, and also by increasing the varieties of fruits and vegetables produced. This programme has been expanded from a pilot programme into a national programme, and currently reaches more than 700 000 households (Talukder *et al.*, 2000).

Iodine deficiency in populations living in iodine-depleted environments cannot be corrected by dietary diversification, and iodine has to be supplied to such populations from an external source, either through supplementation with iodised oil, or through fortification of a commonly eaten food (Mannar & Dunn, 1995).

2.4.4. Genetically modified foods

A relatively novel strategy to combat vitamin A deficiency is the production of genetically modified crops with a high ß-carotene content. An example is genetically engineered "golden" rice which has a carotenoid content of 1.6 µg/g in the endosperm (Ye *et al.*, 2000). Genetically modified foods could become an important complementary strategy to accelerate progress in eliminating vitamin A deficiency (Reddy, 2000). Doubts regarding the ability of this approach to overcome vitamin A deficiency have, however, also been expressed (Nestle, 2001).

2.5 FOOD FORTIFICATION

The are two major challenges with regard to food fortification. The first is identifying an appropriate food product to be fortified (fortification vehicle), and the second is selecting an appropriate fortificant, i.e. one that is both bioavailable, and does not cause organoleptic changes in the vehicle to be fortified.

2.5.1 Criteria for selecting a fortification vehicle

The aim of fortification is to add the micronutrient to a dietary item (food or condiment) that is regularly consumed by the targeted population at a level that will correct an existing dietary deficiency without posing risks of overdosing those who habitually consume large quantities of the fortified product (Sommer & West, 1996). When selecting the vehicle for fortification there are several criteria that should be considered. A panel of the US National Academy of Sciences has put forth a list of such criteria, which is summarised below (Solomons, 1995).

- The vehicle should be consumed by most of the target population.
- There should be little variation in the day-to-day per capita consumption of the vehicle.
- The fortification should have little effect on the organoleptic characteristics and acceptability of the vehicle.
- The added nutrient should be stable under proper conditions of storage and use.
- The nutrient should be biologically available from the vehicle.
- There should be no significant change in the cost of the fortified food.
- Fortification should be economically feasible through an industrial process.
- There should be reasonable assurance against excessive intake.

2.5.2 Examples of vehicles in literature

The selection of a vehicle will depend largely on whether the fortification is aimed at the population as a whole, or whether targeted at certain segments of a population. If fortification is targeted at national level a food that is consumed by most of the population should be used as a vehicle for fortification. Because food consumption patterns vary from country to country, each country should identify its own appropriate vehicles. A survey by Melse-Boonstra *et al.* (2000) in remote areas of Indonesia, for example, identified monosodium glutamate and salt as food items that are consumed on a daily basis by almost all households, whereas consumption of fortified noodles was related to socio-economic status, being the highest in those with better socio-economic status. In South Africa, according to The National Food Consumption Survey (2000), maize, sugar and bread were consistently consumed in most households, and were thus

identified as possible vehicles for micronutrient fortification.

Food vehicles that have been used for iron fortification include cereal products (Cook & Reusser, 1983; Hurrell, 1997b), sugar (Viteri *et al.*, 1995), fish sauce (Garby & Areekul, 1974), curry powder (Ballot *et al.*, 1989), and infant formulas (Hurrell, 1997b). Foods that are coloured and have strong tastes e.g., fish sauce or curry powder, are particularly suitable for iron fortification because they permit the use of more reactive iron compounds (Nestel, 1993). Vehicles that have been used for vitamin A fortification include margarine (Solon *et al.*, 1996), soybean oil (Dutra-de-Oliveira *et al.*, 1998), dairy products (Figueira *et al.*,1969), sugar (Arroyave *et al.*, 1981), monosodium glutamate (Muhilal *et al.*, 1988), noodles (Chavasit & Tontisirin, 1998; Melse-Boonstra *et al.*, 2000), wheat flour (Solon *et al.*, 2000), other grain products (Rubin *et al.*, 1977), tea (Brooke & Cort, 1972), and formula foods. Salt is the vehicle most commonly used for iodine fortification and has been successfully applied in many countries (Van der Haar, 1997). The feasibility of other vehicles, such as sugar (Eltom *et al.*, 1995), water (Pandav *et al.*, 2000) and bread (Clements, 1970) has, however, also been examined.

For targeted fortification, a food preferentially consumed by the at-risk group should be chosen as a vehicle (Hurrell,1997b). Infants, for example, are most commonly targeted via infant formulas and commercial infant cereals. Preschool children can be targeted by fortifying food items specifically attractive to that age group. In a recent study by Sari *et al.* (2001) iron-fortified candies were used to improve the iron status in preschool children. School feeding programmes offer excellent opportunities for targeting school-aged children, and have the additional benefit of regulated intake of the fortified food. Food items that can serve as potential carriers in schoolchildren include chocolate flavoured drinks (Hurrell *et al.*, 1991), cookies (Walter *et al.*, 1993), and soup (Kruger *et al.*, 1996). Specific cultural groups can also be targeted for example, a curry powder fortified with iron had been used to improve the iron status of an Indian community in South Africa (Ballot *et al.*, 1989). It is more difficult to find a vehicle that is specifically aimed at non-pregnant women of child-bearing age, and this group will most probably only be reached by fortification that is targeted at the population as a whole.

2.5.3 Fortificants

(i) Compounds used for iron fortification

Finding the ideal iron fortification compound remains a challenge. The selection of a fortificant represents a compromise between a choice of chemically reactive compounds that cause organoleptic changes in the vehicle to which they are added, but are of high bioavailability, and inert compounds, which do not cause organoleptic changes, but are poorly absorbed (Bothwell,1999). Optimisation is therefore necessary, which means selecting the iron compound with the highest potential absorption without causing subsequent organoleptic problems in the food vehicle (Hurrell, 1997b). **Table 2.1** gives the bioavailability, as well as other characteristics, of the various iron compounds most commonly used for food fortification (Hurrell, 1997b). The compounds with the highest bioavailability are those that are soluble in water or in dilute acid, and therefore also in gastric juice. Ferrous sulphate is used as the gold standard and has been given an arbitrary value of 100. The bioavailability of all other compounds are measured and expressed relative to that of ferrous sulphate.

Ferrous sulphate

A disadvantage of the more soluble iron compounds, such as ferrous sulphate, is that they can react with substances in foods, causing discolouration of the fortified product. Off-flavours can also develop due to the metallic taste of the soluble iron itself, particularly in beverages. A further problem is that these compounds promote fat oxidation and can therefore significantly reduce the shelf life of the products being fortified. Ferrous sulphate is a relatively inexpensive compound, and is widely used to fortify infant formulas, pasta and cereal flour that are stored for short periods (Hurrell, 1997b).

Ferrous fumarate

Ferrous fumarate has the same bioavailability as ferrous sulphate (Hurrell *et al.*, 1989), but because it is less soluble in water it is less reactive, and associated with fewer organoleptic changes when used as a fortificant in foods. Ferrous fumarate is only slightly more expensive than ferrous sulphate, and has been suggested for use in infant cereals (Hurrell *et al.*, 1989) and

Table 2.1: Characteristics of iron compounds commonly used to fortify food.

	Approximate iron content (%)	Average relative bioavailability[a]		Approximate relative cost[a]
		Rat	Man	
Freely water soluble				
Ferrous sulphate $7H_2O$	20	100	100	1.0
Dried ferrous sulphate	33	100	100	0.7
Ferrous gluconate	12	97	89	5.1
Ferrous lactate	19	-	106	4.1
Ferric ammonium citrate	18	107	-	2.1
Poorly water soluble/ soluble in dilute acid				
Ferrous fumarate	33	95	100	1.3
Ferrous succinate	35	119	92	4.1
Ferric saccharate	10	92	74	5.2
Water insoluble/ poorly soluble in dilute acid				
Ferric orthophosphate	28	6-46	25-32	4.1
Ferric ammonium orthophosphate	19	-	30-60	-
Ferric pyrophosphate	25	45-58	21-74	2.3
Elemental iron powders:				
electrolytically reduced	98	44-48	5-100	0.5
carbonyl reduced	98	39-66	5-20	1.0
hydrogen reduced	97	24-54	13-148	0.2
Protected compounds				
NaFeEDTA	14	-	28-416	6.0
Haemoglobin	0.34	-	100-700	-

[a] Relative to ferrous sulphate $7H_20$

Source: Hurrell, 1997b

chocolate drink powders (Hurrell *et al.*, 1991).

Poorly soluble compounds

The compounds that are poorly soluble in both water and dilute acid e.g., ferric orthophosphate, ferric pyrophosphate, and elemental iron powders, does not dissolve in gastric juice, and are therefore the least bioavailable. However, these compounds produce few organoleptic changes and are therefore the most often used compounds in food fortification. Elemental iron powders are also the least expensive. Their relative bioavailability can vary between 5 and 148%, depending on particle size, surface area, porosity, and the manufacturing process (Roe & Fairweather-Tait, 1999). The bioavailability of electrolytically reduced iron with a small particle size (< 10 μm) was, however, shown to be similar to that of ferrous sulphate (Rios *et al.*, 1975).

Protected compounds

The bioavailability of all iron fortification compounds is subjected, to the same extent as the iron naturally present in food, to the influence of inhibitors or enhancers of iron absorption. Many vehicles contain substances that can inhibit absorption e.g., phytic acid in cereals, calcium and casein in milk, and polyphenols in chocolate drinks. There are, however, several methods by which fortification iron can be protected from absorption inhibitors; these include the addition of *ascorbic acid*, the use of chelated iron compounds such as *NaFeEDTA* or *ferrous bisglycinate*, and the use of *haemoglobin* or dried blood.

Addition of ascorbic acid: Ascorbic acid is a potent enhancer of iron absorption and can increase iron absorption several-fold (see "*Enhancers of iron absorption*" under **section 2.2.2**). A maximum absorption of iron from infant formula fortified with ferrous sulphate was obtained at an ascorbic acid-to-iron ratio of 13:1 (Stekel *et al.*, 1986). Ascorbic acid is, however, not very stable and special packaging material may be required to protect the added vitamin C from degradation during storage (Hurrell, 1997b). The use of ascorbic acid in fortification may therefore not always be practical, and an alternative solution is to use chelated iron compounds, in which the iron is protected from the effect of inhibitors in the stomach.

NaFeEDTA: The absorption of iron from NaFeEDTA (sodium iron ethylenediamine tetra acetic acid) is similar to the absorption from ferrous sulphate given together with vitamin C (INACG, 1993), and can be two- to three-fold the absorption from ferrous sulphate without ascorbic acid in meals containing large amounts of phytates (Layrisse & Martinez-Torres, 1977; Mendoza *et al.*, 2001). The efficacy of NaFeEDTA in improving iron status has been demonstrated in several intervention trials. In South Africa fortification of a curry powder with NaFeEDTA resulted in a significant improvement in the iron and haemoglobin status of a female Indian population over a period of two years; iron deficiency anaemia in this population decreased from 22% to 5% (Ballot *et al.*, 1989). Fortification of sugar with FeNaEDTA in a semi-rural Guatemalan population also resulted in a significant increase in iron stores; the impact of this fortification programme was evaluated over 32 months (Viteri *et al.*, 1995). NaFeEDTA has also successfully been used to fortify fish sauce in Thailand (Garby & Areekul, 1974).

Being a strong metal chelator, concern has been expressed over the possible negative influence of long-term NaFeEDTA intake on the metabolism of other essential minerals. Studies have, however, shown that NaFeEDTA, at the levels normally used during fortification, actually increases the absorption of zinc, and has no effect on calcium absorption (Davidsson *et al.*, 1994b).

Na_2EDTA or $CaNa_2EDTA$ are recognised food additives that are widely used to prevent oxidation and colour changes in food, and can, in a similar way to ascorbic acid, be used as an absorption enhancer. MacPhail *et al.* (1994) showed that adding sodium EDTA to a rice meal, fortified with ferrous sulphate, significantly increased iron absorption. A maximum absorption was obtained when the EDTA to iron ratio was 1:2; at higher doses iron absorption was inhibited, and no effect was observed in meals containing no inhibitors. El Guindi *et al.* (1988) also showed iron absorption from an Egyptian flatbread, fortified with ferrous sulphate, to be better in the presence of Na_2EDTA. Sodium EDTA, unlike vitamin C, has the advantage of being stable during storage and processing.

Ferrous bisglycinate: The potential of iron amino acid chelates as iron fortificants, such as ferrous bisglycinate, has been recognised relatively recently. Ferrous bisglycinate is formed by the binding of one molecule of ferrous iron to two molecules of glycine (Allen & Ahluwalia, 1997) and prevents iron from binding to inhibitors in food, or from precipitating as insoluble ferric hydroxide in the pH of the small intestine (Bovell-Benjamin *et al.*, 2000). Ferrous bisglycinate has been shown to be effective in the treatment of anaemia (Pineda *et al.*, 1994; Iost *et al.*, 1998; Pineda & Ashmead, 2001), and has a bioavailability two to four times that of ferrous sulphate (Olivares *et al.*, 1997; Bovell-Benjamin *et al.*, 2000; Layrisse *et al.*, 2000). However, when ferrous sulphate is given together with ascorbic acid, the bioavailability of these two compounds is in the same range (Olivares *et al.*, 1997). A study in female subjects has shown that ferrous bisglycinate appears to be better tolerated than ferrous sulphate with regard to gastric symptoms such as bloating, constipation and nausea (Coplin *et al.*,1991). Ferrous bisglycinate has been successfully used in the fortification of milk products, infant foods, maize, and bread (Iost *et al.*, 1998; Bovell-Benjamin *et al.*, 2000; Layrisse *et al.*, 2000).

Haemoglobin: The iron in haemoglobin is naturally protected from the major inhibitors of iron absorption in the intestinal lumen. Iron is contained within the porphyrin ring of the haem molecule and is taken up intact into the mucosal cells where it is released (Hurrell, 1997b). As a food additive, haemoglobin is added in the form of dried red blood cells and gives the fortified product a dark brown colour. In Chile bovine-haemoglobin fortified cookies were given to children as part of the Chilean school feeding programme over a period of three years. Both haemoglobin and serum ferritin were significantly higher in the children who received the fortified cookies than those who did not (Walter *et al.*, 1993). In another study, an infant cereal fortified with haemoglobin and fed to breastfed infants aged 4-12 months, also markedly reduced iron deficiency anaemia compared to a control group fed regular solid foods (Hertrampf *et al.*, 1990). Cultural and religious beliefs, the possibility of bacterial or viral contamination, as well as technical difficulties in collecting, drying, and storing the animal blood may, however, limit its usefulness as an iron fortificant (Hurrell, 1997b).

(ii) Compounds used for vitamin A fortification

Retinol palmitate

Retinol palmitate is the vitamin A fortificant most commonly used in food fortification, and has been used to fortify sugar in Guatemala (Arroyave *et al.*, 1981), monosodium glutamate in the Philippines and Indonesia (Solon *et al.*, 1985; Muhilal *et al.*, 1988) and wheat flour in the Philippines (Solon *et al.*, 2000). The Guatemalan sugar fortification programme was evaluated over a period of two years and resulted in a significant improvement in vitamin A status in preschool children, especially in those with low serum retinol levels at baseline (Arroyave *et al.*, 1981). In the intestinal lumen retinol palmitate is hydrolysed to retinol and absorbed by carrier mediated diffusion or as free diffusion; the absorption of vitamin A is considered to be a non-saturable mechanism (Biesalski, 1997), and uncontrolled excessive intake may therefore lead to vitamin A toxicity.

Beta-carotene

Beta-carotene, the pre-curser of vitamin A, has been used as a safe colourant in foods and beverages for many years, and also has the potential for use as a vitamin A fortificant. The amount of ß-carotene that is converted to vitamin A in the intestinal mucosa depends on the vitamin A status of the individual (Ribaya-Mercado *et al.*, 2000), which, when adequate, has an inhibitory effect on the enzyme that cleaves carotene into retinol (Villard & Bates, 1986). The risk of vitamin A toxicity with over-consumption, especially in pregnant women, therefore is eliminated if ß-carotene is used as a vitamin A fortificant.

The bioavailability of ß-carotene from pure, powdered crystalline ß-carotene is higher than the bioavailability from vegetables (Bulux *et al.*, 1998). Synthetic ß-carotene was used as a fortificant in a wafer given to lactating women in Indonesia, and was more effective in improving the vitamin A status of these women than the same amount of ß-carotene given in the form of dark-green leafy vegetables (De Pee *et al.*, 1995). Synthetic ß-carotene has also been used to fortify soybean cooking oil in Brazil, and studies have shown that the bioavailability of ß-carotene in the oil was not influenced by heat treatment (Dutra-de-Oliveira *et al.*, 1998).

Red palm oil

Red palm oil is a rich natural source of carotenoids, and can be used as an alternative vitamin A fortificant. Crude palm oil is obtained from the mesocarp of the oil palm fruit (Nagendran *et al.*, 2000) and contains 500-700 ppm of carotenoids, of which 56% and 35% comprises ß- and α-carotene, respectively (Scrimshaw, 2000). It also contains large amounts of tocopherols and tocotrienols (800-1000 ppm), which are powerful antioxidants and can prevent oxidative deterioration of the oil (Nagendran *et al.*, 2000). Crude palm oil has a very strong taste and odour and is not very stable, due to free fatty acids and other impurities, but can be refined through a process of deodorisation and de-acidification to produce an edible oil (refined red palm oil) that is odourless, tasteless and very stable, and in which 80% of the carotenes and vitamin E originally present in the crude palm oil is retained (Nagendran *et al.*, 2000).

Red palm oil, being moderately saturated (1% myristic acid, 44% palmitic acid, 5% stearic acid, 40% oleic acid, and 10% linoleic acid), does not require hydrogenation for most food uses, and is therefore free of *trans* fatty acids (Cottrell, 1991). Several studies have shown palm oil to be non-cholesterolemic (Ng *et al.*, 1991; Zhang *et al.*, 1997). Red palm oil has been shown to be effective in improving the vitamin A status of vitamin A-deficient subjects. In India, red palm oil was given to 7-9-year-old schoolchildren in the form of a sweet snack, and resulted in a significant improvement in their vitamin A status (Manorama *et al.*, 1997). Red palm oil can also be incorporated in various other food products such as margarines, shortenings, and other edible oils, thereby raising the ß-carotene content of these products significantly (Nagendran *et al.*, 2000).

(iii) Compounds used for iodine fortification

The two compounds most often used in iodine fortification are those of potassium iodide (KI) and potassium iodate (KIO_3). Both are extensively used in industrialised countries for the iodisation of refined table salt (Jooste *et al.*, 1995).

Potassium iodide

Potassium iodide is cheaper than potassium iodate but less stable, and can easily be lost through oxidation if the salt is subjected to conditions such as moisture in the salt, humid or excessively aerated environments, exposure to sunlight, exposure to heat, acid reaction in the salt, or the presence of impurities. Losses can be minimised if the salt is very pure and dry, or by the addition of stabilisers and drying agents (Mannar & Dunn, 1995).

Potassium iodate

In developing countries where the salt is usually less refined and storage conditions not always optimal, as well as in tropical or sub-tropical climates, potassium iodate is the preferred compound to be used in iodine fortification. Potassium iodate is more stable under adverse climatic conditions and does not require stabilisers (Mannar & Dunn, 1995).

2.5.4 The vehicle and fortificants evaluated in this thesis

The two main challenges with regard to food fortification thus are: (i) to identify a suitable vehicle that will reach the population that the fortification is aimed at; and (ii) to choose fortification compounds that are both bioavailable and does not cause organoleptic changes in the vehicle to be fortified. In the research that follows a biscuit, given to primary school children during the school day, was examined as a vehicle for targeted fortification, and the efficacy of ferrous fumarate, ß-carotene, and potassium iodate as fortificants in improving micronutrient status evaluated in a randomised controlled trial; ascorbic acid, to enhance the absorption of iron, was given together with the biscuit in the form of a vitamin C-fortified drink. In subsequent studies the use of alternative fortificants was explored: (i) ferrous bisglycinate as an alternative iron fortificant in order to eliminate the need for an iron absorption enhancer, and (ii) red palm oil, a natural source of carotenoids, as an alternative vitamin A fortificant, which concomitantly will simplify the fortification process; the latter was tested and compared with synthetic ß-carotene in a randomised controlled trial.

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Chapter 3

GENERAL METHODOLOGY

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3.1 STUDY DESIGN

Three different study designs were employed to answer the questions posed in this thesis, i.e. (i) the randomised placebo-controlled design, (ii) the longitudinal study design and (iii) a design to assess equivalence between two treatments.

3.1.1 The randomised placebo-controlled trial

The randomised placebo-controlled trial is the standard by which new techniques and treatments are evaluated in clinical medicine. Subjects are randomly allocated to either an experimental group receiving the active treatment, or to a control group receiving the placebo treatment. The key goal of randomisation is to create through chance assignment treatment groups that, on average, will be comparable with respect to measured and unmeasured factors that may influence outcome. The most important process for randomisation to be unbiased is the generation of an unpredictable assignment sequence and the concealment of that sequence until after allocation has occurred. Other processes that are important for reducing bias are double-blinding (i.e. masking the treatment allocation from subjects, laboratory technicians, and all others involved in the study), and also the practice of applying exclusion criteria *before* randomisation takes place (Schulz, 1996). If randomisation is performed and applied properly, it can usually be assumed that most confounding factors (known and unknown) will be equally present in both groups. Data from randomised controlled trials are usually analysed on an "intention-to-treat" basis (Lewis & Machin, 1993); subjects are analysed according to their randomised treatment, irrespective of whether they actually received that treatment. Although this type of analysis gives more conservative results, it mirrors what will happen when the treatment is used in practice.

3.1.2 The longitudinal study

In the longitudinal study the same cohort of individuals are followed over a period of time (Dawson-Saunders & Trapp, 1994). A disadvantage of the longitudinal design is that there is no control group

to control for confounding factors.

3.1.3 The equivalence trial

The aim of the equivalence trial is to show the therapeutic equivalence of two treatments. The new treatment is compared with the standard treatment (active comparator), which has been evaluated against a placebo before. Expectations are that the new treatment will be better than the standard treatment. However, more often than not, the new treatment is simply expected to match the efficacy of the standard treatment, but will have advantages in terms of safety, convenience, or cost. The design of the equivalence trial should mirror the design of the earlier successful trial of the active comparator as closely as possible. The sample size for the equivalence trial, however, needs to be roughly four times the sample size for the randomised placebo-controlled trial. Analysis strategies for the equivalence trial should not concentrate on an "intention-to-treat" analysis only, but should include a range of approaches to show similarity; analysis should be based on confidence intervals. Absolute equivalence can, however, never be demonstrated; it is only possible to assert that true difference is unlikely to be outside a predefined range which depends on the size of the trial, the results of the trial, and the specified probabilities of error. If the confidence interval for the observed difference between the two treatments lies within this predefined range, equivalence can be assumed (Jones *et al.*, 1996).

3.2 STUDY POPULATION

The studies were carried out in two neighbouring primary schools (the Ndunakazi and Intongela Primary Schools) located in a rural mountainous area in KwaZulu-Natal, South Africa. These two schools serve communities characterised by low socio-economic status. A previous cross-sectional nutritional survey in the area revealed a high prevalence of micronutrient deficiencies, not only in the preschool children, but also in the primary school children; with 51% of the primary school children being subclinically deficient in vitamin A, 22% anaemic, and 22% presenting with goitre (Oelofse *et al.*, 1999). According to a pilot dietary intake assessment undertaken in this community (unpublished data), focussing on foods rich in iron and in vitamin A/ß-carotene and on foods

containing goitrogenic substances, animal products were infrequently consumed; *imifino* (a variety of locally grown dark green leafy vegetables) was the only ß-carotene rich food consumed on a regular basis; and goitrogenic foods, such as cabbage and soya, were often consumed. A coarse non-iodised salt was the only salt available from the shops in the area; the iodisation of salt, however, became compulsory in South Africa approximately six months after the start of the first intervention study, and resulted in the use of iodised salt from then onwards. A school feeding programme, in which the children received a cooked meal five days per week, had been in operation at one of the schools for two years prior to the baseline assessment of the first study. Meals were prepared by a member of the community and usually consisted of soy beans, rice, and vegetables (mostly cabbage and potatoes). This programme had, however, been discontinued shortly before the start of the present study (for reasons unrelated to the study) and has not been resumed.

3.3 SAMPLE SIZE

Data from a previous iron fortification study in primary school children were used to calculate sample size (Kruger *et al.*, 1996). In this study the mean baseline ferritin and haemoglobin concentrations were 26.2 µg/L and 11.5 g/dL, respectively. The treatment effect obtained for serum ferritin was 4.74 µg/L (standard deviation 12.0), and for haemoglobin 0.3 g/dL (standard deviation 0.6). Based on this it was calculated that a sample size of 100 per group would be adequate to show a ~20% increase in serum ferritin, and 80 per group to show a ~3% increase in haemoglobin, at a 5% significance level, with 80% power. Provision for a dropout rate of 20% was made. Because serum retinol usually has a lower standard deviation than serum ferritin it was assumed that a sample size large enough to detect a significant treatment effect for serum ferritin would also be large enough to detect a significant treatment effect for serum retinol.

3.4 NUTRITION MONITORS

Selected people from the community were trained by a member of the research team (PhD candidate) as fieldworkers (nutrition monitors) to run the project on a day-to-day basis. Tasks included receiving and storing the delivered biscuits; distributing the biscuits to the respective treatment

groups; ensuring compliance per treatment group; and keeping record of compliance, the reasons for non-compliance, as well as the reasons for absence from school (see **section 3.5**). The monitors were supervised by the headmaster of the school. However, a member from the research team visited the project on a monthly basis to make sure that all procedures were strictly adhered to, and to discuss any problems that might have arisen during the past month.

3.5 PROCEDURE FOR DISTRIBUTING BISCUITS AND MONITORING OF COMPLIANCE

Children were allocated to different treatment groups either by using random tables or by systematic randomisation from alphabetical class lists (see respective chapters for more detail). Biscuits were distributed daily during the school week during the first two hours of the school day, with the children inside their respective classrooms. For each class the names of all the children from a specific treatment group (A, B or C) appeared on the specific compliance sheet for that particular group; pink-coloured sheets were used for group A, blue-coloured sheets for group B, and yellow-coloured sheets for group C (when there was a third group). An example of the compliance sheets used is given in **Appendix A**. Logistical and financial constraints prevented the trial from being double-masked. During distribution and consumption of the biscuits the different treatment groups were seated in different sections of the classroom: the name of each child was read out from the compliance sheet, and the child then moved to the appropriate section of the classroom. The respective biscuits (A, B or C) were then handed out to the respective groups from containers that were clearly marked with A, B or C. Each child received three biscuits. Consumption of the biscuits took place under close supervision; the monitor had to make sure that all three biscuits were eaten, that the biscuits were not exchanged with classmates, and that the biscuits were not hidden and kept for later consumption. Compliance was indicated with a tick mark ("✓") next to the child's name under the appropriate column for that day. If the child did not eat his/her biscuits, the reason for not eating the biscuits was indicated by a specific code from a code list (**Appendix B**). If the child was absent an "**A**"was used, which was replaced, when the child was back at school, with the appropriate code for the reason for absence from a separate code list (**Appendix B**; also see **section 3.10** on morbidity data collection). Compliance was defined as the number of days that a child received the

biscuit, expressed as a percentage of the total number of potential biscuit days; potential biscuit days did not include weekends, school holidays or public holidays.

3.6 ASSESSMENT OF MICRONUTRIENT STATUS

3.6.1 Collection of blood/urine

Blood (7 mL) was obtained from each child by venipuncture, of which 2 mL was transferred to an EDTA tube (for a full blood count), and the rest to a Gel and Clot Activator tube. The latter was centrifuged, the serum removed and stored in 1.5 mL Eppendorf tubes at –80°C until assayed. Care was taken throughout the procedure to protect the blood samples from direct sunlight. All samples were analysed within one month. Casual urine specimens, for the determination of urinary iodine excretion, were collected in a 20 mL containers, which were tightly sealed with screw tops and stored at 4°C until assayed.

3.6.2 Serum ferritin

Serum ferritin reflects the amount of iron in storage and is the first indicator of iron status to decline during iron depletion (Cook & Finch, 1979). Serum ferritin was determined by an immuno-radiometric assay (Ferritin MAb Solid Phase Component System, Becton Dickinson and Company, NY, USA). In this assay an excess amount of antibodies, labeled with [^{125}I], are used to form a radioactive complex with the ferritin in the serum; there is a direct relationship between the measured amount of radioactivity and the serum ferritin concentration. Radioactivity was measured using an Auto Gamma 500C counting system (United Technologies Packard, IL, USA). Two external control samples (FERR/MYO T Control, Roche Diagnostics, Basel, Switzerland; and Ligand 1,2,3, Chiron Diagnostics Ltd, Halstead, Essex, UK) were included with each assay, as well as an internal control sample that was obtained from pooled serum. The coefficient of variance for this method is 5% intra-assay, and 12% inter-assay. In healthy individuals serum ferritin is a reliable measure of the amount of iron in storage, but in the presence of inflammation or infection, serum ferritin may be falsely elevated (Witte, 1991), and may remain so for a period of three weeks following the infection (Hulthén *et al.*, 1998). High serum ferritin concentrations therefore do not

always reflect adequate iron status. On the other hand, because a low concentration of serum ferritin is characteristic of iron deficiency *only* (Dallman *et al.*, 1980), a serum ferritin concentration in the deficient range can always be interpreted as a sign of iron depletion; a cut-off value of below 10 or 12 µg/L is normally used to indicate iron depletion. According to the classification used by Cook and Skikne (1989), all individuals with serum ferritin levels below 20 µg/L will respond to oral iron therapy; those with serum ferritin levels between 20 and 60 µg/L may respond, while those with values above 60 µg/dL rarely show a response. For the purpose of this study a cut-off value of < 20 µg/L was used to indicate marginal iron stores.

3.6.3 Serum iron and transferrin saturation

Serum iron and transferrin saturation (TS) reflects the amount of iron in circulation (Gibson, 1990). Serum iron was determined spectrophotometrically with a RA-1000 Technicon automated system (Technicon, Tarrytown, NY, USA), using a colorimetric method without deproteinisation (Boehringer Mannheim, Mannheim, Germany). Total iron binding capacity (TIBC) was determined, after transferrin in serum was saturated with iron and the uncomplexed iron precipitated with magnesium carbonate, using the same colorimetric method. TS was calculated by expressing serum iron as a percentage of TIBC. An external control sample (Technicon TEST point, Bayer Corporation, Tarrytown, NY, USA) was included with each assay. The coefficient of variance for this method is 2% intra-assay, and 5% inter-assay. TS values above 16 % are considered as normal (Gibson, 1990).

3.6.4 Full blood count

A full blood count was performed by means of an automated cell counter (Coulter STKS, FL, USA), within 12 hours of blood collection by the Department of Haematology, University of Natal, Durban. Haemoglobin represents the main functional compartment of iron status (Cook & Skikne, 1989) and shows a decline only during the third and final stage of iron depletion (Cook & Finch, 1979). For the purpose of this thesis, the WHO cut-off point of < 12 g/dL for children aged 6-14 years was used to define anaemia (WHO, 1972).

3.6.5 Serum retinol

Serum retinol was determined by a reversed-phase HPLC method, which is based on the method described by Catignani and Bieri (1983). The assay was performed under dimmed light. After deproteinisation of 250 µL of serum with ethanol and extraction with hexane, the aliquot was evaporated under nitrogen and the residue dissolved in methanol. The latter was then injected onto a 5µ C_{18} reversed phase column (250 x 4.6 mm, Luna; Phenomenex, Höshbach, Germany). *All-trans*-retinol was used as external standard and *α*-tocopherol acetate as internal standard. The absorption of retinol and *α*-tocopherol acetate was measured at 325 nm and 285 nm respectively, using a programmable UV detector. The mobile phase comprised 100% methanol. A control sample, obtained from pooled serum, was included with each assay. The coefficient of variance for this method is 3% intra-assay, and 4% inter-assay. Serum retinol levels below 10 µg/dL are considered to be deficient and levels between 10 and 20 µg/dL as marginally or subclinically deficient. When more than 20% of a population has serum retinol levels below 20 µg/dL the vitamin A deficiency in that population is regarded as a *severe* public health problem (WHO, 1996). Serum concentrations of retinol above 30 µg/dL are considered adequate (Flores *et al.*, 1991).

3.6.6 Urinary iodine

Urine was digested with chloric acid under mild conditions and iodine spectrophotometrically detected by its catalytic action on the reduction of ceric ammonium sulphate in the Sandell-Kolthoff reaction (Dunn *et al.*, 1993). A control sample, obtained from pooled urine, was included with each assay; this laboratory also participates in an international quality control programme (EQUIP program, CDC, Atlanta, GA, USA). The coefficient of variance for this method is below 5% intra-assay, and between 5 and 8% inter-assay. Urinary iodine excretion reflects recent iodine intake and because of variation from day to day, urinary iodine should be used for making population-based estimates only, and is usually expressed as a median value. According to the WHO/UNICEF/ICCIDD (1994) classification, a median value of <20 µg/L indicates a *severe* public health problem; a median value between 20 and 49 µg/L a public health problem of *moderate* severity; and a median value between 50 and 99 µg/L a *mild* public health problem.

3.6.7 Thyroid size

Thyroid size was determined by a medical practitioner by means of visual inspection and palpation, with the neck in the normal position. The goitre classification system suggested by WHO/UNICEF/ICCIDD (1994) was used, i.e. grade 0 = not palpable or visible; grade 1 = palpable, but not visible; grade 2 = visible. A total goitre rate (goitre grades 1 and 2) of between 5 and 19.9% in primary school children (age range 6-12 years) indicates a *mild* public health problem, a goitre rate between 20.0 and 29.9 % a public health problem of *moderate* severity and a rate of ≥ 30% a *severe* public health problem.

3.6.8 C-reactive protein

C-reactive protein (CRP), an acute phase protein, was measured in serum as an indicator of infection by means of particle-enhanced nephelometry (Behringwerke AG, Marburg, Germany). In healthy persons the CRP concentration in serum is below 5 mg/L, but can rise within hours after the onset of acute infection or inflammation (Pepys, 1981). Serum C-reactive protein is a more sensitive and reliable indicator of acute inflammatory processes than the white blood cell count or the erythrocyte sedimentation rate (Hanson & Wadsworth, 1980).

3.6.9 Summary of the cut-off values

Table 3.1 gives a summary of the cut-off values used in this thesis for indicating micronutrient deficiencies at individual level, as well as the WHO criteria that are used to indicate the level of public health significance of the deficiency.

Table 3.1: The cut-off values that were used in this thesis to define micronutrient deficiencies.

At individual level	Level of deficiency indicative of a public health problem
Vitamin A deficiency: [a] serum retinol < 10 µg/dL *Subclinical vitamin A deficiency:* serum retinol ≥ 10 - < 20 µg/dL SI units 10 µg/dL = 0.35 µmol/L 20 µg/dL = 0.70 µmol/L	[a] *Mild public health problem:* ≥2% - < 10% of a population with serum retinol < 20 µg/dL *Moderate public health problem:* ≥ 10% - < 20% of a population with serum retinol < 20 µg/dL *Severe public health problem:* ≥ 20% of a population with serum retinol < 20 µg/dL
Anaemia: [b] haemoglobin < 12 g/dL SI units 12g/dL = 120 g/L	[c] *Mild public health problem:* [*] prevalence of anaemia 5 - < 20% *Moderate public health problem:* prevalence of anaemia 20 - < 40% *Severe public health problem:* prevalence of anaemia ≥ 40% [*] anaemia = haemoglobin < 11.5g/dL
Iron deficiency: [d] serum ferritin < 10 µg/L *Marginal iron deficiency:* serum ferritin ≥ 10 - < 20 µg /dL	*Level not defined*
Urinary iodine excretion: [e] cannot be interpreted at individual level	[e] *Mild public health problem:* median urinary iodine 50-99 µg/L *Moderate public health problem:* median urinary iodine 20-49 µg/L *Severe public health problem:* median urinary iodine < 20 µg/L
Goitre: [e] the presence of an enlarged thyroid (visible or palpable)	[e] *Mild public health problem:* goitre rate 5 - 19.9% *Moderate public health problem:* goitre rate 20 - 29.9% *Severe public health problem:* goitre rate ≥ 30%

[a] WHO (1996); [b] WHO (1972); [c] UNICEF/UNU/WHO (2001); [d] Cook & Skikne (1989); [e] WHO/UNICEF/ICCIDD (1994).

3.7 ASSESSMENT OF ANTHROPOMETRIC STATUS

The weight of each child was measured, in light clothing and without shoes, to the nearest 0.05 kg on an electronic load cell scale. The accuracy of the scale was checked against an object with a known weight on each day that anthropometric measurements were taken. Height was measured to the nearest 0.1 cm using a wooden board with a fitted measuring tape, a fixed foot plate and a movable head board; height was measured without shoes, with the feet flat, the heels together, the legs straight, the knees together, the shoulders relaxed, the heels, buttocks, shoulder blades and back of head touching the measurement board, and the head looking straight forward (Jelliffe & Jelliffe,1989). Height-for-age and weight-for-age were expressed as Z-scores using the National Center for Health Statistics (NCHS) median as reference (Hamill *et al.*, 1979). Children with height-for-age and weight-for-age Z-scores more than two standard deviations below this reference median, respectively, were classified as stunted and underweight. The birth date of each child was obtained from the school register.

3.8 ASSESSMENT OF COGNITIVE FUNCTION

Cognitive function was assessed in conjunction with the Child Development Programme of the Human Sciences Research Council of South Africa. Children were tested on an individual basis; a series of nine simple tests were administered to each child and each interview lasted approximately 30 minutes. Tests were conducted outside the classroom, with the child and the test administrator seated at a table under a tree some distance away from the school building. The tests, based on the guidelines described by Connolly and Grantham-McGregor (1993), were designed to measure intellectual skills required for schoolwork, such as reaction time, short-term memory, attention span and perceptual-motor co-ordination. The tests were designed for use in this particular age group and were not culturally biased. For five of the tasks the time it took the child to complete the task was measured with a stopwatch and recorded; for the remaining four tasks the amount of the task completed in a set time was recorded.

The tasks included the following:

- ***Reading numbers:*** The child was asked to read numbers as fast as he/she could from a list of 30 double-digit numbers; the time it took the child to complete the task was recorded.
- ***Verbal fluency:*** The child was asked to say as many words he/she could think of denoting things to eat, things to drink, things in the kitchen, things to wear, and things in the house. The scores were the number of items generated for each category in one minute. The subscores were summed to get a total verbal fluency score.
- ***Counting letters:*** The task administrator showed the child a page containing 80 letters arranged in 10 rows. The child was asked to count the number of times the letter B appeared on the page; the time taken to complete the task was recorded.
- ***Cancelling letters:*** Using the same sheet as above, the child was asked to strike out all occurrences of the letter A; the time taken to complete the task was recorded.
- ***Digit copying:*** The child was given a sheet of paper containing 60 single-digit numbers arranged in rows. The child was asked to copy each number underneath itself, working from left to right; the time taken to complete the set was recorded.
- ***Writing crosses:*** The child was given a clean sheet of paper and asked to write as many crosses as he/she could in 10 seconds; the score was the number of crosses completed in the 10 seconds.
- ***Digit span forward:*** The child was asked to repeat a series of digits given to him/her verbally. After each successful repetition an extra digit was added. The score was the length of the longest list of digits that the child was able to repeat correctly.
- ***Digit span backward:*** This test was the same as the previous one, except that the child was required to say the numbers backwards.
- ***Counting backwards:*** The child was asked to count backwards out loud from 30 to 1; the time taken to complete the task was recorded.

3.9 ASSESSMENT OF PARASITIC INFESTATION

Stool samples were collected prior to the first anthelmintic treatment for the first study only. Consistency of the stool was recorded and a weighed subsample (usually between 0.5 and 1 g) was preserved with 10% formalin. The latter was filtered and prepared by the formal-ether method for microscopic examination and identification of helminth eggs. Intensity of infestation was expressed as eggs per gram (epg) of faeces.

3.10 COLLECTION OF MORBIDITY DATA

For each day a child was absent, the reason for absence was obtained from the mother or child as soon as the child was back at school, and recorded by the nutrition monitor, using the compliance sheets (**Appendix A**). Each illness or reason for absence was assigned a specific code from a code list (**Appendix B**). Disorders such as colds, influenza, chest infection and cough were grouped together under the term respiratory-related illnesses; and diarrhoea, vomiting and nausea under the term gastrointestinal-related symptoms. The first four weeks of the intervention was regarded as a "running-in" phase and morbidity data for this period were not included in the analyses. No morbidity data were collected for weekends and holidays.

3.11 STATISTICAL ANALYSIS

The statistical methods used in each of the three studies will be described separately under the respective chapters.

3.12 ETHICAL APPROVAL AND CONSENT

Ethical approval for the studies described in this thesis was obtained from the Ethics Committee of the Medical Research Council of South Africa (**Appendix C**). Permission was also obtained from the KwaZulu-Natal Department of Education, the headmasters of the Ndunakazi and Intongela Primary Schools, as well as the local community leaders. Informed consent was obtained from the parents or guardians of all participants in these studies (**Appendix D**).

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Chapter 4

EFFECT OF A MICRONUTRIENT- FORTIFIED BISCUIT ON MICRONUTRIENT STATUS, COGNITIVE FUNCTION, GROWTH AND MORBIDITY

CHAPTER 4

EFFECT OF A MICRONUTRIENT-FORTIFIED BISCUIT ON MICRONUTRIENT STATUS, COGNITIVE FUNCTION, GROWTH AND MORBIDITY

4.1 INTRODUCTION

Of the various strategies used for combatting micronutrient deficiencies, the food-based strategy is probably the most sustainable and desirable approach (Underwood, 2000). However, changing the dietary habits of a population is not easy and may take years to show an effect. Food fortification, on the other hand, offers a solution that gives more immediate results, and large segments of a population can simultaneously be reached by this strategy. Food fortification can, however, also be targeted at specific segments of a population.

4.1.1 Objectives of the study

The primary objective of this study was to evaluate the effect of a biscuit, fortified with iron, iodine and ß-carotene, on the vitamin A, iron and iodine status of primary school children, living in an area with a known high prevalence of micronutrient deficiencies (Oelofse *et al.*, 1999). Because micronutrient deficiencies can also have an effect on cognitive function (Bautista *et al.*, 1982; Pollitt, 1993; Shrestha, 1994), growth (Chwang *et al.*, 1988), and morbidity (Baynes & Bothwell, 1990; Sommer, 1990), these indicators were assessed as secondary outcomes.

4.2 SUBJECTS AND METHODS

4.2.1 Study population and design

The study population comprised all grade 1 to 5 children, aged 6-11 years, attending the Ndunakazi Primary School (n=280). Based on a previous iron fortification study in primary school children (Kruger *et al.*, 1996), it was calculated that a sample size of 100 per group would be adequate to show a 3% increase in haemoglobin and a 20% increase in serum ferritin, at a 5% significance level, with 80% power. Blood was obtained from 252 children, who were then, after stratification by school grade, randomly assigned to one of two groups. Alphabetical class lists were used for

the randomisation process, with every alternative name appearing on the list being allocated to a specific group; randomisation took place *after* the exclusion of the children from whom a blood sample was not obtained. The two groups were then randomly assigned to two different treatment categories: one group received a fortified biscuit (n=126; intervention group), whereas the other group received an unfortified biscuit (n=126; control group). The two types of biscuits were similar in macronutrient composition (**Table 4.1**), taste and appearance. To enhance the absorption of iron, a vitamin C-fortified cold drink (150 mL) was given to the intervention group; the control group received a placebo cold drink. The biscuits and cold drinks were distributed daily during the school week, during the first two hours of the school day. Each child received three biscuits weighing 15 g each. No intervention took place during school holidays, weekends or public holidays; the biscuits were provided for a total of 215 days or 43 weeks. Compliance was closely monitored and recorded as described in Chapter 3. Only the project leader was aware of group allocation (single-blind study).

Table 4.1: Macronutrient composition of the biscuit and cold drink.

Macronutrient	**Biscuit** (45 g)[a]	**Cold drink** (150 mL)
Protein (g)	4.1	-
Carbohydrate (g)	26.9	13.7
Fat (g)	7.7	-
Energy (kJ)	810	230

[a] Three biscuits weighing 15 g each

Micronutrient status was assessed at baseline and after 6 months and 12 months of intervention; anthropometric status and cognitive function at baseline and at 12 months. To exclude parasitic infestation as a confounding factor, all children were dewormed (400 mg albendazole) at four-monthly intervals for the duration of the study; the first treatment took place after the baseline collection of stool and blood samples, but before the start of the intervention.

4.2.2 Fortification

The biscuit (shortbread-based) was designed to provide 50% of the Recommended Dietary Allowances (RDA) for iron (5 mg iron in the form of ferrous fumarate), iodine (60 µg iodine in the form of potassium iodate), and ß-carotene (2.1 mg) for children aged 7-10 years (National Research Council, 1989). The cold drink (sugar-based; prepacked in plastic sachets) was to provide 90 mg vitamin C. As it was initially uncertain how the baking process will affect the stability of iodine in the biscuit, 60 µg iodine was added to the cold drink as well. The estimated cost of fortification for the biscuit and the cold drink amounted to R4.40 (~ 0.7 US$) per child per school year, each (cost and Rand:Dollar exchange rate (6:1) as at May 1997); the cost of the cold drink included the additional cost of a special packaging material used to prevent the oxidation of the vitamin C.

Table 4.2: The analysed composition of the biscuit and cold drink. [a]

Nutrient	**Biscuit** (45 g) [b]		**Cold drink** (150 mL)	
	Fortified	Unfortified	Fortified	Unfortified
Iron (mg)	5.9 (1.1)	1.2 (0.5)	-	-
ß-carotene (mg)	2.0 (0.6)	trace	-	-
Iodine (µg)	95.4 (27.5)	32.9 (7.2) [c]	39.0 (5.4)	trace
Vitamin C (mg)	-	-	110 (18)	trace

[a] Mean (SD) based on analyses of 9 batches of each type of biscuit (Aspland & James Ltd., Consultant Analysts, United Kingdom) and 5 batches of each type of cold drink (Medical Research Council, South Africa); [b] Three biscuits weighing 15 g each; [c] The iodine in the unfortified biscuit was due to a marine oil that was used in the baking process.

4.2.3 Quality control

A new batch of biscuits and cold drinks was provided once every six weeks. Each new batch was analysed for micronutrient content to ensure that levels were maintained throughout the study. Ten biscuits were randomly drawn from each batch, ground, mixed and a sample sent to Aspland & James (Consultant Analysts, United Kingdom) for analysis. Cold drinks were analysed for vitamin C and iodine content by the Medical Research Council (South Africa). The analysed composition

of the biscuit and cold drink is shown in **Table 4.2**. The iodine in the unfortified biscuit was due to a marine oil that was used in the baking process. Shelf life in terms of micronutrient composition of both the biscuits and cold drinks was at least three months; there were also no organoleptic changes during this period.

4.2.4 Measurements

Micronutrient status was assessed in terms of serum retinol, serum ferritin, serum iron, transferrin saturation, urinary iodine and thyroid size. A full blood count was also performed. Stool samples were analysed to determine the prevalence of parasitic infestation in this study population. Anthropometric measurements included height and weight and were expressed as Z-scores. Cognitive assessments were done on grade 2 (n=51), grade 3 (n=47) and grade 4 (n=37) pupils only; children from grade 1 were too young to perform the tasks and there were too few children in grade 5 taking part in the study. Morbidity was assessed and recorded daily during the school week. A detailed description of all methods and procedures used in this study is given in Chapter 3. Information on the acceptability of the biscuit and cold drink, as well as information on breakfast and snacking patterns during the school day, was obtained by means of a short questionnaire, administered at the baseline, 6- and 12-month assessments (**Appendix E**).

4.2.5 Statistical analysis

Analyses were performed with the SAS software program (SAS Institute Inc., Cary, NC). Data was analysed on an intention-to-treat basis. Changes from baseline to 6 and/or 12 month for all variables in the intervention group were compared with that in the control group using the Wilcoxon 2-sample test. The Wilcoxon signed rank test for paired data was used to compare pre- and post-intervention values within each group. The effect of fortification on the prevalences of micronutrient deficiencies was evaluated using repeated measures analysis of variance for categorical data. Morbidity data was compared using the chi-square test. Spearman correlation coefficients were used to test for the association between white blood cell counts and serum ferritin or retinol. P values < 0.05 were considered statistically significant.

4.3 RESULTS

One hundred and fifteen children in the intervention group and 113 in the control group completed the study. Leaving the area was the main reason for dropping out of the study (6.3%); other reasons were failure to obtain blood from the child at the follow-up assessment (1.2%), or the child being absent during the week of blood sampling (2%). The trial profile is given in **Figure 4.1**.

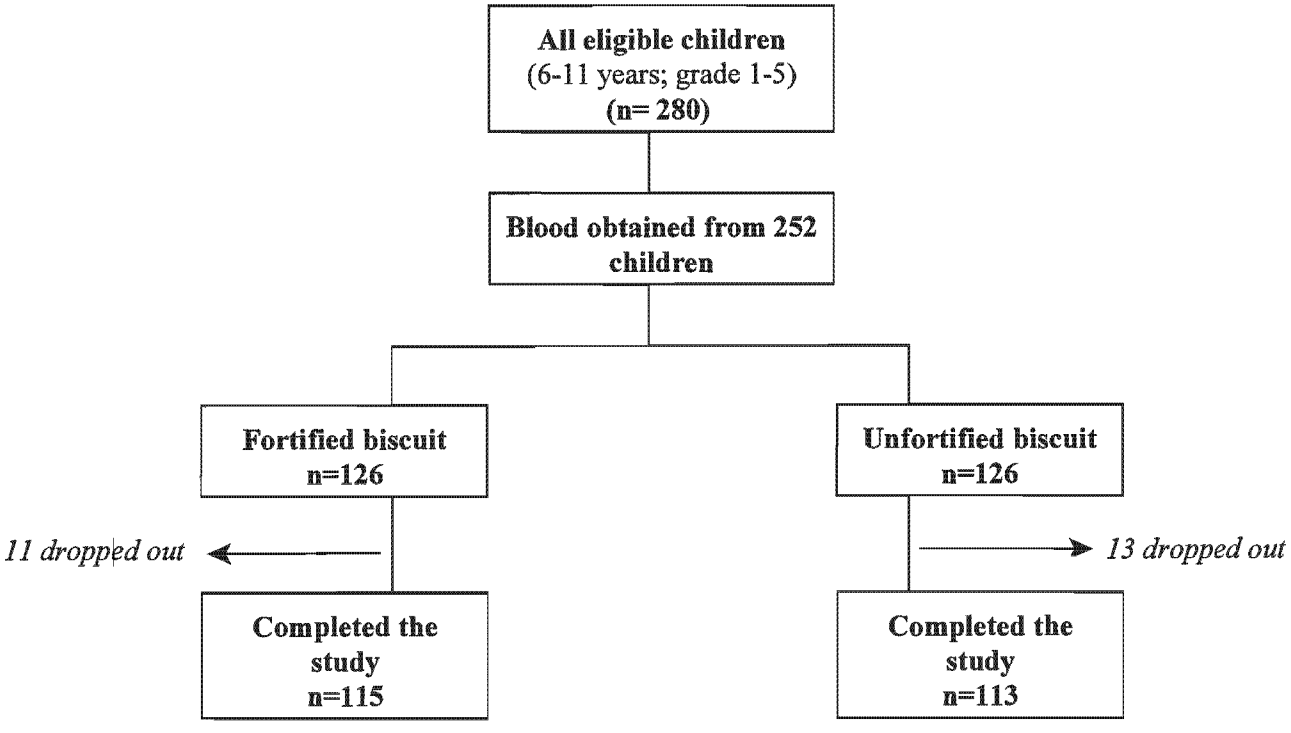

Figure 4.1: Trial profile of the 12-month intervention study.

4.3.1 Baseline characteristics

4.3.1.1 *Characteristics of the intervention and control groups at baseline*

Baseline characteristics of the intervention and control groups are given in **Table 4.3**. There were no significant differences with regard to age, sex, height, weight, the prevalence of subclinical vitamin A deficiency, anaemia, goitre or parasitic infestation between the two groups. Only a small percentage of the children were stunted and almost none underweight. About one-third were infected with one or more parasite, mostly *Trichuris trichiura*; parasite load was, however, low, with only 4% and 2% of the children having faecal egg counts in excess of 1000 eggs per gram of stool for *Trichuris* and *Ascaris*, respectively, and none for hookworm (*Necator americanus*).

Table 4.3: Baseline characteristics of the intervention and control groups.

	Intervention group (n=115)	**Control group** (n=113)
Male/female	61/54	57/56
Mean age (years)	8.8 (2.0) [a]	8.5 (1.9)
Mean height (cm)	125.2 (11.4)	123.7 (10.8)
Mean height-for-age Z-score	-0.98 (0.94)	-0.96 (0.85)
Stunted (%) [b]	14.8	9.7
Mean weight (kg)	26.4 (6.3)	24.9 (5.6)
Mean weight-for-age Z-score	-0.50 (0.77)	-0.61 (0.76)
Underweight (%) [c]	0	3.5
Subclinical vitamin A deficiency (%) [d]	39.1	40.7
Anaemia (%) [e]	29.6	24.5
Goitre (%) [f]	20.0	22.1
Infected with ≥ 1 parasite (%)	34.3	32.7
- *Trichuris trichiura* (%)	27.3	24.8
- *Ascaris Lumbricoides* (%)	6.1	6.9
- *Necator americanus* (%)	6.1	1.0

[a] mean (SD); [b] height-for-age Z-scores < – 2SD of the NCHS median; [c] weight-for-age Z-scores < –2SD of the NCHS median; [d] serum retinol <20 µg/dL; [e] haemoglobin <12 g/dL; [f] palpable and/or visible.

4.3.1.2 *Distribution of micronutrient levels at baseline*

The distribution of serum retinol, serum ferritin, haemoglobin and urinary iodine at baseline of the intervention and control groups combined is shown in **Figures 4.2 to 4.5**. Very few children (1.3%) were vitamin A deficient (serum retinol < 10 µg/dL); however, 40% had serum retinol concentrations below 20 µg/dL, a level regarded by the World Health Organization as a *severe* public health problem (WHO, 1996). Almost 30% of the study population had serum ferritin levels below 20 µg/L (indicative of marginal iron stores), and is, according to Cook and Skikne (1989), likely to respond to iron therapy. Twenty-seven per cent of the children were anaemic, using the World Health Organization criteria (WHO, 1972). The distribution of urinary iodine was skewed toward the lower concentrations, with only 3% of the children having had a urinary iodine

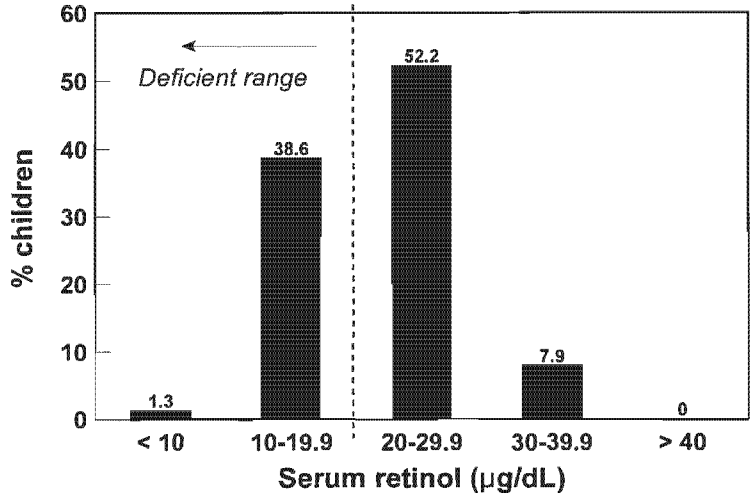

Figure 4.2: Distribution of serum retinol at baseline in the intervention and control groups combined.

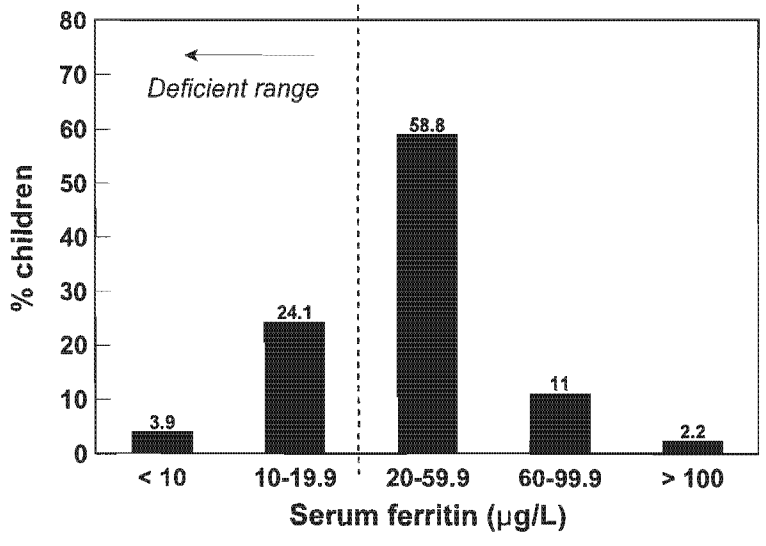

Figure 4.3: Distribution of serum ferritin at baseline in the intervention and control groups combined.

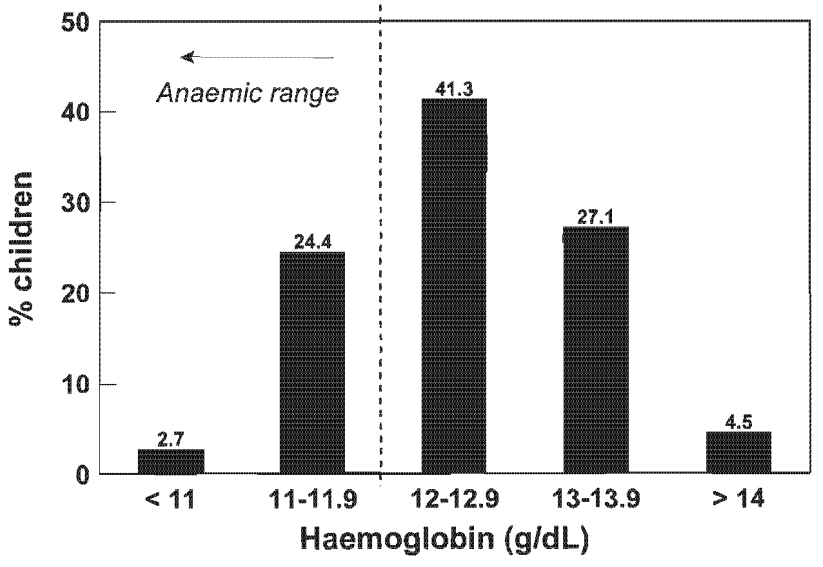

Figure 4.4: Distribution of haemoglobin at baseline in the intervention and control groups combined.

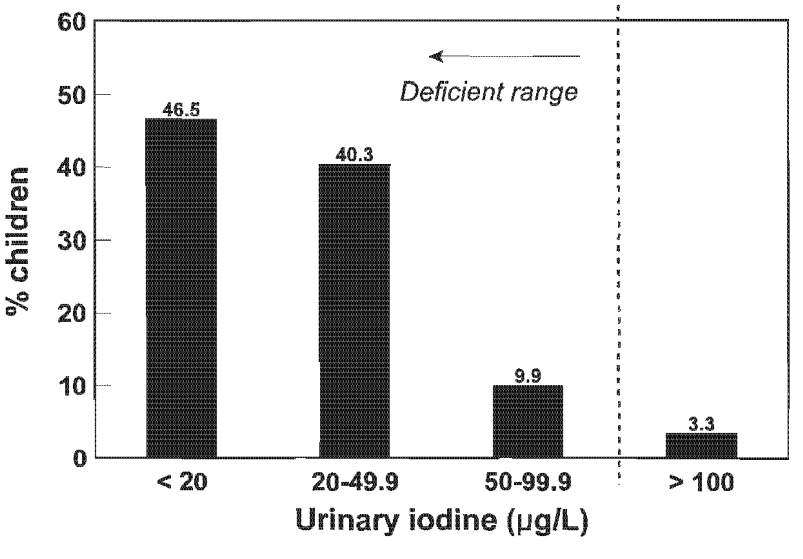

Figure 4.5: Distribution of urinary iodine at baseline in the intervention and control groups combined.

Table 4.4: Micronutrient status of subjects before and after 6 and 12 months of intervention.

	Serum retinol (μg/dL)	Serum ferritin (μg/L)	Serum iron (μmol/L)	Transferrin saturation (%)	Haemoglobin (g/dL)	Urinary iodine (μg/L)	White cell count ($x10^9$/L)
Intervention group (n=115)							
Baseline	22.1 (5.2)	29.2 (13.1, 56.7)	12.7 (5.7)	20.9 (11.4)	12.52 (0.81)	20 (8, 47)	8.02 (2.54)
6 months	25.0 (6.1) [a]	34.1 (18.8, 65.9) [b]	17.2 (7.3) [a]	26.7 (11.4) [a]	12.36 (0.83) [c]	148 (49, 254) [a]	7.73 (2.29)
12 months	26.2 (6.2) [a]	33.6 (19.2, 56.9) [b]	17.5 (6.9) [a]	26.0 (10.4) [a]	12.86 (0.85) [a]	225 (113, 289) [a]	7.81 (2.35)
Control group (n=113)							
Baseline	21.6 (5.7)	31.3 (15.9, 73.0)	12.1 (5.7)	21.0 (11.9)	12.57 (0.84)	20 (7, 68)	8.25 (2.44)
6 months	22.2 (6.3)	24.2 (14.2, 55.3) [a]	13.7 (4.9) [c]	21.1 (7.9)	12.34 (0.74) [b]	35 (12, 108) [a]	7.55 (2.16) [b]
12 months	21.7 (5.5)	22.7 (12.5, 44.2) [a]	12.6 (5.0)	18.1 (7.4) [c]	12.63 (0.79)	137 (42, 267) [a]	7.32 (2.15) [a]
Intervention effect [d] ***(baseline-6 months)***	*2.4 (1.0 -3.9) P = 0.001*	*11.5 (7.2-15.8) P < 0.0001*	*2.9 (0.8-4.9) P = 0.015*	*5.7 (1.9-9.5) P = 0.005*	*0.07 (– 0.11-0.26) P = 0.423*	*98 (78-119) P < 0.0001*	
Intervention effect ***(baseline-12 months)***	*4.0 (2.5-5.5) P < 0.0001*	*13.9 (9.5-18.3) P < 0.0001*	*4.3 (2.2-6.4) P < 0.0001*	*8.1 (4.5-11.7) P < 0.0001*	*0.27 (0.09-0.46) P = 0.0015*	*68 (45-92) P < 0.0001*	

All values expressed as mean (SD), except for serum ferritin and urinary iodine which are expressed as median (10^{th}, 90^{th} percentile).

[a] $P<0.0001$, [b] $P<0.005$, [c] $P<0.05$ compared to baseline (Wilcoxon signed rank test for paired data).

[d] Difference (95% CI) in mean change from baseline to 6 or 12 months between intervention and control groups (Wilcoxon 2-sample test).

concentration in the adequate range; median urinary iodine excretion was 20 µg/L, indicating a public health problem bordering on *severe* (WHO/UNICEF/ICCIDD, 1994).

4.3.2 Effect of the intervention

4.3.2.1 *Micronutrient status*

The micronutrient status of the intervention and control groups before and after 6 and 12 months of intervention is presented in **Table 4.4**. A significant treatment effect compared to the control group was found for serum retinol, serum ferritin, serum iron, transferrin saturation, urinary iodine and haemoglobin over the 12-month intervention period. Serum ferritin, serum iron and transferrin saturation, however, appeared to reach a stable median in the intervention group after 6 months. A decrease in serum ferritin was observed in the control group. Haemoglobin showed a slight decrease at 6 months in both the intervention and control groups. At 12 months urinary iodine excretion was significantly increased in both groups, though the increase in the intervention group was significantly greater than that in the control group.

Table 4.5: Changes in serum retinol and ferritin over the12-month intervention grouped according to concentrations at baseline.

		Intervention group		**Control group**	*P*-value [a]
Serum retinol at baseline (µg/dL)	n		n		
< 20	45	+ 6.5 (5.7) [b]	46	+ 2.2 (4.2) [c]	$P < 0.0001$
20-30	60	+ 3.1 (5.6) [b]	59	− 1.1 (4.8)	$P < 0.0001$
> 30	10	− 0.5 (6.9)	8	− 3.2 (6.7)	*NS*
Serum ferritin at baseline (µg/L)					
< 20	32	+ 10.9 (8.2) [b]	32	+ 1.2 (5.9)	$P < 0.0001$
20-60	73	+ 4.1 (15.6) [e]	61	− 8.5 (11.1) [b]	$P < 0.0001$
> 60	10	− 23.0 (16.5) [d]	20	− 33.9 (22.9) [b]	*NS*

Values expressed as mean (SD). [a] Difference in change between intervention and control groups (Wilcoxon 2-sample test). [b, c, d, e] Change significantly different from baseline (Wilcoxon signed rank test for paired data): [b] $P < 0.0001$, [c] $P < 0.001$, [d] $P < 0.01$, [e] $P < 0.05$.

In **Table 4.5** the changes in serum retinol and in serum ferritin over the 12-month intervention period are grouped according to concentrations at baseline. The greatest improvement in serum retinol was seen in the children with low retinol concentrations (< 20 μg/dL) at baseline, i.e. those who needed it most benefited most from the intervention. There was no improvement in the children with adequate vitamin A status (serum retinol > 30 μg/dL). Similarly, it was the children with marginal iron stores (serum ferritin < 20 μg/L) that showed the greatest response, while the intervention had little or no effect on those with adequate iron status.

The prevalence of low micronutrient concentrations before and after 6 and 12 months of intervention is shown in **Figures 4.6 to 4.9**. The percentage of children with low serum retinol levels (< 20 μg/dL) in the intervention group decreased from 39.1% before intervention to 12.2% after 12 months of intervention, while remaining around 40% in the control group (**Figure 4.6**). No children had serum retinol values below 10 μg/dL at the 12-month assessment in either group. The percentage of children in the intervention group with low serum ferritin concentrations (< 20 μg/L) decreased from 27.8% to 13.9%; in the control group the percentage increased (**Figure 4.7**). Only one child (0.9%) in the intervention group and two children (1.8%) in the control group had a serum ferritin concentration below 10 μg/L at 12 months. The prevalence of anaemia (haemoglobin < 12 g/dL) decreased from 29.6% to 15.6% in the intervention group and from 24.5% to 19.4% in the control group (**Figure 4.8**). Only one child in each group (0.9%) had a haemoglobin concentration below 11 g/dL after the 12-month intervention period. More than 95% of the children in both groups had a urinary iodine excretion below 100 μg/L at the baseline assessment (**Figure 4.9**). This prevalence dropped to 5.4% in the intervention group and to 34.6% in the control group after 12 months. Repeated measures analysis of variance for categorical data showed a significant interaction between time and treatment for serum retinol (P=0.0002), serum ferritin (P=0.0005) and urinary iodine ($P < 0.0001$).

The prevalence of goitre, which was 20% and 22.1% at baseline in the intervention and control groups, respectively, was not reduced by the 12 months of iodine intervention.

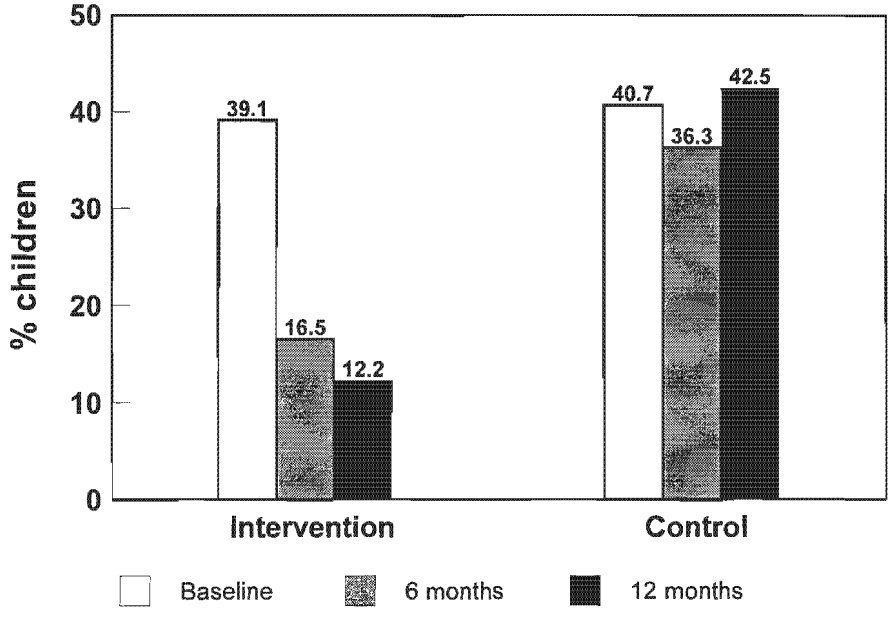

Figure 4.6: The prevalence of low serum retinol concentrations (< 20 μg/dL) at baseline, 6 months and 12 months in the intervention and control groups.

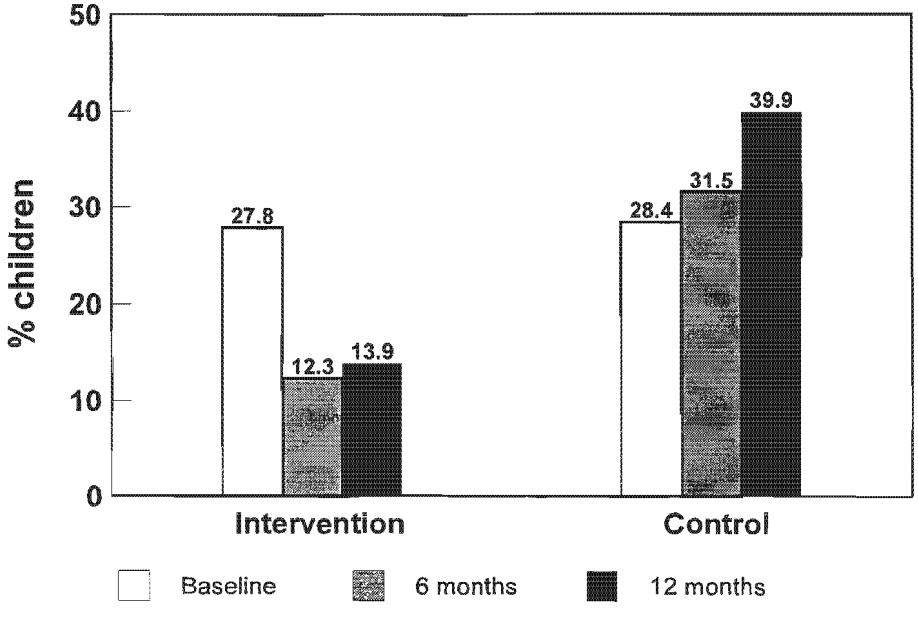

Figure 4.7: The prevalence of low serum ferritin concentrations (< 20 μg/L) at baseline, 6 months and 12 months in the intervention and control groups.

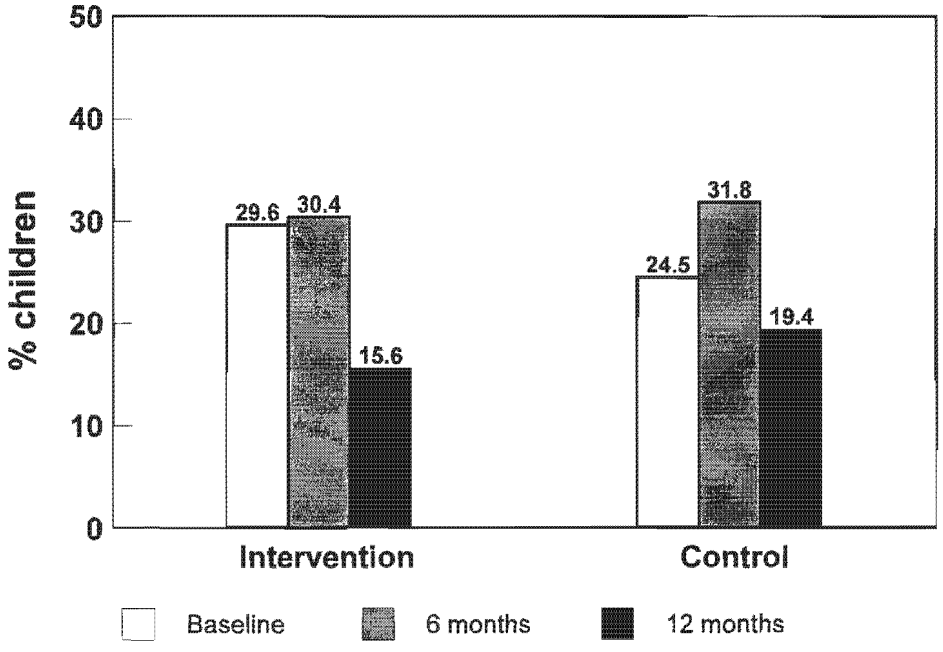

Figure 4.8: The prevalence of low haemoglobin concentrations (< 12 g/dL) at baseline, 6 months and 12 months in the intervention and control groups.

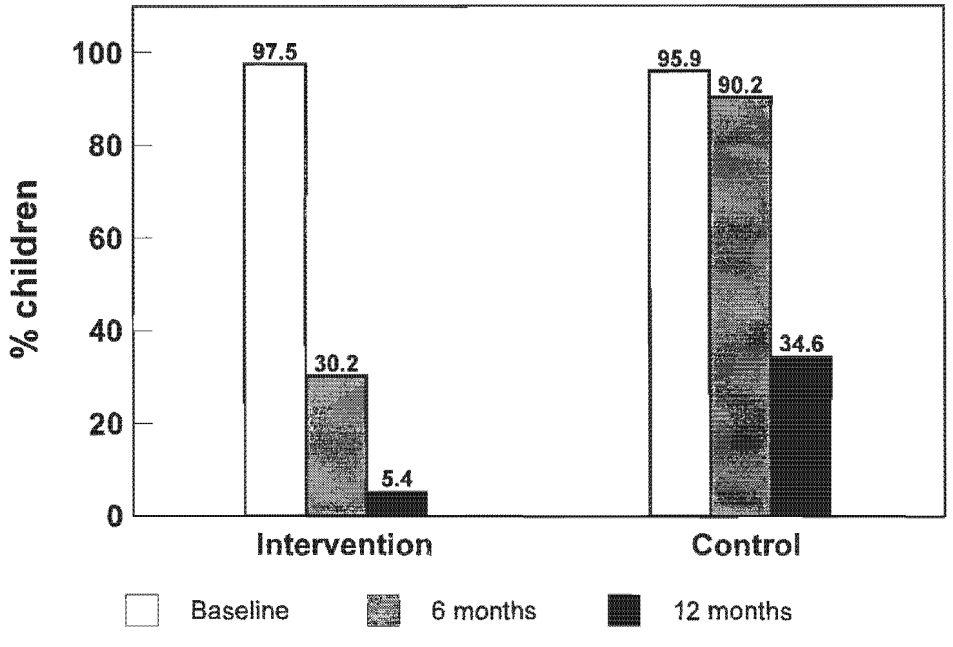

Figure 4.9: The prevalence of low urinary iodine concentrations (< 100 µg/L) at baseline, 6 months and 12 months in the intervention and control groups.

A weak but significant correlation was found between serum ferritin concentrations and white blood cell counts in the control group at the baseline assessment (r=0.24; *P*=0.0131), but not at the other assessments. A decrease in median white cell count was observed over the 12-month period, which was significant in the control group (**Table 4.4**). No correlation was found between serum retinol and white blood cell counts in either group at any of the assessments.

4.3.2.2 *Anthropometric status*

For the study population as a whole, there was no significant difference between the intervention and control groups in the change in height and weight, or height-for-age and weight-for-age Z-scores over the 12-month intervention period. However, in the children with low serum ferritin levels at baseline, a positive effect was observed on the changes in height and height-for-age Z-scores (**Table 4.6**), but not for the weight and weight-for-age Z-scores. Vitamin A status at baseline had no effect on the changes in any of the anthropometric indicators.

Table 4.6: Change in height and height-for-age Z-scores over the 12-month intervention period of children with low and adequate iron stores at baseline.

	Change in height (cm)				**Change in height-for-age Z-score**			
	n	*Baseline ferritin < 20 µg/L*	n	*Baseline ferritin ≥ 20 µg/L*	n	*Baseline ferritin < 20 µg/L*	n	*Baseline ferritin ≥ 20 µg/L*
Intervention	32	5.43 (1.40) [a]	83	5.01 (1.08)	32	−0.05 (0.32) [a]	83	−0.14 (0.19)
Control	32	4.69 (1.19)	81	5.08 (1.58)	32	−0.17 (0.20)	81	−0.12 (0.27)

[a] $P < 0.05$ compared to control group (Wilcoxon 2-sample test).

4.3.2.3 *Morbidity*

Fewer school days (expressed per 100 children) were missed in the intervention than in the control group as a result of respiratory-related illnesses and significantly fewer as a result of gastrointestinal-related symptoms (**Figure 4.10**). There were no cases of measles, chickenpox , or mumps during the study period.

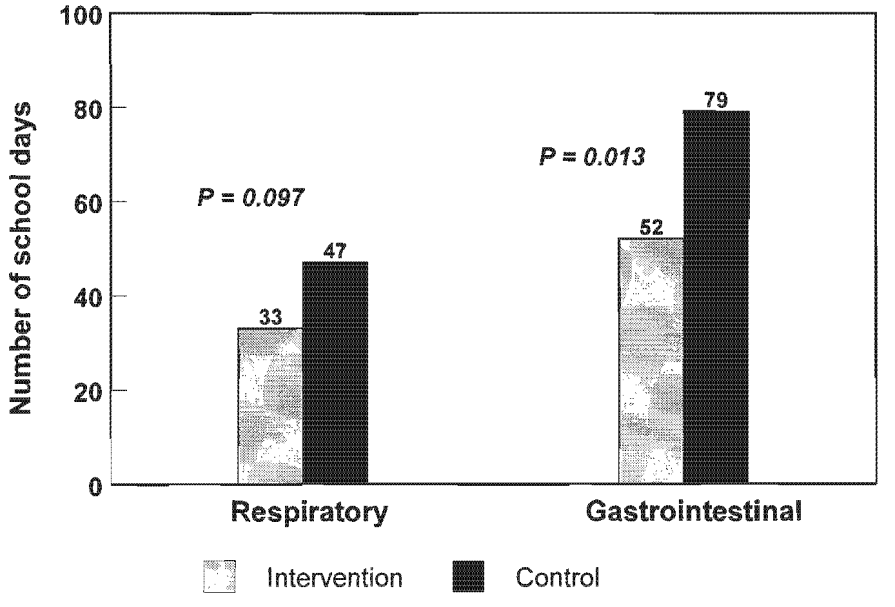

Figure 4.10: Number of school days missed (per 100 children) as a result of respiratory- and gastrointestinal-related symptoms.

4.3.2.4 *Cognitive function*

The baseline cognitive scores for the various tasks, together with the change from baseline to 12 months, for the intervention and control groups are presented in **Table 4.7**. There were no significant differences between the intervention and control groups in baseline scores for any of the tasks. There was a significant between-group treatment effect only for the digit span forward task. When, however, the children who presented with goitre at baseline were analysed separately, significant treatment effects were also found for the verbal fluency task (P=0.027) and the digit span forward task (P=0.025). The children with low serum ferritin at baseline showed significant treatment effects for the reading numbers task (P=0.016), as well as for the digit span forward task (P=0.042).

Table 4.7: Change in cognitive scores from baseline to 12 months.

Task	Intervention			Control		
	n	Baseline	Change	n	Baseline	Change
Verbal fluency (number of items)	67	46.3 (8.7)	1.73 (9.12)	68	45.7 (9.0)	1.68 (9.56)
Digit copying (time; seconds)	65	108.7 (35.0)	–21.3 (26.2)	68	108.0 (34.2)	–22.7 (26.2)
Writing crosses (number of crosses)	67	11.3 (2.7)	0.97 (2.68)	68	11.6 (2.4)	1.25 (2.21)
Counting letters (time; seconds)	67	28.1 (12.5)	–3.94 (14.3)	68	32.2 (14.3)	–5.90 (15.3)
Cancelling letters (time; seconds)	67	43.7 (15.9)	–10.3 (14.7)	68	45.5 (18.9)	–11.7 (16.6)
Reading numbers (time; seconds)	64	48.5 (26.1)	–13.0 (21.3)	64	45.8 (18.0)	–10.3 (11.5)
Digit span forward (number of digits)	67	4.8 (1.0)	0.61 (1.01) [a]	68	5.0 (0.82)	0.25 (0.83)
Digit span backward (number of digits)	62	3.1 (0.8)	0.55 (1.26)	66	3.2 (1.0)	0.59 (1.25)
Counting backwards (time; seconds)	47	63.3 (25.0)	–21.5 (22.9)	43	66.5 (37.2)	–25.5 (29.9)

Values expressed as mean (SD). [a] Significantly different from change in the control group, $P < 0.05$ (Wilcoxon 2-sample test); no significant difference between intervention and control groups in baseline scores for any of the tasks.

4.3.3 Compliance and acceptability of the biscuit

Mean compliance, measured by means of record sheets was 92.4% and 93.4% in the intervention and control groups, respectively; absence from school was the main reason for "non-compliance". The taste of both the biscuit and the cold drink was acceptable to all of the children and 74% indicated at the 12-month assessment that they would prefer more than the three biscuits they were receiving.

4.3.4 Eating patterns before and during the school day

Eating patterns before and during the school day are summarised in **Table 4.8**. Most of the children at the baseline assessment (approximately 90%) reported eating breakfast before coming to school; this percentage remained so throughout the study. Reasons for not eating breakfast were mainly "late for school", "not hungry" or "no food available"; none of the children gave the biscuit handed out at school as a reason for not eating breakfast. Very few children reported bringing food to school. However, 70% reported bringing money to buy something to eat during the day; items usually bought were potato crisps, sweets, cold drinks, cookies, sandwiches, fried fish or fruit and were either purchased from the local shop or from fellow students.

Table 4.8: Eating patterns before and during the school day.

	Baseline	**6 months**	**12 months**
Eat breakfast (%)	89.4	90.0	94.3
Reasons for not eating breakfast	*late for school; not hungry; no food available*		
Bring food to school (%)	4.4	0.8	2.2
Buy something to eat at school (%)	72.9	62.2	73.3
Items usually bought	*potato crisps, sweets, cold drinks, cookies, sandwiches, fried fish, fruit (apple, banana, orange)*		

4.4 DISCUSSION

This study has shown that fortification of a biscuit with iron, iodine and ß-carotene resulted in a significant improvement in the micronutrient status of primary school children from a poor rural community. Prior to the intervention a feeding scheme, consisting mainly of soy beans, rice, cabbage and potatoes, had been in operation at the school for a period of two years. Yet, despite this scheme, a substantial number of children at the baseline assessment presented with micronutrient deficiencies at biochemical level. This implies that the school feeding scheme did not supply enough micronutrients to prevent or eliminate existing micronutrient deficiencies and strengthens doubts raised by some authors that school feeding programmes may not always be effective (Walker and Walker, 1986; Reitsma *et al.*, 1994).

The intervention resulted in a drop in the prevalence of low serum retinol concentrations from a level regarded as a severe public health problem to a level almost no longer regarded a problem (WHO,1996). The study by De Pee *et al.* (1995) has shown ß-carotene, used as a fortificant in a wafer, also to be effective in improving serum retinol concentrations. Their study, however, showed no improvement in vitamin A status when the same amount of ß-carotene was given in the form of dark-green leafy vegetables. The authors suggest that ß-carotene from dark-green leafy vegetables is poorly absorbed because it is trapped in a complex matrix within the plant cell. This low bioavailability may also be a reason that 40% of the children in the present study had subclinical vitamin A deficiency at the baseline assessment, despite their regular consumption of *imifino*, a dark-green leafy vegetable, providing approximately 700 µg RE per 100 g cooked portion (see **section 3.2** for the dietary intake of the study population).

Although the prevalence of anaemia at baseline in both groups combined was 27%, and 32% of the children had low TS values, very few children (4%) were iron depleted (ferritin < 10 µg/L) and only 3% had both low TS and low ferritin values. It is possible that serum ferritin may have been falsely elevated due to the presence of infections (Baynes, 1996), thereby obscuring the real prevalence of iron deficiency. The drop, for no apparent reason, in serum ferritin from baseline to 12 months in the control group, as well as the concomitant drop in white cell counts, strengthens

this speculation. Helminth infections may have contributed to a higher infection rate at baseline, as the children were dewormed only after blood had been drawn. Data on C-reactive protein, a more sensitive indicator of infection, were unfortunately not available for the baseline assessment.

It is difficult to explain the drop in haemoglobin at 6 months; a decrease was, however, also experienced in the control group. Seasonal haemodilution, which has been described elsewhere (Lee *et al.*, 1987), and which was also observed in a previous intervention study by this group (Van Stuijvenberg *et al.*, 1997), might have played a role. Serum ferritin, serum iron and TS appear to have reached a plateau after 6 months of intervention. Absorption of iron is known to be inversely related to the size of body iron stores (Baynes & Bothwell, 1990). It is therefore possible that less iron was absorbed as the iron status of the study population improved. It may, however, also have been due to a mucosal block to iron absorption resulting from prior iron administration (O'Neil-Cutting & Crosby, 1987). As we do not have data on the intervention effects before 6 months, it is not known at what stage of the intervention this plateau was reached.

Median urinary iodine excretion in the intervention group increased significantly from a level regarded as a *moderate to severe* public health problem to a level no longer regarded a public health problem (WHO/UNICEF/ICCIDD, 1994). The slight increase in the control group at 6 months can be explained by the fact that the unfortified biscuit also contained some iodine; this was due to a marine oil used in the baking process. The mandatory iodisation of salt (40-60 ppm) which came into effect in South Africa shortly after the 6-month assessment had been completed, offered the unique opportunity to use the second half of this study to evaluate the impact of the iodisation of salt on this study population. At 12 months, the median urinary iodine concentration in the intervention group increased further to > 200 µg/L, whereas in the control group it rose to a concentration similar to that observed in the intervention group after the first 6 months of the study. The salt iodisation programme was thus as effective as the biscuit in raising urinary iodine concentrations, and has the additional benefit of reaching a much wider population. We found no reduction in the prevalence of goitre in either group; 12 months of fortification might, however, have been too short to reverse an already enlarged thyroid. No reduction in the prevalence of

goitre, despite a significant increase in urinary iodine excretion, was also found in a study by Jooste *et al.* (2000) evaluating the effectiveness of mandatory iodisation of salt over a period of 12 months.

The intervention had no effect on the anthropometric status of the group as a whole. This was not unexpected, as very few children were stunted or underweight at the baseline assessment, and the growth rate in children in this age category is much slower than during the first few years of life. However, a positive effect on the height and height-for-age Z-scores was demonstrated in the children with low serum ferritin values at baseline, which suggests that iron may have been a limiting factor in the growth of these children. This is in agreement with a previous study by our group where iron fortification combined with anthelmintic therapy had a significantly greater effect on the anthropometric indices of children with low iron stores at baseline (Kruger *et al.*, 1996).

The high prevalence of micronutrient deficiencies, despite the low prevalence of stunting and underweight, emphasises the danger of relying on anthropometry as the only indicator of nutritional status; the term "hidden hunger", as micronutrient malnutrition is often referred to, very appropriately describes the situation in this population prior to the intervention.

The cognitive tests administered to the children were designed to measure a range of mental processes and fine motor skills (e.g., verbal learning, visual memory, arousal, attention, retrieval, eye-hand perception, and co-ordination) that are thought to be affected by nutritional deficits. A significant improvement compared to the control group was found for the digit span forward task, which is a measure of short-term memory and attention. Cognitive development can be influenced by factors other than micronutrient deficiencies e.g., parasitic infestation (Connolly & Kvalsvig, 1993) and short-term hunger (Simeon & Grantham-McGregor, 1989). In the present study, both groups received anthelmintic therapy and the confounding effect of short-term hunger was eliminated by the placebo biscuit, which supplied an equivalent amount of macronutrients. A confounding factor beyond our control, however, was the compulsory iodisation of salt during the second half of the study. Iodine-related improvements in cognitive function that might have

occurred in the intervention group would have been partially masked by improvements in the control group. The best way to demonstrate the effect of micronutrient supplementation on cognitive function would be to include only children with micronutrient deficiencies in a study, because an effect is not expected in children who are micronutrient replete. The present study included both micronutrient-deficient and micronutrient-replete children, thus diluting possible intervention effects. When the children with micronutrient deficiencies at baseline were analysed separately, intervention-related effects were also found for the reading numbers task in the children with marginal iron status at baseline, and for the verbal fluency task in the children with goitre at baseline. Improvement in verbal fluency in schoolchildren supplemented with iodine was also observed in the studies of Shrestha (1994) and Van den Briel *et al.* (2000).

Both the deficiencies of iron and vitamin A can increase susceptibility to infections (Baynes & Bothwell, 1990; Sommer, 1990). The fewer school days missed in the intervention group as a result of respiratory- and gastrointestinal-related illnesses could have beneficial effects on learning should the children receive the fortified biscuits for several years as part of a school feeding programme. A shortcoming of this methodology, however, was that our records did not include morbidity data for weekends and holidays, and that for a diagnosis the nutrition monitor had to rely on information provided by the mother or the child. The morbidity trends observed in this study are nevertheless promising and merit further investigation.

In this study it was demonstrated that a biscuit can be used successfully as a vehicle for fortification in school feeding. The fortified biscuit resulted in a significant improvement in micronutrient status and also appeared to have had a favourable effect on the morbidity, anthropometric status and cognitive function of the schoolchildren in this community. The effectiveness of feeding schemes has often been questioned and, according to Beaton and Ghassemi (1982), they may disrupt the balance the child has with an unfavourable environment. The use of a fortified biscuit in school feeding has a major advantage over other school feeding options in that a biscuit is regarded as a snack rather than a meal, and is therefore unlikely to replace meals given to the child at home. The fact that there was no decrease in the number of

children who reported eating breakfast before coming to school further strengthens this notion. Additional advantages of using a micronutrient-fortified biscuit in school feeding, compared to other school feeding options, are that it needs no preparation, is easy to distribute, has a long shelf life and can be monitored easily. The cold drink used in the study served merely as a carrier for vitamin C to enhance the absorption of iron. The use of a more bioavailable form of iron as a fortificant e.g., an iron chelate (Layrisse & Martinez-Torez, 1977; Pineda *et al.*, 1994), could eliminate the need for vitamin C, and thus the cold drink, and reduce the cost of the intervention by half.

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Chapter 5

LONG-TERM EVALUATION OF THE EFFECTIVENESS OF THE BISCUIT INTERVENTION IN MAINTAINING IMPROVED MICRONUTRIENT STATUS

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LONG-TERM EVALUATION OF THE EFFECTIVENESS OF THE BISCUIT INTERVENTION IN MAINTAINING IMPROVED MICRONUTRIENT STATUS

5.1 INTRODUCTION

In the previous chapter the results of a randomised controlled trial, evaluating the effect of a biscuit fortified with iron, iodine and ß-carotene on the micronutrient status of primary school children from a poor rural community, are described. Compared to a control group, consumption of this biscuit resulted in a significant improvement in vitamin A, iron and iodine status; cognitive function, anthropometric status and morbidity also appeared to have been favourably affected.

In the above study the biscuit intervention was evaluated over a period of 12 months. If, however, a fortified biscuit programme is to be implemented in primary schools, children may be exposed to this intervention for a period of several years. The effect of long-term exposure to such a programme in terms of virtual elimination of micronutrient deficiencies, compliance, acceptability and sustainability, is not known. To answer this question the biscuit intervention was continued and the micronutrient status of the experimental group followed for a further 18 months.

5.1.2 Objectives of the study

The primary aim of the present study was to evaluate, in a *longitudinal study*, the effect of the biscuit intervention over a period of 2.5 years (30 months). In addition, data from three subsequent *cross-sectional* surveys, conducted in the same school at 33, 42 and 45 months after the start of the original intervention, are reported; during this time the fortified biscuit continued to be distributed at the school.

5.2 SUBJECTS AND METHODS

5.2.1 Study population and design

The study population comprised the 115 children who formed the experimental arm of the randomised controlled trial (RCT), evaluating the effect of the micronutrient-fortified biscuit over a period of 12 months. After completion of the trial the biscuit intervention was continued and the children from the experimental group followed in a *longitudinal study* for an additional 18 months; during this time the control group from the original trial was also given a fortified biscuit. The original study lasted from May 1995 to June 1996; blood was drawn before the start of the intervention and again after 6 (November 1995) and 12 months (June 1996) of intervention. Of the 115 children who completed the randomised controlled trial, the micronutrient status of 108 was assessed again in February 1997, when the school reopened after the summer holiday break, but before the biscuit programme for that year was resumed; and again in November 1997, i.e. after a total of 30 months (2.5 years) on the biscuit programme. A schematic presentation of the various time-points during the longitudinal study at which blood was drawn is given in **Figure 5.1**.

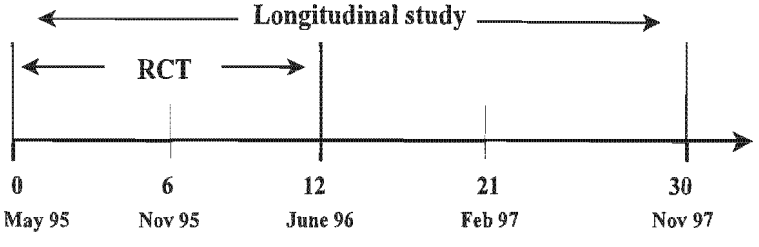

Figure 5.1: The various time-points (months) at which blood was drawn over the 30-month study period.

The biscuits were continued to be distributed daily during the school week and compliance continued to be monitored daily by the nutrition monitors. Again, no intervention took place during school holidays, weekends or public holidays; the biscuits were supplied for a total of 441 days during the 30-month intervention period. The children continued to be dewormed at regular intervals.

5.2.2 Fortification

The composition of the biscuit was the same as for the previous trial (Chapter 4), i.e. providing 50% of the RDA for children aged 7-10 years (National Research Council, 1989) for iron, iodine and ß-carotene. The vitamin C-fortified cold drink, to enhance the absorption of iron, was continued to be provided; the cold drink, however, no longer contained iodine. Each new batch of biscuits and cold drinks continued to be analysed for micronutrient content to ensure that levels were maintained throughout the study.

5.2.3 Additional cross-sectional data

Data from three additional cross-sectional surveys conducted in the same school at 33, 42 and 45 months after the start of the biscuit intervention are also reported (**Figure 5.2**). The data were collected as part of another study which evaluated the effect of an ω3-fatty acid supplement on the cognitive function of primary school children (Tichelaar *et al.*, 2000), and to make sure that micronutrient deficiencies as confounding factors were excluded, the micronutrient-fortified biscuit was continued to be supplied to all the children. This opportunity was used to obtain information on the vitamin A and iron status of the children from this school for a further 15 months.

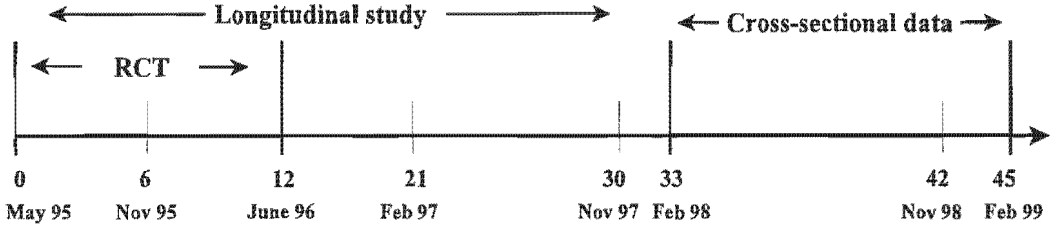

Figure 5.2: The various time-points (months) at which blood was drawn over the 45-month study period.

Two hundred and fourteen children were assessed in February 1998 at the start of a new school year and at a follow-up assessment in November 1998, and 223 again in February 1999, the beginning of the 1999 school year. Because they were not necessarily the same children as those surveyed in the original biscuit study, this phase of the study was no longer longitudinal, but rather *cross-sectional*; the estimated sample overlap between the original cohort and the February

1998 and February 1999 samples was 26% and 17%, respectively. For all three surveys, grade 1 pupils (i.e. new school entrants who had not been exposed to the biscuit intervention the year before) were excluded from the data set; all children thus had exposure to the biscuit programme for at least one year. Children continued to be dewormed at regular intervals.

There were certain differences with regard to the fortification of the biscuit during the cross-sectional phase of the study: (i) due to the mandatory iodisation of salt which came into effect on 1 December 1995, the biscuit supplied to the children in this study was no longer fortified with iodine; (ii) instead of the ferrous fumarate, which was used as the iron fortificant during the first 30 months, a chelated form of iron (ferrous bisglycinate) was used as fortificant during this latter phase of the study; (iii) vitamin C to enhance the absorption of iron was thus thought no longer to be required and the vitamin C-fortified cold drink no longer supplied.

5.2.4 Measurements

Micronutrient status at each time-point was assessed in terms of serum retinol, serum ferritin, serum iron, transferrin saturation and urinary iodine excretion. A full blood count was performed and serum C-reactive protein also determined. No data on serum iron, transferrin saturation and urinary iodine were, however, available for the cross-sectional phase of the study. A detailed description of all the methods is given in Chapter 3. Acceptability of the biscuit, as well as breakfast patterns, continued to be monitored by means of a short questionnaire administered at the various assessments (**Appendix E**).

5.2.5 Statistical analysis

Data were analysed using the SPSS for Windows software package (version 9.0, SPSS Inc., Chicago, IL, USA). Results are presented as means and standard deviations; however, where values are not normally distributed, medians, together with the 10^{th} and 90^{th} percentiles, are given. Comparisons with regard to micronutrient status were made in relation to the immediately-preceding time-point, except for the second and third surveys, which were compared to baseline (these two surveys were part of the original randomised controlled trial where the efficacy of the biscuit was demonstrated against a control group, and are repeated as such in order to set the

scene for what is to follow). White blood cell counts at each assessment were compared to baseline. The Wilcoxon signed rank test for paired data was used to compare longitudinal data and the Mann-Whitney *U* test to compare cross-sectional data. *P*-values < 0.05 were considered statistically significant.

5.3 RESULTS

Table 5.1 gives the mean or median levels of the biochemical indicators of micronutrient status at the various time-points during the longitudinal study, as well as at the three subsequent cross-sectional surveys. Serum retinol improved significantly during the first 12 months of intervention. However, at the 21-month assessment in February 1997, which took place shortly after the school reopened after the summer holiday, there was a significant decrease in serum retinol; biscuits were not distributed during school holidays. Retinol concentrations increased again during the next nine months, but were significantly lower in the subsequent cross-sectional survey carried out directly after the summer holiday; this pattern was repeated in the two further cross-sectional surveys, conducted before and after the holiday period. These fluctuations are also reflected in **Figure 5.3**, where the prevalence of low serum retinol levels is given over time.

There was also a significant improvement in serum ferritin, haemoglobin and transferrin saturation during the first 12 months of intervention. However, when the school reopened after the summer holiday all three variables returned to pre-intervention levels (**Table 5.1**). Haemoglobin showed a gradual deterioration at each subsequent assessment over the next two years. Serum ferritin, apart from an increase at the 42-month assessment at the end of the school year, also deteriorated. Transferrin saturation increased again between 21 and 30 months, but not by the same magnitude as during the first 12 months of intervention; transferrin saturation was not measured in the three subsequent cross-sectional surveys. The prevalences of low serum ferritin and haemoglobin levels over this period are given in **Figures 5.4 and 5.5**. White blood cell counts showed a gradual decline over time, with the values at the fourth, fifth, sixth, seventh and eighth surveys being significantly lower than that at baseline (**Table 5.1**).

Table 5.1: The biochemical indicators of micronutrient status at various time-points during the longitudinal study of 2.5 years, and at three subsequent cross-sectional surveys conducted in the same school during the extension of the biscuit programme.

	Longitudinal study					Cross-sectional data		
	May 1995 0 months (n=108)	**Nov 1995** 6 months (n=108)	**June 1996** 12 months (n=108)	**Feb 1997** 21 months (n=108)	**Nov 1997** 30 months (n=108)	**Feb 1998** 33 months (n= 214)	**Nov 1998** 42 months (n= 214)	**Feb 1999** 45 months (n= 223)
Age (years)	8.8 (2.0)	9.3 (2.0)	9.9 (2.0)	10.6 (2.0)	11.3 (2.0)	9.3 (1.7)	10.0 (1.7)	9.5 (1.7)
Serum retinol † (μg/dL)	22.1 (5.3)	25.1 (6.2)[a]	26.1 (6.3)[a]	23.1 (5.9)[d]	25.4 (5.9)[d]	20.8 (6.0)[d]	26.5 (7.3)[d]	21.9 (6.6)[d]
Serum ferritin † (μg/L)	28.6 (13.1, 58.5)	34.0 (18.4, 66.3)[b]	33.8 (19.2, 57.3)[b]	26.2 (13.9, 51.3)[d]	23.7 (14.6, 45.7)[NS]	27.7 (10.9, 55.3)[NS]	34.4 (10.2, 72.1)[e]	22.7 (8.3, 46.7)[d]
Transferrin saturation † (%)	21.2 (11.5)	26.9 (11.6)[a]	26.0 (10.4)[a]	18.8 (8.1)[d]	22.1 (8.5)[e]	-	-	-
Haemoglobin † (g/dL)	12.55 (0.81)	12.41 (0.79)[c]	12.91 (0.81)[a]	12.32 (0.84)[d]	12.20 (0.85)[NS]	12.10 (0.75)[NS]	11.98 (0.74)[e]	11.59 (0.79)[d]
Urinary iodine † (μg/L)	20 (8, 47)	146 (49, 254)[a]	225 (112, 289)[a]	230 (75, 279)[NS]	232 (76, 779)[NS]	-	-	-
White blood cell count (x 10^9/L)	8.0 (2.5)	7.7 (2.2)[NS]	7.8 (2.3)[NS]	7.5 (2.1)[c]	7.5 (3.2)[b]	7.2 (2.3)[b]	6.9 (2.6)[a]	6.3 (2.1)[a]

All values expressed as mean (SD), except for iodine and ferritin which are expressed as median (10^{th}, 90^{th} percentile).
† Improvement at 12 months (June 1996) significant compared to a control group (Chapter 4).
[a] $P < 0.0001$, [b] $P < 0.005$, [c] $P < 0.05$ compared to baseline; [d] $P < 0.0001$, [e] $P < 0.005$ compared to *immediately-preceding* time-point.
NS= not significant compared to *immediately-preceding* time-point, or to baseline for white blood cell counts.

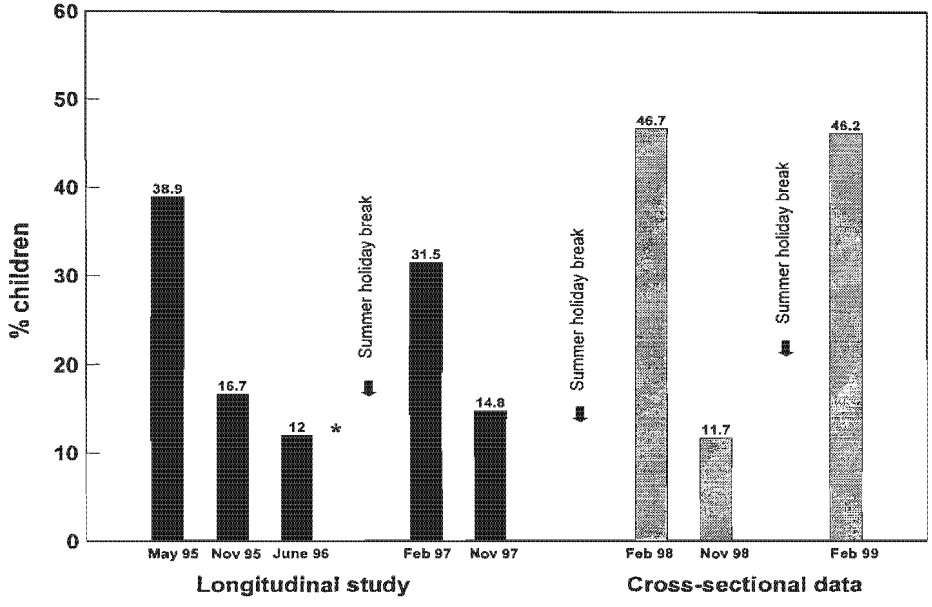

Figure 5.3: The prevalence of low serum retinol levels (< 20 μg/dL) during the longitudinal study of 2.5 years, and at three subsequent cross-sectional surveys conducted in the same school. *Improvement at 12 months significant compared to a control group (Chapter 4).

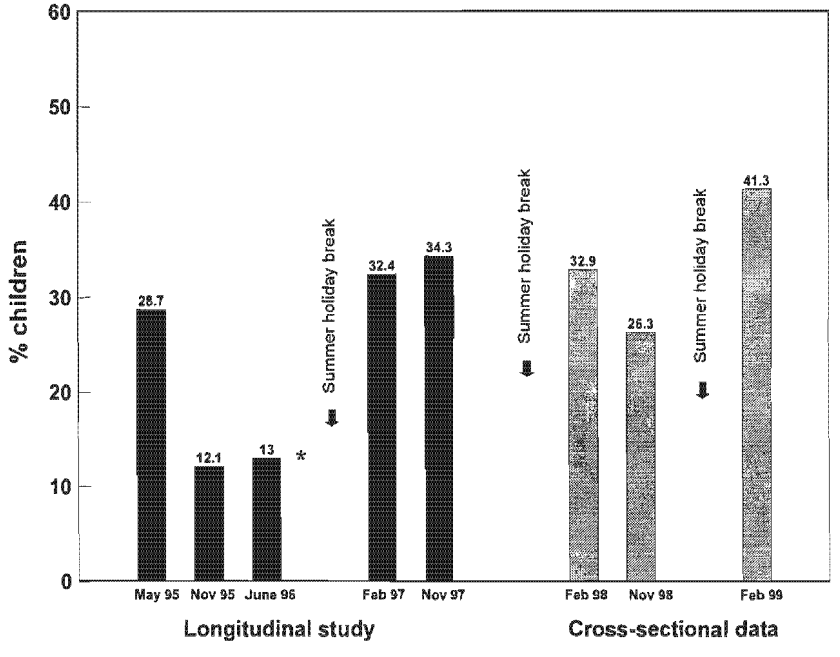

Figure 5.4: The prevalence of low serum ferritin levels (< 20 μg/L) during the longitudinal study of 2.5 years, and at three subsequent cross-sectional surveys conducted in the same school. *Improvement at 12 months significant compared to a control group (Chapter 4).

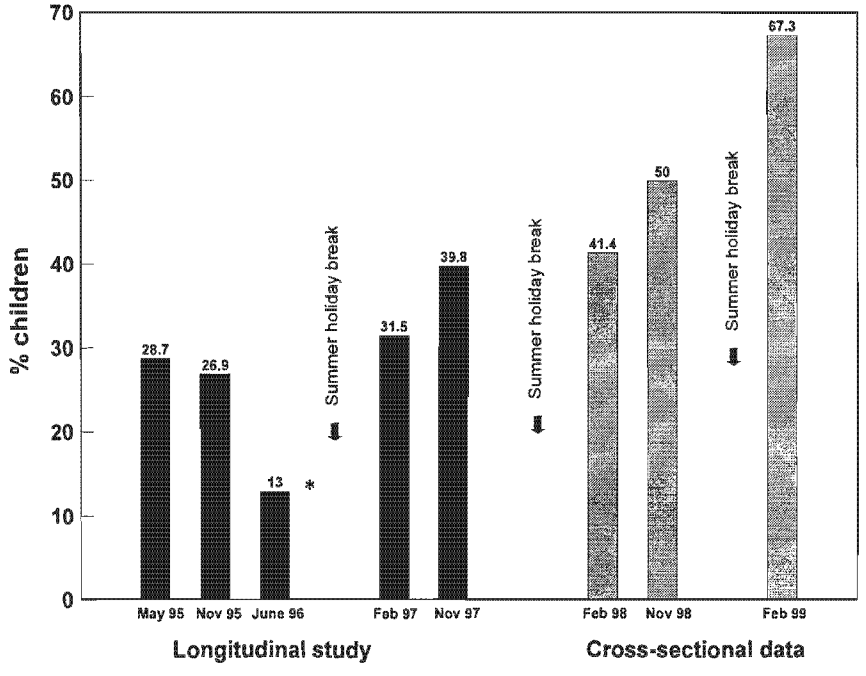

Figure 5.5: The prevalence of low haemoglobin levels (< 12 g/dL) during the longitudinal study of 2.5 years, and at three subsequent cross-sectional surveys conducted in the same school. *Improvement at 12 months significant compared to a control group (Chapter 4).

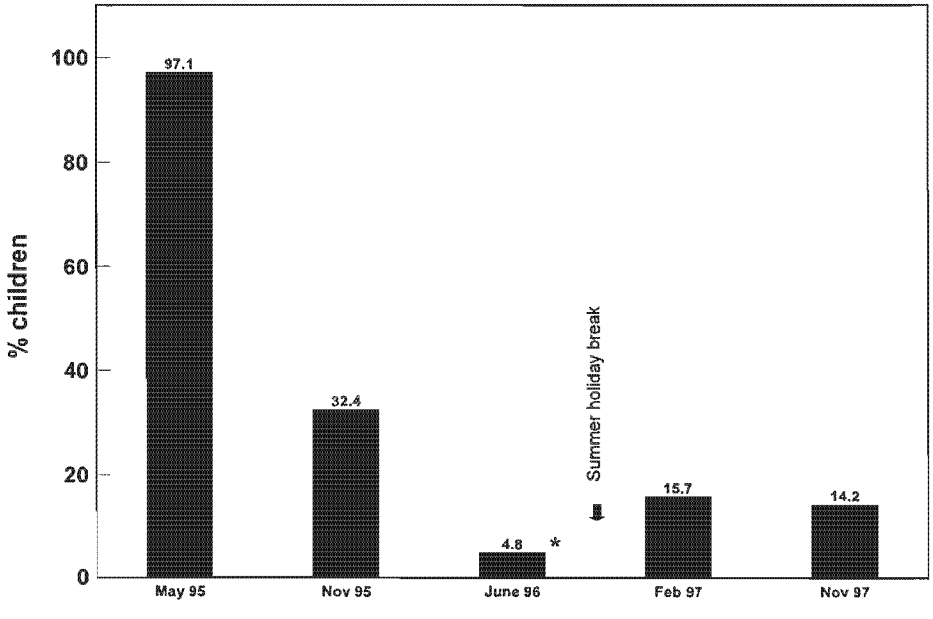

Figure 5.6: The prevalence of low urinary iodine levels (< 100 μg/L) during the longitudinal study of 2.5 years. *Improvement at 12 months significant compared to a control group (Chapter 4).

Because the majority of the children (70%) were not anaemic at the baseline assessment and therefore may have diluted the response to the intervention, we also report the iron status of the 30% that were anaemic (haemoglobin < 12 g/dL) separately for the 2.5 year period (**Table 5.2**). Again there was an increase in all the indicators of iron status over the first 12 months, which was accompanied by a significant decline after the summer school holiday. Only serum iron and transferrin saturation showed a significant increase during the following nine months, though this did not match the magnitude seen during the first 12 months of intervention; serum ferritin and haemoglobin remained unchanged.

Table 5.2: The iron status of the children with baseline haemoglobin concentrations < 12 g/dL during the 2.5-year longitudinal study.

	May 1995 0 months (n=31)	**Nov 1995** 6 months (n=31)	**June 1996** 12 months (n=31)	**Feb 1997** 21 months (n=31)	**Nov 1997** 30 months (n=31)
Haemoglobin (g/dL)	11.63 (0.36)	11.76 (0.60) ^{NS}	12.28 (0.66) ^{a}	11.73 (0.70) ^{b}	11.60 (0.59) ^{NS}
Serum ferritin (μg/L)	32.0 (9.9, 52.6)	30.3 ^{NS} (15.3, 59.7)	38.5 ^{NS} (17.8, 50.3)	23.0 ^{d} (9.8, 57.5)	23.6 ^{NS} (13.8, 47.4)
Serum iron (μmol/L)	10.4 (4.8)	17.2 (7.4) ^{a}	16.6 (6.3) ^{a}	12.1 (7.1) ^{c}	14.7 (5.8) ^{d}
Transferrin saturation (%)	17.3 (8.3)	27.2 (11.0) ^{a}	26.3 (11.2) ^{a}	18.2 (8.4) ^{c}	23.3 (10.6) ^{d}

All values expressed as mean (SD), except for ferritin which is expressed as median (10^{th}, 90^{th} percentile).
^{a} $P < 0.0001$ compared to baseline; ^{b} $P < 0.0001$, ^{c} $P < 0.005$, ^{d} $P < 0.05$ compared to *immediately-preceding* time-point; NS=not significant compared to *immediately-preceding* time-point or to baseline for 2nd and 3rd surveys.

The distribution of serum ferritin concentrations during the 2.5-year longitudinal study is illustrated in **Table 5.3**. Only 1.9% (two children) had serum ferritin concentrations above 100 μg/L at the baseline assessment and there was no increase in the number of children in this category over the next 30 months (the highest concentrations for the five respective assessments were 122.6 μg/L, 118.2 μg/L, 142.8 μg/L, 112.3 μg/L and 101.3 μg/L). Not a single child had consistently high levels of serum ferritin throughout the 30-month study period and in all cases

ferritin levels above 100 µg/L were associated with either elevated C-reactive protein or white blood cell concentrations.

Table 5.3: Distribution of serum ferritin during the 2.5-year longitudinal study.

	% children				
	May 1995 0 months (n=108)	**Nov 1995** 6 months (n=108)	**June 1996** 12 months (n=108)	**Feb 1997** 21 months (n=108)	**Nov 1997** 30 months (n=108)
Serum ferritin					
< 10 µg/L	5.6 %	0.9 %	0.9 %	5.6 %	4.6 %
10 -19.9 µg/L	23.1%	11.2 %	12.0 %	26.9 %	29.6 %
20 -59.9 µg/L	62.0 %	75.7 %	78.7 %	60.2 %	61.1 %
60 -99.9 µg/L	7.4%	10.3 %	6.5 %	6.5 %	3.7 %
> 100 µg/L	1.9 %	1.9 %	1.9 %	0.9 %	0.9 %

Urinary iodine improved significantly from baseline to 12 months (**Table 5.1**), and the prevalence of low urinary iodine dropped from 97.1% before the intervention to 4.8% after the first 12 months of intervention (**Figure 5.6**). This low prevalence was maintained for the rest of the longitudinal study. Urinary iodine was not measured at the subsequent cross-sectional surveys.

Compliance, defined as the number of days that a child received the biscuit expressed as a percentage of the total number of potential biscuit days, was 92.4% during the first 12 months (Chapter 4) and 80% during the last nine months of the longitudinal study. Reason for non-compliance was mostly absence from school (8%) and unavailability of the biscuit (12%), which was due to problems experienced with the supply and delivery of the biscuits by the manufacturing company. Compliance during the cross-sectional phase of the study was 95%. The taste of both the biscuit and cold drink was acceptable to all (100%) of the children at both the 12- and 30-month assessments; 74% of the children at the 12-month assessment (Chapter 4) and

78.5% of the children at the 30-month assessment indicated that they would prefer more than the three biscuits they were receiving.

Eighty-nine per cent of the children at the baseline assessment, and 90%, 94%, and 90% at the 6-month, 12-month and 30-month assessments, respectively, reported eating breakfast before coming to school in the morning; none of the children at any of the assessments gave the biscuit handed out at school as a reason for not eating breakfast.

5.4 DISCUSSION

This study evaluated the effect on micronutrient status of a micronutrient-fortified biscuit, given to primary school children, over a period of 2.5 years. In addition, cross-sectional data from three subsequent surveys conducted in the same school over a further 15-month period are reported. The biscuit was shown to be effective in improving the iron, iodine and vitamin A status of these children during the first 12 months of intervention (Chapter 4); both vitamin A and iodine deficiencies improved from levels regarded as a severe public health problem to levels no longer regarded a significant problem (WHO/UNICEF/ICCIDD, 1994; WHO, 1996). However, at the 21-month assessment, which took place when the schools reopened after the school holidays, a significant decline to pre-intervention status had occurred in serum retinol, serum ferritin, transferrin saturation and haemoglobin.

In South Africa, the academic school year usually lasts from late January to early December, followed by a long summer break of approximately 6-7 weeks. The biscuit programme was stopped in the middle of November 1996 (after the end-of-year school exams when there is usually a dramatic decline in school attendance in this community) and had not been resumed at the time of the February 1997 survey. There was therefore a period of ~10 weeks during which the children were not exposed to the biscuit programme.

The biscuits supplied 50% of the RDA for vitamin A per day in the form of ß-carotene. A recent survey of the dietary intake of the children in this school showed the median vitamin A intake to

be 60% of the RDA; this included the 50% of the RDA that was supplied by the biscuit (Faber *et al.*, 1999). Meals given to the child at home therefore contributed very little to the vitamin A intake of the child. It is therefore not surprising that serum retinol returned to pre-intervention levels during the time that the biscuit was not supplied. A similar drop in serum retinol was observed in the two subsequent cross-sectional surveys, which were also undertaken directly after the summer holidays for two consecutive years. No data were collected after the winter school holidays, which lasts approximately four weeks. It is therefore not known whether micronutrient levels also revert to pre-intervention levels after shorter periods without the fortified biscuit.

The ß-carotene supplied by the biscuit was probably just enough to maintain serum retinol levels from day to day, but not sufficient to replenish stores or to maintain existing stores during periods when the biscuit was not consumed. There are indications from studies in rodents (Thatcher *et al.*, 1998) that previously fed ß-carotene present in the tissues is rapidly lost and not conserved for later use as a source of vitamin A, not even during periods of compromised vitamin A status; whether these results are applicable to humans, however, is not known.

Several options are available to overcome the problem of serum retinol levels that are not maintained during the long school holiday breaks. Increasing the amount of ß-carotene added to the biscuits is one solution. If, however, ß-carotene cannot be stored for later use as a source of vitamin A (Thatcher *et al.*,1998), this exercise would be futile, and the use of vitamin A as fortificant, rather than ß-carotene, would be a better option; excessive intake of vitamin A in this case would be unlikely, because the number of biscuits given to the children in a school setting is regulated. An alternative long-term approach in a community such as this might be to supplement the biscuit programme with nutrition education, as well as a local home gardening programme encouraging the production and consumption of ß-carotene-rich foods.

All the indicators of iron status declined to pre-intervention levels after the holiday break at 21 months, in the group as a whole and in those who were anaemic at the start of the study. The dietary survey (Faber *et al.*, 1999) undertaken in this community showed that the median intake

of iron (which included the 5 mg supplied by the three biscuits) was 109% of the RDA; the diet at home thus supplied 60% of the RDA. However, because of low bioavailability of the iron (mostly of plant origin and with low vitamin C intake), this amount may not have been enough to sustain blood levels during the holiday period. Only serum iron and transferrin saturation improved during the following nine months, but not by the earlier magnitude; there was no response in haemoglobin or serum ferritin, not even in those who were anaemic. A vitamin C-fortified cold drink to enhance the absorption of iron was provided together with the biscuit. This practice was meticulously adhered to during the first 18 months of the study. However, due to logistical problems with the manufacture and supply of the vitamin C-fortified cold drink, it was provided on a less frequent basis during the last nine months. This may have resulted in poorer absorption of iron from the biscuit. It has been demonstrated that ascorbic acid, given in the same meal, can increase iron absorption several fold (Derman *et al.*, 1977; Siegenberg *et al.*, 1991).

A more bioavailable form of iron, i.e. an iron amino acid chelate (ferrous bisglycinate), was used as fortificant during the cross-sectional phase of the study, and the cold drink with vitamin C was no longer supplied. Although there was some improvement in serum ferritin at 42 months, haemoglobin deteriorated further. Because all of the children received the micronutrient-fortified biscuit, there was no control group and no conclusions with regard to efficacy of the iron amino acid chelate used as fortificant in the biscuit can be made. In other studies that showed ferrous bisglycinate to be effective in the treatment of anaemia, the chelated iron was either given in the form of tablets (Pineda *et al.*, 1994) or was used as fortificant in products that were not baked for long periods (Name, 1995) or not baked at all (Iost *et al.*, 1998). The biscuit was baked at a temperature of 210°C for ~20 minutes and it is possible that degradation of the chelate at this temperature might have taken place. According to Albion Laboratories, Inc. (1995) it is not recommended that ferrous bisglycinate is heated by itself above 153°C, because decomposition of the amino acids and subsequent oxidation of the iron can take place. When added to products that are subsequently cooked, higher ambient temperatures may be tolerated, because the degree of destruction will depend on the internal temperature of the food being cooked. An ambient

temperature above 200°C may, however, have been too high for the ferrous bisglycinate to have survived in the biscuits. This, however, needs further investigation.

With any iron intervention the risk of iron overload should be considered. In Africa, iron overload may be caused by excessive intake of iron resulting from brewing beer in iron pots, as well as a genetic predisposition which is unrelated to HLA-linked haemochromatosis (Gordeuk *et al.*, 1992). For this intervention, however, the risk of iron overload is unlikely. The fortified biscuit intervention is targeted at primary school children only and the amount of iron contributed by the biscuits is only 5 mg per day (supplied for approximately 50% of the year only). This amount is negligible compared to intakes that are usually associated with iron overload. Furthermore, the results of our study show a general deterioration in iron status over time, with very few children having serum ferritin levels in excess of 100 µg/L. The latter were more likely to be the result of infection (Witte, 1991), as not a single child had consistently high levels of serum ferritin throughout the study, and in all cases ferritin levels above 100 µg/L were associated with either elevated C-reactive protein or white blood cell concentrations.

The general deterioration in serum ferritin over time is difficult to explain. It is possible that the iron deficiency that may have been prevalent at baseline was masked by a higher infection rate at that time, which declined as a result of the intervention, simultaneously resulting in a normalisation of the serum ferritin levels. Hulthén *et al.* (1998) reported a marked shift in serum ferritin towards higher values in adolescents with upper respiratory infections during the month preceding their survey. Data on C-reactive protein were unfortunately not available for all eight surveys. However, mean white blood cell counts do show a gradual deterioration over time, with counts from the fourth survey onwards being significantly lower than the count at baseline. A decline in infection rate may therefore have contributed to the decrease in ferritin levels. However, the gradual decline, also observed for haemoglobin, suggests that the decline in infection rate was not the only explanation for the decrease in ferritin levels and that some deterioration in iron status did indeed take place.

In cohort studies involving growing children the potential confounding effect of age on nutritional variables should be borne in mind. It is, however, unlikely that age had an effect on the results of this study. We analysed a subgroup of children (including only children aged 8-11 years for each of the eight surveys) and found the pattern to be identical to that reported for the whole group (data not shown).This excludes the possibility that age played a confounding role in the fluctuations in micronutrient status observed in this study.

Only iodine status did not return to pre-intervention levels after the school holiday break; median urinary iodine excretion was maintained well above the acceptable level of 100 µg/L throughout the study. This was probably due to the iodisation of salt which became compulsory in South Africa in December 1995, six months after the start of the biscuit intervention. Iodised salt was available in all of the local shops in the area and salt was used by all households in the preparation of food. There is therefore probably no further need to add iodine to a fortified biscuit distributed in South African schools. The effectiveness of the mandatory salt iodisation programme in South Africa was evaluated by Jooste *et al.* (2000) and shown to be effective in virtually eradicating iodine deficiency, as measured by urinary iodine excretion, in a group of schoolchildren within one year.

Distributing the biscuits at school appeared not to have had an effect on breakfast consumption. The percentage of children that reported eating breakfast before the start of the biscuit intervention was around 90%; this figure remained so throughout the 2.5-year study period. None of the children gave the biscuit given at school as a reason for not eating breakfast. This confirms the notion that the biscuit is perceived as a snack and not as a meal, and that it is unlikely to replace meals given to the child at home. After 2.5 years on the biscuit programme the biscuit was still well liked by all of the children and 78.5 % indicated that they would prefer more than the three biscuits they were receiving.

In conclusion, this study has shown that fortification of a biscuit with ß-carotene at a level of 50% of the RDA was enough to maintain serum retinol concentrations at acceptable levels from day

to day, but not enough to sustain levels during a break of ~10 weeks, especially if intake from other sources is insufficient. Long-term solutions such as local food production programmes combined with nutrition education should also be sought. It would also appear that the bioavailability of ferrous fumarate in the biscuit without the vitamin C-fortified drink is inadequate. The effectiveness of using a chelated form of iron as fortificant in the biscuit needs further investigation.

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Chapter 6

THE POTENTIAL OF RED PALM OIL AS A SOURCE OF ß-CAROTENE IN THE BISCUIT

CHAPTER 6

THE POTENTIAL OF RED PALM OIL AS A SOURCE OF ß-CAROTENE IN THE BISCUIT

6.1 INTRODUCTION

The randomised placebo-controlled trial described in Chapter 4 has shown a biscuit fortified with ß-carotene to be a feasible, practical and effective way of improving the vitamin A status of primary school children from a poor rural community. In this biscuit a hydrogenated oil, widely used in the baking industry, was used as baking fat. Hydrogenated oils contain *trans* fatty acids and because of the negative effects of the latter on plasma lipids and lipoproteins (Judd et al, 1994; Khosla & Hayes, 1996), it is preferable that a baking fat not containing *trans* fatty acids is used for school feeding purposes.

Red palm oil has a moderate level of saturation (Cottrell, 1991) and therefore does not require hydrogenation for use as a fat component in foods; as such it is free of *trans* fatty acids. Red palm oil is also a rich natural source of carotenoids (500-700 ppm), of which approximately 50% comprises ß-carotene (Cottrell, 1991). Studies in India have reported improved vitamin A status in 7-9-year-old vitamin A-deficient children, who were given red palm oil in the form of a sweet snack (Mahapatra & Manorama, 1997; Manorama *et al.*, 1997). In addition, red palm oil contains large amounts of tocopherols and tocotrienols (~1000 ppm) which have powerful antioxidant properties (Cottrell, 1991). By substituting the hydrogenated shortening in the biscuit with a baking fat derived from red palm oil, the biscuit will not only be free of *trans* fatty acids, but, because of its natural carotenoid and antioxidant content, there will also be no need to add synthetic ß-carotene and a synthetic antioxidant to the biscuit; concomitantly, quality control with regard to the fortification process will be simplified.

6.1.1 Objective of the study

The objective of the present study was to determine the effect of a biscuit with *red palm oil* as a source of ß-carotene on the vitamin A status of primary school children, and to compare this with

the effect of a biscuit with *ß-carotene from a synthetic source* in a randomised controlled trial.

6.2 METHODS

6.2.1 Study population and design

The study population consisted of all 5-11-year-old children (n=437) attending the Ndunakazi and Intongela Primary Schools. The study was conducted in the same school (Ndunakazi Primary School) where the previous biscuit intervention took place; the effect of previous interventions is unlikely to confound the results of the present study, since the results of the long-term evaluation of the biscuit programme (Chapter 5) showed that the vitamin A status returns to pre-intervention levels after each long school holiday break. A neighbouring school (Intongela Primary School) was included in this study to increase the study population size to allow for subdivision into three groups. Blood was obtained from 437 children, who were, after stratification by school and by school grade, randomly assigned to one of three treatment groups, using random tables. These groups were then randomly assigned to three different treatment categories: (i) a biscuit containing no added ß-carotene (n=146); (ii) a biscuit with synthetic ß-carotene as vitamin A fortificant (n=146); and (iii) a biscuit with a refined red palm oil shortening as a source of ß-carotene (n=145).

The biscuits were distributed daily during the school week, during the first two hours of the school day; each child received three biscuits weighing 15 g each. No intervention took place during school holidays, weekends or public holidays; the biscuits were provided for a total of 59 school days (the equivalent of 12 weeks). Children were assessed at baseline and after three months of intervention. Only the project leader was aware of group allocation (single-blind study). Compliance was closely monitored and recorded daily as described in Chapter 3. To eliminate any likelihood of parasitic infestation affecting ß-carotene absorption, all children were dewormed (400 mg albendazole) prior to the intervention, after the first blood sampling.

6.2.2 Composition of the biscuits

The synthetic ß-carotene was supplied by Roche Products (Pty) Ltd and the refined red palm oil shortening, containing ~ 475 ppm of carotenoids, by Global Palm Products SDN BHD, Johor Bahru Takzim, Malaysia. The synthetic ß-carotene and red palm oil biscuits were designed to provide a similar amount of ß-carotene; analysis of the biscuits distributed in the field showed the ß-carotene content of the two types of biscuits to be 1.17 and 1.23 mg per 45 g serving, respectively. This amounted to approximately 30% of the RDA for 7-10-year-old children, assuming a conversion factor of ß-carotene to retinol of 6:1 (National Research Council, 1989). The biscuits of all three groups were similar in macronutrient composition (32.1 g carbohydrate, 3.6 g protein, 7.2 g fat, 1.0 g fibre and 844 kJ per 45 g biscuit), in iron content (5 mg in the form of ferrous fumarate per 45 g biscuit; 50% of the RDA) and in taste and appearance.

6.2.3 Measurements

Blood measurements included serum retinol, CRP and a full blood count. Anthropometric measurements were taken at the baseline assessment only and included height and weight, which were expressed as Z-scores. A detailed description of all methods and procedures used in this study is given in Chapter 3. Information on the acceptability of the biscuit was obtained by means of a short questionnaire administered at the 3-month assessment (**Appendix E**).

6.2.4 Statistical analysis

Data was analysed on an intention-to-treat basis. The paired t-test was used to compare pre-and post-intervention values within each treatment group. Changes from baseline to the end of the 3-month study period in the two intervention groups were compared with those in the control group using analysis of variance. To estimate treatment effects and the contrast between the two active treatments an analysis of variance was done on the vitamin A measurement after the intervention period; the factors representing the study design i.e. school (two levels) and grade (1-6) were accounted for, and the baseline measurement for vitamin A was used as a covariate to adjust for starting nutritional level. Equivalence in effect between the two intervention groups was determined by using the estimated contrast between the treatment effects of the two groups,

together with a 90% confidence interval; to conclude equivalence this confidence interval should fall within the pre-specified equivalence limits of –2 to 2 µg/dL. Spearman correlation coefficients were used to test for the association of serum retinol with CRP levels and with white blood cell counts; differences in proportions were determined using the chi-square test. A significance level of 0.05 was used.

6.3 RESULTS

One hundred and thirty children in the synthetic ß-carotene group, 133 in the red palm oil group and 137 in the control group completed the study (dropout rates in the respective three groups thus were 11%, 8% and 6%); leaving the area was the main reason for dropping out of the study. The trial profile is given in **Figure 6.1**.

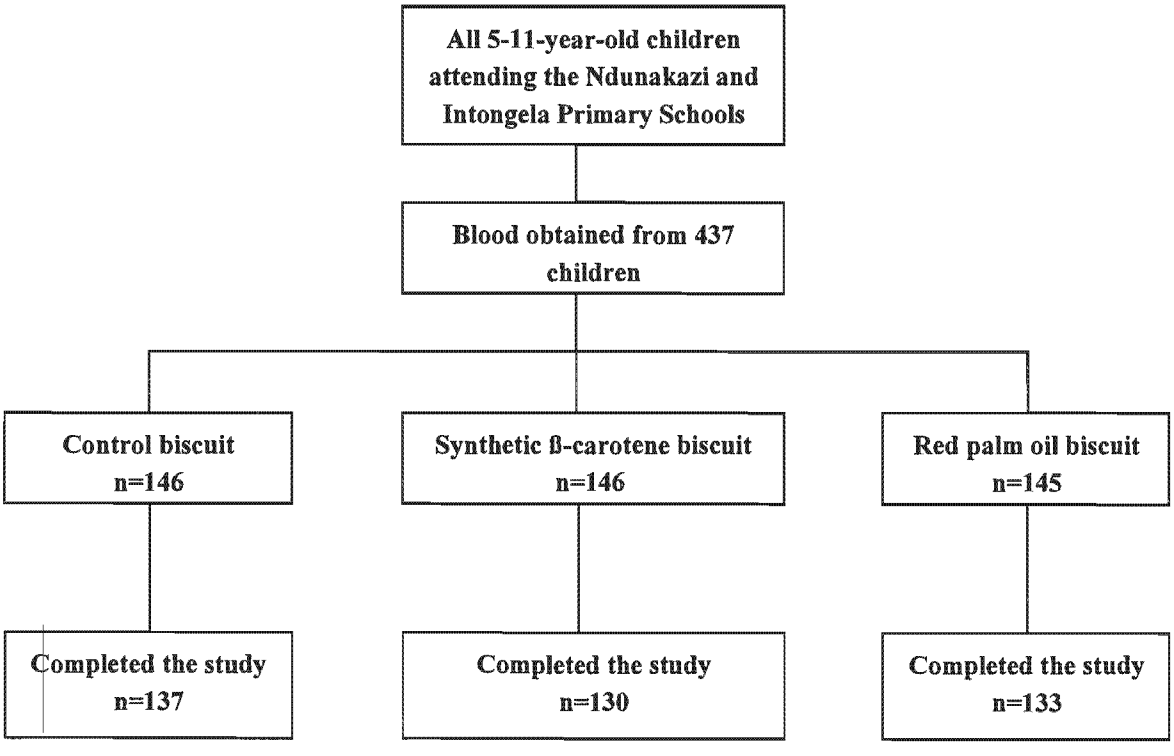

Figure 6.1: Trial profile of the 3-month study.

Baseline characteristics of the control, synthetic ß-carotene and red palm oil groups are shown in **Table 6.1**. One-tenth of children were stunted and very few (3-6 %) were underweight (< –2 SD

of the NCHS reference median for height-for-age and weight-for-age, respectively). Serum retinol concentrations < 20 µg/dL were present in more than 50% of the children, a level regarded as a *severe* public health problem according to WHO criteria (WHO,1996).

Table 6.1: Baseline characteristics of the three treatment groups.

	Control (n=137)	**Synthetic ß-carotene** (n=130)	**Red palm oil** (n=133)
Age (y)	8.7 (2.0) [a]	8.8 (2.0)	8.6 (2.1)
Boys/girls (%)	51.1/48.9	44.6/55.4	48.1/51.9
Height (cm)	125.8 (11.3)	126.3 (10.7)	125.3 (12.0)
Weight (kg)	25.8 (6.4)	26.2 (6.3)	25.2 (6.7)
Stunted (%) [b]	9.6	7.8	10.5
Underweight (%) [c]	4.4	3.1	6.0
Subclinical vitamin A deficiency (%) [d]	52.6	58.5	56.4
Anaemia (%) [e]	44.5	41.9	50.0

[a] mean (SD); [b] height-for-age Z-scores < –2 SD of the National Center for Health Statistics median;
[c] weight-for-age Z-scores < –2 SD of the National Center for Health Statistics median;
[d] serum retinol < 20 µg/dL; [e] haemoglobin < 11.5 g/dL

Mean serum retinol concentrations before and after intervention for the three respective groups are shown in **Table 6.2**. There was a significant improvement compared to the control group in both the synthetic ß-carotene and the red palm oil groups. The estimated treatment effect for the synthetic ß-carotene biscuit was 2.88 µg/dL (95% CI: 1.75 - 4.00) and that of the red palm oil biscuit 2.26 µg/dL (95% CI: 1.14 - 3.37). The estimated difference in treatment effect between the two active treatments was 0.62 µg/dL with a 90% CI: – 0.33 to 1.57; since this confidence interval falls within the pre-specified equivalence limits of –2 to 2 µg/dL, it can be assumed that the effects of the two treatments were equivalent.

Table 6.2: Mean (SD) serum retinol (μg/dL) before and after 3 months of intervention.

	Control (n=137)	Synthetic ß-carotene (n=130)	Red palm oil (n=133)
Baseline	20.6 (5.8)	20.4 (6.0)	20.8 (7.0)
3 months	21.6 (5.9)[a]	24.4 (5.6)[b]	24.0 (6.7)[b]
Change	1.0 (5.2)	4.0 (5.9)[c]	3.2 (4.7)[c]

[a] $P = 0.0248$, [b] $P < 0.0001$ compared to baseline (paired t-test);
[c] $P < 0.005$ compared to change in control group (ANOVA).

In **Figure 6.2** the prevalence of low serum retinol levels before and after 3 months of intervention is shown. The percentage of children with serum retinol levels below 15 μg/dL dropped from 17.5% to 13.1% in the control group, from 17.7% to 4.6% in the synthetic ß-carotene group, and from 15.8% to 6.8% in the red palm oil group (a cut-off value of < 15 μg/dL was used instead of the normal < 20 μg/dL, because the majority of the values for this population lay around 20 μg/dL and, as such, this cut-off value was not sensitive enough for illustrating differences in prevalence changes between the control and intervention groups). The prevalence of subclinical vitamin A deficiency (serum retinol < 20 μg/dL), however, decreased from 58.5% and 56.4% to 21.4% and 32.3% in the synthetic ß-carotene and red palm oil groups, respectively.

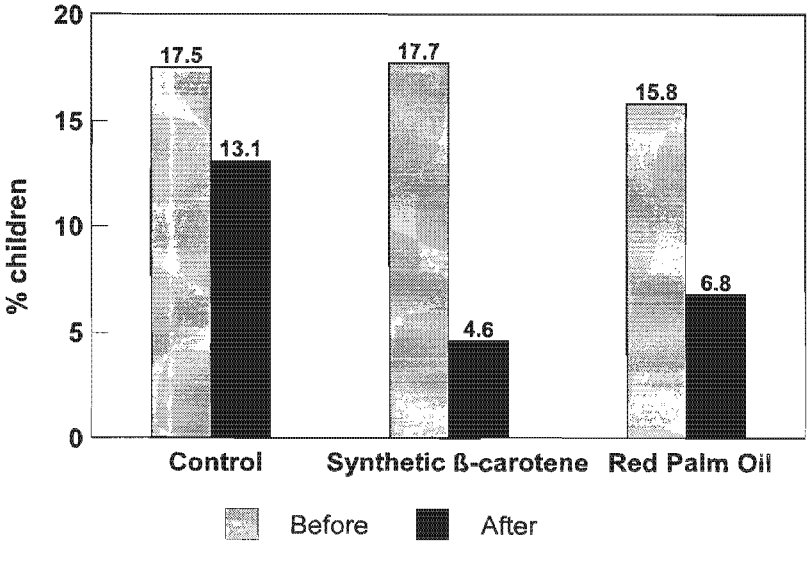

Figure 6.2: The prevalence of low serum retinol concentrations (using < 15 μg/dL as a cut-off) before and after 3 months of intervention.

To show similarity between two treatments, however, it is recommended that more than one analysis approach is followed (Jones *et al.*, 1996). In **Table 6.3** the treatment effect for the two biscuits are shown per school and per class. In five of the classes the synthetic ß-carotene biscuit performed better than the red palm oil biscuit, while in the five other classes the red palm oil biscuit performed better. This is to be expected if two treatments are equivalent.

Table 6.3: Treatment effect per school and per class for the synthetic ß-carotene and red palm oil biscuits.

School	Grade	n	Treatment effect for synthetic ß-carotene biscuit	n	Treatment effect for red palm oil biscuit
N	1	11	6.4 [a]	11	2.2 [a]
N	2	14	2.7	14	3.14
N	3	17	1.83	17	2.62
N	4	16	1.58	14	0.44
N	5	24	4.0 [a]	24	2.43
I	1	15	1.83	19	4.37 [a]
I	2	12	0.91	10	-0.32
I	3	10	0.26	12	0.57
I	4	8	4.13	9	5.27 [a]
I	5	3	11.58 [a]	3	4.55

[a] Treatment effect significant ($P < 0.05$) compared to control biscuit.
N = Ndunakazi Primary School; I = Intongela Primary School.

Mean compliance, i.e. the number of days a child received the biscuit, expressed as a percentage of the total number of potential biscuit days, was 89.1%, 86.9%. and 88.2% in the control, synthetic ß-carotene and red palm oil groups, respectively; absence from school was the main reason for non-compliance. The taste of the biscuits was acceptable to almost all of the children (98.5%), and 51.5% of the children indicated that they would like to receive more than three biscuits per day.

Serum retinol showed a significant negative correlation with CRP levels ($r = -0.283$ $P < 0.0001$) and to a lesser extent with white blood cell counts ($r = -0.133$; $P = 0.008$); 15% of the children with serum retinol concentrations < 20 µg/dL had elevated CRP concentrations (> 5 mg/L), compared to the 4.3% in those with serum retinol concentrations ≥ 20 µg/dL ($P < 0.001$). There was no significant difference in mean CRP concentrations or in mean white cell counts between any of the three groups at either the baseline or the follow-up assessments; median CRP concentrations were 0.8 mg/L in all three groups at both the baseline and follow-up assessments.

The production cost (March 1999) for the red palm oil biscuit, as shown in **Table 6.4**, was 27.68 SA Rand ($4.61 US dollar) per child per school year and slightly lower than that (30.24 SA Rand) for the biscuit in which synthetic ß-carotene was used as a vitamin A fortificant (cost and Rand:Dollar exchange rate (6:1) as at March 1999).

Table 6.4: A comparison of the production cost [a] per child for the three types of biscuits.

	Unfortified biscuit	**Synthetic ß-carotene biscuit**	**Red palm oil biscuit**
Per day (SA cent)	13.3 c	15.1 c	13.8 c
Per school year (SA Rand)	R 26.56	R 30.24	R 27.68
Per school year (US Dollar)	$ 4.43	$ 5.04	$ 4.61

[a] Cost and Rand:Dollar exchange rate (6:1) as at March 1999.

6.4 DISCUSSION

This study has demonstrated that red palm oil, in the form of a baking fat containing ~ 475 ppm of carotenoids, can be successfully incorporated into a biscuit given to primary school children as a snack during the school day. This biscuit was as effective as a biscuit with synthetic ß-carotene in improving the vitamin A status of these children; serum retinol increased

significantly in both the synthetic and red palm oil groups compared to a control group, with the treatment effect being equivalent in these two groups. The biscuit was also well accepted by the schoolchildren with regard to its taste and appearance.

Red palm oil is an excellent alternative for use as a vitamin A fortificant in food products. Not only is red palm oil a very rich natural source of ß-carotene, it also contains a bouquet of other carotenoids. These carotenoids possess, in addition to their pro-vitamin A activity, significant antioxidant properties and have been implicated in the prevention of carcinogenesis (Murakoshi *et al.*, 1992). In addition, red palm oil is a rich source of tocopherols and tocotrienols, which being powerful antioxidants, protect the oil against oxidative deterioration; the shelf life of products in which red palm oil is used, is therefore prolonged without having to add a synthetic antioxidant. Palm tocotrienols have also been reported to have a serum cholesterol lowering effect (Qureshi *et al.*, 1995) and may also play a role in suppressing certain types of cancer, particularly breast cancer (Nesaretnam *et al.*, 1998).

A major advantage of using red palm oil in baked products is that it contains no *trans* fatty acids, unlike the hydrogenated shortening normally used by the baking industry. *Trans* fatty acids have been shown to have cholesterol raising properties (Judd *et al.*, 1994) and have also been implicated in growth retardation (Koletzko, 1992). Another advantage of using red palm oil as a vitamin A fortificant is that it ensures that the correct amount of ß-carotene is added to the baking mixture and that the ß-carotene is evenly distributed throughout the mixture; possible errors with regard to the fortification process are thus eliminated and quality control improved.

The changes in serum retinol observed in this study were smaller compared to the response obtained in the previous study in which synthetic ß-carotene was used as a vitamin A fortificant (Chapter 4). This may be attributed to a shorter study duration (3 months *vs* 12 months), and to the fact that the biscuits supplied only 30% of the RDA for vitamin A, as opposed to the 50% supplied by the biscuit in the previous study. Due to logistical constraints it was not possible for

the present study to be extended beyond three months, and with regard to the fortification level, we were limited by the amount of ß-carotene supplied by the red palm oil shortening. However, the aim of the present study was not to demonstrate an effect, similar to that in the previous study, but to show that the effect of red palm oil in a biscuit is comparable to the effect of a biscuit with synthetic ß-carotene as a vitamin A fortificant. This, in fact, was demonstrated.

An equivalence trial requires a sample size roughly four times that required for the randomised placebo-controlled trial (Jones *et al.*, 1996). Because such a large sample size was not logistically possible, a placebo group was included in the design of the present study so that both treatments could also be compared to a placebo treatment, and the strength of the study design thereby increased. Once a treatment has been proven to have beneficial effects, inclusion of a placebo group in subsequent trials is considered unethical. In the present study a control group could, however, be included, because: (i) vitamin A deficiency is unlikely to be life threatening in school-aged children; (ii) none of the children had eye signs of vitamin A deficiency at the baseline assessment; (iii) the intervention lasted only three months; and (iv) after completion of the trial the placebo group was to be treated with vitamin A.

A slight, but statistically significant, increase in serum retinol also occurred in the control group. This may be attributed to the so-called "placebo effect", an effect often observed in randomised placebo-controlled trials (Kaptchuk, 1998). This may be due to seasonal variation in the availability of ß-carotene-rich foods. However, it is also possible that mere participation in the research project might have created an awareness in the community, that may have led to an increased intake of vitamin A- or ß-carotene-rich foods, or simply to an overall improvement in health practices, which in turn will have led to improved nutritional status. Deworming can also affect vitamin A status; Jalal *et al.* (1998) reported improved vitamin A status in children with a high initial load of *Ascaris lumbricoides* infestation receiving anthelmintic treatment. Anthelmintic treatment was administered to all three groups in this study. It is, however, unlikely that the deworming in the present study contributed to an increase in serum retinol, as a previous

survey among the primary school children of this community (Chapter 4) showed the prevalence and intensity of parasitic infestations to be low [*Trichuris trichiura* (26%), *Ascaris lumbricoides* (6.5%); with only 4% and 2% of the children having faecal egg counts in excess of 1000 eggs per gram for *Trichuris* and *Ascaris*, respectively].

Vitamin A deficiency, can increase susceptibility to and severity of infections (Sommer, 1990). The presence of infection, even at subclinical level, may in turn influence serum retinol levels (Filteau *et al.*, 1993). In this study serum retinol showed a significant negative correlation with the infection indicators, serum C-reactive protein and white cell counts. There was, however, no significant difference in mean levels of C-reactive protein and white cell counts between the three study groups at either assessment. This excludes infection as a possible confounding factor. Other indicators of infection such as α_1-antichymotrypsin or α_1-acid glycoprotein were, however, not measured.

Despite the high prevalence of subclinical vitamin A deficiency at the baseline assessment very few children were stunted or underweight. Information on dietary intake was not collected for this particular study. However, dietary data from a previous cross-sectional survey (Faber *et al.*, 1999) undertaken in this community showed that, despite the energy intake of the children being sufficient (median intake > 80% of the RDA), median vitamin A intake was only 10% of the RDA. This again illustrates that anthropometric data as the only indicator of nutritional status can be misleading and that micronutrient deficiencies, which are often referred to as the "hidden hunger", can be hidden indeed.

Of the various strategies for addressing vitamin A deficiency (Ramalingaswami, 1992), a food-based approach is probably the most sustainable and desirable solution. The bioavailability of ß-carotene from plant sources has, however, been questioned (De Pee *et al.*, 1995). It is suggested that ß-carotene from dark-green leafy vegetables is poorly absorbed because it is trapped in a complex matrix within plant cells. Red palm oil has the advantage that, although a

food, it has no matrix that can hinder bioavailability. Furthermore, by being a fat and providing an oil medium, the bioavailability of ß-carotene is further enhanced (Erdman *et al.*, 1993). By incorporating red palm oil in a biscuit, the food-based and food fortification approaches are combined into one strategy.

The production cost for the red palm oil biscuit was slightly lower than that for the biscuit in which synthetic ß-carotene was used as a vitamin A fortificant. Although the red palm oil shortening may be more expensive than the commercial hydrogenated shortening, the fact that it already contains ß-carotene and antioxidants, and that there is no need to additionally buy and add these components to the mixture, reduces the costs. The time saved by this simplification of the baking process further contributes to the lower production costs.

In conclusion, this study has shown that a biscuit with a red palm oil-based shortening as a source of ß-carotene is as effective as a biscuit with synthetic ß-carotene in improving the vitamin A status of primary school children. This, together with the additional qualities of red palm oil, makes red palm oil an excellent alternative for use as a food fortificant in addressing vitamin A deficiency.

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Chapter 7

GENERAL DISCUSSION AND CONCLUSIONS

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7.1 INTRODUCTION

The aim of the research described in this thesis was to evaluate a biscuit fortified with iron, iodine and ß-carotene as a strategy to address micronutrient deficiencies in primary school children from a poor rural community. The research comprised three phases. In the first phase of the study the efficacy of this biscuit in improving vitamin A, iron and iodine status was evaluated in a randomised placebo-controlled trial. The study stretched over a period of one year, and cognitive function, growth and morbidity were assessed as secondary outcomes. During the second phase of this study the long-term effectiveness of the biscuit programme, in terms of elimination of micronutrient deficiencies, compliance, acceptability and sustainability, was evaluated in a longitudinal study over a period of 2.5 years (30 months). In addition, cross-sectional data on vitamin A and iron status from subsequent studies conducted in the same school at 33, 42 and 45 months after the start of the original biscuit intervention, during which time the fortified biscuit continued to be distributed at the school, are reported. In the third phase, the potential of using red palm oil, a rich natural source of ß-carotene, as an alternative vitamin A fortificant was examined, and compared with ß-carotene from a synthetic source. This study contained elements of both an equivalence trial and a randomised placebo-controlled trial.

7.2 DISCUSSION OF THE MAJOR FINDINGS

7.2.1 Effect of the fortified biscuit on micronutrient status

Intervention with a biscuit fortified with iron, iodine and ß-carotene at 50% of the RDA, given together with a vitamin C-fortified cold drink, resulted in a significant improvement in vitamin A, iron, and iodine status of primary school children over a period of 12 months. Before the intervention the deficiencies of both iodine and vitamin A were present at levels regarded a *severe public health problem*. After the intervention, the median urinary iodine excretion was increased to a level no longer regarded a public health problem (WHO/UNICEF/ICCIDD, 1994), and the prevalence of subclinical vitamin A deficiency reduced to a level almost no longer a public health

problem (WHO,1996). The prevalence of anaemia in the experimental group was halved, as was the prevalence of low serum ferritin levels.

7.2.2 A fortified biscuit as strategy to address deficiencies in schoolchildren

School-aged children are not reached by the supplementation strategies aimed at the preschool child or the pregnant woman, and are therefore often a neglected group in terms of micronutrient interventions. School feeding, however, offers an excellent opportunity for targeted intervention in this segment of the population, especially with regard to fortification. Using the school feeding system as a vehicle for fortification has the additional benefit of regulated intake of the fortified product, thereby minimising the risk of over-consumption. The research described in this thesis has shown that a micronutrient fortified biscuit as school feeding is a feasible, practical and effective way of improving the micronutrient status of primary school children. The biscuit was also well liked by the children. A fortified biscuit has several advantages compared to conventional school feeding options. One is that a biscuit is regarded as a snack rather than a meal, and is therefore unlikely to replace meals given to the child at home. This was confirmed in the first two studies, which showed that the introduction of the biscuit intervention during the school day did not change the breakfast patterns of the children. Furthermore, the biscuit is a compact source of nutrients, that is easy to distribute and that needs no preparation. The biscuit has a long shelf life and therefore does not require regular delivery as, for example, in the case of bread. The quantity of biscuits delivered and distributed can also be monitored easily, which makes the system less vulnerable to abuse or corruption. As a strategy to address micronutrient deficiencies in schoolchildren the biscuit is therefore a feasible and attractive option, that can easily be implemented.

7.2.3 Effect of the fortified biscuit on morbidity

Respiratory- and gastrointestinal-related morbidity appeared also to be favourably affected by intervention with the fortified biscuit. During the intervention period fewer children were absent from school as a result of respiratory- and gastrointestinal-related illnesses than in the control group. In the preschool child the effect of vitamin A supplementation on infectious morbidity and

mortality is well documented (Beaton *et al.*, 1993). In the schoolchild, however, who is less susceptible to infections and in whom vitamin A deficiency is seldom life threatening, the effect of vitamin A supplementation is less defined. The approximately 30% reduction in morbidity demonstrated in this study, despite the small numbers and shortcomings in our method of data collection, is nevertheless promising. If absenteeism as a result of infection-related illnesses in schoolchildren can be reduced by addressing the vitamin A deficiency present in these children, it may have implications for learning and school performance in the long term.

7.2.4 Effect of the fortified biscuit on cognitive function

The cognitive effects observed during the randomised controlled trial, though they were modest, were more pronounced in those children with low iron stores at baseline, and in those with goitre at baseline. Other studies have shown supplementation with both iron and iodine to have positive effects on cognitive function (Soemantri *et al.*, 1985; Shrestha, 1994; Bruner *et al.*, 1996; Van den Briel *et al.*, 2000), and the effects of iodine supplementation appear to be greater than that of iron (Shrestha, 1994). In the present study, possible iodine-related improvements in cognitive function might have been masked by improvements in the control group, caused by the introduction of the salt iodisation programme during the second half of the trial. It is unfortunate that the research for this study was not commenced six months earlier, i.e. one year before the iodisation of salt came into effect. More and more countries implement universal salt iodisation programmes, and it will therefore become increasingly difficult to conduct trials demonstrating the beneficial effects of iodine supplementation in the future.

7.2.5 Effect of the fortified biscuit on growth

The fact that the intervention had no effect on the anthropometric status was not unexpected, as very few children were stunted or underweight at the baseline assessment. The relatively slow growth rate in children in this age category would also further make it difficult to pick up differences in growth between the intervention and control groups. The intervention did, however, appear to have had a positive effect on the linear growth in the children with marginal iron stores at baseline, which suggests iron to have been a limiting factor in the growth in this subgroup of

children. The fact that very few children were stunted or underweight, despite a high prevalence of micronutrient deficiencies underscores the fact that anthropometry as an only measure of nutritional status can be misleading.

7.2.6 Effect of the salt iodisation programme

Due to the introduction of the mandatory salt iodisation programme, the effect of the biscuit intervention on iodine status compared to a control group could only be determined during the first six months of the original randomised controlled trial. The subsequent six months could, however, be used to illustrate the impact of the salt iodisation programme in the control group. During this period the median urinary iodine excretion in the control group increased to a level similar to that observed in the experimental group after the first six months of intervention with the fortified biscuit. The salt iodisation programme therefore appeared to have been as effective as the fortified biscuit in raising urinary iodine concentrations. The effectiveness of this programme, however, continues to be monitored (Jooste *et al.*, 1999; Jooste *et al.*, 2000).

7.2.7 Long-term evaluation of the biscuit intervention

Long-term evaluation of the biscuit intervention in a longitudinal study showed that improved vitamin A status was not maintained during the long summer school holiday breaks when no intervention took place. This observation was confirmed by subsequent data from cross-sectional surveys carried out in the same school. The ß-carotene supplied by the biscuit was probably enough to maintain serum levels from day to day, but not sufficient to replenish stores or to maintain existing stores during periods when the biscuit was not consumed. There are also indications from studies in rodents that previously stored ß-carotene cannot at a later stage be utilised as a source of vitamin A (Thatcher *et al.*, 1998), which would further explain the deterioration in vitamin A status during the periods that the biscuit was not supplied.

Iron status also returned to pre-intervention levels after the school holiday break, but showed no recovery during subsequent intervention periods. Ferrous fumarate was used as iron fortificant, and when given together with the vitamin C-fortified cold drink during the first phase of the

study, it was effective in improving iron status. However, when the vitamin C-fortified cold drink was provided on a less frequent basis, due to logistical problems during the longitudinal follow-up phase, iron status did not improve. To eliminate the need for ascorbic acid, a more bioavailable form of iron, i.e. an amino acid chelate (ferrous bisglycinate) was thus introduced during the subsequent school year (Pineda *et al.*, 1994). This resulted in some improvement in serum ferritin, but haemoglobin deteriorated further. Ferrous bisglycinate performed worse than was expected, and although, due to lack of a control group during this phase, no conclusions could be drawn with regard to its efficacy as fortificant in this biscuit, it is possible that degradation of the amino acid chelate took place as a result of too high temperatures used during the baking process (Albion Laboratories, Inc., 1995). Another biscuit intervention using ferrous bisglycinate as fortificant, and running concurrently with this intervention in the neighbouring Noqomfela Primary School, also failed to show an improvement in iron status (unpublished data).

7.2.8 Red palm oil as an alternative vitamin A fortificant

During the third phase of the study red palm oil, a rich natural source of ß-carotene, was evaluated as an alternative vitamin A fortificant in the biscuit. The results showed that red palm oil, in the form of a shortening containing ~ 475 ppm of carotenoids, was as effective as ß-carotene from a synthetic source in improving the vitamin A status of the schoolchildren. Red palm oil also has various other qualities which make it an attractive alternative for use as a vitamin A fortificant (Nagendran *et al.*, 2000). It contains a bouquet of other carotenoids which, in addition to some pro-vitamin A activity, also have significant antioxidant properties. Red palm oil also is a rich source of tocopherols and tocotrienols which, being powerful antioxidants, protect the oil and the products in which it is used against oxidative deterioration. Tocotrienols have also been shown to have health benefits in humans (Qureshi *et al.*, 1995; Nesaretnam *et al.*, 1998). An important aspect of red palm oil is that it does not require hydrogenation for use as a fat component in foods, and is therefore free of *trans* fatty acids (Cottrell, 1991), unlike the shortening normally used in the baking industry. Red palm oil is not only an attractive alternative for use as a vitamin A fortificant in the biscuit, but also has the potential to be used as fortificant in many other products that require fat as an ingredient. By using a red palm oil-based shortening as a fortificant

the *food-based* and *food fortification* approaches to combat vitamin A deficiency are combined into one strategy.

7.3 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

The conclusions drawn from this research and the recommendations for further research are presented below:

- Baseline data from these studies show that micronutrient deficiencies can be present in a population despite a low prevalence of stunting or underweight. This illustrates that anthropometric status as the only indicator of nutritional status can be misleading, and that micronutrient deficiencies, which are often referred to as the "hidden hunger", can be hidden indeed.

- The research described in this thesis has shown a micronutrient-fortified biscuit to be a feasible, effective and practical way of addressing micronutrient deficiencies in primary school children. Although the biscuit in this study was fortified with iron, iodine and ß-carotene only, it has the potential to serve as a carrier for other micronutrients as well, e.g. B-vitamins and zinc.

- Cognitive function, morbidity and growth also appeared to have been favourably affected by the intervention. The effect on both cognitive function and morbidity may have long-term implications for learning and school performance. There were, however, a few shortcomings with regard to the method of morbidity data collection, and it is recommended that the effect of vitamin A supplementation on morbidity and absenteeism in vitamin A-deficient school-aged children is further investigated.

- Although the status of vitamin A and iron significantly improved as a result of the biscuit intervention, improved status was not sustained during the long summer holiday breaks when the biscuits were not distributed. To overcome this problem with regard to vitamin A, it is

recommended that the biscuit intervention is supplemented with other strategies, such as nutrition education and local home gardening programmes, which encourages the production and consumption of ß-carotene-rich foods.

- Because there is uncertainty regarding the utilisation capabilities of stored ß-carotene, the use of vitamin A as fortificant, instead of ß-carotene, might prevent the decline in vitamin A status observed during holiday breaks. It is therefore recommended that the long-term effectiveness of vitamin A as a fortificant is evaluated in a further study, and that the relative dose response test (RDR) or the modified relative dose response test (MRDR) as a measure of vitamin A stores is included in the assessment.

- The choice of iron compound to be used in the biscuit remains a challenge. Ferrous fumarate without regular consumption of the vitamin C-fortified cold drink appeared to be less effective in improving iron status. To supply vitamin C in the form of a cold drink is not only expensive, but also logistically more complicated. Vitamin C is also easily oxidised, and a special packaging material is required to prevent its degradation during storage. Ferrous bisglycinate was thought to be a promising alternative solution, but appears to be destroyed at the temperature used during baking. Further investigation into the optimum baking conditions is therefore needed. It is also suggested that the feasibility of using NaFeEDTA as alternative iron fortificant is examined.

- A biscuit with a red palm oil-based shortening as a source of ß-carotene was as effective as a biscuit with ß-carotene from a synthetic source in improving vitamin A status. This, together with the additional qualities of red palm oil (i.e. no *trans* fatty acids; rich source of antioxidants), makes red palm oil an attractive alternative for use as a vitamin A fortificant, not only in the biscuit, but also in other food products that require fat as an ingredient.

General conclusion

It can be concluded that a biscuit fortified with iron, iodine and ß-carotene, and given together with a source of vitamin C, is an effective and feasible strategy for addressing micronutrient deficiencies in primary school children. Improved vitamin A and iron status was, however, not maintained during the long school holiday breaks when the biscuit was not supplied, and it is recommended that the biscuit programme is supplemented with other strategies, such as nutrition education and local home gardening programmes. Red palm oil appears to be an attractive alternative for use as a vitamin A fortificant. The choice of iron compound, however, needs further investigation.

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APPENDICES

APPENDIX A

The compliance sheet used for recording compliance and morbidity

(pink sheets were used for group A; blue sheets for group B; and yellow sheets for group C)

Grade 2

Group A

		April																May									
Name	Code	1	2	3	4	5	8	9	10	11	12	15	16	17	18	19	22	23	24	25	26	29	30	1	2	3
John Adams *	201																									
Ann Smith	203																									
Paul Murphey	205																									
Michelle Reynolds	207																									
Mary Peacock	209																									
Andrew Jackson	211																									
Peter Buhr	213																									
Mervyn Fowler	215																									
Lianne Black	217																									
Andrew Fraser	219																									
Jennifer White	221																									
Anna Davies	223																									
Phil Dixon	225																									
Nico Smith	227																									
Colleen Weight	229																									
Gregory Green	231																									
Steve Hulme	233																									
Martin Bradbury	235																									
Jane Wood	237																									
Sonja Johnson	239																									
Mike Richardson	241																									
Sue Miller	243																									
Paul Thompson	245																									
Gary Wells	247																									
Marlene Wilson	249																									
Linda Jordan	251																									
Gordon Anderson	253																									
Jackie du Toit	255																									
Margaret Shephard	257																									

*All names in this table are fictitious

APPENDIX B

Codes that were used for (i) the reasons for non-compliance and (ii) the reasons for absence from school

Reason for not eating biscuit	**Reason for absence from school**
A = child absent	**1** = flu/cold /chest infection /cough
B = child feels sick; does not feel like eating	**2** = diarrhoea/vomiting/nausea
C = child does not like the biscuit	**3** = measles
D = biscuit makes child "feel sick"	**4** = chickenpox
E = biscuit not available	**5** = mumps
F = child received the wrong biscuit	**6** = headache
G = late for school	**7** = ear problem
H = holiday	**8** = scabies/rash/skin infection
I = other (specify)	**9** = injury (e.g. broke arm/leg)
	10 = not sick; reason not related to illness
"A" to be replaced with relevant code from the next column when child returns to school	**11** = left school/attending another school/ passed away
	12 = other (specify)

APPENDIX C
Copies of the letters of ethical approval

MEDIESE NAVORSINGSRAAD
MEDICAL RESEARCH COUNCIL
Kantoor van die President/Office of the President
19070 Tygerberg 7505 • Suid-Afrika/South Africa • (021) 938-0211/938-0911 • Fax (021) 938-0201 •
E-mail OWPROZES@EAGLE.MRC.AC.ZA

22 September 1994

Miss M Kruger
Nutritional Intervention
MRC
PO Box 19070
TYGERBERG
7505

Dear Miss Kruger

YOUR PROTOCOL: "A CLINICAL TRIAL: THE EFFECT OF IRON AND VITAMIN FORTIFIED BISCUITS IN A SCHOOL FEEDING SCHEME ON THE NUTRITIONAL STATUS OF 6-8 YEARS OLD SCHOOLCHILDREN IN LOW SOCIO-ECONOMIC URBAN AND RURAL COMMUNITIES"

Ethical approval is hereby given to proceed with the above study.

We wish you everything of the best with this study.

Yours sincerely

M. P. Keet.

PROF MP KEET
CHAIRPERSON: ETHICS COMMITTEE

MPK/jvd

Copies of the letters of ethical approval (continued)

MEDICAL RESEARCH COUNCIL

MEDIESE NAVORSINGSRAAD

UMKHANDLU WOKUCWANINGA NGEZOKWELAPHA

SOTLA LA PHUPUTSO HO TSA KALAFO

Office of the President

PO Box 19070, TYGERBERG 7505, Republic of South Africa • Francie van Zijl Drive, Parowvalley, Cape Town
Tel: +27 21 938-0211/938-0911 • Fax: +27 21 938-0201
E-mail: OWPROZES@EAGLE.MRC.AC.ZA

15 October 1998

Dr AJS Benadé
Nutritional Intervention
Medical Research Council
PO Box 19070
TYGERBERG
7505

Dear Dr Benadé

PROTOCOL: THE EFFECT OF A SCHOOL BISCUIT, WITH RED PALM OIL AS A SOURCE OF B-CAROTENE AND *FERROUS BISGLYCINATE* AS AN IRON FORTIFICANT, ON THE VITAMIN A AND IRON STATUS OF PRIMARY SCHOOL CHILDREN: A COMPARISON WITH *B-CAROTENE FROM A SYNTHETIC SOURCE* AND *FERROUS FUMARATE* IN A RANDOMISED CONTROLLED TRIAL

Thank you for your revised consent form. Ethical approval is now granted to go ahead.

Wishing you well with your research.

Yours sincerely

PROF PE CLEATON-JONES
CHAIRPERSON: MRC ETHICS COMMITTEE

APPENDIX D

A copy of the form that was used to obtain informed consent

INFORMATION TO PARENT OR LEGAL GUARDIAN

Dear parent / legal guardian
The Medical Research Council wants to undertake a study to determine the effect of a vitamin and mineral enriched biscuit on the nutritional status of primary school children. Supplementary feeding during the school day can contribute to the ability of a child to learn at school. We need your consent for your child to take part in this study. Participation is completely voluntarily. This project has the full support of the Departments of Education and National Health.

During the surveys your child will be asked questions in a one-to-one personal interview about, for example, the house where he/she lives and about his/her eating patterns. This information will be regarded as confidential. Your child's height and weight will also be measured.

It is essential for a nutrition study of this kind to analyse the blood of the children biochemically. For this reason, 10 ml blood (± two teaspoons) will have to be drawn from your child. This procedure will be carried out by a medical doctor or a medical technologist using sterile equipment (only used once and then discarded). It is completely safe although puncturing of the skin is involved.

Your child will be dewormed during the course of the study, a therapy which is not associated with serious side effects. Worms may, however, appear in the stools of your child.

Benefits for the child:
1) Biscuits and cold drinks daily during the school week for *at least* one year;
2) Deworming medication

INFORMED CONSENT

THE EFFECT OF IRON, IODINE AND VITAMIN A FORTIFIED BISCUITS ON THE NUTRITIONAL STATUS OF PRIMARY SCHOOL CHILDREN

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

I have been informed about the advantages and possible adverse effects (i.e. worms that may appear in the stools of the child) that may result from procedures and/or treatment, and I understand what it says.

I understand that participation is voluntary and that I can recall my consent at any time without forfeiting the availability of any future routine medical care.

Nutritional status will be assessed by means of the measurement of height and weight, and analysis of a blood sample. Blood will be drawn by a medical doctor or a medical technologist.

Name of volunteer: _____

Address _____

_____

.....
PARENT OR LEGAL GUARDIAN ***SIGNED THIS ..... DAY OF ..... 19.....***

APPENDIX E (QUESTIONNAIRE)

MICRONUTRIENT FORTIFIED BISCUIT PROJECT
MEDICAL RESEARCH COUNCIL

All information is confidential and for research purposes only

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Name of child: **Date:**
Code:

School grade:

General comments:

To be completed for all assessments

1. Did you eat anything from the time you got up this morning until you came to school?	yes	no	do not know
2. If yes, what did you eat?			
3. If no, why did you not eat anything?			
4. Did you drink anything from the time you got up this morning until you came to school this morning?	yes	no	do not know
5. If yes, what did you drink?			
6. Did you bring something to eat or drink to school today?	yes	no	do not know
7. If yes, what did you bring?			
8. If no, why did you not bring something to eat or drink?			
9. Did you buy anything to eat or drink during the school day yesterday?	yes	no	do not know
10. If yes, what did you buy?			

To be completed at follow-up assessments only

11. Do you like the taste of the biscuit?	yes	no	do not know
12. If no, why not?			
13. Do you like the taste of the cold drink? (if applicable)	yes	no	do not know
14. If no, why not?			
15. How many biscuits would you like to receive per day?			