

**Impact of vitamin A and iron on anaemia and cognitive
functioning of anaemic school children in Tanzania**



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DEDICATION

Dedicated to my parents, the late Aileen Munuo and Justo Mwanri.

To my mother, for whom, herself did not get an opportunity for any formal schooling, but always acknowledged and emphasised continued education as a key for a future quality life.

To my father whose passing was during the course of the current study, for his inspiration and persistent encouragement for hard work and determination as the only way forward for any success.

Although their passing has always been a great sense of loss, their inspiration has always remained the light for the future direction.

PUBLICATIONS

Material based on this thesis has been used to produce the following publications.

- 1 **Mwanri, L. and Worsley, A.** (1999): Infant Feeding in developing Countries: **NUTRIDate** Vol. 10 No. 3
2. **Mwanri, L., Worsley, A., Ryan, P. & Masika, J** (2000). Vitamin A Improves Anemia and Growth in Anemic School Children in Tanzania. *J.Nutr.***130**: 2691-2696
- 3 **Mwanri, L., Worsley, A., Ryan, P. and Masika, J** (2001): School Children and anaemia Prevention: Opportunities and challenges: A Tanzanian case study: Health Promotion International (in press)
- 4 **Mwanri, L., Worsley, A., Ryan, P and Masika, J** (2001): Effects of Vitamin A alone, iron alone or combination on indices of cognitive function and education achievements in anaemic Tanzania school children. *International Journal of Epidemiology* (Submitted)

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SUMMARY

Micronutrient deficiencies are serious problems. They hinder mental and physical development and are important causes of death in developing countries. They also hinder the socio-economic development of the affected population.

Iron deficiency which is often manifested as iron deficiency anaemia (IDA) is the most prevalent nutritional deficiency world-wide. It affects nearly 5 billion people in the world. Nearly 90 percent of anaemia in the world is due to iron deficiency. The problem is much more severe in developing countries, although the developed countries are not spared.

The studies presented in this thesis examined the complex nature of interrelationships between iron deficiency anaemia and its determinants. Both experimental and survey methods were employed. The experimental method was used to investigate the impact of dietary supplements on the biomedical and cognitive functioning of the study subjects. Surveys were also conducted because it was thought that the local context in which the problems evolved must be understood for effective control of the problem of iron deficiency anaemia.

This thesis examined the impact of iron and vitamin A supplementation on the haemoglobin concentrations and cognitive function of 9 to 12 year old anaemic school children from the rural and remote area in Tanzania. The findings suggest that both vitamin A and iron raise their haemoglobin levels. Furthermore, the results suggest that vitamin A in combination with iron may have a role in improving the cognitive function of anaemic school children. This study

showed that relatively small changes in nutritional status can bring about major health and cognitive improvements.

School children, parents and teachers were interviewed to examine their knowledge, attitudes and practices towards anaemia control. The results of these surveys showed that school children's knowledge of causes and prevention of anaemia was poor compared to that of their parents and teachers. Moreover, the results showed that children's dietary intake was poor and could be one amongst many causes of anaemia in these children. However, the parents were willing to participate in activities that would improve their children's health and education, for example via provision of school meals and improvement of school physical environments.

The thesis also examined the iron concentrations and bioavailability in two varieties of maize, one the main staple food in Tanzania and the other, a newly bred iron-rich variety from Zimbabwe. Although the newly bred variety had higher iron concentrations, its bioavailability was lower than the conventionally consumed variety, indicating need for further work in breeding high micronutrient dense and highly bioavailable varieties.

The results of the thesis indicate that the causes of malnutrition and micronutrient deficiencies, especially iron deficiency anaemia, are complex, context dependent and dynamic in nature. While these results identify dietary supplements as effective short-term interventions, the complexity of micronutrient deficiency causation demands a new holistic paradigm for its control. This is described in a set of set of recommendations.

Holistic systems approaches have particular value in addressing micronutrient deficiencies because they consider a variety of causal variables. Relevant factors such as community participation, women's empowerment, improvement of farming systems and storage of foods, plant breeding and possibly the use of dietary supplements in the short term, are all important in addressing micronutrient deficiencies. All these require policies that encourage integrated and well co-ordinated multi-sectoral strategies that promote nutritional outcomes through such means as education, health, information technology, financial support (e.g. micro-credit), political commitments, agricultural research and sustainable development.

DECLARATION

This thesis contains no material which has been previously submitted or accepted for the award of any other degree or diploma in this or any other University. To the best of my knowledge and belief, no material described herein has been previously published or written by another person except where due reference is made in the text.

I give consent to this copy of my thesis being available for loan and photocopying.

Lillian Mwanri

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 Introduction

Malnutrition which consists of three types of nutrition problems predominates in low and middle income countries (World Bank, 1997). The three types of malnutrition are: undernutrition, micronutrient deficiencies and overnutrition. The World Bank describes these as the results of interactions between food intake, disease risk factors, and human behaviour.

Deficiencies of water- soluble vitamins (e.g. thiamine, riboflavin and niacin) and minerals (e.g. iron, iodine and fluorine) and fat-soluble vitamins A and D, which was of concern in the Western World early in the last century, have been substantially reduced as a public health problem. Problems like iron deficiency anaemia and vitamin A deficiencies remain quite common but in western countries not on the same scale as in developing countries.

The developing countries however, have yet to experience relief from the consequential effects of micronutrient deficiencies, particularly from iron, vitamin A and iodine deficiencies which are recognised global public health problems (WHO, 1992; WHO/UNICEF, 1995; WHO/UNICEF/ICCIDD, 1993). The Second World Nutrition Situation report (ACC/SCN, 1992) showed that micronutrient deficiencies continue to

affect a large number of people in developing world. Iron, vitamin A and iodine are essential for normal processes of growth, development, maintenance and resistance to infections (ACC/SCN, 1992). In their absence, individuals, families as well as communities suffer serious consequences expressed as increased mortality, morbidity and disability rates. Micronutrient deficiencies constitute a major constraint on future economic and social development. Micronutrient deficiencies in school aged children may be considered important public health problems which impair health and quality of life during childhood and in adult life.

1.2 Tanzania

The United Republic of Tanzania is the largest country in East Africa, covering 940,000 square kilometres. It is sparsely populated although population density is high in some parts of the country and has been increasing over time (Bureau of Statistics, 1967; Bureau of Statistics, 1978; Bureau of Statistics, 1988; TDHS, 1991/92; WDR, 1997). Average population density is 26 persons per square kilometre. More than 80 percent of the population lives in rural areas; however, the proportion of urban residents has been increasing steadily from 6 percent in 1967 to 18 percent in 1988. Currently, it is estimated that the proportion of urban residents is 24 percent, with an average annual urban growth rate of 6.7 percent (WDR, 1977).

Tanzania has a mixed economy in which agriculture, comprising of crop, animal husbandry, forest, fishery and hunting subsections, is the most important sector. It

provides a livelihood for over 80% of the population and accounts for over 50 percent of GDP and 75% of foreign exchange earnings (Tanzania Bureau of Statistics, 1997). Furthermore, it is the main source of the food supply and provides raw material for the industrial sector. Accordingly, the performance of the agricultural sector is a major indicator of overall economic performance (Tanzania Ministry of Finance, 1996). In recent years, the country has experienced negative trends in economic growth leading to a fall in GDP per capita estimated at US \$180 in 1978 down to US \$ 120 in 1995 (Haub & Yanagishita, 1992; World Development Report, (WDR), 1978, 1997). The general performance of the agricultural sector in 1990-1995 was moderately low compared to 1980-1990 i.e. 4.1% and 4.9% of GDP per capita respectively.

In 1981, Tanzania signed an agreement with the International Monetary Fund (IMF) and the World Bank (WB) to adopt the Structural Adjustment Programs (SAP) (Lugalla, 1995). The IMF and WB recommended the establishment of a competitive export oriented production sector and a social sector reform program. Trade was liberalised and most import controls were lifted. Private foreign investment was encouraged through a wide range of incentives (Tanzania Investment Centre, 1985).

Because of the adoption of stabilisation and adjusted policies sponsored by the IMF and the World Bank, Tanzania has experienced the collapse of local industries with the influx of imported goods; lack of protection of Tanzanians who worked in the private sector, an increase in foreign debt; a widening gap between the rich and the poor; and difficulties for those on fixed-incomes resulting from inflation. These changes have brought about

reductions in social services such as health and education, the scrapping of subsidies on food and agricultural inputs and a push to increase export production. These reform measures have had limited economic success even in other countries in sub Saharan Africa which have also recorded a negative GNP growth rates (World Bank, 1996). Tanzania is presently more dependent than before on international economic trends (Tanzania Bureau of Statistics, 1997). "These adjustment policies" are also blamed for stagnant and even deteriorating human development indicators and declining living standards (Cornia et al., 1987).

The WHO recognises health is a complex interaction between people and the broader environment. Health is not just a result of human activity but also a broader socio-economic environment in which that activity takes place.

However, socio-economic conditions are constantly changing, affecting both human activity, their health and education. Overall, over the last 15 years, two significant changes have occurred in the socio-economic conditions in Tanzania as in other sub Saharan African countries (World Bank, 1996). These changes are the increasing poverty associated with economic crisis and economic adjustment policies, and the AIDS pandemic. They both had significant impacts on the health status and education of individuals and communities in Tanzania (World Bank, 1996, personal observation). The economic crisis of the 1980s and the subsequent adjustment policies have halted both economic and human development in Tanzania. Please (1994) noted that structural adjustment policies affect all members of the society to differing degrees, with health,

education and nutrition programs being severely curtailed with subsequent deteriorations in human development. OXFAM suggests that structural adjustment policies in Africa have resulted in massive social costs, which have been borne disproportionately by poor individuals and communities (Watkins, 1995).

Acquired Immune Deficiency Syndrome (AIDS) is becoming an important nutritional problem world-wide. Mrisho (1994) reported increasing morbidity, mortality and social problems associated with AIDS/HIV infections in Tanzania and other developing countries. For example, she reported that families and communities have to cope with increasing number of malnourished and orphaned children whose parents have died of AIDS pandemic (Mrisho, 1994). Although there has been a reversal of the trends towards better health in the developed countries this does not seem to be the case in Tanzania and other developing countries (Mrisho, 1994).

In his New Year's speech to the people of Tanzania, the President of the United Republic of Tanzania emphasised the importance of AIDS/HIV infections on the health and economic development of the country (Mkapa, 2001). He pointed out that AIDS/HIV is a very serious socio-economic problem with serious and devastating health and economic costs for families and the nation. It increases poverty, workloads and responsibilities of every member of society (Mkapa, 2001). AIDS/HIV infections have serious implications for children in Tanzania, for whom access to education and health facilities is limited in many parts of the country. People who live in extreme poverty with little access to resources are now having to face the socio-economic and health consequences of the

AIDS pandemic. The recognition of these health problems by the president and other government top officials is a good sign that health issues are now becoming a priority, for political and other policy makers.

1.3 The nutritional challenge

Nutritional wellbeing plays important role in health and human development (Iunes & Monteiro, 1993). Iron deficiency is the most common nutritional disorder in Tanzania and in the world (Kavishe, 1991; WHO, 2001). The available data is staggering. The WHO reports as many as 4-5 billion people, 66-80% of the world's population, may be iron deficient; 2 billion people, over 30% of the world's population are anaemic, mainly due to iron deficiency. In developing countries, the condition is frequently exacerbated by malaria and worm infections (WHO, 2001). The WHO further reports that iron deficiency affects more people than any other condition. Iron deficiency is more subtle in its manifestations than other conditions, its overall toll includes prolonged ill health, premature deaths and lost earnings (WHO, 2001). Iron deficiency is the commonest cause of anaemia (WHO, 2001).

It has been reported that iron deficiency anaemia reduces the work capacity of individuals and entire population, bringing serious economic consequences and obstacles to national development. WHO further acknowledges that, treatment of iron deficiency anaemia can raise national productivity levels by 20%. Overall, it is the most vulnerable, the poorest

and the least educated who are disproportionately affected by iron deficiency anaemia, and it is they who stand to gain the most by its reduction (WHO, 2001).

In Tanzania, iron deficiency anaemia, vitamin A deficiency and iodine deficiency are the main micronutrient deficiencies (Ministry of Health, 1999). Micronutrient deficiencies can be caused by low food intakes and/or increased nutrient needs. Iron intake may, for example, be high, but when bioavailability is poor because of presence of factors which inhibit absorption, or when losses are high due to worm infections or pathological menstrual bleeding, iron deficiency may develop. Combinations of strategies need to be employed in combating these deficiencies.

1.4 Outline of the thesis

The studies in this thesis focussed on anaemic school children because they are vulnerable to iron deficiency anaemia (Tanzania Partnership for Child development, 1996). Generally, they are not regarded as at high risk because of their low mortality rates and so there is little emphasis on prevention of iron deficiency anaemia among Tanzanian school children. Nevertheless, high prevalence of iron deficiency in school children with its negative impact especially on mental and physical outcome continues to be a problem particularly in developing countries

After describing the global state of micronutrient deficiency which is the main theme of the thesis, **chapter one** also provides brief background information on the demographic

and the socio-economic challenges in Tanzania in order to provide an understanding of the overall context in which micronutrient deficiencies occur in developing countries.

Chapter two presents the literature review in which an overview of the causes and effects of iron and vitamin A deficiencies are described at length. More information associated with the contexts in which iron deficiency anaemia occurs is also provided. This chapter also describes the aims, objectives and hypotheses of the studies in the thesis.

Chapter three describes research setting and methodological approach of the studies.

Chapter four provides the details of study 1, which explored the impact of supplemental iron and vitamin A on anaemia and growth.

Chapter five presents the results of the second part of the study 1 in which the impacts of supplemental iron and vitamin A on cognition and educational achievement are explored and discussed.

Chapter six describes the knowledge, perceptions, attitudes and practices of anaemia control in school children, parents and teachers who form the school community.

Chapter seven reports the laboratory study which examined the iron concentrations and bioavailability in traditionally consumed maize varieties from Tanzania as well as newly bred cultivars from Zimbabwe which have higher iron densities.

Chapter eight provides a general discussion of the findings of these studies. Recommendations are made for the development of a new paradigm for the control of iron deficiency anaemia which might also be applicable to other micronutrient deficiencies.

Generally, the thesis provides a broad outlook of issues surrounding the development and control of iron deficiency anaemia which may also be applicable to other micronutrient deficiencies. A holistic approach is recommended for the control of iron deficiency anaemia and other micronutrient deficiencies.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The objective of this chapter is to provide an overview of iron deficiency anaemia (IDA). The overview will highlight the role of iron in the body, the mechanisms through which anaemia occurs, its consequences and its associations with cognitive functioning. It will also highlight the importance of anaemia in school aged children and the interrelationship between vitamin A and iron deficiency. In addition, the chapter will also highlight control strategies which have been used previously in a number of developing countries.

2.2 The role of iron in the body and the causes of anaemia

Iron is necessary for the transport of oxygen, for the synthesis of proteins and nucleic acids, for energy production, and for a number of metabolic reactions required for growth and reproduction (Emery, 1991). Most of the iron in the body is present in haemoglobin. However, it is also found in different haem containing enzymes within the mitochondria i.e. in cytochromes, myoglobin, catalase and peroxides. Non-haem iron is also involved in some iron dependent enzymes i.e. succinic dehydrogenase, reduced nicotinamide adenine dinucleotide dehydrogenase and xanthine oxidase (Emery, 1991).

Anaemia is a condition in which a deficiency in the size or number of erythrocytes or in the amount of haemoglobin they contain, limits the exchange of oxygen and carbon dioxide

between the blood and the tissue cells (Mahan and Arlin, 1992). It occurs when the tissue stores of iron are depleted leading to lowered serum iron concentrations, a decrease in transferrin saturation and an increase in erythrocyte protoporphyrin. When tissue stores are seriously depleted, haemoglobin levels decline. Anaemia is the end result of the development of iron deficiency and is indicated by low haemoglobin concentration (Viteri, 1998).

The World Health Organisation (WHO, 2000), has set normal values of haemoglobin concentration according to age, sex and physiological status of individuals. **Table 1** presents the normal values of haemoglobin concentrations according to age, sex and physiological status. Individuals found to have haemoglobin concentrations lower than the indicated level by age, sex and physiological status are described as anaemic. Iron deficiency remains the most common cause of anaemia.

Table 1: Haemoglobin levels indicative of anaemia

Age/sex group	Haemoglobin level(g/l)
Children 6m-5y	<110
Children 6y-11 y	<115
Children 12y-14y	<120
Adult males	<130
Adult females(non pregnant)	<120
Adult females(pregnant)	<110

Source: WHO, 2000

2.3 The development of anaemia

The onset of iron deficiency anaemia is not of sudden occurrence, but an outcome of a series of events. Iron is an essential micronutrient for haematopoiesis, the manufacture of red blood corpuscles containing haemoglobin, as well as for growth in general (Connolly and McGregor, 1993). Iron deficiency arises because of one or a combination of four major factors:

2.3.1 Low dietary intakes of iron and/or poor bioavailability*

The nutritional adequacy of iron depends on both the amount and bioavailability of iron in the diet. Animal based foods are a rich source of iron which is readily available (Gibson, 1994) whereas plant based diets often contain high levels of phytic acid (myoinositol hexaphosphate) and dietary fibre, components known to inhibit the absorption of iron (Hallberg, 1987; Gibson, 1994). Plant based staples such as unrefined maize flour, brown rice, sorghum and certain legumes (eg groundnuts, pigeon peas, kidney beans and cowpeas) have elevated phytate levels (Gibson, 1994).

**Bioavailability can be defined as the proportion of a nutrient ingested which becomes available to the body for metabolic processes, or the proportion of a nutrient capable of being absorbed and available for use or storage, or more briefly the proportion of nutrient ingested that can be used (Castenmiller and West, 1997). Several methods to determine the bioavailability of nutrients can be employed. Food labelling using radioactive substances having been a traditional method to determine iron absorption from provided meals. However, in vitro methods using digestion/Caco-2 cell model cells (details in chapter seven) to assess iron availability from foods is now available. This method uses ferritin formation by Caco-2 as an indicator of iron uptake. Ferritin formation by Caco-2 cells occurs in response to iron uptake at concentrations of available iron greater than that of the culture media to which the cells have been adapted. This methodology circumvents the need for using radioactive iron and thus eliminates the costs and controversies associated with radioactive food labelling (Glahn, et al. 1998).*

In this thesis in vitro iron bioavailability is examined in detail in Chapter seven.

As described before, the nutrition adequacy of dietary iron depends on long-term iron balance, which is favoured by both the amount and bioavailability of iron in the diet. Food iron is present in most diets and is composed of two different pools; haem and non-haem (Hallberg and Bjorn-Rasmussen, 1972; Layrisse et al., 1969). The haem iron pool in the diet is provided by blood, flesh and viscera. The most important is haemoglobin in blood and myoglobin in muscle both of which are readily available. The non haem iron pool is made up of all other sources of iron. It is often found in seeds, and in vegetable tissues bound to phenolic compounds (Viteri, 1998) and its absorption is affected by many dietary compounds. Inhibitors of non-haem iron absorption include phytates, polyphenols, fibers and other compounds present in foods and beverages such as tea and coffee (Gibson, 1994).

Bothwell et al., (1979); Cook, (1990) and Layrisse et al., (1969) indicated that, in developing countries, the content of flesh food which contains haem iron especially in rural diets is often low and so their contribution to body iron is small. Instead, diets are mainly plant based and cereals, starchy roots, and tubers usually are the major sources of iron (Bothwell et al., 1979; Gibson, 1994; Cook, 1990). Hence, diets based on these staples and cereals tend to have low bioavailable iron due to presence of the various inhibitors such as phytates. In Tanzania like many other developing countries, non-haem iron is the most important source of dietary iron where a limited plant centred diet is an economic necessity (UKUMTA, 1994; Tatala, et al., 1998). Thus, in many developing countries inadequate food intake may be a cause of iron deficiency anaemia. This thesis will examine anaemia in school children whose diet is mainly composed of plant foods that provide non-haem iron.

2.3.2 High physiological requirements

The efficiency of iron absorption from the intestinal lumen is regulated by the body's iron status (Viteri, 1998). Greater absorption efficiency is related to greater severity of iron deficiency anaemia (Kavishe, 1993, Viteri, 1998). Physiological requirements are increased during periods of rapid growth in children's early years. Hence infants and children are vulnerable to iron deficiency. Infants in developing countries may have small iron stores at birth as a consequence of their low birth weight and poor nutritional status of the mothers (Hofvander, 1977). Therefore, their dietary requirements for catch-up growth will be higher than those of infants from industrialised countries (WHO, 1997). Male infants and children appear to have higher requirements for iron than females, because of their higher growth rates and greater proportion of muscle per kilogram body weight; (muscles contain a higher content of iron than fat (WHO, 1967).)

Adolescence is another period of rapid growth and physical and psychosocial development. Rapid pubertal musculoskeletal development, together with expanding blood volume create additional requirements for iron in both boys and girls (Viteri, 1998). Adolescent females also have an additional demand created by the onset of menses (Viteri, 1998).

The body's requirements for iron are greater during pregnancy and lactation for the growth and development of foetal and maternal tissues. Pregnancy results in approximately 740g of iron loss which includes 230mg for maternal basal losses and 360mg for the products of conception (270mg for foetus, 90mg for the placenta and the umbilical cord) and about 150mg as pre-partum blood losses (Bothwell, et al., 1979; Hallberg, 1988). In addition, during lactation, 0.3mg/day iron loss occurs (Siimes, et al., 1979). The increases in iron requirements during rapid growth in

infants and adolescents and during pregnancy partially explain the increased risk of iron deficiency and anaemia in these groups (Baynes and Bothwell, 1990).

2.3.3. Chronic iron losses

Excessive blood loss from any cause may exacerbate sub-optimal iron status existing in a population and so may precipitate anaemia. For example, pathological losses from excessive menstrual flow can cause anaemia in women. These menstrual blood losses are highly variable among women but fairly constant within individual women (Hallberg, et al., 1966). Exogenous causes of variation in menstrual blood losses can be due to anovulatory medications and intra-uterine devices which can reduce or increase the losses respectively (Viteri, 1998).

Other losses occur through perspiration, gut mucosal turnover, skin desquamation (particularly in hot and humid climates), intestinal excretion, chronic haemolysis due to genetic factors (e.g. thalassaemia, sickle cell disease), parasitic infections (e.g. malaria, hookworms and schistosomiasis), and other occult blood loss (Viteri, 1998).

The results of blood lost into the gut during hookworm infections in humans have been reviewed by Crompton & Stephenson (1990). It is estimated that an adult woman weighing about 50kg and carrying an infection of hookworms about 250 worms would lose about 10ml of blood containing about 3.7mg of iron per day. If such a woman had a marginal iron intake, the iron loss through hookworm infection could be substantial. If the infection is not adequately treated blood loss may continue for many years, leading to depletion of body iron stores and the development of iron deficiency anaemia. Pawlowski et al., (1991) note that there is also loss of serum proteins, which may result in severe hypoalbuminaemia. Pawlowski et al., (1991) however, suggest that,

whether or not a person infected with hookworms develops anaemia depends on several factors, including the species of hookworms, the worm load, the duration of infection, the body iron stores, dietary iron intake and absorption, physiological iron requirements and other iron losses.

Serious pathological blood losses may also result from chronic gastrointestinal disturbances, such as diarrhoeal diseases. Other intestinal diseases such as peptic ulcers can cause iron deficiency as well (Skikne, 1988). Sometimes the excessive use of particular medications such as aspirin may lead to bleeding and anaemia (Flemming, et al., 1987).

2.3.4 impaired iron utilisation

Chronic and repeated infections have been associated with anaemia (WHO, 1997). For example patients suffering from chronic infections such as tuberculosis, HIV and related infections are often found to have anaemia (WHO, 1997). Catabolic losses have also been found to cause anaemia particularly in young children (WHO, 1997). As mentioned above, chronic hookworm infections may result in severe hypo-albuminaemia which impairs iron utilisation (Pawlowski et al., 1991). Vitamin A deficiency has also been associated with anaemia (WHO, 1991). The interrelationships between vitamin A and anaemia will be discussed in a separate paragraph in this chapter and more details will be given in Chapters four and seven.

2.4 Magnitude of iron deficiency and anaemia

Iron deficiency is the most common nutritional disorder in the world (WHO, 2000). As many as 4-5 billion people, 66-80% of world's population, may be iron deficient (WHO, 2000). About 2 billion people worldwide are anaemic mainly due to iron deficiency (WHO, 2000). The problem

is much more severe in the developing countries but the developed countries are not spared (DeMayer and Adiels-Tegman, 1985). The prevalence of iron deficiency anaemia is approximately 11 percent in developed countries, nearly 1/3 of population in developing countries suffers from it (DeMaeyer and Adiels-Tegman, 1985; Royson, 1982; WHO, 2000). Worldwide, 79 million pregnant women, and 61 million young children were anaemic; and over 290 million school aged children, of whom 150 million attended school were anaemic in 1991. Among adults, close to 600 million adult women and 330 million men were anaemic (Viteri, 1991; 1998). The WHO reports showed similar trends in recent years (WHO, 2000).

2.4.1 Magnitude of anaemia in Tanzania

Tanzania, is one of the developing countries most affected by iron deficiency anaemia (Kavishe, 1991; Mwanri, et al., 2000). However, due to lack of wide spread objective diagnostic facilities, the epidemiological picture regarding the nature and extent of anaemia in Tanzania is very incomplete (Kavishe, 1991). An analysis of hospital data indicated that in 1991, 20-80% of admitted underfives and 18-87% of admitted pregnant women were anaemic. In addition, anaemia was a direct cause of 5% of maternal mortality and an underlying cause in 63 to 73% of maternal morbidity (Kavishe, 1991). These estimates are thought to be gross underestimates of the problem because the cut-off point for the definition of anaemia was a haemoglobin level of < 8.5g/dl not 12g/dl. This level was arbitrarily chosen because of the scarcity of available resources (Kavishe, 1991). It was thought that it is very important to have an overview of the magnitude of the problem in the country.

Kavishe (1991) further argues that the prevalence of anaemia in Tanzania varies from 0 to 100 percent depending on the population group considered and geographical location. He claims that

where anaemia has been typed, it has been found to be mainly due to iron deficiency with other factors such as helminthic infections and poor dietary intakes usually exacerbating the situation.

2.5 Consequences of iron deficiency anaemia

The consequences of iron deficiency anaemia in humans are numerous. There is a wide range of negative impacts on a variety of attributes. Iron deficiency alters physiological functions before the deficiency becomes clinically apparent (WHO, 2000). It is associated with impaired physical growth, as well as potentially adverse effects on neurological functions involving cognitive dysfunction, short attention span and impaired learning capacity (Pollitt et al., 1989; Aladin, 1986; Dallman, 1974). It reduces muscle functions and energy utilisation and therefore decreases physical activity (WHO, 2000). It also affects emotional behaviour, reactions to stimuli, and neuromotor development and functions (Pollitt et al., 1989, Soemantri, 1989).

Another major effect of IDA is lowered immunity. This can result in increased susceptibility to infections; and alterations in the reproductive process (Scrimshaw & SanGiovanni, 1997, Viteri & Torun, 1974; WHO, 1997; Howson, et.al., 1998). More generally, iron deficiency anaemia has profound effects on productivity and, hence, has economic implications for countries in which it is prevalent. It reduces overall quality of life and ultimately slows national development (World Bank, 1994).

2.5.1 Iron deficiency anaemia and associated mental outcomes in children

A substantial number of studies have been conducted to examine the effects of iron deficiency and iron deficiency anaemia on mental outcomes. They have been associated with significantly

poorer performance on psychomotor and mental development scales and behavioural ratings in infants, as well as lower scores on cognitive function and educational achievement tests in pre-school and school-aged children (Webb & Oski, 1973; Pollitt et al., 1982; Lozoff, 1988; Mwanri et al., 2001).

For example, a study to examine the effect of iron supplementation on attention processes was conducted among iron deficient Papua New Guinea infants by Heywood, et al. (1989). The results revealed that iron supplementation affected attention processes but the direction of the effects depended on the presence of other factors such as malaria infections. Improved cooperativeness and increased attention span following short term oral iron supplementation have been observed in other studies (Walter et al., 1983). For example, iron deficient anaemic children in Chile scored higher on developmental indices after 10 days of iron supplementation. This study confirmed the association of impaired iron status with developmental scores. Another study by Lozoff et al. (1982) in Guatemala demonstrated adverse effects of iron deficiency anaemia on infant behaviour. The subjects in Lozoff's study were iron deficient anaemic infants 19-24 months old who exhibited fearfulness, increased body tension and decreased gross body movements. They were less responsive to the examiners and less reactive to ordinary stimuli than non iron deficient children. These behavioural differences disappeared after treatment with oral iron supplements (Lozoff, 1982).

The cognitive benefits of iron treatment have been demonstrated more clearly in pre-school than in infants. For example, Pollitt et al., (1978) in a double blind, iron treatment study compared iron deficient anaemic pre-school children with non-anaemic, pre-school control before introducing iron therapy. At baseline, the iron deficient children performed more poorly than their non-anaemic counterparts on tests of attention and memory control processes. At follow up,

after four months of iron supplementation, there were no significant differences between the groups. This study strongly suggests that iron deficiency is associated with lower scores on cognitive development tests in pre-school children and that performance returns to an optimal level after adequate treatment.

Treatment of iron deficiency anaemia with iron supplements improves school aged children's scores on tests of cognition and educational achievement. For example, Webb et al. (1978) conducted a cross sectional study among 12-14 year olds to assess the relation between the presence of anaemia and school performance, as measured by the Iowa Basic School Skills test. Although the subjects performed poorly relative to national standards, the anaemic children scored significantly lower than the non-anaemic control children. Seshadri et al.(1982) in a double blind intervention study of 5-6 year olds in India demonstrated that iron treated children had improved education achievement scores and these scores were higher than those of the placebo group. The results of these well designed studies suggest a causal association between iron status and school performance.

Similarly, Soemantri, (1989) found that iron supplementation increased rural Indonesian children's scores on a variety of tests. Iron supplemented children without anaemia exhibited improvement on these tests as well. These examples show that there is ample evidence that suggests a positive relationship between iron status and cognitive performance. It is reasonable therefore to propose that, there is a link between iron deficiency and lowered performance on cognitive and developmental tests as well as school performance. It is reasonable then, to expect that the majority of school children in Tanzania and developing countries who are anaemic may perform poorly on tests of cognitive performance. It is hypothesised in this thesis that

supplementing anaemic children's with iron should raise their scores on cognitive performance tests.

2.6 Anaemia in school aged children

World Health Organisation data indicate that 46% of school children in developing countries are anaemic (Viteri, 1998). The reports of the Tanzania Partnership For Child Development (1996) also confirm that anaemia is a widespread and important problem. Indeed, these reports suggest that the prevalence of iron deficiency anaemia in Tanzanian school children is approximately 80 percent. A similar prevalence rate was noted in the work conducted for this thesis (Mwanri, et al., 2000). This is a serious situation, considering the consequences of iron deficiency anaemia particularly on cognitive functioning and productivity as described earlier in this chapter.

The most common causes of iron deficiency anaemia among school children are multi-factorial. In Sub-Saharan Africa, the bioavailability of dietary iron is the most important determinant of anaemia in school aged children (WHO, 1997). Parasitic infections, which are most prevalent in this age group (Tanzania Partnership for Child Development, 1996), may lead to greater than normal requirements for iron because hookworm and urinary and intestinal schistosomiasis cause blood to be lost into the urinary bladder or intestines. The need for iron also increases at puberty with the onset of menses. Genetic disorders such as sickle cell disease and other factors are also cause of anaemia in school aged children (WHO, 2000).

2.7 Vitamin A and iron deficiency anaemia

Vitamin A is required for the maintenance of healthy epithelial tissues, mucus production, growth and adequate immune response as well as for sight (Underwood and Arthur, 1996). Originally discovered as a growth nutrient, vitamin A soon became recognised as an important substance involved in normal vision. Vitamin A is also involved in the formation of specific glycoproteins in disease resistance and may have a specific role in the prevention of specific cancers such as skin cancers (McLaren, 1986).

Nikawa et al. (2000) examined whether vitamin A could improve immune depression in mice with protein deficiency. The results suggested that large oral supplements of vitamin A could preserve mucosal *IgA* levels during protein malnutrition, possibly by stimulating *Th₂ cytokine* production and thereby inducing resistance against infections. Mucosal *IgA* is the principal arm of mucosal immune response that protects the upper respiratory tract against infection by pathogenic organisms. Other studies with animal models have shown that, the mucosal *IgA* response is impaired in vitamin A deficiency (Semba, 1998; Puengtomwatanakul & Sirisinha, 1986; Wiedermann, et al., 1993). In field trials, vitamin A supplements have reduced mortality in preschool children in Indonesia (Sommer et al., 1986), India (Rahmathullah et al., 1990) and Nepal (Keith et al., 1991) by 34%, 54% and 30% respectively. However, another trial in India (Vijayaraghavan et al., 1990) failed to confirm these results, raising concern about the potential impact of vitamin A supplementation on child survival in different places.

Vitamin A deficiency (VAD) is a worldwide nutritional problem, but it is mainly confined to the developing world where diets are restricted (Sommer & West, 1996). The most obvious health consequences of severe vitamin A deficiency involve the visual system, affecting vision in low

light or darkness and dryness (xerosis) and disruption in the integrity of the surface of the conjunctiva and cornea (bitot's spot, cornea clouding, ulceration). Occurrences of these signs are associated with elevated risk of blindness and death. The WHO reported that in 1991 nearly 14 million preschool children had eye damage due to vitamin A deficiency, the majority of whom are located in developing countries (WHO, 1992). Each year, it is estimated that between 250,000 to 500,000 preschool children go blind from vitamin A deficiency with about two-thirds of these children dying within months of going blind (WHO, 1992 & 2000). Vitamin A deficiency has long been associated with mammalian growth (McCollum and Davis,1913) but it has been difficult to demonstrate the effect on the growth of the children.

Clinical vitamin A deficiency has long been linked to poor growth on a cross sectional basis. However, Sommer (1982) has claimed that, often the more severe the eye signs, the more severe the stunting and wasting. Children who develop mild xerophthalmia or Bitot's spots, also show less weight gain and linear growth than the non-xerophthalmic peers. Conversely, Tarwojo, et al. (1982) described the improved weight gain that can accompany spontaneous recovery from xerophthalmia, although catch up linear growth is less evident.

In Sudan, Sedgh, et al. (2000) conducted a prospective study to examine the association between dietary vitamin A intake and survival, health and growth in 8174 children aged 6-72 months who were stunted at the start of the follow up. After 18 months, the findings suggested that diets rich in vitamin A particularly in the form of carotenoids, can improve growth and increase the rate of recovery from stunting among malnourished children. These associations may represent a direct effect of vitamin A nutrition on growth or they may be indirect, mediated by other co-varying nutritional factors that can affect growth such as protein energy malnutrition.

The high prevalence of infections among children living in poor areas of developing countries has been associated with impaired linear growth. Stephensen (1999) has described the impact of repeated infections on weight gain for an individual child. He also illustrated the association between high incidence of infection, particularly after weaning at 6 months of age, and the association of these episodes of infections with weight loss. It has been claimed that acute, invasive infections, which provoke a systemic response (e.g. dysentery and pneumonia) and chronic infections, which affect the host over a sustained period (e.g. helminth infections), have a substantial effect on linear growth (Stephensen, 1999). Many episodes of infections are associated with lack of catch-up growth, which might allow the child to regain his or her expected growth trajectory. Knowledge of the association of episodes of infection with linear growth deficit might be applied in promoting the use of vitamin A in programs that aim at improving children's health.

In pregnant women VAD causes night blindness and may increase the risk of maternal mortality (WHO, 2000). Recent WHO reports claim that VAD is a public health problem in 118 countries especially in Africa and South East Asia, hitting hardest young children and pregnant women in low- income countries (WHO, 2000).

Vitamin A is present in food in two forms. The various carotenoids (Predominantly B-carotene) that serve as provitamins occur mainly as plant material, such as carrots, green leafy vegetables, red palm oil, yellow and orange vegetables and fruits. Preformed retinol (usually in the ester form) is found in foods of animal origin, for example liver, and dairy products.

The ability of developing countries to provide a diet which is variable enough to ensure adequate vitamin and mineral intake is limited. Populations without access to varied diets which contain abundant amounts of fish or animal products, fresh vegetables, fruits and oils are susceptible to

vitamin A deficiency. These products provide adequate vitamin A or pre-cursors to vitamin A. Unfortunately, foods high in starch, such as rice, maize and wheat are low in vitamin A and its precursors. Generally, such foods are the staples which are mostly used in areas that can least afford to combat the problem (ACC/SCN, 1997).

2.7.1 Interrelationship between vitamin A and iron

A few studies have shown evidence of an interrelationship between vitamin A and iron. These have been demonstrated both in laboratory animals and humans. For example, Findlay and Mackenzie (1922) reported haemopoetic changes (gelatinous degradation in bone marrow) in rats fed a vitamin A deficient diet. Consequently, Roodenburg et al. (1995) demonstrated that supplemental vitamin A enhanced recovery from iron deficiency in rats with chronic vitamin A deficiency. Studies in horses (Donoghue, et al., 1981) and rhesus monkeys (O'Toole, et al., 1974) have demonstrated lowered haemoglobin concentration with vitamin A deficiency. The anaemia in rhesus monkey failed to respond to dietary liver, iron and whole blood but was responsive to treatment with vitamin A.

In the mid 1930's studies in children showed that vitamin A deficiency resulted in anaemia and haemosiderosis in the liver and spleen (Blackfan & Wolbach 1933; Sweet & Ka'ng, 1935). It was observed that the correction of vitamin A deficiency caused regeneration of bone marrow, disappearance of haemosiderin from the spleen and liver, and an outburst of erythroblast activity. These findings suggest that vitamin A deficiency may interrupt some steps in the transfer of iron from storage in the liver to incorporation of haemoglobin in haemopoetic tissues.

In human volunteers, maintained on very low vitamin A diets for 357 to 771 days, moderate iron deficiency anaemia was developed which was refractory to medicinal iron but responsive to vitamin A (Hodges & Kolder, 1971). More recently, several studies in humans have demonstrated beneficial effects on iron status via vitamin A supplementation with or without iron supplements. For example, children (Mejia & Arroyave, 1982; Bloem et al., 1989, Wolde-Gebriel et al., 1993) and pregnant women (Surhano et al., 1992) living in areas where vitamin A deficiency is endemic exhibited a positive relationship between vitamin A and iron status. When children with low concentrations of haemoglobin, low serum iron, and low transferrin saturation were supplemented with vitamin A, their haemoglobin levels rose (Mejia, 1988; Mejia and Arroyave, 1982). Similarly, Indonesian children supplemented with vitamin A showed increases in haemoglobin levels (Semba et al., 1992).

Panth, et al. (1990), observed rises in haemoglobin levels at 26-28 weeks of pregnancy which were higher among women supplemented with iron plus vitamin A than iron alone. In another study, Dreyfuss et al., (2000) demonstrated the contribution of hookworms, malaria and vitamin A deficiency in anaemic women from the plains of Nepal. In their study, low serum retinol was most strongly associated with mild anaemia, whereas *P.vivax* malaria and hookworm infection intensity were stronger predictors of moderate to severe anaemia.

These studies provide evidence that, regardless of the mechanisms involved, vitamin A deficiency may contribute to the development of anaemia, particularly where dietary intakes of both vitamin A and iron are low. Therefore, a major aim of this thesis was to investigate whether haemoglobin levels and other biomedical and cognitive variables can be improved via vitamin A supplementation.

2.8 Strategies to prevent iron deficiency anaemia

In general the prevention of iron deficiency anaemia and other micronutrient deficiencies may be addressed by appropriate combinations of the following strategies: increased dietary diversification at individual and community levels as a long term measure as well as supplementation and fortification. The above strategies coupled with health education and promotion approaches can be effective ways to prevent iron deficiency anaemia. Wass (2000) acknowledges that health is created and lived by people within the setting of their everyday life. Therefore health education and promotion which can impart knowledge of a particular health problem to targeted population is crucial in prevention and control of iron deficiency anaemia.

2.8.1 Dietary diversification

Dietary diversification seeks to change people's food behaviour, and in turn, their nutritional status (Smitasiri, 1991). It is well known that there are two kinds of iron in food which differ according to their mechanisms of absorption: haem iron and non-haem iron. Haem iron, present in haemoglobin and myoglobin, is usually consumed as meat. Its absorption is high and largely, independent of iron status. The daily amount of haem iron in the Western type diet is 1 to 1-2mg (Hallberg, 1989).

In diets in developing countries, especially in Africa, staple diets are mainly based on plants and contain high levels of non-haem iron. Therefore, the amount of haem iron consumed is often negligible and can usually be disregarded (Hallberg, 1989; Gibson, 1994). The absorption of non-haem iron from the gut is very poor because it is bound to substances such as tannins, phytates and polyphenols which inhibit absorption of iron (Kohmeier, et al., 1998, Hallberg,

1989). However, cereal staples form major components of meals as an economic necessity in many developing countries, Tanzania included. Thus non-haem iron is the main type of dietary iron (Gibson, 1994, Tatala et al., 1998).

One way to overcome dependence on single cereals is to promote dietary diversification. Another is to make more nutrient dense cereals available for consumption. Initiatives to enrich staple crops with micronutrients through plant breeding are underway (Graham & Welsh, 1996). These initiatives aim to combine favourable agronomic and nutritional characteristics into cultivars of cereals and other food staples in order to correct existing micronutrient deficiencies. This approach is being pursued in several centres of the Consultative Group on International Agricultural Research (CGIAR) (Bouis, 1996). Bouis (1996) suggests that the most sustainable approach to control iron deficiency could be via the ingestion of bioavailable iron in food. It is likely that new micronutrient-dense cereals would provide a long lasting solution for iron deficiency anaemia in impoverished regions.

Underwood and Smitasiri (1999) however observe that, before staples with optimal agronomic, nutritional and organoleptic qualities are available to farmers, several years and substantial resources will be needed to complete the field work necessary for breeding, propagation, bioavailability testing and consumer acceptance. Therefore, breeding programs should be considered to be only one part of a micronutrient prevention strategy. Bouis (1996), on the other hand, argues that, once plant breeding for micronutrient enrichment of dietary staples has been achieved, the recurrent expenditure would be low compared with non-food based strategies. This thesis will explore the potential benefits of micronutrient enriched maize over maize strains which are currently used in the developing world. This will involve the in vitro bioavailability estimation procedure which employs the Caco-2 cell method as described in Chapter seven.

Whatever the nutritional qualities of staple foods, favourable dietary practices must be learned and utilised by food providers, preparers, and those responsible for the distribution of food in the household. The empowerment of communities is essential for achieving these desired dietary practices. Underwood and Smitasiri (1999) have shown that different approaches to communication and education in food and nutrition (e.g., social marketing and reflexive participation) are important processes in the maintenance of the sustainability of the food supply. This is important because the effects of improved iron nutrition (increasing iron reserves and/or reducing anaemia prevalence and incidence) only become evident after several months or years because increments in total iron absorption are moderate and only gradually become perceptible to lay people (Hulten et.al, 1995).

2.8.2 Iron supplementation

In industrialised countries, over the counter medications (in safe dosages not requiring prescriptions) have played a role in controlling iron deficiency. Supplements of various sorts are consumed daily by 25% of the population in the United States (Slesinsk, et al., 1995). Supplements are also, a routine part of public health programs for low income children both in the United States and the United Kingdom (Slesinsk, et al., 1995). The lower prevalence of iron deficiency anaemia in developed countries may be due in part to the public and private accessibility of supplements (Underwood & Simitasiri, 1999).

In developing countries where iron deficiency is endemic, iron supplements are the most important method of combating acute clinical deficiency (Underwood & Smitasiri, 1999). Iron supplements can be given as a measure to combat anaemia. Oral daily tablets of chemical iron, such as ferrous sulphate or ferrous fumarate, for a period of several weeks are often administered

(UNICEF, 1995). Iron supplements have also been used to reduce iron deficiency in specific situations such as pregnancy and early childhood. Reviews of iron supplementation trials prior to 1956 and up to 1983 (Hyttén & Duncan, 1956) and studies from 1966 to 1989 (Sloan et al., 1992) have indicated that iron administration during pregnancy does reduce anaemia. The reduction in anaemia is positively related to the duration of supplementation. The lower the haemoglobin concentrations prior to supplementation, the greater the reduction.

However, Gibson (1994) and Underwood & Simitasiri (1999) argue that although iron supplementation and policies for their use have existed for years globally, evidence of their effectiveness has not been very apparent because of problems such as poor compliance, poor logistics, lack of political and program support as well as inadequate awareness of the seriousness and magnitude of the problem.

Most programs have targeted pregnant women and young children (Stoftzfus & Dreyfuss, 1998). Accordingly, this thesis will describe the potential benefits of iron and vitamin A supplementation in combating iron deficiency anaemia in school aged children, among whom anaemia can have very negative impacts on their physical and cognitive development.

2.8.3 Food Fortification

Fortification is the addition of nutrients to commonly consumed foods so as to maintain or improve their nutritional quality and that of the community's diet (Solon, 1998). Fortification with substances commonly consumed by a target population has been used to improve population nutritional status in wealthy and developed countries (Solon, 1998). The great advantage of fortification is the potential speed with which it can be deployed and the absence of need for a new distribution system or for changes in dietary habits. For example, the efficacy of combating

anaemia in children's schools, homes and in the entire community were investigated. The details of the processes and findings form Chapter six.

Wass (2000) describes other health promotion actions which include:

- (i) Putting health on the agenda of policy makers in all sectors and at all levels; raising their awareness of the health consequences of their decisions; and enhancing their willingness to accept their responsibilities for community health. Health promotion policy requires the identification of obstacles to the adoption of healthy public policies in non-health sectors, and ways of removing them.
- (ii) Creating supportive environments. The link between people and their environment constitutes the basic foundation for a socio-ecological approach towards health. Systemic assessment of the health impacts of a rapidly changing environment is essential to ensure positive benefits for the health of the public. As such supportive environment is critical in maintaining good health.
- (iii) Concrete and community action in setting priorities, making decisions, planning strategies and implementing them to achieve better health is important. The empowerment of communities, their ownership and control of their endeavours is an important process in health promotion. Likewise, in anaemia prevention, the empowerment of parents, teachers and the community, their ownership and control of their own anaemia prevention and control programs is essential. In this thesis health promotion is regarded as an essential strategy for the prevention of iron deficiency anaemia.

2.9 Aims, hypotheses and objectives

2.9.1 To examine the impact of iron and vitamin A on anaemia, growth and cognitive functioning of anaemic school children.

Hypotheses

(i) Anaemic children who receive vitamin A supplements will show similar reductions in anaemia and cognitive function improvements to children who receive iron supplements.

(ii) Anaemic children who receive a combination of iron and Vitamin A supplements will show greater improvements in increases in haemoglobin levels and cognitive function than children who receive single supplement.

2.9.2 To examine the knowledge, perceptions, attitudes and practices of children, parents and teachers, and school communities, regarding anaemia control.

Hypothesis

There will be important gaps in knowledge, as well as preparedness to take part in community anaemia prevention and control activities between teachers, parents and children. Teachers will have greater knowledge of prevention and control of anaemia than parents and children.

2.9.3 To examine the iron bioavailability of a conventionally consumed maize staple from Tanzania and the newly bred cultivars from Zimbabwe.

Hypothesis

The newly bred maize cultivars from Zimbabwe will have higher iron density and more biavailable iron than the traditionally consumed strains from Tanzania.

CHAPTER THREE

THE SETTING FOR THE RESEARCH AND METHODOLOGICAL APPROACH

3.1 The setting of the research

3.1.1 Study area

The fieldwork for the studies described in this thesis was conducted in Bagamoyo District in Tanzania. Bagamoyo is among the six districts of the coast region. Other districts are Kibaha, Kisarawe, Rufiji, Mafia and Mkuranga. It lies between 6° to 7° Longitude East and 38° to 39° Latitude south. It shares borders with Kinondoni district in the Dar-es-Salaam region and the Kibaha district in the coast region. The district also shares its border with the Morogoro region in the west. In the north it shares the border with Handeni and Pangani districts in the Tanga region. The district covers an area of 9842 km². Out of these 855 km² are covered by water (ocean and rivers).

Bagamoyo was purposefully chosen for the study because most of its rural area can be reached throughout the year including the rainy season. The tests of cognitive functioning and educational achievement were adapted in Bagamoyo district, therefore there was no need to make any new adaptations. In addition, all people in Bagamoyo district speak Swahili which avoided any translation problems.

3.1.2 Population

According to the 1988 National Population census, the district had a total population of 173, 885 people (Tanzania Bureaus of Statistics, 1988). There were 85,426 males and 88,259 females. Based on an estimated district growth rate of 2.5% per annum, when the studies were conducted, in 1999, population was estimated to have been 218,983 (Tanzania National Population Policy (NPP), 1992). Bagamoyo district has had long historic contact with the coastal belt. Ethnically, the majority of people of Bagamoyo are Bantu consisting of a mixture of Kwere, Zaramo, Ng'indo and Matumbi tribes. There are also some people of Arabic origin from Persian Gulf who migrated to Bagamoyo on slave trade business.

3.1.3 Climate

Bagamoyo has a tropical climate and is essentially hot and humid. The district has two seasons a year. These are the dry and rainy seasons. There are two rainy seasons: the short rains from October to December and the long rains from March to June. These are monsoonal rains. The average annual rainfall ranges from 800mm to 1200mm. The maximum mean temperature is 30°C and the minimum temperature is 13°C (Mwaseba, 1999).

3.1.4 Economy

The main economic activities in Bagamoyo district are predominantly based on subsistence agriculture and livestock rearing. In the absence of large-scale production, small holders dominate farming in the district. Indeed, ninety percent of the population is engaged in agricultural activities. The average farm size per household is estimated at 3.9 hectares. The main cash crops are cashew nuts, cotton, coconuts and tropical crops such as mangoes, cowpeas, pigeon peas and pineapples.

The staple food crops include maize, rice, cassava, millet, sorghum, beans and sweet potatoes.

Livestock include cattle, sheep and poultry.

3.2 Methodology

3.2.1 Approval and Consent

Approval of the studies reported in this thesis was obtained from the University of Adelaide Human Research Ethics in Australia (**Appendix 1**). In Tanzania, approval was obtained from Ministries of Health (**Appendix 2**). The Tanzania Food and Nutritional Centre (TFNC), which is an approved institution of the Ministry of Health for approval of studies related to nutrition, also consented to the conduct of the studies (**Appendix 3**). Written permission to work in schools was also obtained from the Director of Research and Planning in the Ministry of Education and culture (**Appendix 4**).

District officials were informed of this project and their approval was obtained. A letter of approval was obtained from the office of the District Executive through the district education officer (**Appendix 5**). An officer from the district education office was designated to accompany the study team to each school to provide introductions to the staff, parents and children.

3.2.2 Study designs

The study instruments were written in English and translated into Swahili (the national language of Tanzania). The instruments included forms for anthropometry (weight and height) and physical examinations (**Appendix 6**). There were also three sets of questionnaires for examining children's, parents' and teachers' anaemia knowledge, attitude and practices (**Appendix 7**). All study

instruments had a place to record the basic information such as respondents' identification number, sex, age, class and date of interview or questionnaire/test.

Pre-testing of the questionnaires was conducted in a school which was not involved in the project. The questionnaires were administered by the author to check their completeness and accuracy, and to ensure that the information collected was of high quality. Pre-testing revealed that the questionnaires were well accepted and understood except for a few areas that needed modifications.

3.2.3 Operations

The studies were carried out between February and July 1999. They consisted of:

- (1) A two part clinical trial which investigated:
 - (a) the impact of vitamin A and/or iron on anaemia and growth indices, and,
 - (b) the impact of vitamin A and/or iron on tests of cognitive function and the educational achievements of anaemic school children.

The details of these two parts of study 1 will be the bases for Chapters four and five of this thesis respectively.

- (2) The knowledge, attitudes and practice study, the details of which are the basis for Chapter six.

The clinical trial involved two rounds of measurements. In the first round, baseline measurements were carried out between February and March 1999. In the second round, follow up measurements were conducted between June and July 1999.

Preparations for the administration of the trial plus its associated surveys involved meetings with officials of the Ministries of Health and Education. Their approval was essential for the conduct of the project. Subsequently, there was a felt need to inform and obtain approval from different levels of the Education department. After obtaining these approvals, initial visits were made to schools in the study area. During the visits the study schools were selected and pre-testing of the questionnaires and modification was conducted. Recruitment of study team members and assistants was also conducted during this time.

3.2.4 Selection of schools

A list of schools was obtained from the Bagamoyo District Education office. Three schools that were accessible throughout the year with adequate numbers of children for the clinical trial were selected. The socio-economic features of the schools in Bagamoyo were quite apparent. It was important to obtain study subjects who shared similar socio-economical characteristics. This was to control for different confounding effects that could result due to mixing of subjects from different social strata.

On arrival at the participating schools, the school principals were introduced to the project team by an officer from the local education office. The principals were requested to sign a letter (**Appendix 8**) to give informed proxy consent for members of their school to participate in the study. Meetings were held with school teachers in each study school and the objectives and procedures of the project were explained. This was important to ensure that they understood the aims of the project.

Letters were sent to parents and guardians which requested their written or informed proxy consent for their children to participate (**Appendix 9**). These letters explained the objectives of the project and asked the parents to volunteer for their children in the project. The contacts for information on study and independent complaints procedures were also explained (**Appendix 10**). Informed

verbal consent from parents/guardians who were not able to sign written consents was also accepted.

Children were informed that their participation in the project was voluntary. An objection by any child to take part in the project was respected in all cases even if the parents had given consent. Children had the right to drop out of the project at any time if they so wished.

3.2.5 Selection of children

Selection of children for the trial and associated surveys was done by the investigator and the school principals in each school. Although sample size calculations revealed that 120 children (**Appendix 11**) would be adequate to detect differences at the $p < 0.05$ level of significance, the sample size was set at 136 children in order to cater for any loss of subjects which could occur during the course of the project. Children aged 9-12 years old in years 2 to 5 were the target population. School registries were used to identify the children who met the selection criteria. The names of these children were written on pre-prepared forms for the research team (**Appendix 12**). Each child was then given an identification number (**IDNO**) for the purpose of identification on all data collection forms and tests.

Children were included in the project if they met the following inclusion criteria:

- ◆ They were children of parents/guardians from whom informed consent to participate in the project had been received
- ◆ They had attended school for at least two years.
- ◆ They were able to speak, read and write Swahili (the National Language in Tanzania)
- ◆ They were not using any supplements.
- ◆ They had no obvious illness or chronic conditions.

Two children who had attended school for two years and who did not take supplements were immediately dropped from the studies as one was hard of hearing and the other one had problems with speech. Teachers and parents were informed of their conditions and were advised to seek further medical attention from the district hospital.

A total of 208 children were eligible for inclusion in the studies and were given letters to take to their parents/guardians for consent. Nine parents (4.3 percent) refused to give consent for their children to participate in the studies. Two children (0.96 percent) did not volunteer to participate. Both of these children changed their minds but it was at the time after randomisation had been completed, therefore they could not be included. After receipt of the consents a total of 197 children (94.7 percent) qualified to be included and these were invited to participate in the project. These children were then screened for anaemia. One hundred thirty six (136) children were found to be anaemic and were recruited for the studies.

3.2.6 Deworming

All children recruited for the studies were dewormed for helminthiasis two weeks before it commenced. Two weeks has been reported elsewhere as the duration in which worm clearance occurs after deworming (Hall et. al; 1993). A single dose of oral Albendazole tablets (400mg) was administered to each subject before supplementation. Treatment for worms was necessary because the study area is endemic for helminthiasis (Kihamia et al., 1974) and therefore this prevented further blood loss caused by intestinal worms which could have further aggravated existing anaemia. Children with clinical malaria were not admitted to the project.

3.2.7 Randomisation

After selection of the sample from the population and the measurement of the baseline variables, a randomised double blinded placebo-control trial (**Figure I**) was used to determine the effect of providing Vitamin A and iron supplements on anaemia, weight, height, cognitive functioning and academic achievement of anaemic school children. Randomisation was important in order to eliminate biases, which could influence the treatment allocation process. The trial was blinded in such a fashion that neither the study subjects, teachers, research team members nor the investigator had any knowledge of the individuals' treatment assignments. This was important to eliminate confounding by unintended intervention. A double blind design was particularly necessary because the outcome required judgements on the part of the investigator. This again helped to prevent ascertainment bias from affecting one group more than others.

The randomisation procedure was conducted by an independent scientist in Bagamoyo. Randomisation was done according to school and sex clusters. One hundred and thirty six children were randomised into four treatment groups. Each group had 34 children as follows:

Treatment group I: Was assigned to both vitamin A and ferrous sulphate. 5000 IU (1.5mg) vitamin A in tablet form and 200mg of ferrous sulphate (equivalent to 60mg elemental iron) were given to each child per day for three days each week. These are doses recommended by the WHO (Sommer, 1982) and the International Nutritional Anaemia Consultative Group (INACG, 1981).

Treatment group II: This group was assigned to 5000 IU of vitamin A and placebo for ferrous sulphate (Magnesium stearate, Dextrose monohydrate), both in tablet forms.

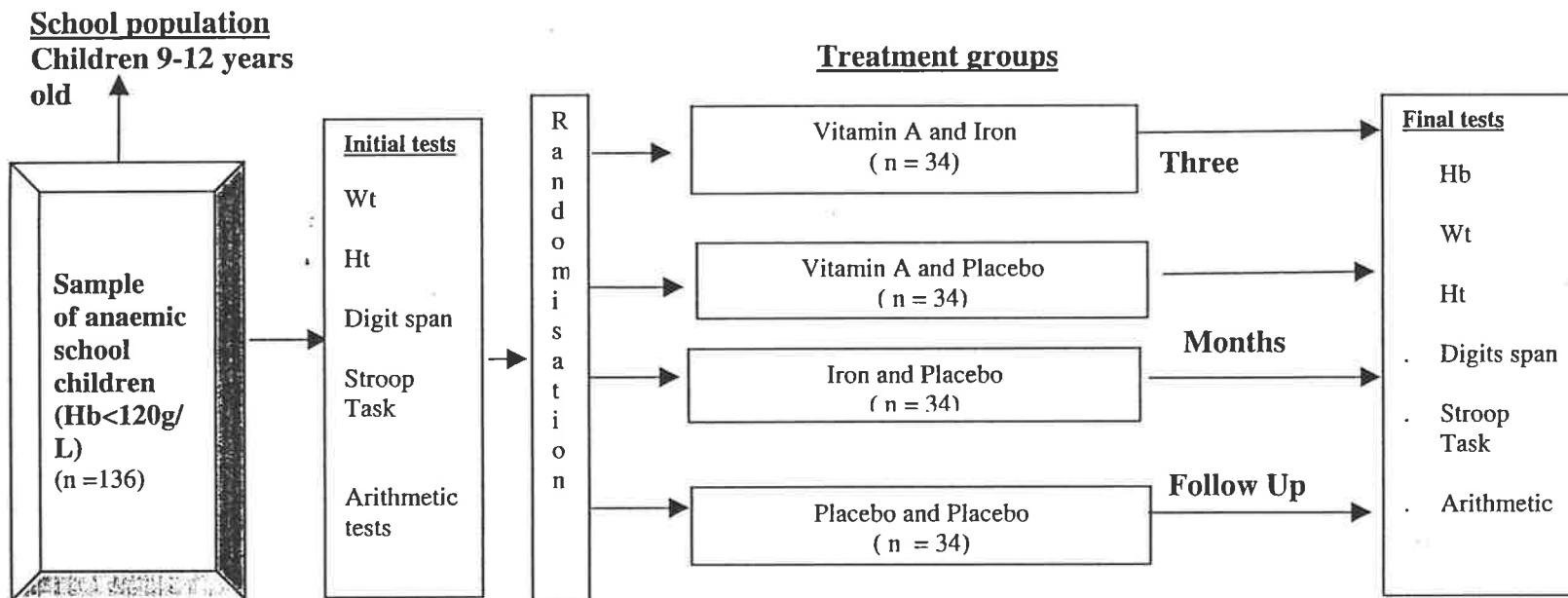
Treatment group III: Was assigned to 200mg of ferrous sulphate and the placebo for vitamin A (Magnesium stearate, Dextrose monohydrate, Hydrogenated Vegetable oil)

Treatment group IV: Was assigned to both the placebo for vitamin A and the placebo for ferrous sulphate as indicated above.

3.2.8 Provision of Supplements

After the baseline measurements had been completed, all treatments including placebo were given along with a maize preparation in gruel, three days a week for three months. This duration has been shown to increase haemoglobin concentration (Pollitt et al, 1989; Soemantri, 1989). Supplements were provided during the mid-morning break in school by either the head teacher or the health education teacher. This appeared to be convenient to both teachers and children as it prevented wastage of teaching time. After three months the intervention was terminated and the follow up measurements were taken.

Figure 1: schematic presentation of study 1 design



In addition, children who had received placebo only were treated for anaemia. Special forms were designed to record each child's supplement use for the duration of the study. This allowed monitoring for compliance over the entire period of the study. All but one (99.3%) completed the supplementation according to the protocol.

3.3 *Practical problems encountered during operation of the research*

The preparation of this study was itself a complicated process which involved a lot of difficulties. Obtaining permission from different ministries in Tanzania and at various levels of administration caused a lot of anxiety because of the complexity involved in ownership of the schools and the bureaucratic procedures involved in conducting clinical trials. One might think that the schools in Tanzania are solely in the jurisdiction of the Ministry of Education and Culture, therefore student participation in the study might only require the permission from this Ministry. However, although the Ministry of Education administers and trains school personnel, the local government owns the school buildings and other non-academic properties. Therefore in order to do research in schools these two ministries were pivotal. I had to get permission from the head quarters as well as from the local offices of both ministries. This was not easy, as it involved a long process, involving considerable frustration at times.

In addition, since the study involved the use of human subjects it required the approval of the Ministry of Health, and specific human health ethics committees. The Ethical Committee of the Tanzania Food and Nutrition Centre reviewed the proposal. This committee has its members derived from different organisations and from different backgrounds. The comments from different members were sent to me to be incorporated in the already approved research proposal in Adelaide. I found it hard to incorporate the comments proposed by Tanzania Food and Nutrition Centre ethics committee members without changing the intended objectives and methodology of this study. This difficulty was due to the fact that, some members proposed certain changes while the others suggested contrary changes

The decision to carry out this study in Tanzania was based on many facts. Firstly, I had a lot of experience in the Tanzanian School Health programme; I had headed

the program for three years. Secondly, it was clear that it would be possible to use the transport and other resources from the School Health Programme. Thirdly, it appeared that it would be easy to get approvals and permits for this study. To the contrary, this was not the case. And, contrary to my expectation, the Ministry of Health was unable to provide transport and other resources because of financial constraints. The Ministry of Health only had one vehicle which was fully allocated to the School Health Programme and therefore I contacted other organisations for vehicular assistance. The Tanzania Partnership for Child Development Project offered to let me use its vehicle for the entire period of my field work.

The establishment of a research team in any study is of paramount importance. Although I had worked in the past with a number of people who could be contracted for the job, at the time of this study most of them were occupied with other activities and were not available. Nevertheless, with my previous experience and inclination to work in the area of School Health, I was able to consult colleagues about the possibility of recruiting research team members. I was able to recruit members of research teams who had previous fieldwork experience. I was particularly happy with the team members especially that the psychometric tester had used similar tests before. Previous experience was advantage because I was not able to undertake intensive training of the research assistants due to limited time and resources.

It was a conscious decision to work in Bagamoyo district. This is because Bagamoyo had several qualities which made it possible for research to be undertaken with manageable difficulties: for example in other areas of Tanzania most people speak different languages and dialects whereas in Bagamoyo district in the Coastal Region, the majority of its people speak Swahili, which is the national language of Tanzania. It was important that the subjects of the study and the investigator should speak the same language especially for the testing of their educational achievement and cognitive function. Secondly, most of the roads in Bagamoyo district are passable all year through. Since they are not paved in most places in Tanzania, it would not be possible to conduct this type of research in other districts because they are often impassable. These studies were partly

conducted during the rainy season. In this regard, Bagamoyo district roads are much better than those in other areas. Nevertheless, it took me one hour to travel one way to each of the schools which were about 20 km from Bagamoyo town. To accomplish the task it was necessary to get up very early in the morning, which involved having to carry our locally prepared breakfast or sometimes take coconut juice for breakfasts or lunches.

The trust and consent of the children and parents was essential to the success of this project. Obtaining parental consent was not always straightforward. In these studies parents/guardians were invited to meetings where the aims and procedures of the study were carefully explained. Holding these meetings with parents was crucial as I gained their support and children participated without fear.

The classrooms in each school were in appalling conditions, for example, mud floors, broken walls and leaking roofs. Indeed, there were no adequate rooms for regular classes, let alone the research work. To cope with this situation, the investigations were done under the trees in all schools. Only the tests of cognitive function were done indoors. In two schools, the tests were conducted at school teachers' houses while in the third school the tests were done in the principal's office. For the written mathematics tests, regular classes were interrupted to enable the tests to be done in the classrooms.

3.4 Data Management and Analysis

Data were entered into computer files using the Epi Info computer program. Later, the data were transferred into the Stata computer package version 6.1. The data management encompassed the initial task of creating a dataset, editing to correct errors and inconsistencies and adding internal documentation such as variable and value labels. Variables were given descriptive names of not more than 8 alphanumeric characters. By labelling data, variables and values, a dataset which was more self explanatory was obtained. The data were inspected, and where required, the variables

were converted and new variables created. Different sub-datasets for example anthropometric, cognitive tests and haematological data were merged accordingly by using index variables to form a complete data set using the Stata spread sheet.

After the dataset had been created, different commands were used to restructure it such as reshaping and collapsing to fit different analytical purposes. Various statistical analyses were conducted for different purposes using the Stata commands. These will be described in the specific sections of the thesis.

CHAPTER FOUR

STUDY 1: THE EFFECTS OF IRON AND VITAMIN A SUPPLEMENTATION ON HAEMOGLOBIN AND GROWTH INDICES IN ANAEMIC SCHOOL CHILDREN IN TANZANIA.

4.1 Introduction

This chapter reports findings from the study which was done to investigate the impact of dietary supplements given with local foods on anaemia and growth in anaemic children in Tanzania where anaemia and growth retardation are public health problems. The supplements were iron and vitamin A.

In developing countries such as Tanzania, anaemia and growth retardation are important public health problems among children living in poverty (UNICEF 1995). Although supplementation policies have existed for many years, supplementation has most often been seen as a short-term measure for combating micronutrient deficiencies, as there is little evidence from field trials in developing countries regarding its efficacy (Gibson, 1994; Darnton-Hill, 1998; Underwood & Smitasiri, 1999).

Because anaemia is associated with peri-natal mortality, supplementation policies in developing countries have mainly focused on pregnant women and young children (Stoltzfus and Dreyfuss, 1998). As such, anaemia in school aged children has not been seen as a priority problem even with its known consequences, as mentioned in Chapter 2, for this age group.

Although something is known about the potential harm that anaemia causes school aged children, there is little information on the extent of benefits of supplementation on anaemia and growth in children of school age.

Growth retardation is one amongst many consequences of anaemia, as described in the literature review in Chapter 2. The extent to which supplements can bring about catch up growth, and how catch up growth in later childhood reduces deficits incurred in early childhood is not well documented. However, the biological potential for catch up growth has well been illustrated in studies that evaluated the responses to clinical intervention with supplementary feeding, treatment of illness or hormone therapy (Golden 1994). Tanner (1981) has advanced the general hypothesis that when undernourished children are exposed to better environmental conditions and good nutrition, the likelihood of catch up is greater, with the degree of recovery depending on the severity of growth retardation and the timing of exposure. Martorell et al. (1994) suggested that catch up may depend on whether undernutrition is associated with delayed maturation, which in turn could allow for a prolonged adolescent growth spurt with greater time for recovery before skeletal growth is complete.

Recently, it has been suggested that there may be a synergistic relationship between vitamin A and iron. Garcia-Casal et al. (1998), Garcia-Casal and Layrisse (1998) and Layrisse et al. (1997) showed in isotopic studies that relatively low doses of vitamin A or β -carotene can double the absorption of endogenous non-haem iron from cereal (staple) meals in anaemic adults in Venezuela. More interactions between vitamin A and other essential

micronutrients which are largely missing from the diets in developing countries have been reported in several studies. For example, Christian and West (1998) showed that zinc in Retinol Binding Protein (RBP), increases lymphatic absorption of retinol and its inter- and intracellular transport, whereas vitamin A affects the synthesis of a zinc dependent binding protein and so the absorption and lymphatic transport of zinc. The interaction of these two essential nutrients when delivered to people deficient in both was shown by Udomkesmalee et al. (1992). They observed synergistic activities of these two nutrients on eye parameters and RBP. These findings suggested that vitamin A supplementation may have a crucial role in controlling iron deficiency anaemia.

4.2 Methods

4.2.1 Estimation of anaemia

Haemoglobin concentrations were used to detect anaemia. This constituted the use of a finger prick to obtain a drop of blood. Sterile lancet prickers in a soft touch lancet device were used, the subjects' skin being swabbed in alcohol before hand. A finger prick blood sample was taken and placed into Hemocue cuvettes (Hemocue, UK) for reading. Haemoglobin concentrations (Hb) were measured using a portable battery operated haemoglobinometer (Hemocue Ltd), which is a simple technique for photometric detection of haemoglobin. This method is easy to use and it serves as a direct diagnostic test for anaemia. It provided an immediate measure (taking approximately 15-45 seconds) for haemoglobin concentrations. A child was defined as anaemic if the haemoglobin concentration was less than 120g/l (WHO, 1995)¹. A qualified public health nurse and the candidate were involved in reading the haemoglobin level for each individual child selected for anaemia screening. Those children found to have anaemia were immediately

¹ WHO definition of anemia for this age group changed after the fieldwork was completed (page 11).

recruited for study. Two children with severe anaemia (haemoglobin concentration $< 80\text{g/l}$) were not recruited for this study but were referred for further investigations to a nearby dispensary and their parents/guardians notified.

4.2.2 Physical examination

Each subject was given a physical examination by the candidate. These involved the following (1) Examination of the eyes for pallor of conjunctiva and of the tongue, both of which are signs of anaemia. (2) Examination of the eyes for signs of xerophthalmia (dry eye due to a deficiency of vitamin A), Bitot's spots (milky white spots on the eye) and corneal scars, all of which are signs of vitamin A deficiency (Kavishe 1991).

4.2.3 Anthropometrical measurements

The children were weighed in light clothing with no objects in the pockets. Each child stood bare-footed at the centre of the balance platform of a Soehnle electronic weighing scale (CMS Weighing Equipment, London, UK) with their feet placed together while relaxed but still. Each child's body weight was recorded to a precision of 0.1kg.

Height was measured using a fixed based stadiometer (CMS weighing Equipment, London, UK). Each child was positioned standing straight on the platform, bare-footed, with feet together, knees straight, and heels, buttocks and shoulder blades in contact with vertical surface on the stadiometer. Arms were allowed to hang loosely at the sides with palms facing the thighs. Height was recorded to a precision of 0.1cm.

All measurements were taken first at baseline and then at follow up, three months after treatment, by the same persons.

4.2.4 Data Analysis

For each of the three outcome variables: haemoglobin, weight and height the following were calculated:

- (i) mean changes within treatment groups over the three month follow-up period and
- (ii) comparison of mean changes between pairs of treatments. Each within-treatment change and between treatment comparison was specified a priori. The Bonferroni method was used to control for Type 1 error such that within each group of comparisons the overall Type 1 error was limited to 0.05 and the joint coverage of the confidence intervals was 95%.

Both the changes within treatments and the differences in change between treatments were estimated from an analysis of covariance model, one model for each of haemoglobin, weight and height. The models were adjusted for baseline measurements. In the case of haemoglobin, the model underlying the analyses was:

$$\Delta\text{Haemoglobin}_i = \beta_0 + (\beta_2 \times \text{VitA}_i) + (\beta_3 \times \text{Fe}_i) + (\beta_4 \times \text{VitA_Fe}_i) \\ + \beta_1 \times (\text{Hb0}_i - \text{mean Hb0}) + \beta_5 \times (\text{Wt0}_i - \text{mean Wt0}) \\ + \beta_6 \times (\text{Ht0}_i - \text{mean Ht0}) + \varepsilon_i$$

where: $\Delta\text{Haemoglobin}$ is the change in Hb (follow-up minus baseline);
 Hb0 , Wt0 , Ht0 are, respectively, the haemoglobin, weight and height measured at baseline, and, for convenience in the model, are centred at their overall means; Vitamin A, Iron, VitA_Fe are dummy variables indicating treatment with Vitamin A, Iron, or both Vitamin A and Iron, respectively (the reference group is therefore Placebo);
 β_0 through β_6 are coefficients to be estimated;
 ε is the error term, assumed iid $\sim N(0, \sigma^2)$;
 i is a subscript denoting this model holds for each of the 135 subjects.

The ANCOVA models showed no evidence of interaction between baseline covariates and treatment groups.

Regression diagnostics showed no important violations of the assumptions of the linear model. Tests of contrasts of interest are based on the usual Wald statistics formed from appropriate linear combinations of estimated coefficients and standard errors derived from elements of the full variance-covariance matrix of the model.

As noted above, the Stata statistical package (Stata Corporation, College Station Texas) was used for all analyses.

4.3 Results.

4.3.1 Baseline characteristics.

Of 136 anaemic children aged between 9-12 years recruited in this study, 70 (51.5%) were girls and 66 (48.5%) were boys. None of the children examined showed clinical signs of vitamin A deficiency, although most of the children had clinical signs of anaemia such as pale conjunctivae, tongues and gums.

Differences among groups in demographic and nutritional characteristics at the beginning of the study, while not statistically significant, showed the need for adjusted comparisons (**Table 2**).

TABLE 2: Characteristics of subjects according to age, sex and haemoglobin concentrations (g/L), weight (kg) and height (cm) measurements before supplementation¹

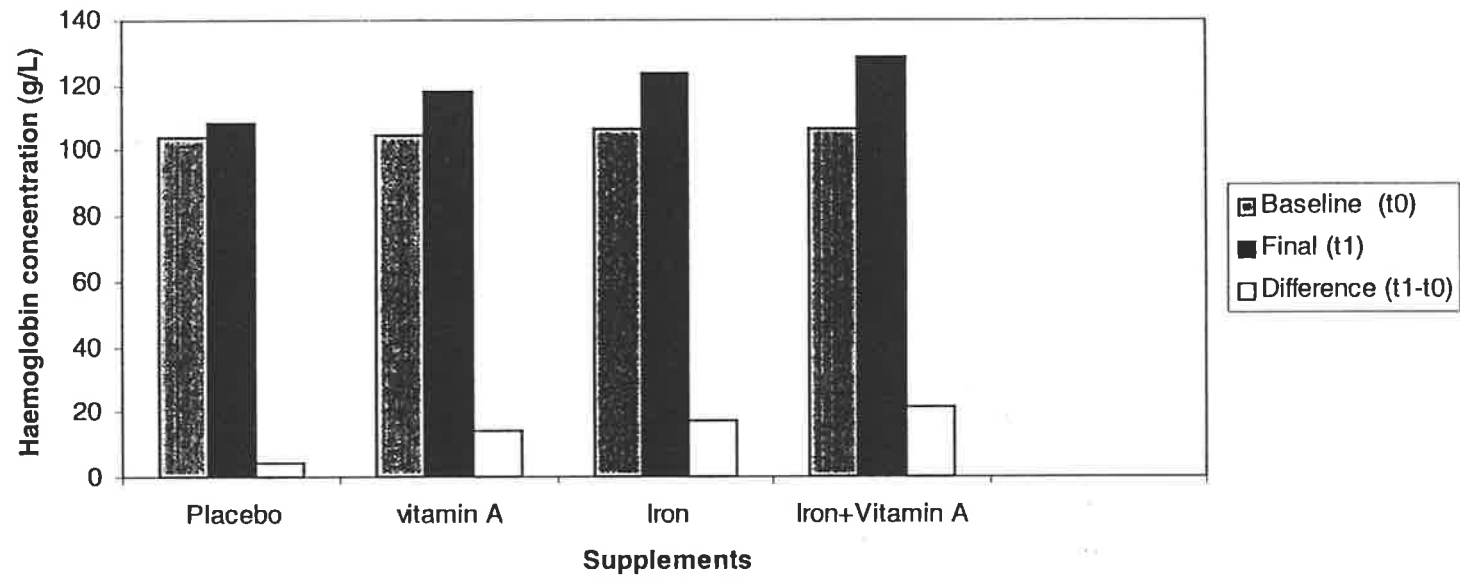
Characteristic	Supplement groups			
	Placebo (n=34)	Vitamin A (n=34)	Iron (n=34)	Vitamin A + Iron (n=34)
Age(y)	10.9±1.1	11.0±1.1	4.3 11.1±1.0	10.0±0.8
Sex: M/F	16/18	17/17	16/18	17/17
Haemoglobin (g/L)	103.9 ± 10.5	104.3 ± 0.8	106.1 ± 8.8	106.5 ± 10.5
Weight (kg)	26.0±3.7	26.7±5.2	27.2±3.9	26.6±4.4
Height (cm)	130.1±6.3	129.5±6.8	133.0±8.2	130.9±6.7

¹Values are means ± standard deviation of 136 children enrolled to the study and randomised into four treatment groups each with 34 children.

4.3.2 Effects of supplements on Haemoglobin.

At three months follow up 135 subjects were re-examined. **Figure 2** shows the mean haemoglobin concentrations at baseline and at follow up. The differences in mean change in haemoglobin concentration between follow up and baseline are also shown. It appeared that all groups had increased haemoglobin levels although the major increase occurred in the group that received both iron and vitamin A supplements.

Figure 2: The effect of haemoglobin (g/L) after three months of supplementation



Adjusted for baseline, haemoglobin levels in each group revealed significant increases over time. The most substantial increase was seen in the combined vitamin A and iron group (adjusted change 22.1g/L; 95% CI: 19.64, 24.62) (Table.3) and the group also showed the greatest change in haemoglobin relative to that in the placebo group (adjusted differences in change 18.5g/L; 95%CI: 14.81, 22.23) (Table 4)

TABLE 3: Mean change in haemoglobin (g/L) within randomised groups from baseline after 3 months treatment.

Randomised Group	Mean change in Haemoglobin from baseline at 3 months ¹	95% CI ²	P ²
Placebo	3.6±1.0	1.15, 6.07	0.001
Vitamin A	13.5±1.0	11.00, 16.00	<0.0001
Iron	17.5±1.0	15.00, 20.00	<0.0001
Vitamin A and Iron	22.1±1.0	19.64, 24.62	<0.0001

¹ mean changes ± standard error (follow -up minus baseline): values are adjusted for baseline haemoglobin, weight and height, each centred at their respective mean.

² 95% confidence intervals and two-tailed P values are corrected for multiple comparisons using the Bonferroni method.

TABLE 4: Difference between treatment groups in mean change in haemoglobin (g/L) from baseline after 3 months treatment

Comparison Groups	difference in mean change in hemoglobin ¹	95% CI ²	P ²
Vitamin A vs Placebo	9.9±1.4	6.19, 13.57	<0.0001
Iron vs Placebo	13.9±1.4	10.14, 17.59	<0.0001
Vitamin A+ Iron vs Placebo	18.5±1.4	14.81, 22.23	<0.0001
Iron vs Vitamin A	4.0±1.4	0.19, 7.77	0.0337
Vitamin A+ Iron vs Vitamin A	8.6±1.4	5.00, 12.38	<0.0001
Vitamin A+ Iron vs Iron	4.7±1.4	0.94, 8.37	0.006

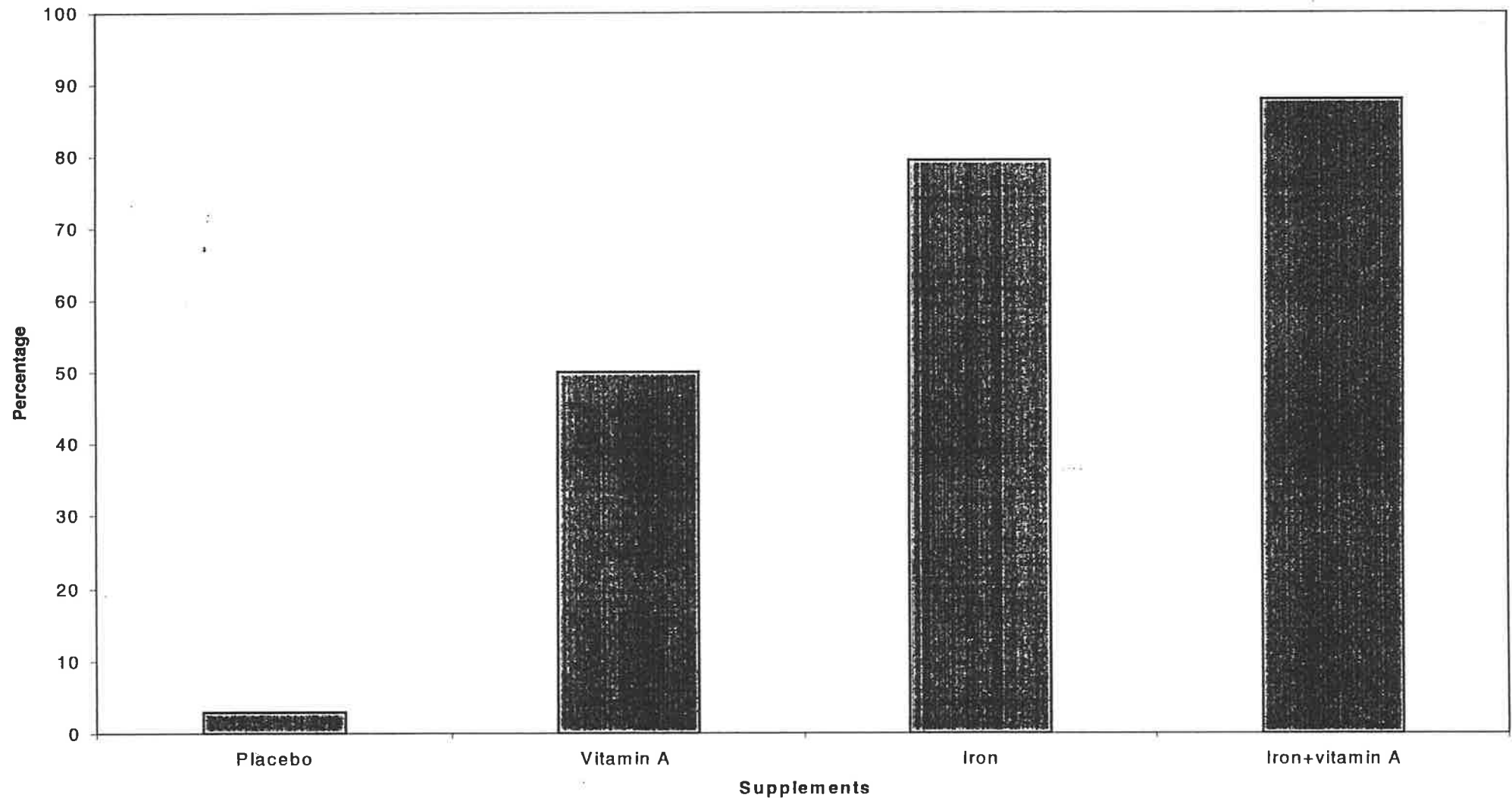
¹ differences in mean changes ± standard error: values are adjusted for baseline haemoglobin, weight and height, each centred at their respective mean.

² 95% confidence intervals and two-tailed P values are corrected for multiple comparisons using the Bonferroni method.

The combined group also showed significant improvements relative to each of the vitamin A alone and iron alone groups.

To further explore the data we calculated the proportion of children recovering from anaemia after three months of treatment (**Figure 3**). Eighty eight percent (88%) of children who received both vitamin A and iron were not anaemic ($Hb \geq 120g/L$) after three months of supplementation compared to only 3% in the placebo group. Seventy nine percent (79%) of children who received iron alone recovered from anaemia while only 50% of children who received vitamin A alone were found to have no anaemia at the end of the study.

Figure 3: Percentage of children without anaemia after three months of supplementation



These results indicate that there was a 9% advantage of correcting anaemia when children were supplemented by both combined vitamin A and iron compared to treatment by iron alone. A test for linear trend in proportions across treatment groups was significant ($\chi^2 = 15.2$ on 1 df, $P = 0.0001$).

4.3.3 Effects of Supplementation on Weight.

Figure 4 shows the mean weights at baseline, follow up and the differences in mean weight between the period of follow up and at baseline.

Mean changes in weight for each group, adjusted for baseline, after three months are shown in Table 5. There were significant increases in weight over time in each group, the most substantial was seen in the combined vitamin A and iron group (0.9kg; 95% CI: 0.73, 1.04)

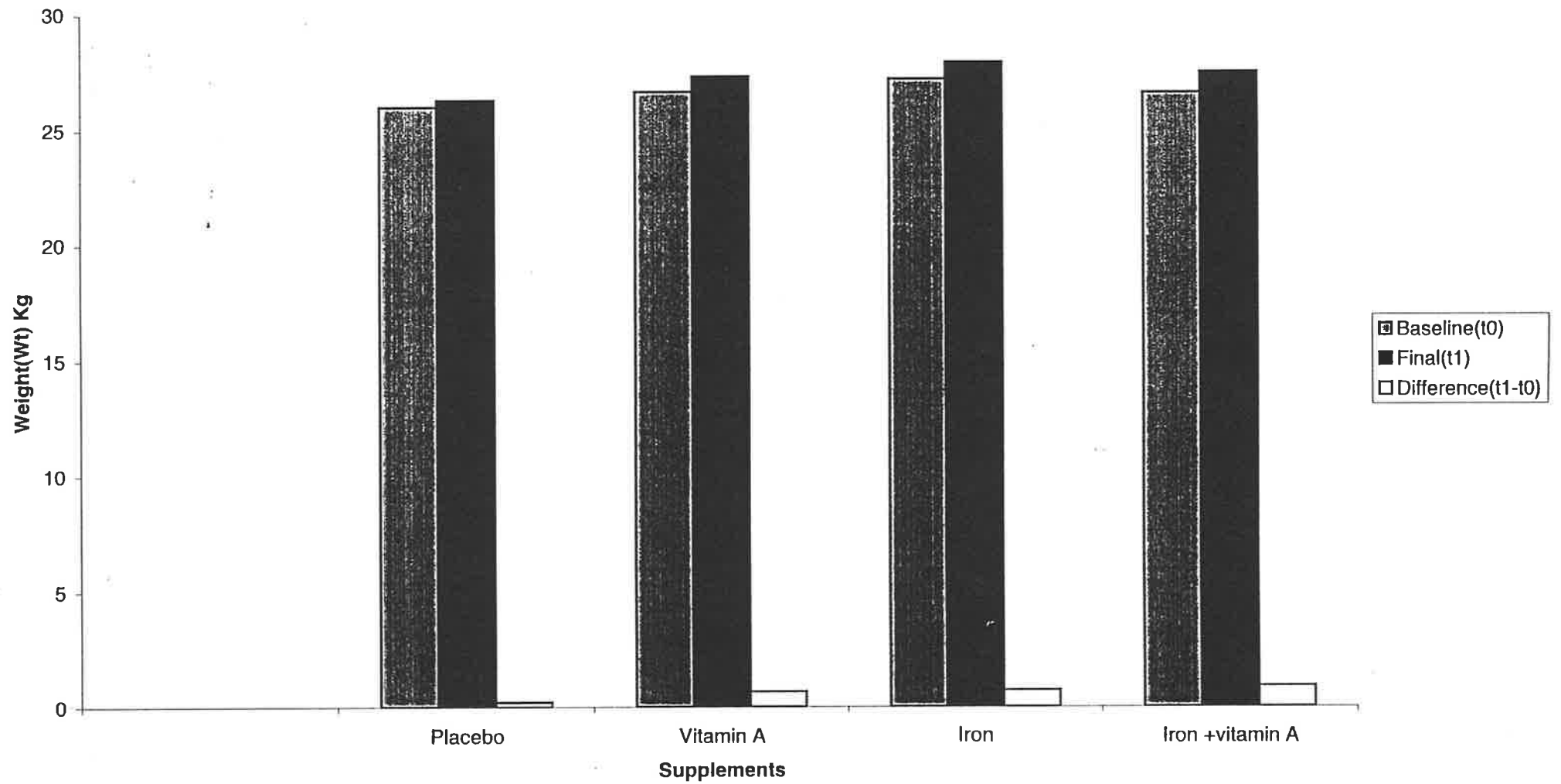
TABLE 5: Mean change in weight (kg) within randomised groups from baseline after 3 months treatment.

Randomized Group	mean change in weight from baseline at 3 months ¹	95% CI ²	P ²
Placebo	0.2±0.1	0.08,0.38	0.0008
Vitamin A	0.6±0.1	0.50, 0.80	<0.0001
Iron	0.7±0.1	0.54, 0.85	<0.0001
Vitamin A and Iron	0.9±0.1	0.73, 1.04	<0.0001

1 mean changes ± standard error (follow-up minus baseline): values are adjusted for baseline haemoglobin, weight and height, each centred at their respective mean.

2 95% confidence intervals and two-tailed P values are corrected for multiple comparisons using the Bonferroni method.

Figure 4: The effect of supplements on weight (k) after three months of supplementation



Comparison of the changes in mean weight over time between the treatment groups showed significant increases in mean weight in each actively treated group compared with placebo (Table 6). The increase in weight in the combined treatment was greater than either vitamin A or iron alone.

TABLE 6: Difference between treatment groups in mean change in weight (kg) from baseline after 3 months treatment.

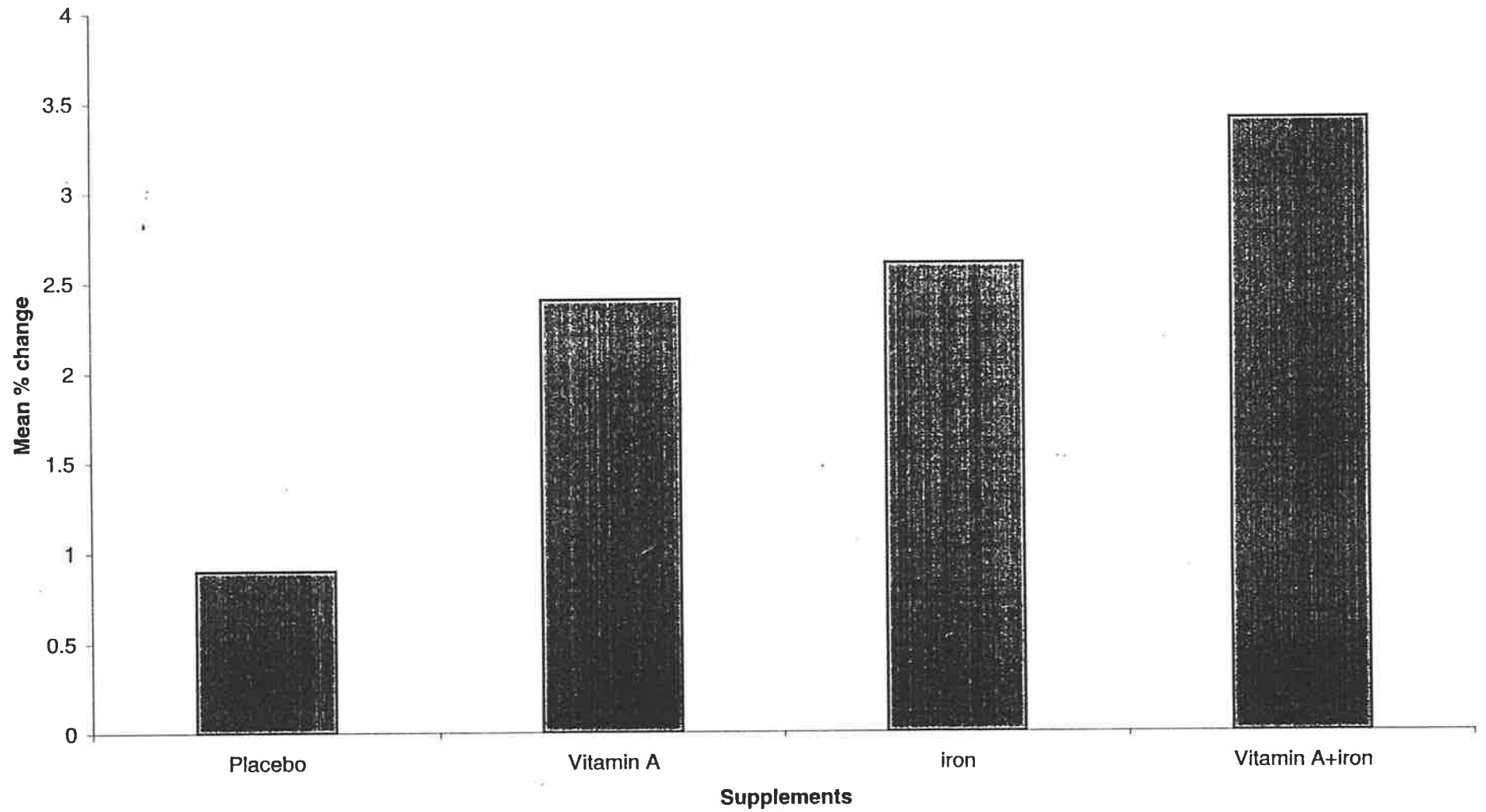
Comparison Groups	difference in mean change in weight ¹	95% CI ²	P ²
Vitamin A vs Placebo	0.4±0.1	0.19, 0.65	0.0000
Iron vs Placebo	0.5±0.1	0.24, 0.69	<0.0001
Vitamin A+ Iron vs Placebo	0.7±0.1	0.43, 0.88	<0.0001
Iron vs Vitamin A	0.0±0.1	-0.18, 0.27	1.0000
Vitamin A +Iron vs Vitamin A	0.2±0.1	0.01, 0.46	0.0386
Vitamin A + Iron vs Iron	0.2±0.1	-0.04, 0.42	0.1707

¹ differences in mean changes ± standard error: values are adjusted for baseline haemoglobin, weight and height, each centred at their respective mean.

² 95% confidence intervals and two-tailed P values are corrected for multiple comparisons using the Bonferroni method.

Whilst the combined treatment with vitamin A and iron compared to vitamin A alone produced a significant weight increment (0.2kg; 95% CI: 0.01, 0.46) there was no significant difference in mean weight increase between the group that was treated with combined vitamin A and iron and the group treated with iron alone (95% CI: -0.04, 0.42). However, when exploration of the proportion of weight increment was conducted, the findings showed a remarkable increase in weight gain between baseline and follow up (Figure 5)

Figure 5: Mean percentage change in weight at follow up



4.3.4 Effect of supplements on height.

As shown in **Table 7**, there were slight increases in mean height over time in each group, the least increase occurring in the placebo group. As with the changes in haemoglobin and weight, height increased most in the group which received both vitamin A and iron (0.5cm; $P < 0.0001$; 95% CI: 0.42, 0.65).

TABLE 7: Mean change in height (cm) within randomised groups from baseline after 3 months treatment.

Randomized Group	mean change in height from baseline at 3 months ¹	95% CI ²	P ²
Placebo	0.1±0.0	0.03, 0.26	0.0063
Vitamin A	0.4±0.0	0.28, 0.51	<0.0001
Iron	0.4±0.0	0.29, 0.52	<0.0001
Vitamin A and Iron	0.5±0.0	0.42, 0.65	<0.0001

1 mean changes ± standard deviation (follow-up minus baseline): values are adjusted for baseline haemoglobin, weight and height, each centred at their respective mean.

2 95% confidence intervals and two-tailed P values are corrected for multiple comparisons using the Bonferroni method.

The change in height for each of the three actively treated groups was significantly greater than that in the placebo group (**Table 8 & Figure 6**). However, there was no significant difference in the change between combined supplementation and iron alone.

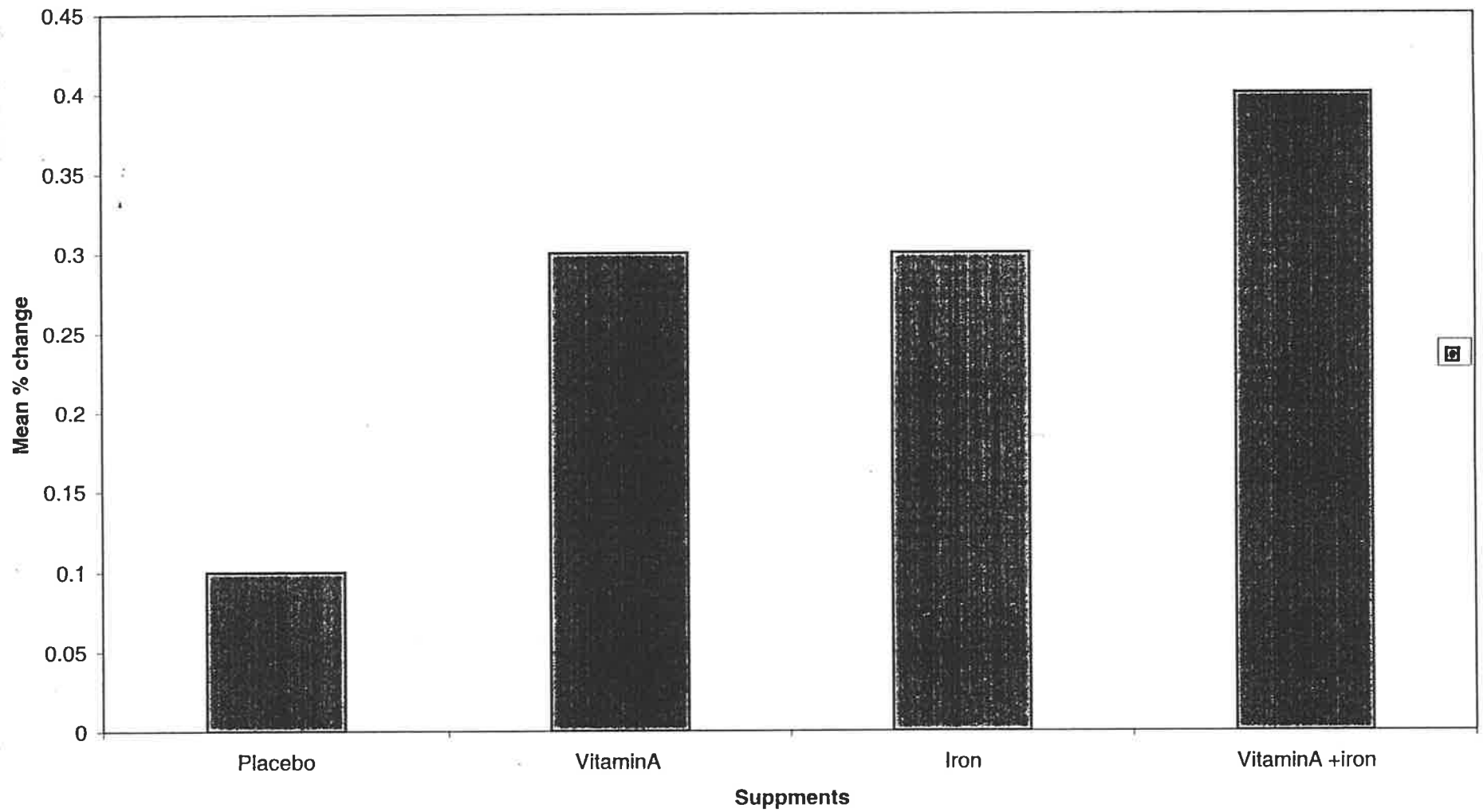
TABLE 8: Difference between treatment groups in mean change in height (cm) from baseline after 3 months treatment.

Comparison Groups	difference in mean change in height ¹	95% CI ²	P ²
Vitamin A vs Placebo	0.2±0.1	0.08, 0.42	0.0009
Iron vs Placebo	0.3±0.1	0.08, 0.43	0.0007
Vitamin A + Iron vs Placebo	0.4±0.1	0.22, 0.56	<0.0001
Iron vs Vitamin A	0.0±0.1	-0.17, 0.18	1.0000
Vitamin A + Iron vs Vitamin A	0.1±0.1	-0.03, 0.31	0.1837
Vitamin A + Iron vs Iron	0.1±0.1	-0.04, 0.3072	0.2454

1 differences in mean changes ± standard error: values are adjusted for baseline haemoglobin, weight and height, each centred at their respective mean.

2 95% confidence intervals and two-tailed P values are corrected for multiple comparisons using the Bonferroni method.

Figure 6: Mean percentage change in height at follow up



4.4 Discussion

This study was the first of its kind to investigate the effect of vitamin A given with local foods in rural anaemic schoolchildren of low socio-economic background in a developing country.

The results of the study have revealed that supplementation with iron or vitamin A either singly or in combination had major effects on the anaemic status and growth of these children. After the combined treatment only 12% remained anaemic compared to 97% of the children who received placebo. Similarly, supplementation with iron or vitamin A either singly or in combination had a significant effect on weight and height compared to the children who received placebo

Iron deficiency occurs when insufficient iron is absorbed to meet the body's requirements (Viteri, 1998). Iron and vitamin A deficiencies are widespread in many developing countries and may co-exist (Bloem et al. 1989, Mejia and Arroyave 1982). Many programs have been implemented to reduce iron deficiency anaemia including iron supplementation, fortification and dietary modification for example in Tanzania. Similar, but separate programs have been implemented to combat vitamin A deficiency (Sommer, 1998).

The relative simplicity and low cost of the present intervention strongly suggest that vitamin A and iron supplements have a place in the prevention of iron deficiency anaemia and growth retardation. Rather than being seen as an exclusive alternative to long term changes

in the food supply, supplementation may actually be used to enhance normal diets and bring about major improvement in children's health.

The mechanism by which combined vitamin A and iron led to improvements in haemoglobin concentration is not established in this study. Possible mechanisms include improved iron absorption from the maize based food, through increased mobilisation of iron from the tissue stores through increased receptor synthesis, or decreased sequestration resulting from decreased (probably sub-clinical) infections, or increased erythropoiesis, or formation of a complex between vitamin A and non-haem iron, keeping it soluble in the intestinal lumen and preventing the inhibitory effects of inhibitors of iron absorption.

In developing countries, growth retardation arises primarily as a result of malnutrition and infection. Rapid rates of catch up growth have been described in extremely severe and prolonged cases of growth retardation from children recovering from severe malnutrition, provided that epiphyseal fusion has not occurred (Ashworth and Millward 1986). Furthermore, Ashworth and Millward (1986) review many instances where catch-up growth has occurred. For example, these authors cite the case of 3-year-old who had suffered from severe anorexia for more than half of her life, yet achieved full catch up in height, weight and skeletal maturation by 5 years of age.

In other settings, researchers have observed prolonged growth spurts resulting in reduction of adult height deficits (Cameron and Kgamphe 1993), hypothesised as a result of delayed pubertal age. In a non intervention longitudinal study of 2 to 12 year old Filipino children,

Adair (1999) described the potential for catch-up growth in children into preadolescent years. These observations suggest a considerable degree of possible catch-up growth at different age groups in childhood when there is removal of growth-retarding factors. The findings of the study suggest that dietary supplementation has some potential to bring about catch-up growth in later childhood in undernourished children.

In addition, the findings provide the strongest evidence that vitamin A may have a useful role in combating vitamin A deficiency, iron deficiency anaemia as well as growth retardation. The consistency in improvements in anaemia and growth among the treatment groups suggests that supplementation programs are likely to bring about major reductions in the numbers of anaemic and malnourished children.

4.5 Conclusion

1. Supplementation with Vitamin A improved recovery from Anaemia and improved levels of growth retardation.
2. The role of vitamin A in combating micronutrient deficiencies, which remain a serious global and public health scourge, requires further investigation.

CHAPTER FIVE

STUDY I: THE EFFECTS OF IRON AND VITAMIN A ON INDICES OF COGNITIVE FUNCTION OF ANAEMIC TANZANIAN SCHOOL CHILDREN

5.1 Introduction

This chapter describes another aspect of the study which was conducted on the same children who are described in chapter four. From the review work in chapter 2, it was expected that anaemic children would have lower cognitive functioning and poor academic achievement as a result of anaemia and poor growth. The aim of the second part of study 1 was to determine whether supplemental vitamin A and iron alone or in combination fed with local foods would have beneficial effects on cognitive functioning as well as the educational achievement of anaemic school children.

Beyond establishing that a child has impaired cognitive functioning of a particular type, it is important to consider the consequences of this impairment for everyday activities. For example a child with impaired short term memory would have problems performing basic activities which require short term memory, such as remembering prices that a shopkeeper has told him/her, or retaining the formulae that a mathematics teacher has been teaching in his/her class. Short term memory has been linked with difficulty in learning previous information as well as inability to learn new information or recall on going events (Mishkin, 1995)

Material based in this chapter has been produced for publication "The effects of iron alone, vitamin A alone or in combination on indices of cognitive function of anaemic school children" International Journal of Epidemiology (Submitted for review)

The consequences of anaemia for populations are similar to those of Iodine Deficiency Disorders (IDD) i.e. widespread cognitive dysfunction as well as impaired socio-economic development. Realisation of the benefits of iodine supplementation on iodine deficiency disorders (IDD), especially on cognitive functioning and socio-economic progress led to a worldwide movement for its eradication (Hetzel, 1989). It is likely that iron and vitamin A deficiencies may have similar profound societal effects.

5.2 Tests of cognitive functions and academic achievement

The battery of tests included measures of auditory short memory, speed processing and ability to switch mode of thinking as well as a test of academic achievement. All these tests have been adapted and modified for use in Tanzania (Tanzania Partnership for Child Development, 1997) and were conducted in Swahili, which is the language spoken in Tanzania. The tests used were:

5.2.1 Digit-Span

This is a test which measures verbal short-term memory (Wechsler, 1992) (**Appendix 14**). The digit span test comprises of two lists of numbers of increasing length, each with a pair of trials. It involves immediate recall of increasingly longer strings of digits that are read to the children. The forward items of the digit span test measure auditory short-term memory whereas the backset includes immediate processing of information. The forward set had to be recalled as they were given (eg 3-8-6; 3-4-1-7; 8-4-2-3-9...) and a backset had to be recalled in reverse order (eg 5-7-4 recalled as 4-7-5) (**Appendix 14**).

The numbers were read to each child at the speed of one per second from a list of series of numbers written on the score sheet. The tester said the numbers in exactly the same manner for each child, in order not to help or hinder the child's performance. Care was taken to not group the numbers when reading them, or to differentiate or vary intonation. The child was asked to listen carefully and say the numbers exactly in the same order. Each child was allowed to practice with a few numbers before the exercise started. The reading was repeated once when the child did not hear well. When the child said different numbers, she/he was told to say exactly what the tester had said. The test was stopped when the child failed all three trials at same level.

Whenever the tester read a wrong number by adding or reading fewer numbers, it was indicated by a note where the mistake had happened. If a tester read a number which is not in the list but the item was exactly the same length, a tick was written to indicate whether the child was correct or wrong, but the error was noted as well.

For the backset, the child was told that she/he was going to say more numbers but was asked to say the numbers backwards. Three examples were given and in circumstances where a child failed to give any correct answers the test was discontinued.

“Scoring was done by putting a tick (✓) under the column with x if the answer was wrong and a tick (✓) under the column with tick if the answer was correct. Whenever child failed to say the numbers correctly but self-corrected it was regarded as wrong. The total number done correctly was scored. The highest level scored was also recorded. The entire test took about 6 minutes ie 2-3 minutes for forward set and 2-3 minutes for backset (**Appendix 14**).

5.2.2 Stroop Task

The Stroop test taps basic psychological processes useful in the study of human neuropsychological and cognitive processes (Golden, 1978). Golden further narrates that the basic dimension tapped by Stroop which has been associated with cognitive flexibility, resistance to interference from stimuli, creativity, psychopathological and cognitive complexity plays a role in many interrelated cognitive processes which determine an individual's ability to successfully cope with cognitive stress and to process complex input. The Stroop may offer a link between studies in such basic areas of neuropsychology, neurophysiology, personality, and cognitive processes in psychopathology. The Stroop offers researchers in these diverse fields a reliable and basic measure of important underlying processes which are basic to cognition (Golden, 1978).

Stroop test has a number of versions with varying structures, instructions and administration formats and have been used in both clinical and experimental work (Golden, 1978) and can be used in screening test or as an effective part of a more general test battery.

The current test version was adaptation of Stroop test and was designed for more general test battery applications (UKUMTA, 1997). The task involved the child naming an object as quickly as possible in the first instance, then switching so that the object was given an alternative name (UKUMTA, 1997) (**Appendix 15**). In this test, there were four trials each consisting of a page with 48 boxes containing a tick (✓) or a cross (✗). There were two trials with “forward” instructions, where children were asked to touch

the box and say as quickly as possible whether it contained a tick or a cross. There were also two "backward" trials where children did the reverse, saying "tick" if the box contained a cross and saying "cross" if the box contained a tick. The errors made and time taken to read each page were recorded and scored.

This test measures children's speed of processing and also their ability to switch modes of thinking, in other words to switch from one conflicting activity to another. This is a useful ability in many situations, where a child knows one course of action but will then have to learn to do another course of action in some situations (Bruner, 1996). For example in map reading, one reads the map on the paper but has to translate what is seen on the map to the real physical features of the landscape. Changing gears while driving a motor vehicle is another important example in daily life where the ability to switch the focus of attention is essential. It is then suggested that the basic ability tapped by Stroop is the ability to select relevant information from one's environment in a flexible manner, to adapt to new circumstances and to more effectively function in general.

5.2.3 Test of Educational Achievement

Written and oral arithmetic tests were used for education achievement assessment. Both tests have been adapted from Wide Range of Achievement Test (WRAT -III) and modified for use in Tanzania (Tanzania Partnership for Child Development (UKUMTA), 1997).

Children in grade two were given an oral arithmetic test which was administered individually (Tanzania Partnership for Child Development (UKUMTA), 1997)

(Appendix 16). An arithmetic test was given orally to each individual grade two child recruited in the study to assess how well he or she was achieving academically. This test consisted of the following:

1. 14 dots which form a line. The child was supposed to count these and earn marks from correct counts.
2. Numbers 3, 5, 6 and 71. A child was asked to read the number and correct reading was marked.
3. A child was asked to show three fingers, and she/he earned marks if correctly performed.
4. Simple arithmetic problems were given to the child and she/he earned marks accordingly.

A total of the above determined the arithmetic performance of each child.

On the other hand, children in grades three to five were given the written form of the arithmetic test (Appendix 17). This set of tests comprised several sets of arithmetic which included additions, subtractions, fractions, multiplication and divisions. This version of written arithmetic was given during class but each child completed it individually under examination conditions. Oral and written arithmetic tests were used to assess how well the children were achieving academically.

The cognitive function and educational tests were conducted during regular school hours after the subjects had received some snacks (locally made buns) fifteen minutes before administration of the tests to alleviate short-term hunger. All tests except the written mathematics test were administered to individual children in a quiet room to minimise

interruption that could hinder performance. The same tests were given at the end of the supplementation phase under similar conditions.

5.3 Data Analysis

Before deciding to use the any tests, data were examined to ensure that they were not skewed. Data were found to be normally distributed and as such there was no need to do any transformation. Descriptive data are expressed as means, standard deviations (SD) and the result of intervention as mean (SE). Further 2X2 factorial analysis of variance (ANOVA) was conducted, as interaction was assumed. In the absence of significant interaction the main effect of vitamin A versus placebo and iron versus placebo was tested. If an interaction was shown, then the effect of vitamin A in the presence and absence of iron and the effect of iron in the presence and absence of vitamin A was tested. The alpha level for statistical significance was set at $p < 0.05$ after adjusting for type 1 error using Bonferroni method.

For the digit span tests the primary outcomes were the mean longest set of numbers that a child could recall and the mean number of correct responses obtained. The forward and reverse items were analysed separately.

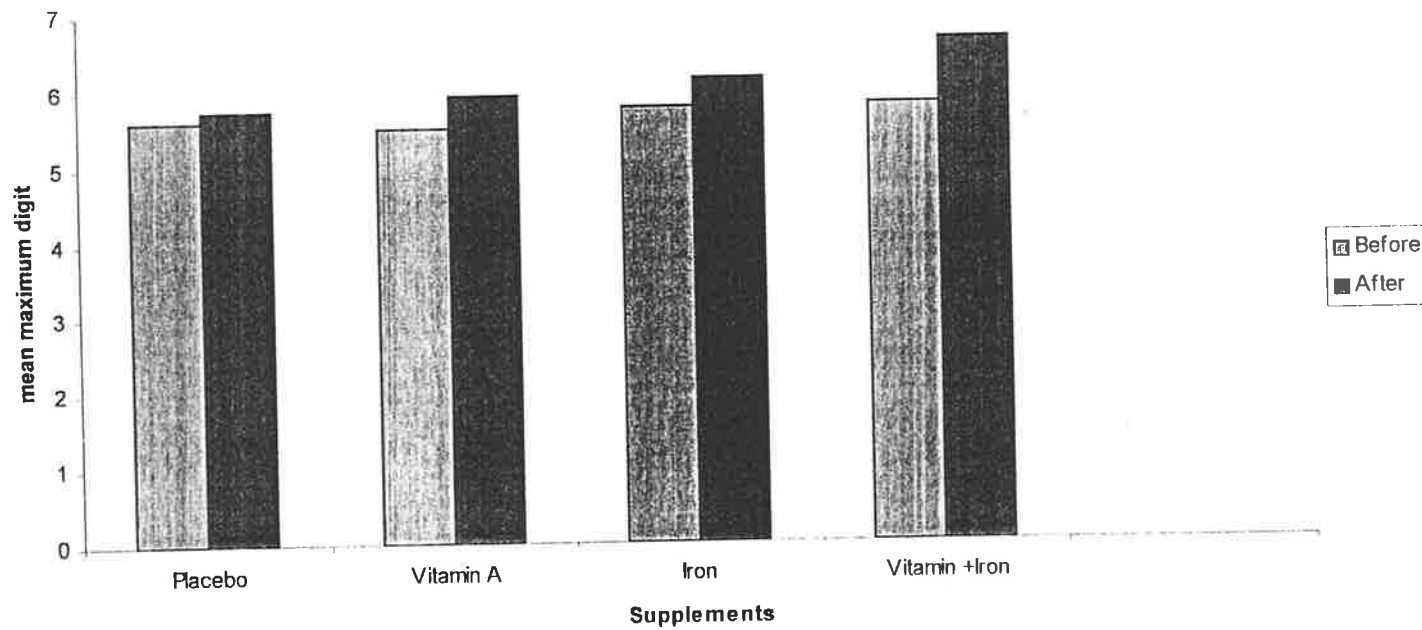
The Stroop test was analysed in terms of mean numbers of errors and the time which was taken for the children to complete the test. Errors and the time spent for forward and contrast items were analysed separately.

The percentages of scores obtained for both oral and written arithmetic tests were additional primary outcomes.

Non parametric and parametric tests revealed no interaction between vitamin A and iron.

Linear regression models were then used to compare changes from baseline in the primary outcomes among treatment groups. These outcomes were the maximum number of digits recalled, the correct number of responses obtained, time spent, and errors made. Total scores for both written and oral arithmetic tests among treatment groups were also compared. All the primary outcome variables were adjusted for the respective baseline measures. As noted in the previous chapters, the Stata statistical package was used for all analyses.

Figure 7: Mean digit span: maximum digit forward and backward before and after supplementation



5.4 Results

5.4.1 Effects of supplements on digit span test scores

After three months the children who had been given different types of active supplement were compared to those given the placebo before and after supplementation (**Figure 7**).

The mean percentage changes (relative to baseline) for each treatment group in the maximum number of digit recalled at follow up testing was also computed (**Figure 8**). It appears that children who received active supplements improved their digit recall after three months of treatment. The main effect of iron for digits recalled, that is the difference in change in maximum digits recalled was 0.33 and it was statistically significant ($p > 0.007$). The main effect of vitamin A for maximum digit recalled in the forward item was 0.38 and it was also significant ($p > 0.002$). With regard to reverse digit recall, the placebo group deteriorated in their recall of the number of digits over time (**Figure 8**).

The actively treated groups scored more correct answers for both forward and backward items. In the contrast, the placebo group deteriorated in their correct recall of forward items (**Table 10**).

Table 9 presents the test scores of the subjects at baseline and after supplementation. Digit span and Stroop task tests were performed by all children. However the tests of educational achievement which included oral and written arithmetic tests were performed by selected children. While all children in grade two (56 children) performed the oral arithmetic test, 80 older children in grades three to five completed the written arithmetic test.

TABLE 9: Test scores by randomised treatment groups before and after supplementation¹

	<u>Treatment group</u>							
	Placebo (n=34)		Vitamin A (n = 34)		Iron (n = 34)		Vitamin A + Iron (n = 34)	
	t ₀	t ₁	t ₀	t ₁	t ₀	t ₁	t ₀	t ₁
<u>Digitspan(n=136)</u>								
<i>Recall</i>								
Forward								
Reverse								
<i>Correct number</i>								
Forward	5.6±0.6	5.7±0.8	5.5±9.3	5.9±0.9	5.8±0.9	6.1±0.9	5.8 ± 0.8	6.6±0.9
Reverse	3.2±1.0	3.1±1.4	2.9±1.3	3.3±1.1	3.1±1.6	3.7±0.9	3.5±0.87	4.2±1.0
	6.7±1.5	6.6±2.0	6.6±2.3	7.0±2.0	6.9±2.3	7.7±1.9	7.3±2.2	8.7±2.3
<u>Stroop (n=136)</u>	3.4±1.5	3.4±1.8	2.9±1.7	3.7±1.6	3.6±2.1	4.5±1.9	4.2±1.5	5.1±1.7
<i>Errors</i>								
Forward								
Contrast								
<i>Time(Sec)</i>								
Forward	3.8±2.7	6.2±4.9	4.4±3.2	4.9±2.7	5.0±6.7	4.9±3.8	4.6±3.7	3.7±3.3
Contrast	3.7±3.2	3.9±2.9	3.4±2.4	3.9±1.7	3.2±2.5	3.4±1.8	3.4±2.6	3.1±1.9
	0.4±0.1	0.4±0.1	0.4±0.1	0.4±0.1	0.4±0.1	0.3±0.1	0.4±0.1	0.3±0.1
<u>Arithmetic(%)[*]</u>	0.6±0.7	0.4±0.1	0.5±0.1	0.5±0.2	0.4±0.1	0.4±0.1	0.5±0.1	0.4±0.1
Written (n=79)								
Oral (n=56)								
	26.6±12.7	32±12.4	29.1±11.5	32.3±15.7	32.8±8.7	35.1±11.4	30.4±13.1	34.5±14.6
	91.6±7.9	92.1±7.9	93.8±7.0	94.1±8.6	95.2±5.7	95.2±5.8	92.8±14.2	93.6±11.6

¹Values are means ± standard deviation

t₀ = Test scores at baseline

t₁ = Test scores after three months of supplementation

* Passmark = 50%

Figure 8: Digit span: mean percentage change in maximum digits recalled at follow up

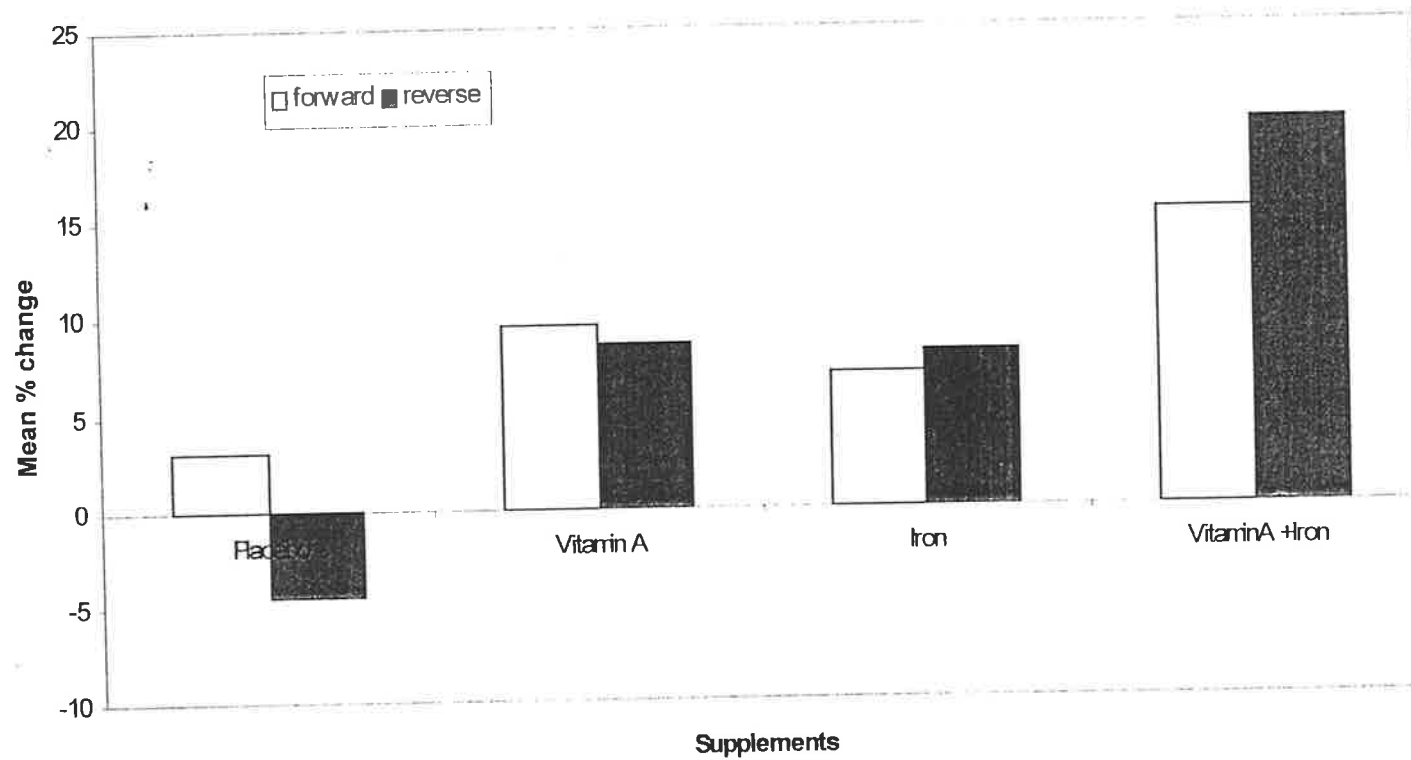


TABLE 10 Results of linear regression of mean changes (relative to placebo) of test scores on randomised treatment group, after adjusting for baseline score.

	Treatment group								
	Vitamin A			Iron			Vitamin A + Iron		
	Coef.	P	95% CI	Coef.	P	95% CI	Coef.	P	95% CI
Digit Span									
Recalled									
Forward	0.24	0.138	-0.08, 0.55	0.26	0.102	-0.05, 0.58	0.77	0.000	0.45, 1.08
Reverse	0.41	0.053	-0.00, 0.83	0.61	0.004	0.20, 1.02	0.91	0.000	0.50, 1.33
Correct Digits									
Forward	0.50	0.125	-0.14, 1.12	0.90	0.006	0.26, 1.53	1.65	0.000	1.01, 2.29
Reverse	0.61	0.063	-0.03, 1.26	0.94	0.004	0.30, 1.58	1.16	0.001	0.51, 1.82
Stroop Task									
Errors									
Forward	1.48	0.079	-0.17, 3.13	1.77	0.037	0.11, 3.43	2.77	0.001	1.10, 4.43
Contrast	-0.03	0.946	-1.04, 0.98	0.44	0.386	-0.57, 1.46	0.83	0.107	-0.18, 1.85
Time									
Forward	-0.00	0.908	-0.02, 0.02	0.01	0.279	-0.01, 0.03	0.02	0.030	0.00, 0.05
Contrast	-0.04	0.227	-0.20, 0.02	0.02	0.533	-0.04, 0.08	0.02	0.502	0.04, 0.08
Arithmetic									
Written	-1.67	0.556	-7.31, 3.96	-2.34	0.385	-7.66, 2.99	-0.74	0.787	-6.17, 4.69
Oral	0.79	0.76	-4.40, 5.97	0.01	0.996	-5.83, 5.85	0.89	0.748	-4.64, 6.42

Regression coefficient gives difference in mean change of score between supplement group and placebo, adjusted for baseline measures.

5.4.2 Effect of supplements on Stroop task scores

In the Stroop task the groups that received iron supplementation had fewer errors for the forward item set after three months of supplementation (**Figure 9**). However, the combined group (iron plus vitamin A) showed the greatest reduction in forward item errors compared with change in errors seen with placebo ($p=0.001$) (**Table 10**).

Figure 10 shows the mean change in time (relative to baseline) for each treatment group in the time taken to perform the Stroop task at follow up testing. After three months of supplementation, the children performed faster for both forward and backward items. However only the combined group showed significant improvement in speed for the forward items ($p=0.030$) relative to that seen with placebo (**Table 10**).

5.4.3 Effects of supplements on educational achievement test scores

Table 10 shows that children in grades three to five performed poorly on the written arithmetic test both at baseline and after supplementation. However it appears that there were slight improvements in test scores in all groups, except on the oral arithmetic test for the iron only group. Moreover, compared to placebo, the improvements in both written and oral arithmetic test scores were not statistically significant in any of the groups (**Table 10**).

Figure 9: Stroop task: mean errors forward and backward before and after supplementation

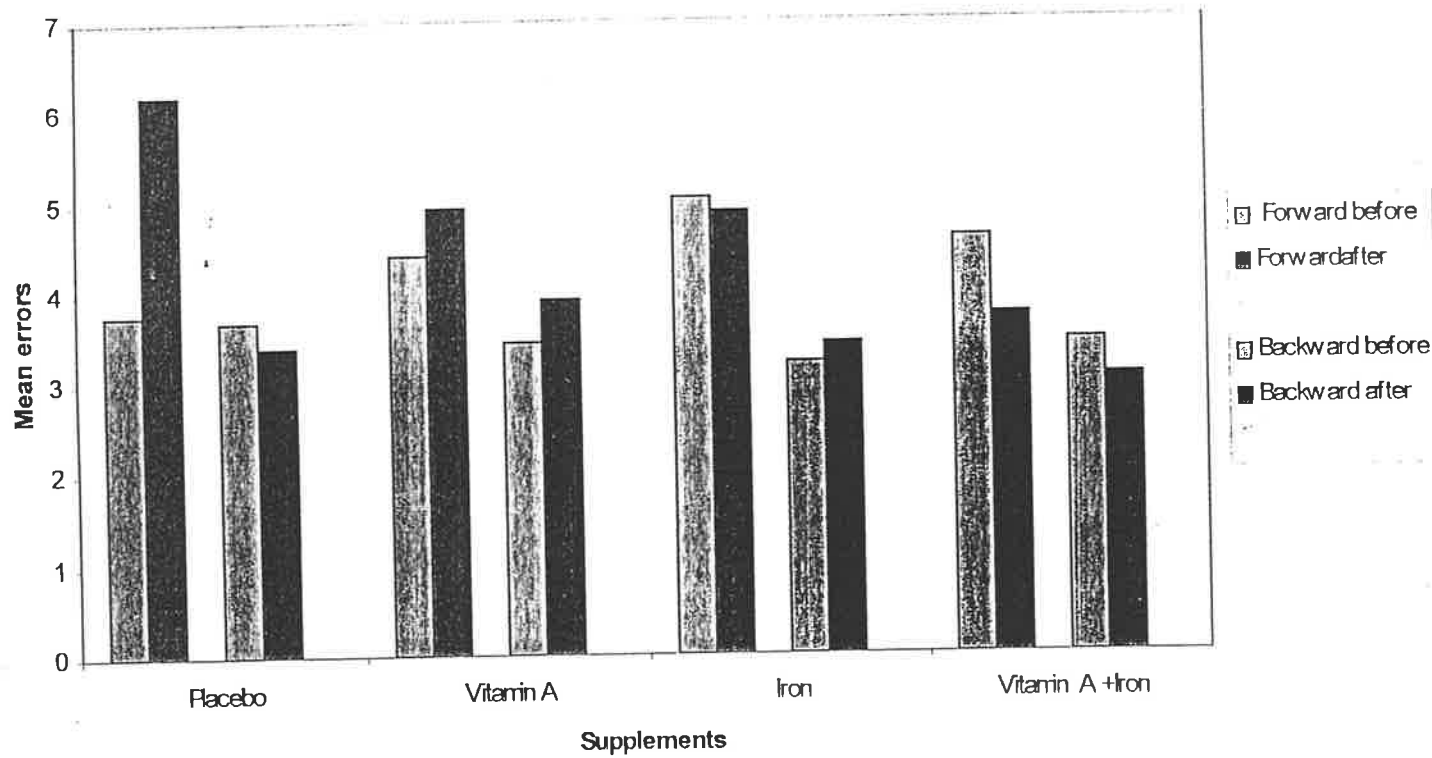
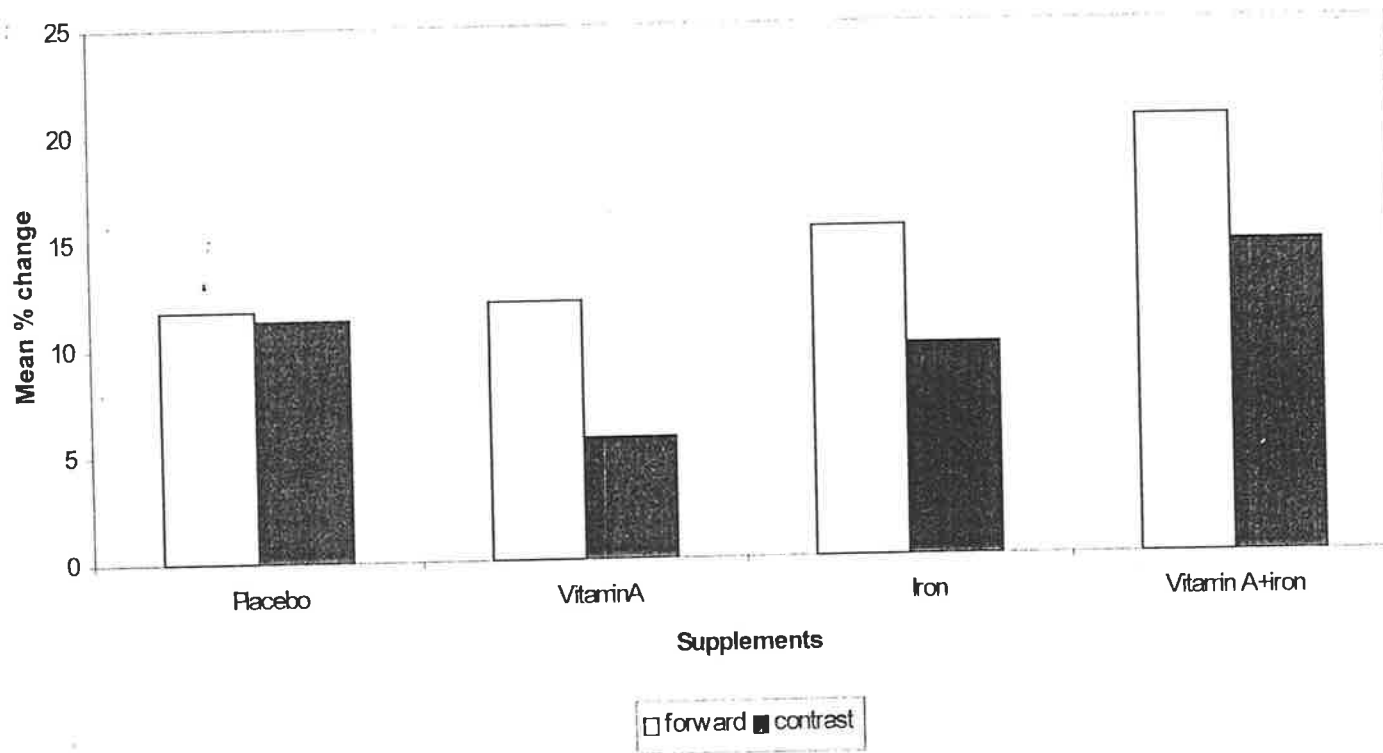


Figure 10: Stroop task: mean percentage change in time achieved at follow up



5.5 Discussion

The results of this study have important implications for several issues including the impact of micronutrients on cognitive functioning, educational achievement and public health in developing countries.

5.5.1 Cognition

The study shows that anaemic school children's low scores on tests of cognitive function can be increased significantly when anaemia is reduced. The beneficial effects of iron supplementation on cognition have been demonstrated among iron deficient adolescent non- anaemic American girls who were treated for eight weeks with ferrous sulphate tablets. Iron supplementation showed positive effects on both verbal learning and memory (Christian & West, 1998).

However, the present study shows larger iron effects as well as potentiation of iron effects by vitamin A. These findings are novel. There is evidence that vitamin A has a synergistic effect on the absorption of other micronutrients that are deficient in developing countries (Udomkesmalee, 1992; Vallar & Baddeley, 1982). The separate and joint effects of iron and vitamin A on biomedical indices have been reported in chapter four. It is likely then, that vitamin A could be used to reduce anaemia as well as to improve the cognitive functioning of anaemic children in developing countries. The form in which vitamin A is delivered is a matter of debate. This will be discussed in details in the general discussion.

5.5.2 Educational achievement

This study did not show significant improvement in educational achievement scores. However, it can be argued that the present intervention was carried out for only three months, which is not really enough time to improve educational achievement. Moreover, it can be speculated that the tests of educational achievements used in this trial are not necessarily sensitive to show improvements in the first place, and so the treatment trial was not in a position to inform us whether there was any improvements. In addition, factors such as poor classroom conditions, socio-economic status and teaching conditions which prevailed in these schools could have affected the outcome. Furthermore, at the time of this study, the children could not attend lessons all the time because it was raining and there were no adequate classrooms. Indeed, the children had to take turns in attending lessons, thus could impend their learning. All these factors have to be taken into account as possible causes of the failure to improve in educational achievement.

5.5.3 Public Health significance

These results suggest that supplementation with iron or vitamin A either singly or in combination can have major effects on the cognitive functioning of anaemic children. Certainly one would expect children with short-term memory deficits to be impaired in everyday tasks that require its utilisation. Common examples of tasks requiring short-term memory include retention of lists of items for shopping, assignment of daily responsibilities, simple counting and exchange of information in daily life activities. Short-term memory is fundamental for a "normal life". For

example, reports of difficulties in language development following short-term memory impairment have been documented (Garrett, 1975). Most theories of sentence comprehension assume that a working memory system is used to retain the partial results of phonological, semantic and syntactic processing until a final interpretation of the sentence is constructed (Garrett, 1975). In this regard, improved cognitive functioning, especially short-term memory, is fundamental aspect in normal life.

5.6 Conclusions and implications.

Vitamin A and iron may have a significant role in the improvement of cognitive functioning among anaemic children. However, other factors, such as social and dietary deprivation, are also likely to have important influence on the educational achievement of these Tanzanian children. Despite the criticisms about the efficacy of supplementation, it is worth mentioning that supplementation programs are likely to bring about rapid, major reductions in the numbers of anaemic children and thus improve levels of cognition. Improvements in school children's cognitive functions may bring about improvement in the future social and economic development in developing countries. However, further studies should be conducted for a longer duration to examine the role of vitamin A and iron in cognition and in the educability of anaemic children who are unable to attain the full potential benefits of education.

CHAPTER SIX

STUDY 2: SCHOOLS AND ANAEMIA PREVENTION: KNOWLEDGE, ATTITUDES AND PRACTICES OF STUDENTS, PARENTS AND TEACHERS.

6.1 Introduction

This chapter describes three surveys which were conducted among three groups of people associated with schools in Bagamoyo District. These groups were: anaemic children (described in chapter three), their parents and health education teachers in the entire Bagamoyo district of Tanzania.

In order to be able to control and prevent any health condition (in the current context, anaemia), children, parents and other members of the community usually require sound knowledge of that particular condition in order for them to take preventive measures. Likewise, parents and children need to have sound knowledge of nutrition in order for them to be able to consume foods rich in iron which will lead to the prevention of iron deficiency anaemia. Knowledge is one amongst several factors required to promote health in order for people to increase control over, and to improve their health (WHO, 1996). The others are building healthy public policy, creating supportive environments, strengthening community action and reorienting health services (Ottawa Charter, 1986).

Parents' health knowledge has been found to be positively correlated with children's health and nutrition status in developing countries (Glewwe, 1999). For example, in his survey, Glewwe (1999) found mothers' knowledge to be correlated with raising healthy children in Morocco.

Similarly, Variyam et al. (1999) used U.S. food consumption data to examine the effects of maternal knowledge on dietary intakes of children, and found that maternal nutritional knowledge influenced children's dietary patterns. This may significantly reduce the probability of acquiring certain chronic diseases in the future.

Home environment and household characteristics such as hygiene, parents' education, household income and number of children living in the same house appear to be important determinants of nutrition knowledge and dietary intake (Ottawa Charter, 1986; Variyam et al., 1999; Gibson et al., 1998). As noted above, nutrition knowledge affects health-related choices and as such may influence the probability of acquiring certain nutrition conditions.

Schools should provide sound nutrition education and should be entry points for promoting children's as well as the entire community's health. In 1997 the World Health Organisation (WHO, 1997) initiated the Global School Health Initiative, "The Health Promoting School", which focuses on the creation of comprehensive school based activities to improve children's as well as communities' health. The initiative describes a health promoting school as a school which strives to strengthen its capacity as a health setting for living, learning and working. Various health problems or risks may be identified and prioritised and used as entry points in order to effectively address important health concerns within the schools and the community. This initiative recognises the need of the community to understand the importance and feasibility of improving health through schools.

The physical environment of the school has been identified as an essential factor in children's learning (Long-Shan et al., 2000). For example good sanitary facilities and a safe water supply are essential elements in promoting children's health. Schools can also develop the environment,

motivation, services and support necessary to contribute to the integrated promotion of health behaviours which can be a lifelong asset (Orloske & Leddo, 1981, McKenzie & Williams, 1982). Healthy behaviours are crucial for the prevention of iron deficiency anaemia.

This chapter examines the state of the factors described above in the Bagamoyo district where anaemia has been described as endemic (chapter 1). The aims were to:

1. Examine children's, parents' and teachers' knowledge of the symptoms, causes and prevention of anaemia.
2. Investigate children's, parents' and teachers' attitudes towards anaemia control and prevention practices.
3. Examine the environmental and living conditions both at home and school that can promote healthy living.
4. Investigate parents' and teachers' attitudes towards school meals and their willingness to provide them.

6.2. Methods

The three cross sectional surveys were conducted in the Bagamoyo area in March 1999. As noted above, the surveys involved the children who have previously been described in chapters three and four who were involved in study 1, their parents, and teachers.

6.2.1. Children's survey

A questionnaire was designed to obtain information on the children's knowledge, attitudes and practices regarding anaemia. The questionnaire was pre-tested on children in another

school which was not involved in the study. Each child was interviewed in private by a trained interviewer. The questions asked included:

- ◆ Have you heard of anaemia?
- ◆ Where did you get this information from?
- ◆ How harmful is anaemia?
- ◆ Which are the symptoms of anaemia?
- ◆ How do people get anaemia?
- ◆ What could be done to prevent anaemia?

Additional questions were asked about their meals and diet in order to estimate roughly the pattern and quality of diets eaten by them at home and at school for the previous 24 hours:

These questions included:

- ◆ Did you eat any breakfast before school this morning?
- ◆ What did you have for breakfast?
- ◆ What did you eat yesterday for breakfast?
- ◆ What did you eat yesterday for lunch?
- ◆ What did you eat last night for dinner?
- ◆ Did you eat any vegetables for dinner?
- ◆ Do you normally have something to eat when you are at school?
- ◆ What do you usually eat at school?
- ◆ How often do you eat meat or fish with your meals?
- ◆ How often do you eat vegetables with your meals?
- ◆ Do you eat fruits with your meals?
- ◆ How often do you eat fruit or drink fruit juice?
- ◆ Please can you tell me what foods you should eat to prevent anaemia?

The data were analysed by calculating the percentages of children's the responses to the various questions.

6.2.2. Parents' survey

A similar questionnaire was designed for parents. However, additional information was sought to ascertain their social economic status; details of foods their children ate at school; their opinions about their children's diets' while at school, and in particular, whether they approved the provision of meals for their children at school. They were also asked about the ways in which the provision of school meals could be organised. They were also asked about their opinions on how the provision of school meals could be organised. Additional questions asked were:

- ◆ What is your occupation?
- ◆ How many children live in your house?
- ◆ How many children are enrolled in school?
- ◆ What is the roof of your house made of?
- ◆ What is the wall of your school made of?
- ◆ What is the floor of your house made of?
- ◆ In your house, do you have the following?
 - Television,
 - Radio,
 - Bicycle,
 - Motor cycle/car?
 - Domestic animals e.g. goats, sheep, chicken etc.
- ◆ Does your household have its own latrines?
- ◆ Where does the water you drink at home come from?

- ◆ What type of lighting do you use at night?
- ◆ What do you use to cook food at home?

Questions about their educational level and type of education received were asked to ascertain whether the parents' educational background had any association with other outcome measures.

The data were analysed by calculating the percentage of parents' responses to each question.

6.2.3 Teachers' survey

A third questionnaire was sent to the health teachers in all schools in the district. It included similar questions to those asked of the pupils but additional questions were included to establish the number of pupils enrolled, the presence or absence of latrines (including the number of pits); the presence or absence of vegetable or fruit gardens in the school; the provision of school meals; the teachers' willingness to participate in school meal provision and their opinions on the ways in which schools could participate in school meal provision. The additional questions included:

- ◆ How many children are enrolled in your school?
- ◆ Does your school have toilets?
- ◆ How many pits are there?
- ◆ Does your school have vegetables/fruit gardens?
- ◆ Does your school provide meals to its children?
- ◆ Are you willing to participate in the provision of school meals?
- ◆ What are your opinions on the ways that schools could participate in provision of school meals.

The data were analysed similarly to the children's and parents' responses.

6.3 Results

6.3.1 Children's demographic characteristics and sources of information about anaemia

Out of 136 children who were invited to participate in the study, 131 were interviewed, consisting of 70 girls and 61 boys. The majority of children had heard about anaemia (73%) and learnt about it from school (82%). However only 57% cited teachers as sources (Table 11). Parents appeared to be sources of information for only 18% of the children.

TABLE 11: Characteristics of children and source of information (n=131; F/M =70/61)

Response	Percentage
Heard of anaemia	73
Learnt from school	82
Teachers source of information	57
Learnt from parents	18
Hospital to be attended when sick	66
Hospital as source of information	7
Local health workers as source of information	47
Friends as source of information	5

6.3.2 The home environment, parents' literacy status and their recalled sources of information

Most of the houses were of poor quality, made of thatched roofs and mud walls (Table 12). The majority had uncovered floors and a few had roofs made of corrugated iron sheets. The majority of the community members owned latrines most of which were uncovered. Many households shared communal taps for water supply, but a few only had shallow wells, which were not safe for drinking. Firewood appeared to be the major source of energy. Most households had a radio (73%) and bicycles (62%). The majority of parents (88%) domesticated animals which were used for cash as well as for food. There were approximately 5.5 children per household (range 1 to 15).

TABLE 12: Characteristics of parents, home environment and sources of information (n=90)

Response	Percentage
<u>Education</u>	
Attended any formal schooling	79
Able to read Swahili	73
Able to read English	1
Religious reading	9
Not able to read at all	21
<u>Housing condition</u>	
Thatched roof	67
Muddy walls	70
Un-cemented floors	72
Corrugated iron sheet roofs	33
Bricked walls	16
Cemented floors	28
<u>Sanitary facilities</u>	
Own latrines	91
Do not own latrines	9
Availability of tap water	78
Access to shallow well	16
<u>Household energy</u>	
Firewood	84
Other sources	16
<u>Household possessions</u>	
Radio	73
Bicycles	62
Television set	2
Motorcar	1
Small scale animal husbandry	88

However, the children living in the household did not necessarily belong to the parents but they were related, for example, nephews and nieces. The mean number of children living with their own parents was 3.6 per household. Twenty one percent of parents interviewed did not have any formal education and as such they did not know how to read or write.

6.3.3 Teachers' responses and their sources of information.

Teachers in 76 schools (out of 98 invited) participated in the study. Sixty-three responding teachers were males (83%) and 13 (17%) were females.

Almost all (99%) of them had heard about anaemia. Their main sources of information were teachers' colleges (52%), hospitals (86%) self reading (58%) and friends (41%). All the teachers had sound knowledge of anaemia; and most thought it was very harmful to health. About eight percent reported they had been treated for anaemia in their lifetime.

6.3.4 Children's, parents' and teachers' knowledge of symptoms of anaemia

The children had less knowledge of the symptoms of anaemia than the parents and teachers (Table 13). For example, only 28% of children knew that dizziness was a symptom of anaemia compared to 72% and 78% of parents and teachers respectively.

TABLE 13: Children, Parents and Teachers' knowledge of symptoms of anaemia.

SYMPTOMS	<u>Percentage Responses</u>									N/A*		
	YES			NO			DON'T KNOW			C	P	T
	C (n=131)	P (n=90)	T (n=76)	C (n=131)	P (n=90)	T (n=76)	C (n=131)	P (n=90)	T (n=76)	(n=131)	(n=90)	(n=76)
Tiredness	41	73	72	23	14	28	9	9	0	27	3	0
Red eyes	14	*	1	50	*	99	9	*	0	27	*	0
Loss of energy	40	76	78	23	11	22	10	10	0	27	3	0
Dizziness	28	72	89	34	14	11	11	10	0	27	3	0
Palpitation	29	53	66	33	34	34	11	10	0	27	3	0
Hand tremor	24	36	17	38	53	82	11	8	0	27	3	0
Vomiting	19	*	3	43	*	97	11	*	0	27	*	0
Loss of vision	*	53	*	*	33	*	*	11	*	*	3	*
Sadness	*	23	*	*	64	*	*	10	*	*	3	*

C =Percentage of children responded.

P =Percentage of parents responded.

T= Percentage of teachers responded.

*Not asked this question.

N/A= Not applicable.

Bolded figures indicate low knowledge by particular respondent group.

Similarly, only 41% of children knew that tiredness was a symptom of anaemia compared to 73% and 72% of parents and teachers respectively.

6.3.5. Knowledge of causes and prevention of anaemia.

Again, the children had less knowledge than the parents and teachers (Table 14). For example, only 14% of them knew that chronic illness was a cause of anaemia, compared to 53% of parents and 63% of teachers.

TABLE 14: Children, parents and teachers' knowledge of causes of anaemia

Percentage Responses

Causes	YES			NO			DON'T KNOW			N/A		
	C (n=131)	P (n=90)	T (n=76)	C (n=131)	P (n=90)	T (n=76)	C (n=131)	P (n=90)	T (n=76)	C (n=131)	P (n=90)	T (n=76)
Low dietary intake	50	79	88	11	8	12	11	10	0	28	3	0
Eating lots of rice	5	3	3	50	84	97	18	10	0	27	3	0
Parasitic infections	27	55	*	34	32	*	12	10	*	27	3	*
Chronic illnesses	14	53	63	39	23	37	20	21	0	27	3	0
Eating lots of meat	2	2	3	59	85	97	11	10	0	28	3	0
Walking long distances	*	18	*	*	70	*	*	9	*	*	3	*
Sickle cells disease	*	*	57	*	*	43	*	*	0	*	0	

C= Percentage of children responded.

P= Percentage of parents responded.

T= Percentage of teachers responded.

*Not asked this question.

N/A= Not applicable

Bolded figures show low percentage of knowledge by the respondent group.

Similarly, the children had less knowledge about the prevention of anaemia than parents or teachers. In general, the teachers had better knowledge about anaemia than the parents (Table 15).

TABLE 15: Children, Parents and Teachers' knowledge of prevention of anaemia

PREVENTION MEASURES	Percentage Responses			NO			DON'T KNOW			N/A		
	YES C (n=131)	P (n=90)	T (n=76)	C (n=131)	P (n=90)	T (n=76)	C (n=131)	P (n=90)	T (n=76)	C (n=131)	P (n=90)	T (n=90)
Using toilet	18	52	51	37	35		17	10	0	27	3	0
Drinking bottled water	13	*	5	42	*		18	*	0	27	*	0
Treating malaria	22	56	72	34	31		17	10	0	27	3	0
Regular deworming	27	52	78	29	33		17	12	0	27	3	0
Visits to traditional healer	12	*	*	42	*	*	19	*	*	27	*	*
Drinking coke	*	54	*	*	37	*	*	6	*	*	3	*
Drinking beer	*	49	11	*	41		*	7	0	*	3	0
Drinking coffee	*	30	*	*	59	*	*	8	*	*	3	*
Using honey	*	38		*	53		*	6	0	*	3	0
Eating meat, fruits and vegetables	*	83	96	*	6	4	*	8	0	*	3	0

C= Percentage of children responded.

P= Percentage of parents responded.

T= Percentage of teachers responded.

* Not asked this question.

N/A= Not applicable.

Bolded figures show low knowledge by the respondent group.

6.3.6. Children's, parents' and teachers' dietary habits

Over half of the children (59%) had nothing to eat before coming to school (Table 16). For the other 41%, the most commonly consumed breakfast included black tea and bread, porridge made from maize flour, black tea with cassava, and sometimes, plain black tea only.

TABLE 16: Responses of Children's food Practices

Factor	Percentage responses			
	Yes		NO	
	n	%	n	%
Breakfast before school today	54	41	77	59
Ate tea and bread	26	20		
Ate porridge	9	7		
Ate tea and cassava	18	14		
Ate black tea	1	1		
Breakfast before school yesterday	93	71	38	29
Ate tea and bread	50	38		
Ate tea and cassava	21	16		
Ate porridge	16	12		
Black tea	6	5		
Had something to eat at school	35	27	96	73
Ate buns	17	13		
Ate groundnuts	4	3		
Ate iceblocks	14	11		
Had lunch yesterday	124	95	7	5
Ate ugali and vegetables	62	47		
Ate ugali and meat	40	31		
Ate rice and vegetable	10	8		
Ate rice and meat	8	6		
Ate pumpkins	1	1		
Ate futari	3	2		
Had dinner last night	129	98	2	2
Ate ugali and vegetables	47	35		
Ate Ugali and meat	58	44		
Ate rice and vegetables	13	10		
Ate rice and meat	8	7		
Ate futari	2	2		
Had vegetables last night	60	46	71	54
Type of vegetables:	21	16		
Cassava	7	5		
Pumpkins	14	11		
Sweet potato leaves	10	8		
Amaranth	4	3		
Mlenda	5	4		
Legume				

n = number of responses Yes or No

During the school day, the majority of children (73%) did not have anything to eat in school.

Even for those who said they had something to eat during school hours the nutrient value of what was eaten was poor as it consisted of small locally made buns, chewable groundnuts and in one school, ice blocks sold by teachers who owned refrigerators.

Consistent with the children's reports, 17% of parents reported that their children ate something while in school. However, only 10% provided their children with some sort of food to be eaten at school. Seven percent provided their children with money to buy snacks from the school vendors. The majority of parents (93%) supported the provision of meals at school. Indeed, many of them (84%) were willing to participate in providing school meals. They suggested that parents could provide assistance in meal preparation and other contributions such as foodstuff.

6.3.7. The children's meal/food consumption outside school

Vegetables, some meat and fish were consumed by the majority of children (84%) at least once a week. However, only about 6% reported daily consumption of beef and vegetables. Although all children reported eating fruits, the majority only had access to seasonal fruits (73%) (Table 17).

TABLE 17: Frequency of children's consumption of green vegetables, fish/meat and fruits

Food item	Frequency of consumption			
	Daily	Weekly	Monthly/rarely	Seasonally
Green vegetables	8 (6%)	110 (84%)	13 (10%)	–
Fish/meat	8 (6%)	110 (84%)	13 (10%)	–
Fruits	3 (3%)	23 (18%)	9 (7%)	96 (73%)

All parents reported that they and their children ate vegetables, or meat with their main meal (ugali) made from maize flour. The majority ate vegetables on a weekly basis (66%). While most (81%) reported eating meat on a weekly basis, many of them ate fruits when they were in season (77%, Table 18).

TABLE 18: Frequency of parents' consumption of vegetables, fish/meat and fruits

Food item	Frequency of consumption			
	Daily	Weekly	Monthly/rarely	Seasonally
Green vegetables	14 (16%)	59 (66%)	16 (18%)	—
Fish/meat	9 (10%)	73 (81%)	8 (9%)	—
Fruits	2(2%)	15(17%)	4 (4%)	69 (77%)

It appears that the teachers consumed fruits and vegetables more frequently than the children's families (Table 19).

TABLE19: Teachers frequency of consumption of vegetables, fish/meat and fruits

Food item	Frequency of consumption			
	Daily	Weekly	Monthly/rarely	Seasonally
Green vegetables	27 (36%)	48 (63%)	1(1%)	—
Fish/meat	13 (17%)	54 (71%)	9 (12)	—
Fruits	11 (14%)	36 (47%)	22 (29%)	7(9%)

6.3.8. The schools' physical environment.

School enrolments ranged from 50 to 1133 children. All schools but one claimed to have pit latrines for excreta disposal, but they were of poor quality. Most of the latrines were for temporary use with no cement floor. Furthermore, most of the latrines had no roofs to protect the superstructures and users from rain or sunlight. Most pits had no covers, making them places for housefly breeding, which could pose a risk for disease transmission. The walls were built of grass or coconut leaves. In most schools there were far fewer pits than recommended by the World Health Organisation (WHO) (one pit for every twenty-five girl students or one pit for 30 boys). Only seven schools (9%) met this requirement.

Forty five percent of the schools had vegetable gardens. However, they were activated only during the rainy season because of the scarcity of water during the dry season. Although 13% of schools provided school meals, this was possible only for a short time after the harvesting season. Most schools sold their produce because the amount produced was too little to feed the school population even for a short period of time. Most of the teachers, like the parents, were ready to participate in school meal provision. They suggested ways in which school meals could be provided including, support from the parents, both financial and in kind; and assistance from the government.

6.4. Discussion

6.4.1. Knowledge of anaemia

The results of this survey show that the children had fairly poor knowledge of anaemia compared to parents or teachers. Common causes of anaemia such as parasitic infections and chronic illness were known by only a small percentage of school children compared to parents and teachers.

Although the children were supposed to be taught nutrition from grade one, their basic knowledge of symptoms, causes and prevention of anaemia was the lowest among the three groups of people interviewed. These results highlight the need for more resource allocation to programs that would improve the health and education of school children. These resources are needed for health education materials, teachers' capacity building and training. Moreover, there is a need for the establishment of school lunch programs in order for children to learn about health issues as is the case in some Thai schools (Mwanri, 1994).

Even the parents' knowledge was, in some instances, questionable. For example, 35% of parents reported that use of toilets does not prevent anaemia and 13% did not know about it. That is 48%

of parents did not acknowledge the importance of toilets for anaemia prevention. This could be a result of currently available health education, which does not relate the use of latrines with the prevention of anaemia. The available public health education materials (in illustrations, or radio programs) overemphasise the consumption of balanced diet as a preventive measure against anaemia and overlook other related issues such as hygiene and water supply which can be indirect causes of anaemia (via worm infections, houseflies transmitting dysentery when they fly from uncovered pit latrines to food ready to be eaten).

Almost 50% of parents reported that drinking beer/coke would prevent anaemia, which could be a result of attractive promotion programs held by beer and soft drink companies, which are conducted very intensively, down to the village levels throughout the country. Such poor knowledge of symptoms, causes and preventive measures make it very difficult to effectively control and prevent anaemia.

Although the teachers' knowledge of symptoms, causes and prevention of anaemia was the highest of the three groups, the reliability of their knowledge was somewhat doubtful. For example, 49% did not know whether the use of toilets would help prevent anaemia. Based on these facts, it is evident that there is a need to develop activities which will ensure that parents and children are provided with adequate education in order to raise their knowledge of anaemia. The present results suggest the need for review of current anaemia education programs in the country.

6.4.2. The domestic physical and socio-economic environment.

The environment in which children live strongly influences the learning of children (Saini, 2000). It may also be the source of health problems which may affect how they interact and learn (Wass,

2000). Overcrowding in the homes of these children (mean 5.5 children /household) could pose a risk of health problems such as respiratory tract infections which could lead to poor school attendance, and in turn, poor learning. Moreover, overcrowding could lead to poor sleeping patterns which may ultimately, lead to poor concentration in school work. The poor quality of their latrines and the sharing of the communal water supply are likely to pose considerable risks of diseases such as intestinal worms and recurrences of diarrhoeal diseases. Improvement of the home environment and living conditions is important in order to improve the health and learning of these people.

Better parental education, especially maternal education, has been shown to increase health and nutrition knowledge which in turn can increase the quality of children's diets (Variyam, 1999; WHO, 1999). Since a substantial number of parents did not read or write, there is a need for deliberate efforts to improve their literacy level.

6.4.3. The role of schools and teachers in the prevention of anaemia.

The importance of safe hygienic school environments which allow children to work and play is self evident (Long-Shan et al., 2000). Schools should be able to provide an environment which promotes health not only to children but to the entire school community. Schools could act as role models in sanitation as well as in all matters pertaining to health and education. As such, they should have high standard sanitary facilities, especially pit latrines which are important in the prevention of diarrhoeal conditions such as dysentery which could lead to severe anaemia. The government could allocate some funds to subsidise parents' efforts for building simple but effective latrines for excreta disposal. In this regard, parents could contribute human labour, just as they provide it for building classrooms, while the government contributes building materials

for such activities. The existence of such collaboration would be consistent with the WHO notion of Health Promoting Schools.

At the curriculum level, teachers should be able to teach material relevant to local situations in order for children to acquire knowledge which they can use for the prevention of locally prevalent diseases. The current curriculum does not emphasise teaching children about the local issues which affect their health. Teachers should be free to tailor their teaching to specific local health issues which are relevant to their wellbeing.

6.4.4. Dietary intake

The children and parents appeared to have iron deficient diets. Dietary intakes of food such as meat, vegetables and fruits which are rich in iron are important in the prevention of anaemia (WHO, 1997). In addition, the low frequency of food consumption is another determinant of anaemia prevention. Not only did children have poor intakes of iron rich foods, but a substantial number of children did not have anything to eat before going to school or during school hours. This suggests that many are hungry for much of the day. The provision of meals at school would improve children's nutritional status and reduce the incidence of short term hunger at school (Walker et al, 1997). Studies in developing countries have shown that undernutrition and hunger among school children may affect their performance in school (Pollitt, 1990).

In their responses, both parents and teachers acknowledged the need for children to have meals during school hours. Most were willing to participate in the provision of meals. The parents' willingness to improve the health and nutrition of their children is a resource which can be employed to achieve better health and nutrition among these malnourished children. This could

be done by developing consultation strategies which would involve parents, teachers and school communities as a whole in participating in activities that would promote health in schools. For example, in China, the involvement of parents and the entire community in a deworming program helped transform the health behaviour of school children and influenced school policy formulation that favoured not only children's health but that of the entire school community (Long Shan et al., 2000). This Chinese example demonstrates that governments cannot conduct children's health promotion programs in isolation from the local community. Partnerships are required between schools, the local community and central government. The health and welfare of school children should be a responsibility of the school community (parents and teachers) as well as government. Governments have to ensure that they involve key partners in these issues and set up a policy framework for the provision of school meals, as is the case of health promoting schools.

6.4.5. Development of partnerships for improving school health and education.

To achieve maximum community partnership and commitment and to be able to work in partnership with local and central governments, schools should be advised by parents' boards. These would empower parents to address school meal provision and other issues related to school children's welfare. This approach had been used in the 1970's in Tanzania and anecdotal information suggests that children's health status then was better than now. In the above example from China, the deworming program became successful and influenced family priorities after parents and other community members became actively involved in the program (Long Shan et al., 2000). This suggests that partnership with parents, local communities and the government can be developed to foster activities that will promote the health and learning of school children.

The running costs for school meal programs can be relatively high. Partnerships between communities, local government (especially district and village councils) and central government, may allow some cost sharing in a manner appropriate to the local situation. For example in Thailand, school lunches are provided by all schools but the delivery strategies differ in different regions and schools (Mwanri, 1994). In the cities, parents contribute a certain amount of money to schools and schools use the money to buy foodstuffs, pay the cooks and other workers involved in school meal provision. In the rural areas, parents donate foodstuffs and volunteer to prepare and serve meals.

In Tanzania, the organisation of these services could be adapted to local conditions. For example, communities could involve volunteers such as parents or carers in meal preparation while other parents, who cannot volunteer their time, could contribute foodstuffs. Local government might contribute additional foodstuffs and cooking utensils, while the central government could provide kitchen facilities, and in an event that some schools fail to organise community volunteers, the employment of a part time cook. Alternatively, parents could contribute a certain amount of foodstuff, particularly maize flour, during the harvesting season allowing the school to store it for use for the whole year. In addition, parents could allow their children to bring firewood to school for cooking. Since the majority of schools have gardens which are not used in most instances due to lack of water, ways of collecting rain water during rainy seasons could be devised allowing the schools to collect water for use in the gardens in the dry season.

In all these options, the school principals should be the accounting officers. At the central government level school issues should be co-ordinated by a team of representatives from different sectors. These should include health, education, community development and agriculture. They

would advise government and schools on children's health promotion. At the curriculum level, health should be emphasised from the early years of schooling, across the curriculum.

6.5. Conclusion

Children are the future backbone of every nation, hence their current health and education are of paramount importance. It is reasonable to expect that schools and homes provide suitable conditions for healthy living and learning. As such there is a need to develop strategic plans to ensure the current meagre resources have maximum effects on the health and education of children.

Programmes should be designed to promote health and education. Consistent with WHO's Health Promoting Schools' Initiative, these programmes should not only target the children, but the parents, teachers and the wider community. Processes for involving teachers, parents and local communities in planning, and maintaining order are important for the long term sustainability of health promotion activities.

CHAPTER SEVEN

IN VITRO BIOAVAILABILITY OF IRON FROM MAIZE STAPLES.

7.1 Introduction

This chapter reports the results of the laboratory study which investigated iron concentrations and the in vitro bioavailability of iron from maize samples from Tanzania and Zimbabwe.

As described in chapter two, the onset of iron deficiency anaemia is not of sudden occurrence, but an outcome of a series of events (Viteri, 1998). Iron deficiency arises because of a combination of four major factors, among them, dietary iron bioavailability. Maize grains are the main staples consumed in Eastern and Southern Africa (Bazinger, 1997). Since the main diet consumed by children in Bagamoyo district (study 1) is maize it appeared important to investigate the bioavailability of iron in these staples to determine whether poor bioavailability of dietary iron is one among the major causes of anaemia in these communities. The results of these assessments may assist in making recommendations for the control of iron deficiency anaemia related to dietary iron bioavailability.

In addition, since there are now newly bred varieties of maize cultivars which are known to have higher iron concentrations than the conventionally consumed cultivars (Graham & Welsh, 1996), it was important to assess their iron's bioavailability in order to be able to compare them with the traditional ones and recommend the breeding of new cultivars with high iron density. Selective plant breeding for high iron density maize may well be among one of the most cost effective means of controlling iron deficiency anaemia in the country.

Bioavailability is one amongst many crucial determinants of iron adequacy in the body (Viteri, 1998). As defined in chapter two, iron bioavailability is the proportion of ingested iron which is absorbed from the intestinal lumen and transferred into the circulation. Iron bioavailability is determined by a complex mixture of factors, but the major important ones are dietary factors and body iron status.

7.1.1 Dietary factors: Types of iron

As noted above in chapter two, there are two principal forms of iron:

- Haem iron: iron in the form of haemoglobin or myoglobin, found in meat, poultry and fish (Cook, 1990; Layrisse, 1973; Viteri et al. 1978).
- Non-haem iron: iron found in non-animal sources, including plant foods, supplements and fortified foods (Bothwell et al., 1979; Layrisse et al., 1969).

Haem iron is much better absorbed than non-haem iron (Viteri, 1998). Although the haem iron content of a mixed diet is typically 10% or less of the total iron consumed, contributes approximately one third of the iron absorbed (Cook, 1990; Viteri et al.,

1978). Unfortunately, there is no figure which expresses exactly the degree of bioavailability of either haem or non-haem in the whole diet.

However, in single food studies a reasonable approximation is that haem iron is typically 20% or more absorbed, whereas absorption of non-haem iron is usually 5% or less (Cook, 1990; Layrisse et al., 1969). Non-haem iron is also significantly affected by other dietary factors (iron absorption inhibitors and iron absorption enhancers). Unlike non haem iron, haem iron is absorbed via a different mechanism and is not affected by other factors in the meal (Layrisse et al., 1969).

7.1.1.1 Iron absorption inhibitors

Several substances have been described as iron absorption inhibitors. These are: phytates, polyphenols, calcium, soy products, and cow's milk. Phytate is a potent inhibitor of non-haem iron, and it decreases the absorption by 50-80%. It is found in a range of foods, particularly cereal foods, legumes and nuts. The phytate content of cereals is high in unrefined forms. Moreover, the inhibitory effect of phytate is dose dependent. Polyphenols inhibit non-haem iron absorption and are found in tea, coffee, and some vegetables. Calcium, both in foods and in supplements, has been shown to inhibit haem and non-haem absorption in single meals. Other inhibitors are herbal infusions that produce polymers and insoluble, unabsorbable iron chelates (Viteri, 1998).

7.1.1.2 Iron absorption enhancers

Ascorbic acid, can increase non-haem iron absorption by two to three times (Cook, 1990). The enhancing effect of ascorbic acid is dose dependent, but it diminishes at high levels (Viteri, 1998). The absorption effect of ascorbic acid greatly depends on the meal matrix, its potency being least when a variety of foods is eaten, particularly meat. Other enhancers are cysteine, alcohol and some organic acids such as lactic acid, tartaric and citric acids which have been reported to increase non-haem iron absorption (Viteri, 1998).

7.1.2 Body iron status

Body iron status significantly affects iron absorption (Bothwell et al., 1979; Cook, 1990; Layrisse et al., 1969). When iron stores are replete, dietary iron absorption decreases to a level sufficient to cover the basal iron losses (Cook, 1990; Bothwell et al., 1979). As such, no accumulation of iron stores by dietary iron absorption occurs in normal people (Bothwell et al., 1979; Cook, 1990). However, in some circumstances in which normal homeostatic mechanisms are impaired such as haemochromatosis, iron overload can occur (Halliwell et al. 1992; Herbert, 1992).

7.2 Iron bioavailability and anaemia

As described previously, iron deficiency anaemia is the most widespread nutritional disorder in the world, but it affects more people in the developing world (Viteri 1998). The mechanisms which cause iron deficiency anaemia are complex. In part, this is because of the poor bioavailability of dietary iron in food and poor availability of food. This paucity of dietary iron is exacerbated by the high consumption of plant foods which contain high levels of phytates. These bind to iron to form insoluble complexes which interfere with iron absorption (Disler et al., 1995; Stahl et al., 1998; Viteri, 1998).

Efforts to improve the bioavailability of food iron and other nutrients especially from plant based foods have not been very successful due in part, to the lack of a simple means of estimating bioavailability (Glahn, 1998).

Recently, a laboratory model (Caco-2 cell) has been developed which simulates food digestion with a human intestinal cell line. The Caco-2 cell model resembles the human intestinal epithelial cells that line the inner surface of the small intestine. It allows food "digestion" to occur and at the same time nutrients are taken up by the Caco-2 cells (Glahn et al., 1998). The Caco-2 cells have been used for studying the digestion of staple foods like rice, corn, wheat, beans, and food supplements including cereals (Glahn et al., 1998). To find out how much of a food's iron is available to the Caco-2 cells, ferritin, iron storage protein is measured in the cells.

In previous chapters it was noted that many people in developing countries consume mainly plant-based staples. Maize (corn) is the main staple consumed in Tanzania, particularly for the majority of people in rural areas. Corn is also the main staple food consumed in Eastern and Central Africa (Bazinger, et al., 1997). Like many staples, the iron content in this staple is of non-haem origin and binds to iron inhibitors, mainly phytates which interfere with its bioavailability. In addition to inhibiting the bioavailability of iron, the iron content of conventional maize is also known to be low (Graham & Welsh, 1996). Efforts are underway to breed cultivars with higher densities of iron and other micronutrients, for example in Zimbabwe (Graham & Welsh 1996). It is likely that these biotechnologies which may enhance iron concentrations and iron bioavailability would be useful for the control of iron deficiency anaemia in Tanzania and elsewhere in the developing world.

7.3 Maize iron bioavailability study

The current study analysed the iron concentrations and their bioavailability from the two cultivars of maize grains described above.

Glahn et al's. (1998) novel the Caco-2 cell model, is an in vitro laboratory model which resembles human intestinal epithelial cells that line the inner surface of the small intestine and absorb nutrients from the food eaten. The model combines digestion with cell culture. To achieve digestion and cell culture, a dialysis

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membrane is attached to a plastic insert (transwell inserts) specifically designed to fit inside each half dollar-sized culture wells. A dialysis membrane separates the well into an upper and a lower chamber. This protects the culture from digestive enzymes and microorganisms.

The food sample and enzymes that digest it are placed in the upper chamber for a period of three hours, which is about the time spent for digestion to occur in humans. The dialysis membrane prevents the digestive enzymes and microbes from reaching the Caco-2 cells in the lower chamber. This mimics the role of the mucus layer that protects the epithelial cells of human digestive tract. Nutrients and minerals pass through the filtering membrane to the waiting nutrient absorbing the Caco-2 cell cultured in the well below. The amount of ferritin, an iron storage protein in the cell is then measured to find out how much of the food's iron is available to the Caco-2 cells. By comparing the amount of iron absorbed by the cells to the amount originally present in food, a determination of nutrient bioavailability can be made. Glahn et al.(1998) claim that the amount of ferritin formed is a highly sensitive and accurate measure of iron uptake. The Caco-2 cell model appeared to be a valid simulator of human digestion as it has been accepted by some nutritionists as a model for human absorption (Glahn et al., 1998).

Therefore the aim of study 3 was to determine the concentration of iron in both cultivars mentioned above and to assess its bioavailability using the Caco-2 cell method.

As noted in chapter four, in recent years, Garcia-Casal et al. (1998) have reported that the addition of vitamin A and β -Carotene in varying concentrations to human diets can improve non haem iron absorption from staple based diets. However, Glahn et al. (1998) have claimed that such studies are too costly and slow in establishing the bioavailability of nutrients in humans.

Since the Caco-2 cell model has been claimed to mimic the human digestive system and demonstrated to be relatively cheaper and faster for bioavailability studies, it would appear that, adding vitamin A at varying concentrations would enhance the bioavailability of iron in vitro if Caco-2 model is a sound simulation of nutrient absorption in humans (See study 1 on Garcia-Casal et al).

Thus, the current study also attempted to test the hypothesis that addition of vitamin A in varying concentrations would lead to increased bioavailability of iron from maize staples in the Caco-2 cell simulation. We adapted the in vitro digestion/ Caco-2 cell model for this purpose.

7.4 Material and Methods

7.4.1 Collection of maize samples and determination of iron concentration

Different maize cultivars were obtained from different places in Tanzania namely:

- Mbeya A (Southern mountainous area)
- Mbeya B (Lowland areas)
- Kilimanjaro A (mountainous areas)
- Kilimanjaro B (Lowland area)
- Mororogoro A (Mountainous areas)
- Morogoro B (lowland areas).

New cultivars, known to have higher iron concentrations than the Tanzanian varieties, were also obtained from Zimbabwe. While the maize from Tanzania were collected by the candidate, the maize cultivars from Zimbabwe were received in Adelaide via post and had been sent under quarantine conditions. The maize cultivars from Zimbabwe were labelled as follows:

- entry 1 (Catete stock #DCJ),
- entry 2 (high Fe +Zn stock # 295 1-21),
- entry 3 (low Fe + Zn F1 stock # 295 22-42),
- entry 4 (stock # 295-7),
- entry 5 (stock # 295-2)
- entry 6 (stock 295-15) entry 7 (stock 295-1).

Each collected sample of maize was weighed and the weight recorded before grinding (**Appendix 19**).

Weighed maize seeds were ground by a hammer mill into powder form and stored in water and air proof plastic bags.

7.4.2 Determination of iron concentrations

The iron concentrations of the traditionally used maize from Tanzania and the newly bred iron rich cultivars from Zimbabwe (Bazinger, 1997) were compared.

The details of the procedure to determine iron concentrations is given in **appendix 20**.

After determination of the iron concentrations of each sample, two types of cultivars (Morogoro A and Entry 1 Catete stock #DCJ) were chosen for the estimation of their iron bioavailability. The sample of maize from Morogoro A was chosen because it was a sample collected from maize used to feed the children in study 1 of this thesis. The maize sample from Catete (Zimbabwe) was chosen because it was found to have the highest iron concentration of all the maize samples (**Table 20**). This was important because the amount of iron in the diet is an important factor which partly determines its bioavailability (Viteri, 1998). If the selected high density iron sample also provided more bioavailable iron, then it would suggest the utility of plant breeding for the amelioration and prevention of iron deficiency anaemia.

The absorption of non-haem iron depends not only on the amount of iron but also on the presence of enhancers or inhibitors of absorption in a food (Bothwell et al., 1979; Cook, 1990; Layrisse et al., 1969). Therefore, it was important to determine the concentration of phytates in the samples. As such, the two samples which were chosen for in vitro analysis were analysed to determine the concentrations of phytate. These findings are given in

Table 21

7.4.3 Caco-2 iron bioavailability method

The Caco-2 cell iron bioavailability method was laborious work which involved several procedural steps. First, media and transwell inserts were prepared in which the chemical reactions took place (Caco-2 cells). Second, the samples were prepared (the two maize samples, plus controls in the laboratory setting). Third, the various reagents were prepared as follows:

- ascorbic acid (this was used as control alone and for all samples)
- Fe solutions (as control in the laboratory setting)
- Pepsin and pancreatic/bile solutions (Digestive enzymes and the method to mimic the gastric juice)
- vitamin A (different concentrations used to assess whether differing concentrations affect bioavailability differently)

The details of the methods of preparations of these reagents are given in **Appendix 22**.

The above methods were applied three times (rounds) to determine the bioavailability of the two samples of maize cultivars.

7.4.4 Statistical analysis

Statistical analysis of the data was performed using the software package Genstat Release 4.1. Prior to analysis, the data were log transformed to achieve equal variances. As each replication of the experiments was in triplicate, a block analysis of each series of experiments was performed. A one way analysis of variance (ANOVA) was performed to compare the blocks. P values were considered to be significant if $p < 0.05$.

7.5 Results

7.5.1 Iron concentrations from different cultivars

The iron concentrations of the different samples of maize cultivars from Tanzania and Zimbabwe are summarised in **table 20**. The highest concentration of iron in cultivars from Zimbabwe was in Catete. Although the maize from Morogoro A did not have the highest iron concentrations among the maize samples from Tanzania, it was selected for Caco-2 assessment because it was a sample taken from maize used to prepare the foods provided in the clinical trial of school children described in Chapters four and five.

Table 20: Iron Concentrations from different maize cultivars

Sample ID	Tube No.	Wt digested (g)	Fe ug/g	Av Fe ug/g	SD
Entry 3a	1	0.5029	17.4	17.4	0.29
	b	0.5000	17.1		
	c	0.5064	17.7		
Entry 4a	4	0.5012	26.4	27.6	1.71
	b	0.5031	28.9		
	c	0.5007	28.9		
Entry 6a	7	0.5009	27.3	26.8	3.07
	b	0.5007	25.3		
	c	0.5009	23.9		
	d	0.5029	30.9		
Entry 1a	11	0.5020	29.8	31.5	1.59
	b	0.5030	32.9		
	c	0.5031	31.9		
Entry 7a	14	0.5000	23.9	26.2	2.05
	b	0.5030	27.1		
	c	0.5000	27.7		
MoroB: a	17	0.506	14.5	15.0	0.58
	B	0.5026	14.8		
	C	0.5019	15.6		
KilimaA: a	20	0.5052	18.9	19.6	1.15
	B	0.5054	18.9		
	C	0.5050	20.9		
MoroA: a	23	0.5054	18.3	18.4	0.81
	b	0.5000	17.7		
	c	0.5050	19.3		
KilimaB: a	26	0.5003	18.1	19.3	1.08
	B	0.5027	19.6		
	C	0.5029	20.2		
MbeyaB: a	29	0.5040	19.9	22.6	2.46
	b	0.5026	24.8		
	c	0.5034	23.1		
Mbeya A:	32	0.5029	22.4	24.0	2.81
	b	0.5049	27.2		
	c	0.5031	22.4		
Entry 5: a	35	0.5004	21.7	21.4	0.42
	b	0.5038	20.9		
	c	0.5008	21.7		
Entry 2: a	38	0.5018	21.6	22.3	0.59
	b	0.5060	22.6		
	c	0.5010	22.7		
Control	41	0.5014	37.0	36.5	
Control	42	0.5033	36.1		

Sample ID= Maize samples from Tanzania and Zimbabwe. Each sample had 3 sub-samples.

Tube No. = Each sub sample was placed in a pre labelled test tube

Wt digested = Weight of maize sample analysed

Fe ug/g =Iron concentration in each sub sample

Av Fe ug/g = Average iron concentration per sample

SD = Standard deviation of the sample

7.5.2 Phytates concentrations in the samples

Table 21 summarises the average amount of phytates present in the two samples of maize. The results indicate that, cultivars from Zimbabwe which are known to have high concentrations of iron have three fold greater concentrations of phytates than the cultivars from Tanzania.

Table 21: Phytate concentration in each of the samples

Sample	Total phytates $\mu\text{g}/25\text{ml}$	Phytates mg/g sample	Average phytates mg/g sample
Standard 1a	356.1		
Standard 2a	354.3		
Standard 1b	392.4		
Standard 2b	320.8		
Catete 1a	140.1	9.94	
Catete 1b	166.9	11.83	$\bar{x} = 10.7$
Catete 2a	134.3	9.53	$\%cv = 9.6$
Catete 2b	164.8	11.69	
Morogoro A 1a	44.6	3.16	
Morogoro A 1b	51.9	3.68	$\bar{x} = 3.5$
Morogoro A 2a	78.9	5.56	$\%cv = 7.2$
Morogoro A 2b	52.42	3.72	

Each sample was prepared in four sub-samples to control for any errors that could occur in the laboratory setting. The total average (\bar{x}) of phytate concentrations (mg) from each sub-sample, formed the basis for amount of phytates content per sample (gram)

7.5.3 Iron uptake from the digests

Table 22 & Figure 11 present the amount of ferritin absorbed by each digest sample. When different concentrations of vitamin A were used to determine their effects on iron absorption, both samples of maize from Tanzania and Zimbabwe demonstrated relatively higher iron absorption when only 10 μ M of vitamin A was added as opposed to when 50 μ M of vitamin A was added. The highest concentration of ferritin was absorbed from the sample made of iron alone. This was expected since this digest contained the most iron. Comparison of the amount of iron absorbed when vitamin A was added to the Fe alone sample showed that vitamin A inhibited iron absorption to a certain extent although higher absorption occurred when 50 μ M of vitamin A was added compared to when only 10 μ M of vitamin A was added.

When ascorbic acid only was used, the maize from Tanzania generated more ferritin than the maize from Zimbabwe

Table 22: Ferritin absorption from different treated and digested samples

Summary of Ferritin production results

Sample	Round 1	SD r1	Round 2	SD r2	Round 3	SD r3	Average Ferritin	
							ng/mg protein	SD
AA only	20.9	19.5	28.3	4.34	75.8	5.38	41.67	26.26
AA +Vit A L1	27.6	4.89	20.3	5.17	72.8	24.44	40.23	24.93
AA +Vit A L2	26.9	4.54	28.8	4.31	61.2	48.74	38.97	22.97
Fe only	336.9	14.88	226.2	51.03	647	343.63	403.37	231.08
Fe + Vit A L1	243.8	31.61	224.3	76.95	238.7	116.51	235.60	92.19
Fe + Vit A L2	289.7	39.74	265.4	16.23	326.9	204.48	294.00	132.17
Morogoro Only	61.9	16.54	64.5	2.79	193.8	104.19	106.73	69.10
Morogoro + Vit A L1	20.7	11.94	43.9	21.18	160.5	36.87	75.03	55.77
Morogoro + Vit A L2	14.5	4.65	57.3	19.11	110.3	41.89	60.70	38.91
Entry 1 only	35.5	6.12	43.7	5.25	168	33.57	82.40	60.60
Entry 1 + Vit A L1	38.6	4.88	50.7	10.55	171.2	46.77	86.83	60.56
Entry 1 + Vit A L2	12.4	7.83	40	9.73	111.2	64.33	54.53	40.91

Legends

AA=Ascorbic acid alone

AA+VAL1=Ascorbic acid + Vitamin A at 10µM

Fe+AA= Iron +Ascorbic Acid

Fe+VAL1= Iron + Vitamin A at 10µM

Fe+VAL2=Iron + Vitamin A at 50µM

Mor+AA= Morogoro A maize +Ascorbic acid

Mor +VAL1= Morogoro A maize + Vitamin A at 10µM

AA+VAL2= Ascobic Acid + Vitamin A at 50µM

Mor+VAL2= Morogoro A maize + Vitamin A at 50µM

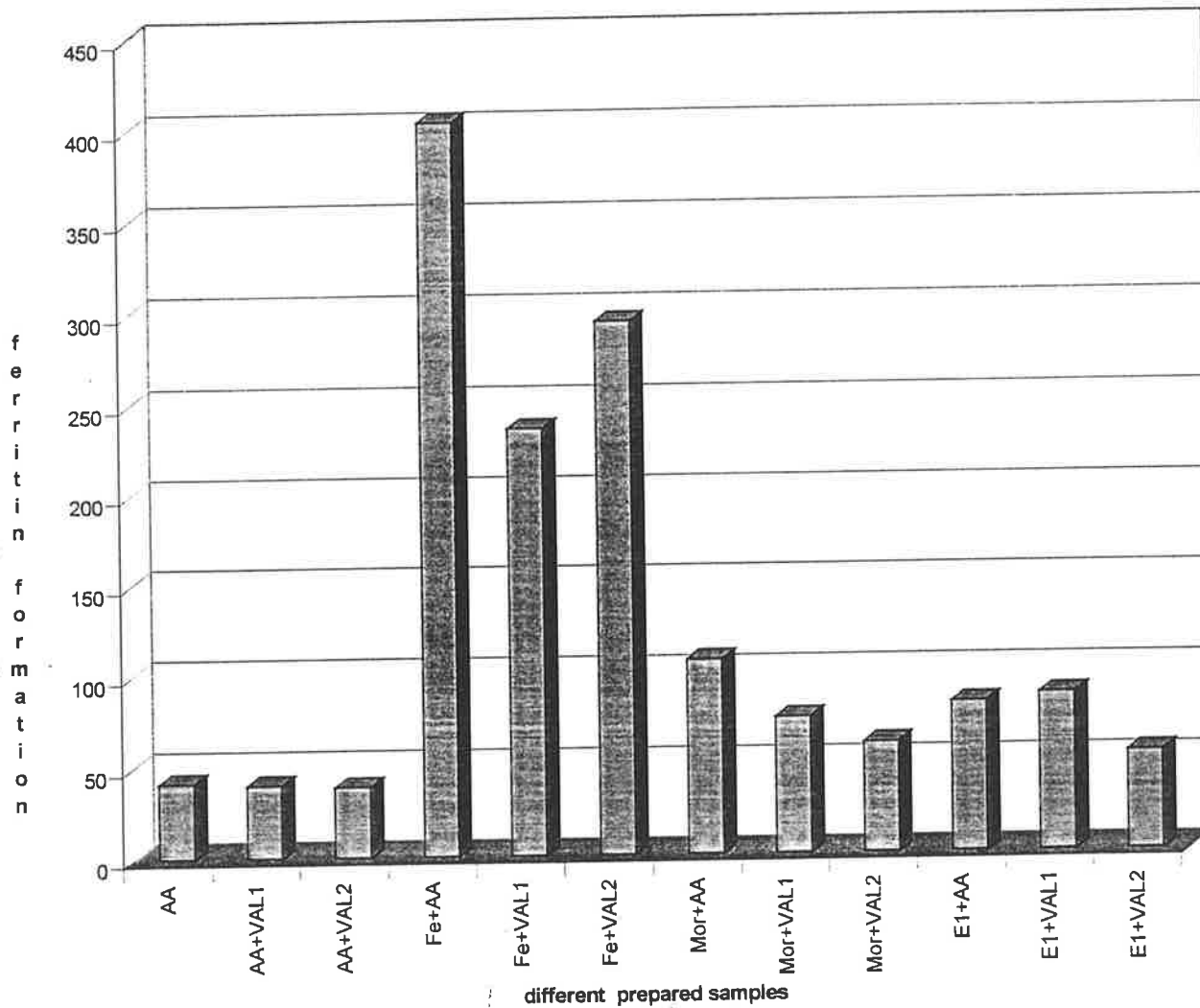
E1+AA=Entry 1 + Ascorbic acid

E1+VAL1= entry 1 + Vitamin A at 10µM

E1+VAL2=Entry 1 + Vitamin A at 50µM

* Ascorbic acid was used as control, but was also added in all samples.)

Figure 11: Ferritin absorption from different treated and digested samples.



Legends

- AA=Ascorbic acid alone
 - AA+VAL1=Ascorbic acid + Vitamin A at 10µM
 - AA+VAL2= Ascorbic Acid + Vitamin A at 50µM
 - Fe+AA= Iron +Asocrbic Acid
 - Fe+VAL1= Iron + Vitamin A at 10µM
 - Fe+VAL2=Iron + Vitamin A at 50µM
 - Mor+AA= Morogoro A maize +Ascorbic acid
 - Mor +VAL1= Morogoro A maize + Vitamin A at 10µM
 - AA+VAL2= Ascorbic Acid + Vitamin A at 50µM
 - Mor+VAL2= Morogoro A maize + Vitamin A at 50µM
 - E1+AA=Entry 1 + Ascorbic acid
 - E1+VAL1= entry 1 + Vitamin A at 10µM
 - E1+VAL2=Entry 1 + Vitamin A at 50µM
- * Ascorbic acid was used as control, but was also added in all samples.)

7.6 Discussion

The use of Caco-2 cells has been described as an attractive alternative to studies involving human and animal models (Gangloff et al.; 1996). However, the current study which investigated the bioavailability of iron from the two maize cultivars and tested the hypothesis that the addition of vitamin A would lead to increased availability of iron by Caco-2 cells from the corn samples did not support this claim.

In addition, the results of this study indicated that, although the caco2-cell model simulates digestion, it appeared that under controlled conditions iron up take by Caco2-cells does not exactly mimic human epithelial cells.

Although in the current study the Caco-2 cell model demonstrated that more ferritin (a measure of iron absorption) was formed when iron alone was used, addition of vitamin A in the iron alone decreased iron absorption compared to when ascorbic acid alone was used. This was in contrast to the effects of vitamin A on nonhaem iron availability which was demonstrated in studies involving humans (Garcia-Casal et al., 1998).

Although the maize from Zimbabwe had a higher iron concentrations than the maize from Tanzania, the ferritin formation was not as high as the maize from Tanzania. This could indicate that, the newly bred maize cultivars may have improved concentrations but not easily available as intended. This could be due to the fact that because the maize from Zimbabwe had higher concentrations of phytates than the maize from Tanzania the high

phytate levels could have affected the absorption. It was noted earlier in this chapter (7.1.1.1) that the inhibition of iron absorption by phytates is dose related.

When vitamin A was added in both samples of maize from Tanzania and Zimbabwe, it appeared that increasing levels of vitamin A would not increase the amount of ferritin absorbed by the cells. Both samples revealed decreased ferritin formation when higher concentrations of vitamin A were added. As noted earlier in chapter four, studies in humans have revealed that vitamin A and β -Carotene can enhance the absorption of non-haem iron (Garcia-Casal et al., 1998). The current study of in vitro digestion and iron bioavailability did not support this generalisation

7.7 Conclusion

1. The Caco-2 cell model does not necessarily mimic human epithelia cells and therefore does not simulate iron absorption in humans.
2. Phytates are indeed a problem; especially in population where plant based diets are main sources of iron.
3. Increased level of maize iron concentrations, as a measure of controlling iron deficiency anaemia may not necessarily be a quick fix of the problem because it is likely that, newly bred staples may have higher levels of iron inhibitors (such as phytates in this investigation) which may affect bioavailability.

4. Plant breeding applications may be one amongst many means of micronutrient prevention and control, but they need more fieldwork and trials in order to ensure that the newly bred micronutrient enriched staples are also highly bioavailable. Emphasis should also be placed in the crops and staples which are widely consumed in developing countries where iron deficiency anaemia and other micronutrient deficiencies are major public health problems.

CHAPTER EIGHT

GENERAL DISCUSSION

8.1 Introduction

The causes of malnutrition and micronutrient deficiencies especially iron deficiency anaemia are complex and context dependent, as well as dynamic in nature (Pinstrup Andersen, 1991). Such complexity demands a holistic approach to ensure that the determining causes of micronutrient deficiencies are addressed on a sustainable basis.

The studies presented in this thesis examined some of the complex interrelationships between iron deficiency anaemia and its determinants via experimental and survey methods. The latter approach examined the local context in which the problems evolved. This was thought to be necessary for effective control of these health and nutritional problems.

8.2 Relationship between vitamin A and anaemia.

The role of vitamin A in anaemia has been reported in earlier studies especially in pre-school children and pregnant women (Semba et al., 1992; Mejia et al., 1979; Mejia & Chew, 1979; Bloom et al., 1990). However, in this thesis, investigation of the effects of vitamin A and iron supplementation on biomedical status and cognitive functioning of anaemic school children was conducted for the first time. The results suggest that vitamin A supplementation of

anaemic children improved their haemoglobin concentrations (chapter four) and several aspects of their cognitive functioning (Chapter five). Although the age groups under which the investigations were undertaken were different from the previous studies, the findings were consistent with the findings of studies conducted in pre-school children and pregnant women (Mejia et al., 1979; Mejia & Chew, 1979; Bloom et al., 1990). In the current study both iron and vitamin A supplementation improved haemoglobin concentrations to differing degrees. The proportion of children who became non-anaemic after supplementation was 50% in the vitamin A-supplemented group, 79% in the iron supplemented group, and 88% in the group supplemented with both, but only 3% in the placebo group. This provides a strong evidence that supplementation with vitamin A and iron in iron deficient populations of children, can contribute to the control of anaemia. It also suggests that inclusion of vitamin A rich foods in the diets of the children may be central in any sustainable food-based approach to the elimination of iron deficiency anaemia.

8.3 Vitamin A and growth

Ashworth and Milliward (1986) have reported very rapid rates of catch-up growth in children recovering from severe malnutrition. Weight gains up to 20 times faster than those of normal children have been reported in these children. However, in the presence of stunting, the average rate of weight gain is considerably slower, approximately three to four times faster than would be expected for chronological age. Complete catch-up growth in situations where growth retardation has been extremely severe or prolonged, may occur prior to epipheseal fusion. Vitamin A may play a central role in this form of recovery from malnutrition.

Iron deficiency anaemia along with infectious diseases have been associated with poor growth (Kavishe, 1993; Stephensen, 1999). For example, enteric infections such as diarrhoea and helminthic infections can lead directly to malabsorption of nutrients. Stephensen et al., (1994) noted that even after nutrients are absorbed, they may still be lost as a result of infection with a large number of pathogens that cause direct nutrient loss, most commonly into the gut or urine. This phenomenon may be associated with protein loss; one of the proteins lost may be retinol-binding protein (RBP), the serum transport protein for vitamin A. Thus vitamin A may be directly excreted into urine with losses increasing as the severity of infection increases. The interaction of vitamin A and RBP has been mentioned in chapter four.

Consistent with the increase in haemoglobin concentrations, the current study found that vitamin A supplementation was associated with linear growth in anaemic children. Although these findings seem to be the first to be reported for school aged children, they are consistent with the findings of a study conducted in Indonesia, in which vitamin A supplementation in pre school children was correlated with attained weight and height (Haji et al., 2000). Similarly, in their longitudinal study in Sudan, Sedgh et al., (2000) found major reversal of stunting associated with intakes of vitamin A -containing foods.

Although the present results suggest that the rate of recovery was greater in children supplemented with both vitamin A and iron, it is important to note that the increased growth (weight and height) of the vitamin A only -supplemented group were significantly greater than the placebo group. Thus, these findings suggest that vitamin A can improve growth and increase the rate of recovery from stunting among anaemic malnourished children. The

probable mechanisms of action under which vitamin A works have been described in chapter four.

8.4 Iron and vitamin A supplementation and cognition

There is strong evidence that among school age children, initial low scores of tests of cognition or school achievement due to iron deficiency anaemia can be improved and in some instances reversed after iron treatment (Seshadri et al., 1982; Soemantri et al., 1985; Groner et al., 1986; Kayshap & Gopaldas, 1987; Seshadri & Gopaldas, 1989; Pollit et al., 1989). The current study which was carried out for only twelve weeks showed that both supplementation with iron and vitamin A improved the children's cognitive test scores but not their educational achievement. Iron supplements were associated with greater improvements than vitamin A alone.

However, supplementation with combined iron and vitamin A brought about more marked improvements than either supplement given singly. Although, to the best of the candidate's knowledge, the role of vitamin A in cognition has not been documented previously, the findings strongly suggest that iron deficiency anaemia is causally associated with lower scores on cognitive function tests in school children, and secondly, that increases in haemoglobin concentrations via iron and vitamin A supplementation can improve anaemic children's performance on these tests. As such, the findings show that iron and vitamin A have a role in raising haemoglobin concentrations and therefore, cognition in anaemic school children. The mechanism through which vitamin A improves cognitive functioning was probably through reduction of iron deficiency anaemia, though this requires further examination.

Iron deficiency anaemia has previously been associated with poorer performance on tests of educational performance (Pollitt et al., 1989). The results of the current study are consistent with this finding. The inability of the present study to demonstrate the effects of supplementation on the educational performance test scores may be explained as follows: Firstly, it appears that the period of three months was probably too short a period of time to demonstrate any improvements in educational performance. Secondly, it can be argued that cognitive function tests could be more sensitive than the educational achievement test. Thirdly, it may be argued that iron deficiency anaemia may be associated with poor educational achievement, but the effect may not be reversible with treatment. Seifer (2001) demonstrated that other factors such as social and economic influences may play more important roles in educational achievement. Further studies with larger sample sizes would be needed to demonstrate such an effect.

The findings described in this thesis suggest that vitamin A has a role in counteracting the consequences of iron deficiency anaemia. However, further longer term studies are suggested to investigate the impact of vitamin A on educational achievement and the mechanisms in which growth retardation is reversed. It would also be worthwhile to investigate the mechanisms through which vitamin A contributes towards improving cognitive functioning of anaemic children.

8.5 Iron deficiency anaemia and iron bioavailability

Study 3 looked at various maize cultivars including the newly bred varieties from Zimbabwe to determine the iron concentrations as well as in vitro bioavailability following in vitro digestion using the Caco-2 model technique. The newly bred cultivars from Zimbabwe appeared to have higher concentrations of iron compared to the traditionally consumed varieties from Tanzania. In addition to iron concentrations, the current study investigated whether, newly bred maize with high iron density would have higher iron bioavailability compared to conventionally consumed maize. Although the maize from Zimbabwe had higher iron concentrations than the conventional maize from Tanzania, it also had higher phytate concentrations. Moreover, consistent with the previously described dose related absorption relationship (Viteri, 1998), the phytate levels in Zimbabwean maize appeared to have affected its iron absorption. These are new findings which to the best of candidate's knowledge, have not been described before.

The newly bred maize cultivars from Zimbabwe are still under experimental investigations. Based on the amount of iron content of the newly cultivated maize, it is reasonable to suggest that, breeding for micronutrient enriched staples may be a useful approach towards the elimination of micronutrient deficiencies. However, substantial resources are needed to complete the fieldwork and trials in order to ensure that the newly bred micronutrient enriched staples are also highly bioavailable. Therefore, this may probably not be a quick fix for iron deficiency anaemia in developing countries. It appears that, there is a need to lobby governments and potential supporters for agricultural policies that will strengthen research for

micronutrient enrichment of staples. This would be one option, among several, for the long-term solution of micronutrient deficiencies in developing countries. However, other measures should be taken to promote other forms of anaemia control. These include community nutrition and health promotion approaches.

8.6 Health promotion as an integrative solution for control of iron deficiency anaemia.

It is evident that, regardless of possible development of plant breeding, health promotion has an essential role in the control of iron deficiency anaemia and other micronutrient deficiencies. However, it is important to note that there is a need to utilise a mix of interventions according to feasibility and objectives. Wass (2000) argues that health promotion goes beyond health care. It puts health on the agenda of policy makers in all sectors and at all levels, directing them to be aware of health consequences of their decisions and accept their responsibilities for health. She suggests that health promotion policy combines diverse but complimentary approaches including legislation, fiscal measures, taxation and organisational change. She emphasises that the co-ordination of health, income and social policies that fosters greater equity. This is important because the problem of micronutrient deficiencies is a manifestation of biological and social processes in society, rather than an isolated medical problem.

Parents, teachers and children's knowledge of causes, symptoms and preventive measures was elicited in study 2. Their willingness and attitudes towards anaemia prevention were also investigated. Other information related to living conditions and dietary practices was also

collected. The collection of this information was important because societies are complex. It was recognised that this information would give us a hint of some of local contexts in which health and nutrition problems evolved.

The survey of children, parents and teachers showed that they had poor knowledge of symptoms, causes and prevention of anaemia, especially, the children. The children's poor knowledge may be explained in a number of ways: Firstly, the primary school curriculum in Tanzania is developed at the central level in the Ministry of Education head quarters in Dar-Es-Salaam. In the absence of flexibility, teachers may not be able to include important local health issues in their teaching. Secondly, poor availability of education and information materials, and poor motivation for learning associated with poor supportive learning environments both at school and home could be another factor. Thirdly, the school environment under which children learn is very poor as illustrated in chapter six. This kind of environment may not facilitate learning, so even if children were taught under such conditions, then retention of knowledge could be poor. For example, in the study area, among the several causes of anaemia, intestinal worms, including hookworms and ascariasis are known to be highly prevalent. The transmission of these involves safe excreta disposal, handwashing, hygiene education, shoe wearing and poor living conditions. Knowledge of these factors is important for school children, parents and teachers if they are to control infections. These findings suggest the need for provision of more appropriate health education materials and modification of current health education policy in primary schools to allow greater flexibility in children's health education in Tanzania.

The poor living environments at home as well as at school, demonstrated in the quantitative data and observed during the fieldwork, do not reach minimal WHO criteria for health promoting schools. Inadequate sanitary facilities, lack of potable safe water, overcrowding, poor cooking facilities and poor housing were associated with low socio-economic background in the current study population. Pawlowski et al. (1991) have observed that a general rise in the standard of living conditions played a major role in the spontaneous decline of severe hookworm infection in developed countries where it was once prevalent but is now rare. With economic progress, hookworm anaemia declined or disappeared from many areas such as Japan, Europe and the United States (Pawlowski et al., 1991).

Therefore it is likely that the complete control of iron deficiency anaemia in developing countries will require radical but gradual economic progress coupled with low cost interventions and supporting infrastructure such as water supply, sanitary disposal facilities, better housing conditions and better road networks to allow transfer of a wide variety of foodstuff from one area to another. When living standards are improved, the supporting environment will be improved or vice versa. For example, shoes will be widely worn, minimising the hookworm infections, a potable water supply will be available to enable handwashing after visiting the toilets and before handling food, and better housing will enable prevention of vector borne diseases such as malaria which is among the major causes of iron deficiency anaemia. The attainment of these outcomes requires community involvement and identification of obstacles to the adoption of healthy public health policies in non-health sectors such as housing, water and sanitation. The need for a holistic approach to iron deficiency anaemia and other micronutrient deficiencies control programs across economic and social

sectors clearly emerges. Social interventions should involve more consciousness of micronutrient deficiencies, as well as combined efforts towards the control of other health and nutrition problems.

Exploration of the dietary practices of the participants demonstrated that the parents and the children consumed iron deficient diets. This is likely to be a major determinant of anaemia in this population. Moreover, the majority of the children went to school hungry and they were provided with no food while at school. A few schools did provide meals but the impression obtained is that the commonest meal provided was porridge. The nutritional value of this type of meal is questionable because in most cases it is made of maize flour, water and some sugar. Even for those schools where meals are provided, it is usually only during the harvest period when food security at home is also high.

According to the National School Health Policy (School Health Program, Ministry of Health, 1994), school meals are supposed to be provided by the communities. Those schools which did provide meals used produce from their gardens. This suggests that communities could be mobilised to contribute food and to prepare it for schools. It was observed that parents already provide labour for the construction of the classrooms. Perhaps, communities could also be mobilised to construct storage facilities so that schools can provide meals when food availability at home is lowest, for example, during dry seasons. The provision of school meals is likely to motivate children to go to school and consequently reduce truancy and drop out rates. It may also reduce the number of children who buy unhygienic and non-nutritious food from vendors in school compounds.

Wass (2000) discusses community action as one part of health promotion. The present survey demonstrated that parents as well as teachers were willing to participate in activities that would enhance children's health. Their positive attitude towards activities that would enhance the health of their children can be used as an entry point for health promotion, among the school community, especially in anaemia control. As in the example of the Grameen Bank in Bangladesh, parents', especially women's willingness and participation in social interventions can be a useful factor in bringing about social change (Grameen Bank, 2001).

8.7 RECOMMENDATIONS

The findings suggest a need for a new paradigm in the control of anaemia. A holistic approach is required to address the issues related to iron deficiency anaemia and other micronutrient deficiencies. The following are some of the recommendations for the way forward towards controlling the micronutrient deficiencies in Tanzania and other developing countries in a sustainable manner in which the conventional strategies would be augmented by others such as plant breeding and community development. The new paradigm would emphasise community involvement and fostering of intersectional partnership.

(i) Village development and women empowerment.

It is evident from the findings and my own experience that prevailing poverty is an important factor contributing to micronutrient deficiencies. Closer consideration of the village settings in Tanzania suggests that overall economic development of the villages would alleviate the nutritional problems. From my own observations, this is particularly true bearing in mind that

even in some village settings of Tanzania where there is better economic infrastructure there are fewer burden of disease in the population. It can be argued that poverty dictates a lower intake of iron rich meats and vegetables leading to iron deficiency anaemia. Parasitic infections, particularly hookworm disease, and other chronic infections which are highly prevalent in poor communities, increase susceptibility to nutritional deficiencies especially anaemia (Pawlowski et al., 1991).

So the way forward would be to improve the socio-economic status of the village people in developing countries. It has been observed that increases in economic base especially for women would lead to improved living standards (Grameen Bank, 2000). It can therefore be argued that participation of women as beneficiaries, providers and mediators in economic development can bring about a lot of success. This would probably mean that families would have better access to food availability, improved water supply, better environmental sanitation and the like.

Most women are hard working, modest and family oriented. They have multiple roles in the families and their contribution to the economic development of their households and communities are recognised. For example, Wandel & Holmboe-Ottesen (1988) in their study in rural Western Tanzania showed increases in stocks of maize and beans in households when women spent more time in the field. Husbands stayed longer in the field when the wife spent more time cultivating. In addition, women often brought children along to the field for breast-feeding, and they prepared something for the weaned children when they were left with someone else. When they left the field, they carried firewood at their heads while carrying

their young children on their backs. On their arrival at home they prepared food for the entire family and fed the animals. Empowering them economically would result in each member of family gaining benefits from the empowerment. Economic empowerment can be obtained through micro credits. In Bangladesh for example, majority of the Grameen bank's clients are women. It has been shown that micro-credit is an invaluable tool in alleviating poverty, promoting self sufficiency and stimulating economic activity in some of the world's most destitute and disadvantaged communities (Grameen Bank, 2000).

(ii) Introduction of information communication technology (ICT)

Lack of resources in schools, have been observed in this thesis as one amongst many reasons why the children had low knowledge of anaemia. Likewise, knowledge has been linked with economic development in many circumstances (Wolfensohn, 2000).

In developed countries, children acquire knowledge not only in the classrooms, but through other sources such as the Internet, radio, television and other information communication technologies. In fact, it appears that information communication technology (ICT) can be a major spur to economic development. The linking of the villages in developing countries with developed countries through information technology should be explored. Wolfensohn (2000) argues that the use of information and communication technology can improve education and overall economic development. This can provide sustainable solutions for training, education resources, and school to school partnership, which can bring students from developing countries online with global community. Wolfensohn (2000) further describes the

development spur in Uganda and other developing countries resulted from linking these countries with other developed countries. It is reasonable to argue that information communication technology can assist the children in developing countries to enter an information age and participate effectively in the global economy. This will in the end help develop their own communities through interchange of knowledge facilitated by this technology.

(iii) Improvement in governance, leadership and peace.

In many local settings in Africa, village development is constrained by other socio-economic challenges. Good governance has been acknowledged among other things to be critical in any development processes (WHO, 1999). In order to achieve what has been planned for village development, villages and local governments need to demonstrate good leadership, commitment and transparency. Good governance associated with partnership development among the community members and their leaders will need to be built if micronutrient deficiencies are to be controlled and prevented. Good governance was discussed as an important factor for disease control in Africa in the 21st century (WHO, 1999).

A peaceful environment is critical for the development of population health. For example, in some African and other developing countries, where wars are prevalent micronutrient malnutrition is also an important problem. The success of many interventions is based explicitly on the attainment of peace to maximise outcomes and impacts of investments (WHO, 1999).

(iv) Reinforcement of plant-based approaches and cropping systems diversification

The potential for home garden projects to add to the households food supply and income in developing countries should not be ignored. Home gardens have potential to not only improve the food supply and income, but also would provide for micronutrients which are mostly deficient in most foods. Vegetable gardens for example are important sources of iron and vitamin A. To meet the nutritional requirements of the entire family, households can grow different fruits, vegetables and staples in their gardens. In Tanzania, many fruits and vegetables are seasonal. This was revealed by the investigations in this thesis and my own observations. To cater for the dry seasons when water availability is poor, it is essential to develop local means of preserving vegetables and/or water supplies via wells or tanks.

It can be argued that, animals have a critical role in the food production system. Animals can provide power for cultivation, iron rich food, and other human resources e.g. clothing. Furthermore, small animals such as rabbits, chickens and fish are easy to look after and are major sources of nutrient e.g. eggs from chickens. My own experience provides evidence for this. Recalling from my primary school age when we used to keep these at school and regularly they would be a great source of food for lunches which were provided in school. Farming and home rearing of these animals coupled with other interventions can improve dietary quality, generate income as well as enhancement of agroecosystem in developing countries (Combs et al. 1995).

8.8 Conclusion

In this thesis aspects of anaemia control were addressed in the developing world context. The findings from these studies suggest that:

- 1 Vitamin A alone or in combination with iron can improve haemoglobin concentrations significantly.
- 2 Vitamin A in combination with iron can improve weight and height of malnourished children.
- 3 Vitamin A in combination with iron can improve cognitive function test scores but not educational achievement scores
- 4 Iron concentrations and bioavailability in the main staples may be one amongst the causal factors of anaemia. However, the findings suggest that plant breeding to produce high iron staples may not be a quick fix in the control of anaemia in developing countries.
- 5 The findings also provide information that children have relatively poor knowledge of causes and prevention of anaemia, which may have been a result of inadequacies in the school curriculum and educational material. Other factors such as poor dietary intakes, poor physical environments at home and in schools may possibly have been the causal factors for anaemia. However, communities in Tanzania are willing to participate in school activities that would improve the health of their children.

These findings suggest a complexity causation which demands a new paradigm in the control and prevention of anaemia. Holistic systems approaches are suggested because they consider a

variety of causal variables. Relevant factors such as community participation, women's empowerment, improvement of farming systems and storage of foods, plant breeding and possibly the use of dietary supplements in the short term, are all important in addressing iron deficiency anaemia and other micronutrient deficiencies. All these require policies that encourage integrated and well co-ordinated multi-sectoral strategies that promote nutritional outcomes through such means as education, health, information technology, financial support (e.g. micro-credit), political commitments, agricultural research and sustainable development.

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APPENDICES

Appendix 1: A letter of approval of the study from the University of
Adelaide Human Research Ethics Committee in Australia



H/27/98

THE UNIVERSITY OF ADELAIDE

Secretariat, Office of the Vice-Chancellor

Applicant: PROFESSOR T WORSLEY
Department: PUBLIC HEALTH
Project Title: THE IMPACT OF VITAMIN A - CAROTENE AND IRON
SUPPLEMENTS ON ANAEMIA AND COGNITIVE
FUNCTIONS OF SCHOOL CHILDREN, ACCEPTABILITY
AND ADOPTABILITY OF IRON DENSE MAIZE GRAIN IN
TANZANIA

THE UNIVERSITY OF ADELAIDE HUMAN RESEARCH ETHICS COMMITTEE

Project No: H/27/98
APPROVED for the period until 31 December 1999
noting that this research will be conducted by Dr Lillian Mwanri.

Dr CE Mortensen
Convenor

27 AUG 1998

Appendix 2: A letter of approval of the study from the Ministry of Health in
Tanzania

THE UNITED REPUBLIC OF TANZANIA
MINISTRY OF HEALTH

Telegrams: "AFYA", DAR ES SALAAM
Telephone: 20261
(All letters should be addressed to
the Principal Secretary)
In reply please quote:



P.O. Box 9083
Dar es Salaam

HED/51/92/VOL III/131

February 27, 1999

Mganga Mkuu wa Mkoa
Ofisi ya Mkuu wa Mkoa Pwani,
PO Box 30080
KIBAHA.

**Yah: UTAMBULISHO WA DR. LILIAN MWANRI KAMA MTAFIGI WA AFYA
YA JAMII**

Mtajwa hapo juu ni mtumishi wa wizara hii ambaye kwa sasa yupo masomoni Australia kwa Shahada ya Uzamili katika fani ya afya ya jamii.

Mojawapo ya masharti ya kupata shahada hiyo ni kufanya utafiti katika eneo la afya ya jamii. Yeye amechagua kufanya utafiti wa kutafuta athari za vitamin A katika upungufu wa damu na njia za fahamu za watoto wa shule.

Anakusudia kufanya utafiti huo katika Wilaya zilizopo katika mkoa wa Pwani. Kwa nakala ya barua hii, naomba Waganga Wakuu wa Wilaya wampatie msaada katika kurahisisha utafiti wake. Utafiti huo iwapo utahusu utoaji wa damu au matumizi ya dawa ya aina yeyote, Dr. Lillian atapaswa kutoa uthibitisho wa kibali kutoka Taasisi zilizoruhusiwa kisheria kutoa vibali vya aina hiyo.

Tutashukuru kwa ushirikiano wenu.

Nakala kwa: Waganga Wakuu wa Wilaya za; Kisarawe, Kibaha, Bagamoyo, Rufiji, Mafia na
Mkuranga.
" " Dr. Lilian Mwanri



TANZANIA FOOD AND NUTRITION CENTRE

OCEAN ROAD, NO.22

P. O. BOX 977 DAR ES SALAAM TANZANIA

Telephone: 255-51-118137/9

Telegraphic Address: "LISHE"

Fax: 255-51-116713

Telex: 41280.

E-mail: tfnc@costech.gn.apc.org

Your Ref. No

Our Ref. No

Date:

TFNC/24/119 vol.II/184

April 13, 1999

Dr. L. Mwanri
UKUMTA
Ocean Road Hospital
P.O. Box. 9383
Dar es Salaam

Dear Dr. Mwanri

Subject: *Research and Ethics Clearance of a research proposal titled: 'The impact of vitamin A on anaemia and cognitive functions of school children in Tanzania'.*

Your first and second revised versions of your proposal which had incorporated many of the comments raised by reviewers communicated to you earlier on became very handy when the Research and Ethics Committee met on April 7, 1999. I am therefore pleased to inform you that during that meeting the Committee (TFNC-REC) granted research and ethics clearance to the above referenced proposal.

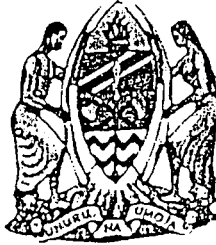
This memorandum may serve as evidence of the Committee's approval as far as research and the use of human subjects is concerned. Along with this clearance are recommendations from the Committee. We hope that you will use these recommendations to improve upon the proposal, before undertaking the research.

The Committee wishes you all the best.

Appendix 4: A letter of introduction and approval to conduct the studies in schools from the Ministry of Education and Culture in Tanzania

THE UNITED REPUBLIC OF TANZANIA
MINISTRY OF EDUCATION AND CULTURE

Cable: ELIMU DAR ES SALAAM.
Telephone: 110146, 110150/2, 111679.
Telex: 42741 Elimu Tz.
Fax: 0811 - 337563.
Email: escc@intafrica.com



P. O. Box 9121,
DAR ES SALAAM.

Reply please quote:
Ref. No. ED/SDP/CONSULT/3/22/63

Date: February 15, 1999

Dr. Lilian Mwanri,
Ministry of Health,
P.O. Box 9083,
DAR ES SALAAM.

YAH: KIBALI CHA KUFANYA UTAFITI KATIKA SHULE ZA MSINGI

Husika na barua yako ya maombi ya kufanya utafiti katika shule za msingi katika wilaya ya Babamoyo.

Baada ya kusoma msuada wako (Protocol) nimeridhika kuwa utafiti huo utakuwa wa manufaa kwa Taifa. Hivyo unaruhusiwa kufanya utafiti huo katika shule nne utakazo chagua wilayani Bagamoyo.

Pamoja na kibali hiki kutolewe unaagizwa kupata vibali vifuatavyo kwa maandishi yenye saina za wahusika:

1. Kibali kutoka kwa Afisa elimu wa wilaya ya Bagamoyo
2. Kibali kutoka kwa kila mwalimu mkuu wa shule zitakazo husishwa
3. Kibali cha wazazi wa watoto watakaohusika kwenye utafiti huo
4. Kibali cha kila mtoto atakaye husishwa kwenye utafiti huo kulingana na sheria za nchi zinavyoagiza hasa katika masuala ya umri wa watoto hao.

Baada ya kufanya utafiti huo tutahitaji kupata nakala tatu z ripoti yako.

Nakutakia ufanisi.

for MANENT SECRETARY

- Nakala:
1. Mkurugenzi wa Elimu (Msingi)
 2. Afisa elimu wa Wilaya, Bagamoyo
 3. Wizara ya Afya

Appendix 5: A letter of introduction and approval to conduct the studies in
schools the Bagamoyo District Executive Office

HALMASHAURI YA WILAYA YA BAGAMOYO

OFISI YA MKURUGENZI MTEMDAJI WILAYA,
S.L.P. 59,
BAGAMOYO.

Kumb. Na. BC/DC/E.10/3/VOL.II/142

4/4/1999

Walimu Wakuu Shule za Msingi,
Mataya, Konge na Kaclo,
Wilayani Bagamoyo.

YAN: KIBALI CHA KUFANYA UTAFITI KATIKA
SHULE ZA MSINGI

Hudika na mada ya hapo jam yohudika.

Kwa barua hii naporzia kutugulisheni kuamba Ofisi ya
Mkurugenzi Mtemdaji Wilaya inotoa kibali cha Dr. Lillian Mwarri
kutenbelea katika Shule zenu na kufanya utafiti.

Kibali hicho kinatolwa kuanzia tarehe 4/4/99 hadi tarehe
31 Julai, 1999 tunaomba muwape ushirikiano wa kutosha.

Kny

Appendix 6: Haemoglobin, anthropometry and physical examination tool

HAEMOGLOBIN, ANTHROPOMETRY AND PHYSICAL EXAMINATION:

Subject's name: _____

NO BASIC INFORMATION REQUIRED	DATA	CODES TO USE
1. Subject's identification number	_ _ _	Enter unique ID no
2. Subject's sex	_	1= Boy; 2= Girl
3. Subject's age	_ _	Enter age in years
4. Subject's class	_	Enter Class no.
5. Enter today's date	_ _ - _ - _ _	Enter as dd-mm-yy

BLOOD AND HAEMOGLOBIN

6. Haemoglobin concentration	_ _ _	enter gram/litre
------------------------------	-------	------------------

ANTHROPOMETRY

7. Weight	_ _ . _	enter to 0.1 of kg
-----------	---------	--------------------

8. Height	_ _ _ . _	enter to 0.1 of cm
-----------	-----------	--------------------

PHYSICAL EXAMINATION

8. Any symptoms or signs of vitamin A deficiency?	_	1 = yes; 2 = No
---	---	-----------------

If no enter 9 in Q8 and go to Q10.

If yes, go to Q9

9. Enter symptoms or signs of deficiency	_	1=Night blindness 2=Xerosis 3=Bitot's spots 4=Corneal ulcer 5=Blindness
--	---	---

10. Any symptoms of iron deficiency	_	1= Yes; 2= No
-------------------------------------	---	---------------

11. Enter symptoms and/or signs of iron deficiency

11.1. Angular stomatitis

11.2. Pale conjunctiva

11.3. Pale gums

_	1= Yes; 2= No
---	---------------

_	1= Yes; 2= No
---	---------------

_	1= Yes; 2= No
---	---------------

Appendix 7: The Knowledge Attitude and Practices Study Instruments

7.1 Children's Questionnaire

Subject's name: _____

Name of the School _____

NO	BASIC INFORMATION REQUIRED	DATA	CODES TO USE
1.	Subject's identification number	_ _ _	Enter unique ID no
2.	Subject's sex	_	1= Boy; 2= Girl
3.	Subject's age	_ _	Enter age in years
4.	Subject's class	_	Enter Class no.
5.	Enter today's date	_ _ - _ _ - _ _	Enter as dd-mm-yy

No.	QUESTIONS	CODES	ANSWERS or CODING
-----	-----------	-------	-------------------

6.	Have you ever heard of anaemia?	_	1= Yes; 2=No
----	---------------------------------	---	--------------

Question: Where did you get this information from?

7.	School	_	1= Yes; 2=No; 3=I don't know
8.	Home	_	1= Yes; 2=No; 3 = I don't know
9.	Hospital visit	_	1= Yes; 2=No;3 = I don't know
10.	Self reading	_	1= Yes; 2=No;3 = I don't know

Question: Who told you about anaemia?

11.	Teacher(s)	_	1= Yes; 2=No;3= I don't know
12.	Health worker(s)	_	1= Yes; 2=No; 3;I don't know
13.	Parents	_	1= Yes; 2=No; 3=I don't know
14.	Friend(s)	_	1= Yes; 2=No; 3 = I don't know
15.	Others (specify) _____	_	1= Yes; 2=No; 3 = I don't know

16.How harmful is anaemia ?

(1) not harmful	_
(2) fairly harmful	
(3) very harmful	
(4) I don't know	

Question: Which of these are symptoms of anaemia?

17.	Tiredness	_	1= Yes; 2= No; 3=I don't know
18.	Red eyes	_	1= Yes; 2= No; 3=I don't know
19.	Loss of energy	_	1= Yes; 2 =No; 3=I don't know
20.	Dizziness	_	1= Yes; 2 =No;3=I don't know
21.	Palpitation	_	1= Yes; 2= No; 3=I don't know
22.	Shaking	_	1 =Yes; 2 =No; 3=I don't know
23.	Vomiting	_	1 =Yes; 2 =No; 3=I don't know

Question: How do people get anaemia

24.	By eating inadequate meat, fish and green vegetables	_	1 =Yes; 2 =No; 3= I don't know
25.	If infected with malaria/hookworm/schistosomiasis	_	1= Yes; 2 =No; 3= I don't know
26.	Chronic illnesses such as sickle cell disease	_	1= Yes; 2 = No;3= I don't know
27.	By eating a lot of meat, fish and eggs	_	1= Yes; 2 = No;3= I don't know

28. Have you ever been treated for anaemia? 1= Yes;2= No;3= I don't know
29. Where do people get treatment from?
1. Hospital;
 2. school;
 3. traditional healer;
 4. Others (Specify) _____

Question: What could be done to prevent anaemia?

30. Eat a lot of rice 1=Yes; 2= No; 3= I don't know
31. Use toilets for excreta disposal 1=Yes; 2 =No; 3= I don't know
32. Drink bottled water 1=Yes; 2 =No; 3= I don't know
33. Treat malaria infection 1=Yes; 2 =No; 3= I don't know
34. Regular deworming of school children 1=Yes; 2 =No; 3= I don't know
35. Regular visits to traditional healers 1=Yes; 2 =No; 3= I don't know

Now I want to ask you questions about the food that you eat.

36. Did you eat any breakfast before you came to school this morning? 1= Yes; 2= No

37. What did you eat yesterday for breakfast? _____
38. What did you eat yesterday for lunch? _____
39. What did you eat last night for your dinner? _____

40. Did you eat any vegetables with your dinner last night? 1=Yes; 2= No

41. What vegetables did you eat? _____
- _____
- _____

42. Do you usually have something to eat when you are at school? 1= Yes; 2= No

43. What do you usually eat at school? _____
- _____
- _____
- _____

- 44 How often do you eat meat or fish with your meals?
1. Once or twice everyday
 2. Once or more per week
 3. Rarely
 4. None

45. How often do you eat green vegetables with your meals?
1. Once or twice everyday
 2. Once or more per week
 3. Rarely
 4. None

46. Do you eat fruits with your meals? 1 = Yes; 2 = No

47. How often do you eat fruit/drink fruit juice?
1. Everyday
 2. Once or more per week
 3. Once or more per month
 4. Only during fruit seasons

Question: Please can you tell me what foods you think you should eat to prevent anaemia?

48. Meat

49. Honey

50. Fruits

51. Green vegetables

52. Beer

53. Oranges

54. Bottled water

| _ | 1 =Yes; 2= No; 3= I don't know

| _ | 1 =Yes; 2= No; 3= I don't know

| _ | 1 =Yes; 2= No; 3= I don't know

| _ | 1 =Yes; 2= No; 3= I don't know

| _ | 1 =Yes; 2= No; 3= I don't know

| _ | 1 =Yes; 2= No; 3= I don't know

| _ | 1 =Yes; 2= No; 3= I don't know

7.2 Parents Questionnaire

NO BASIC INFORMATION REQUIRED DATA CODES TO USE

Subject's name (Name of the pupil whose parent/guardian is interviewed): _____

Subject's School Name _____

Subject's identification number | _ | _ | _ | Enter unique ID no

Enter today's date | _ | _ | - | | _ | _ | - | | _ | _ | Enter as dd-mm-yy

Subject's sex | _ | 1= M; 2= F

Subject's age | _ | _ | Enter age in years

No.	QUESTIONS	CODES	ANSWERS or CODING
1.	Do you know how to read? If Yes, Which language do you read If no enter 9 in the box and go to Q2	_ _	1= yes; 2= No 1 = Kiswahili; 2= English; 3 = Arabic; 4 =others (specify) _____
2.	What type of School have you attended?	_	1= Government 2= Religious
3.	How many years of education have you completed?	_	1= incomplete primary education 2= Completed primary education 3= Secondary/higher education
4.	Have you ever heard of anaemia?	_	1= Yes; 2=No
Question: Where did you get this information from?			
5.	School	_	1= Yes; 2=No;3=I don't know
6.	Health campaigns	_	1= Yes; 2=No;3=I don't know
7.	Hospital visit	_	1= Yes; 2=No;3=I don't know
8.	Self reading	_	1= Yes; 2=No;3=I don't know
Question: Who told you about anaemia?			
9.	Teacher(s)	_	1= Yes; 2=No;3=I don't know
10.	Health worker(s)	_	1= Yes; 2=No;3=I don't know
11.	Heard from the radio	_	1= Yes; 2=No;3=I don't know
12.	Friend(s)	_	1= Yes; 2=No;3=I don't know
13.	Village leader	_	1 = Yes;2=No;3=I don't know
14.	Others (Specify) _____	_	
15.	How harmful is anaemia?	_	1 =not harmful 2 =Fairly harmful 3 =Very harmful 4 =I don't know
Question: Which of these are symptoms of anaemia?			
16.	Tiredness	_	1= Yes; 2= No; 3=I don't know
17.	Loss of vision	_	1= Yes; 2= No; 3=I don't know
18.	Loss of energy	_	1 =Yes; 2 =No; 3=I don't know
19.	Dizziness	_	1 =Yes; 2 =No; 3=I don't know
20.	Hand tremors	_	1 =Yes; 2 =No; 3=I don't know

- 21.Sadness | _ | 1= Yes; 2 =No; 3=I don't know
 22. Palpitation | _ | 1= Yes; 2= No; 3=I don't know

Question: How do people get anaemia ?

- 23.By eating inadequate meat, fish and green vegetables | _ | 1= Yes; 2= No;
 24.By walking long distances | _ | 1 = Yes; 2 =No
 25.If infected with malaria/hookworm/schistosomiasis | _ | 1= Yes; 2 = No
 26.By eating a lot of meat, fish and eggs | _ | 1= Yes; 2 = No
 27.Chronic illnesses such as sickle cell disease | _ | 1= Yes; 2 = No
 28. Have you ever been treated for anaemia? | _ | 1=Yes;2=No; 3=I don't know;
 29. Where do people get treatment from? | _ | 1=hospital; 2=school;
 3=traditional healer
 4=others(Specify)_____

Question: What could be done to prevent anaemia?

30. Eat a lot of rice | _ | 1=Yes; 2= No; 3=I don't know
 31. Eat meat, green vegetables and fruits. | _ | 1=Yes; 2= No; 3=I don't know
 32.Use of toilets | _ | 1=Yes; 2 =No; 3=I don't know
 33. Treat malaria infection | _ | 1=Yes; 2 =No; 3=I don't know
 34.Regular deworming of people in the community | _ | 1=Yes; 2 =No; 3=I don't know

Now I want to ask you questions about the food that you eat.

13. Do you eat any vegetables with your meals | _ | 1=Yes; 2= No
 14. What vegetables do you eat? _____

15. How often do you eat vegetables with your meals?

- | _ | 1.Once or twice everyday
 2.Once or more per week
 3. Rarely
 4. None

- 16.How often do you eat meat or fish with your meals?

- | _ | 1.Once or twice everyday
 2.Once or more per week
 3.Rarely
 4.None

20. Do you eat fruits with your meals?

- | _ | 1 = Yes; 2 = No

21. How often do you eat fruit/drink fruit juice?

- | _ | 1.Everyday
 2.Once or more per week
 3.Once or more per month
 4 Only during fruit seasons

Question: Please can you tell me what foods you think you should eat to prevent anaemia?

- 22.Meat | _ | 1 =Yes; 2= No; 3=I don't know
 23.Honey | _ | 1 =Yes; 2= No; 3=I don't know
 24.Fruits | _ | 1 =Yes; 2= No; 3=I don't know
 25.Green Vegetables | _ | 1 =Yes; 2= No; 3=I don't know
 26.Beer | _ | 1 =Yes; 2= No; 3=I don't know
 27.Coke | _ | 1 =Yes; 2= No; 3=I don't know
 28.Coffee/tea | _ | 1 =Yes; 2= No; 3=I don't know
 29. Does your child eat any meal at school? | _ | 1 = Yes; 2= No
 30. Who provides your child with school meal? | _ | 1 = School
 2 = Parents/guardians
 3 = Buy from school vendors
 9= Not applicable

- 31.Do you approve the provision of school meals by school? | _ | 1=Yes; 2= No

- 32.Are you willing to participate in the provision of school meal? | _ | 1=Yes; 2= No

- 33..What are your opinions on the ways parents could participate in school meal provision?

PART II: SOCIAL ECONOMIC STATUS :

QUESTION	CODE	ANSWERS
1. What is your occupation?	_	1.Farmer 2.Fisherman 3.Civil servant 4.Petty business 5.Others(specify)_____
2. How many children live in your house?	_ _	
3. How many are your own?	_ _	
3.How many children are enrolled in school?	_ _	
4. What is the roof of your house made of?	_	1=Thatch/grass 2=Iron sheet 3=Tiles 4=Others(Specify)_____
5. What is the wall of your house made of?	_	1=Sticks and mud 2=Bamboo sheets 3=Bricks 4=Cement blocks 5=Others(Specify)_____
6. What is the floor of your house made of?	_	1=Earth 2=Cement 3=Burnt bricks 4=Wooden 5=Others(Specify)_____
Question: In your house do you have		
7.A Television	_	1=Yes; 2= No
8.A radio	_	1=Yes; 2= No
9.A bicycle	_	1=Yes; 2= No
10.A motor cycle or car?	_	1=Yes; 2= No
11. Domestic animals eg (Goats, Sheep and Chicken) ?	_	1=Yes; 2= No
12.Does your household have its own latrine?	_	1=Yes; 2= No
13. Where does the water you drink at home come from?	_	1=Tap 2= Bore hole 3= Rainwater 4 =River 5 =Pond/dam 6 =Others(Specify)_____
14. What type of lighting do you use at night?	_	1= Lantern 2=Pressure lamp 3= Electricity 4=Kerosine lamp 5=Others(Specify)___
15. What do you use to cook your food at home	_	1=Wood 2=Charcoal 3=Electricity 4=Kerosine stove 5=Other(Specify)_____

7.3 Teachers Questionnaire

Name of School _____

School Identification number _____

1 Have you ever heard of anaemia? | _ | 1= Yes; 2=No

Question: Where did you get this information from?

2.College | _ | 1= Yes; 2=No; 3=Don't know

3.Home | _ | 1= Yes; 2=No; 3=Don't know

4.Hospital visit | _ | 1= Yes; 2=No; 3=Don't know

5.Self reading | _ | 1= Yes; 2=No; 3=Don't know

Question: Who told you about anaemia?

6.College trainer(s) | _ | 1= Yes; 2=No

7.Health worker(s) | _ | 1= Yes; 2=No

8.Friend(s) | _ | 1= Yes; 2=No

9.Others (please specify) _____

10.How harmful is anaemia? | _ |

1. not harmfu
2. fairly harmful
3. very harmful
4. I do not know

Question: Which of these are symptoms of anaemia |

11.Tiredness | _ | 1= Yes; 2= No

12.Vomiting | _ | 1= Yes; 2= No; 3=I do not know

13.Loss of energy | _ | 1 = Yes; 2 =No; 3=I do not know

14.Red eyes | _ | 1 = Yes; 2 =No; 3=I do not know

15.Dizziness | _ | 1= Yes; 2 =No; 3=I do not know

16.Palpitation | _ | 1 = Yes; 2 =No; 3=I do not know

17.Hand Tremors | _ | 1 = Yes; 2 =No; 3=I do not know

Question: How do people get anaemia

18.Eat a lot of rice | _ | 1.= Yes; 2= No; 3=I do not know

19.By eating inadequate meat,
fish and green vegetables | _ | 1 = Yes; 2 =No; 3=I do not know

20.If infected with malaria/hookworm/schistosomiasis | _ | 1= Yes; 2 = No; 3=I do not know

21.Chronic illnesses such as sickle cell disease | _ | 1= Yes; 2 = No; 3=I do not know

22.By eating a lot of meat, fish and eggs | _ | 1= Yes; 2 = No; 3=I do not know

23. Have you ever been treated for anaemia? | _ | 1=Yes;2=No; 3=I don't know

24. Where do people get treatment from? | _ | 1=hospital;
2=school;
3=traditional healer;
4=others

Question: What could be done to prevent anaemia?

25.Drink bottled water | _ | 1=Yes; 2= No; 3=I don't know

26.Use toilets for excreta disposal | _ | 1=Yes; 2 =No; 3=I don't know

27.Eat a lot of boiled rice | _ | 1=Yes; 2 =No; 3=I don't know

28.Treat malaria infection | _ | 1=Yes; 2 =No; 3=I don't know

29.Regular deworming | _ | 1=Yes; 2 =No; 3=I don't know

Now I want to ask you questions about the food that you eat.

30. Do you eat any vegetables with your meals | | 1=Yes; 2= No
31. What vegetables do you eat? _____
32. How often do you eat vegetables with your meals?
 1. Once or twice everyday
 2. Once or more per week | |
 3. Rarely
 4. None
33. How often do you eat meat or fish with your meals?
 1. Once or twice everyday
 2. Once or more per week
 3. Rarely | |
 4. None
34. Do you normally eat fruits? | | 1 = Yes; 2 = No
35. How often do you eat fruit/drink fruit juice?
 1. Everyday
 2. Once or more per week | |
 3. Once or more per month
 4. Only during fruit seasons

Question: Please can you tell me what foods you think you should eat to prevent anaemia?

36. Meat | | 1 =Yes; 2= No;3=I don't know
37. Honey | | 1 =Yes; 2= No;3=I don't know
38. Fruits | | 1 =Yes; 2= No;3=I don't know
39. Vegetables | | 1 =Yes; 2= No; 3=I don't know
40. Beer | | 1 =Yes; 2= No;3=I don't know

Now I would like you to answer the following questions about your school.

41. How many children are enrolled in your school? | | | |
42. Does your school have toilets? | | 1 =Yes; 2= No;
43. How many pits are there? | | | | |
44. Does your school have vegetable and/or fruit garden? | | 1=Yes; 2= No
45. Does your school provide meals to children? | | 1=Yes; 2= No
46. Are you willing to participate in the provision of school meal? | | 1=Yes; 2= No
47. What are your opinions on the ways that school could participate in provision of school meal?

Appendix 8: A consent letter by school principals

BARUA KWA WAALIMU WAKUU WA SHULE

Upungufu wa damu ni tatizo ambalo limeonekana kuwaathiri watoto wengi wa shule za msingi hapa Bagamoyo. Ili kuweza kutatua tatizo hili, Wizara ya Afya ikishikiana na Wizara ya Elimu inafanya utafiti ili kupata taarifa zitakazoweza kupanga mikakati ya kuondoa tatizo hilo. Shule yako imechaguliwa kuwa mojawapo ya zile shule zitakazohusika katika utafiti huu. Tunaomba ushirikiano wako na tutashukuru kwa msaada wako wakati wa utafiti huu. Timu ya utafiti itakuwa hapa shuleni kwenu kwa muda wa wiki chache. Utafiti utachukua muda mfupi na tutajitahidi kutovuruga shughuli za shule za kila siku. Ikiwa ni uthibitisho wa kukubali, tafadhali weka sahihi katika barua hii.

Natanguliza shukrani za pekee.

Dr. Lillian Mwanri.

Mratibu wa utafiti

Mimi _____ ambaye ni mwalimu mkuu wa shule ya _____, nakubali shule yangu ishiriki katika utafiti wa kuwachunguza watoto kuhusu upungufu wa damu.

Sahihi ya Mwalimu mkuu _____.

Tarehe | _ | _ | _ | | _ | _ | _ | | _ | _ | _ |

Appendix 9: A letter to parents asking for proxy consent for their children to participate in the studies

Watoto wengi wa Tanzania wana upungufu wa damu ambao unawaletea kuwa wanyonge na kupelekea uwezo wao wa kimasomo kupungua. Tungependa kufanya utafiti ili kuweza kujua ni jinsi gani tungeweza kupunguza upungufu huu wa damu kwa kutumia vyakula vya mahindi ambavyo vinatumika na watu wengi katika vijiji vyetu. Utafiti huu utafanywa na wizara ya Afya, wizara ya elimu nautamaduni na Chuo Kikuu cha Adelaide huko Australia. Tunaomba idhini yako ili tuweze kufanya utafiti huu.

Mwanzoni mwa utafiti huu watoto wenye umri wa miaka tisa hadi kumi na mbili wataandikishwa kwenye utafiti huu. Mtoto atahitajika kutoa kidole chake cha kati ambapo tone kidogo la damu litachukuliwa ili kuangalia kama ana tatizo la upungufu wa damu. Uchunguzi huu utafanywa na daktari na kwakutumia kipimo maalum ambacho kimetengenezwa kwa kazi hii.

Watoto ambao wataonekana hawana upungufu wa damu hawatafanywa uchunguzi zaidi. Watoto watakaogundulika kuwa na upungufu wa damu watahitaji uchunguzi zaidi kuhusu afya zao na uwezo wao kimasomo. Endapo mtoto atahitajika kuendelea na uchunguzi zaidi, tutampima uzito na urefu wake ili kupima maendeleo ya makuzi yake. Mtoto atafanya zoezi la darasani kuchunguza maendeleo yake ya masomo.

Endapo mtoto ataonekana ana upungufu wa damu atapatata chakula cha shule na dawa ya madini ya chuma na vitamini. Baadhi ya watoto watapatata madini ya chuma peke yake au vitamini peke yake au chakula tu. Uchunguzi huu ni wa hiari kwa kila mtoto, kwani kama mtoto au mzazi hataki kushiriki katika uchunguzi huu hatalizimishwa. Taarifa zozote zinazowahusu watoto au wazazi wao zitatumizwa kwa siri na hazitaandikwa kwa ajili ya watu kusoma.

Kama utahitaji taarifa yoyote kuhusu utafiti huu, tafadhali uliza mwalimu wa shule ya mtoto wako au mratibu wa utafiti huu, Dr. Mwanri ambaye atakuwa katika katika eneo la utafiti.

Ili kuidhinisha mtoto wako achunguzwe, tafadhali tia sahihi au alama ya dole gumba katika mstari ulioochwa hapo chini. Kam hutapenda kusaini barua hii lakini uko tayari mtoto wako achunguzwe, tafadhali mwambie mwalimu mkuu wa shule ili aandike jina la mtoto wako kwa mratibu wa utafiti .

Asante sana kwa msaada wako.

Ninaidhinisha mtoto wangu kushiriki katika utafiti ambapo atachunguzwa kama ana upungufu wa damu. Nimesoma maelezo yaliyotolewa na watafiti na kuyakubali bila kulazimishwa.

Pamoja na kukubaliana na maelekezo ya watafiti, nakubali kutia sahihi barua hii kwa niaba ya mtoto wangu ambaye ni _____.

Appendix 10: Information of the studies and independent complaint procedures for parents/guardians.

Study 's Title: Impact of Vitamin A on Anaemia and Cognitive Functions of School Children in Tanzania.

A: Information which was given in request for informed consent:

Informed consent to conduct the study will be sought from the teachers and the principals within the study site as well as parents and guardians. Meetings will be held with them to ensure they understand the aims of the study, their roles in the study and potential benefits and risks to children who may be selected to participate. Parents and Guardians will be requested to sign a letter like the example given below to give their informed consent for their children to participate in the study. Informed verbal consent will also be accepted.

The basic information about the study to be presented for obtaining informed consent is given below. While the main content of the message will remain the unchanged, the style of letters will be modified according to whom the letter is intended. All letters will be translated into Swahili, a language spoken in Tanzania.

What is the purpose of this study:

Many children in Tanzania, have low haemoglobin and this may make them feel unwell and affect their ability to concentrate and learn. We would like to do a study to try and find out how we can increase the haemoglobin levels of the children by using foods which are used in this village.

The study is being organised by the Ministry of Health, Ministry of Education and Culture and The University Of Adelaide, Australia.

We would like to ask your permission to conduct this study. Before you decide, please read this letter carefully to find out what children would be asked to do.

What happens if you give permission for your child to participate.

At the start of the study, children aged 9 to 11 years will be registered to the study. Each child will be asked to give a middle finger and a small drop of blood will be taken by a small prick to see if he/she has low haemoglobin. This will be carried out by a Doctor and will cause a slight discomfort. There are no risks of infection associated with finger pricking as we use disposable collection devices.

Some children may be found to have high haemoglobin and will not have to do anything more in the study. Other children will be asked to stay in the study to find out more about their health and ability to learn at school. If a child is asked to take part in the rest of the study we will ask to measure her height and weight to see if he/she is growing well. We will also ask a child to do some small tests to see how much he/she is concentrating and learning at school. Children will be asked to be weighed again and measured again after three months to see how much he/she is growing. Children will also be asked to have a finger prick for blood to see how his/her blood haemoglobin has improved and do another tests to see how much he/she is improving in school.

If a child has low haemoglobin he/she will receive some lunch made at school with some vitamin and iron. Groups of children may receive vitamins only and others may receive iron or food alone.

What are your rights and benefits.

Participation in the study is voluntary. Children do not have to take part unless they want to. Children can also drop out of the study at any time if they or their parents want to.

Any information about parents' and children's names, and personal information will be kept confidential and will not be published.

Specific information for parent/guardian consent:

If you have specific questions about the study, please ask the school teacher and Field Co-ordinator (Dr. Lillian Mwanri) who will be visiting your area.

If you would like to let your child to take part in the study please sign your name on the line below.

Please put your thumb print if you can not write your name. If you do not want to sign the form but are happy for your child to participate in the study, please visit the principal of the school who will write down your child's name and pass it to the field co-ordinator.

Thank you for your help.

I hereby give my informed consent to allow my child to take part in the study.

I acknowledge that I have understood the information for request of the Informed consent and I have had the study as far as its effects fully explained to me by research worker. My Consent is given Freely.

In addition, I acknowledge the following on behalf of my child (-----)

Although I understand that the purpose of this study is to improve the knowledge in Public Health, it has been explained to me that involvement may not be of any benefit to her/him.

I have been given the opportunity to have some other parents and members of family and friends present while the study was explained to me.

I have been informed that the information s/he provides will be kept confidential.

I understand that s/he is free to withdraw from the study at any time and that this will not affect medical advice in the management of her/his health, now and in the future

I am aware that I should retain a copy of this Consent Form , when completed, and the relevant information sheet.

Signature-----

Date: -----

Signature of the witness-----

Specific information for teacher consent:

We would very much appreciate your assistance and support during the study. A research team including a medical doctor will visit your school for several weeks of the study. The team will make effort to minimise disruption with your school. As a sign of appreciation for your support of the study we will provide the school with a wall clock which can be put in the staff room, and the children taking part in the study will be provided with ball pens.

B: Contacts for information on study and independent complaints procedure.

If you have any questions or problems associated with the practical aspect of your child's participation in the study, or wish to raise concern or complaint about the study, then you should consult the project field coordinator

NAME: Dr. Lillian Mwanri (who will be in your area)

If you wish to discuss with an independent person matters related to making a complaint, or raising concerns on the conduct of the study, or the policies on research involving human subjects, or your rights as a participant.

Contact:

The Director of Preventive Services,
Ministry of Health,
P.O Box 9083,
Tel (051) 20236
Dar-es Salaam Tanzania.

OR

The Commissioner for Education,
Ministry of Education and Culture,
P.O Box 9121,
Tel. (051)110150/2
Dar-es-Salaam,
Tanzania.

APPENDIX 11: Calculations of the sample size

The sample size required for this study is based on the number of children required to find a significant intervention effect in the tests of cognitive functions. The method of calculating the sample size is given below (Kirkwood, 1990)

The standard deviation for cognitive functions (WISC) were obtained from Simeon and Grantham-McGregor (1989)

Sample size formula:

$$N = \frac{(u + v)^2 ((1+2))}{((1)-(2))^2}$$

Where (1 and 2) are variance of each group observed in Simeon and Grantham-McGregor (1989).

((1)-(2) is the difference in mean score between the group given breakfast and the one which breakfast was omitted.

Calculation was done applying a power of 90 (U = 1.28)

At the 5% level of significance (v = 1.96).

A minimum sample of 30 children per experimental group is required for this study. A total of 34 children was recruited for each experimental group to allow for losses to the study follow-up. (A factor Of 1.3 was applied for design effect and to cater for anticipated losses).

Calculation for Haemoglobin concentration was obtained from data observed by (Seshadri and Gopaldas, 1989) where a sample size of 16 school age children per experimental group gave statistical significant results.

Appendix 12: selection of pupils for impact of vitamin a on anaemia and cognitive functions of school children in Tanzania

School Name _____ School No: | _ |

Date of visit: | _ | | _ | | _ | | _ | (dd-mm-yy)

Ser. No.	Pupil's Name	Class	IDNO	Age	Sex
1.	_____	_	_ _ _	_ _	_
2.	_____	_	_ _ _	_ _	_
3.	_____	_	_ _ _	_ _	_
4.	_____	_	_ _ _	_ _	_
5.	_____	_	_ _ _	_ _	_
6.	_____	_	_ _ _	_ _	_
7.	_____	_	_ _ _	_ _	_
8.	_____	_	_ _ _	_ _	_
9.	_____	_	_ _ _	_ _	_
10.	_____	_	_ _ _	_ _	_
11.	_____	_	_ _ _	_ _	_
12.	_____	_	_ _ _	_ _	_
13.	_____	_	_ _ _	_ _	_
14.	_____	_	_ _ _	_ _	_
15.	_____	_	_ _ _	_ _	_
16.	_____	_	_ _ _	_ _	_
17.	_____	_	_ _ _	_ _	_
18.	_____	_	_ _ _	_ _	_
19.	_____	_	_ _ _	_ _	_
20.	_____	_	_ _ _	_ _	_
21.	_____	_	_ _ _	_ _	_
22.	_____	_	_ _ _	_ _	_
23.	_____	_	_ _ _	_ _	_

Appendix 14: Digit span test: Instructions and score form.

Materials

Two lists of numbers of increasing length, each with a pair of trials.

Instructions

The numbers are to be read at the speed of one second each. It is important for the tester to say the numbers exactly the same, in order not to help or hinder the child's performance. Ensure that the tester does not group the numbers when reading them, or differentiate or vary intonation. The test should stop if the child fails all three trials at one level.

Instructions for forwards digit span

If the child says quite different numbers from the ones read tell him/her once: *"Say exactly the numbers that I have said, not any other numbers"*

"Now I am going to say some numbers. I want you to listen carefully. When I have finished I want you to say those numbers exactly in the same order. Remember to repeat exactly what I have said. Let's do some practice. If I say 2 - 4 what would you say?"

Pause a little for the child to respond. If the child makes a mistake repeat the same example.

"We'll try again. If I say 2 - 4 what would you say?"

If the answer is correct say *"Yes, very good Now if I say 3 - 7 - 8 what would you say?"*

If answered correctly say *"Good. Well done. Now we shall do some more. I want you to repeat exactly what I say. We will start with a few numbers and go on to more numbers. Remember to say the numbers exactly as I have said them."*

Read the series of numbers from the lists written on the score sheet. Repeat a reading once if need be (if the child didn't hear well).

If the tester reads a wrong number by adding numbers or reading fewer numbers, indicate by a note where this mistake happened.

If the tester reads the numbers wrongly by reading a number which is not in the list but the item is exactly the same length, write a tick to indicate whether the child is correct or wrong, but show the error by a note as well.

Backwards

"Now I am going to say some more numbers but this time I want you to say them backwards. For example if I say 9 - 2 you would say 2 - 9 ?"

"Now if I say 4 - 5 what would you say?"

If the child does not say "5 - 4" you should say *"Listen carefully. If I say 4 - 5 you say 5 - 4. Let's practice again. Remember to say the numbers backwards. Now if I say 1 - 3 what would you say?"*

If the child says "3 - 1" or if he or she answers correctly earlier say "Good, you are right". Then continue with the three digit examples. If the child doesn't answer any of the examples correctly for the first time, repeat all the examples once only. Also say this: "Start with the last number then the first number" Demonstrate with your hands and your head "1 - 3" "3 - 1".

If the child fails to answer correctly any of the three examples twice (i.e. six examples in total) discontinue the test.

Three digit examples

It is not necessary for a child to get these correct. If the child has finished all the three digit examples without giving any correct answer, leave the examples and continue with number one.

Now if I say 5 - 6 - 8 what would you say? "

If answered correctly, say "Good, you are right."

Then proceed to question number one.

If the child fails in the example, say "No, you would say 8 - 6 - 5. Now if I say 5 - 6 - 3 what would you say?"

If the child says it wrongly say:

"I said 5 - 6 - 3, you would say 3 - 6 - 5." Demonstrate with your hands.

Let's try the following numbers. Remember to say them backwards: 7 - 2 - 4"

If the child answers correctly proceed to number 1. Item number one only has to be introduced by saying "Now let's start. Remember you have to say the numbers backwards"

Read the series of numbers as shown on the score sheet. If requested a trial may be repeated once (if the child didn't hear well). If the child says the numbers forwards, remind them by saying "Remember to say them backwards"

Scoring

Because children need to be encouraged at all times, say "Good", but put a tick (3) under the column with x if the answer is wrong, and put a tick (3) under the column with 3 if the answer is correct.

If the child fails to say the numbers correctly but self-corrects, that is still wrong.

Score the total number done correctly. The test should be stopped if the child fails on all items of one trial. Also score the highest level reached.

Duration: Forwards 2-3 minutes
 Backwards 2-3 minutes

Tarehe

|_|_|_|_|_|_|_| Jina

Idadi ya namba - Fomu ya matokeo

Mbele

Zoezi: 2-4 3-7-8

Kiwango	Jaribio 1	✓	X	Jaribio 2	✓	X	Jaribio 3	✓	X	Jumla
	3-8-6			6-1-2			4-6-1			
	3-4-1-7			9-1-5-8			6-1-4-9			
	8-4-2-3-9			5-2-1-8-6			7-2-8-1-3			
	3-8-9-1-7-4			7-9-6-4-8-3			9-2-5-1-6-8			
	5-1-7-4-2-8-3			9-8-5-2-1-6-3			5-6-8-4-2-7-1			
	1-6-4-5-9-7-6-3			2-9-7-6-3-1-5-4			7-1-6-8-4-8-3-9			
	5-3-8-7-1-2-4-6-9			4-2-6-9-1-7-8-3-5			2-7-5-8-1-3-9-4-6			
Idadi										
Jumla ya majibu sahihi										
Kiwango cha juu alichofikia										

Nyuma

Zoezi: 9-2 4-5 1-3

5-6-8 5-6-3 7-2-4

Kiwango	Jaribio 1	✓	X	Jaribio 2	✓	X	Jaribio 3	✓	X	Jumla
2	2-5			6-3			8-3			
3	5-7-4			2-5-9			1-3-5			
4	7-2-9-6			8-4-9-3			7-5-2-8			
5	4-1-3-5-7			8-7-4-5-2			3-6-1-9-4			
6	1-6-5-2-9-8			3-6-7-1-9-4			6-4-8-1-3-7			
7	8-5-9-2-3-4-2			4-5-7-9-2-8-1			4-3-6-7-9-2-8			
8	6-9-1-6-3-2-5-8			3-1-7-9-5-4-8-2			2-8-1-4-9-6-3-9			
Idadi										
Jumla ya majibu sahihi										
Kiwango cha juu alichofikia										

Tarehe

Jina la mtoto _____

Mchezo wa alama- Fomu ya matokeo

Mchezo

Muda

Makosa

Ukurasa 1 (Kimbele)

.

Ukurasa 2 (Kimbele)

.

Ukurasa 3 (Kinyume)

.

Ukurasa 4 (Kinyume)

.

Appendix 15: Instructions and the modified Stroop Task test

Materials

3 sheets of paper with approx. 6 x 8 marks (tick or cross).

Instructions

Tell the child that "I want you to touch and tell me if the mark is wrong or right.

Do as follows:

1. Tester will touch each mark and say if it is mistake or correct. Demonstrate three lines and say "*I want you to touch and say the names for all the remaining lines.*" If the child does well, tell him "*Well done*".

2. Tell the child:

"I want you to touch and tell the mark for the whole page as quickly as possible"
(show how to do quickly for two lines and the subject will touch and say all the marks for the whole page.)

The tester should insist on child touching and saying as quickly as possible, before and after; and remind the child whenever necessary not to make mistakes, nor to repeat any marks.

3. Tell the child "*Now I want you to touch and say the marks on the whole test page*"
Remind the child to go quickly and not to make any mistakes nor repeat any marks. If a child does make a mistake let him continue without going back. Ask the child "*Are you ready? OK, start*".

NB:

- Use a stopwatch once the child starts to mention the first word and stop it immediately after telling the last word.
 - Record the time used to cover the whole page until the last mark.
 - Show mistakes on the testing page, write one mark for each mistake and add them up later.
4. Tell the child, "*Now touch and say the marks as they are; on the tick mark say right and on the cross mark say wrong*".

"Ready? Start"

Record mistakes and time.

5. Tell the child *"Now you will do the opposite, for the cross, say right, and for the tick mark say wrong"*. The tester should demonstrate this.

Repeat the same way as for forwards test. Follow all steps exactly.

NB: Tell the child *"Now I want you to touch and say the opposite. For a tick mark you will say "wrong" and for a cross mark you will say "right". Remember to touch and say the opposite as quickly as possible"*

"Ready? Start"

Record time and mistakes.

6. Tell the child *"Now I want you to touch and mention the opposite again"*

Tell the child *"Remember to touch and mention the opposite as quickly as possible without going back or making mistakes while you are doing the whole page"*.

"Ready? Start"

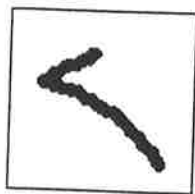
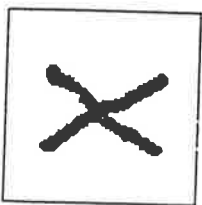
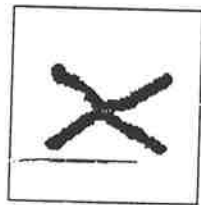
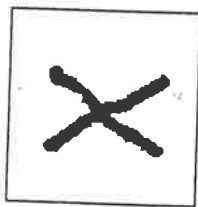
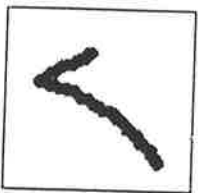
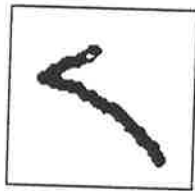
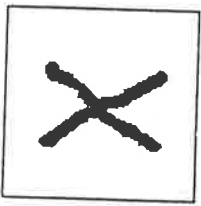
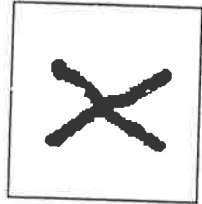
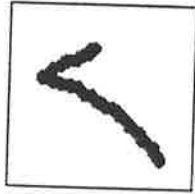
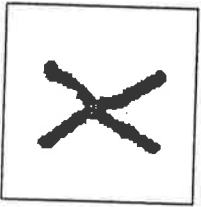
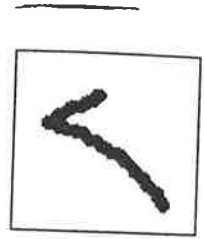
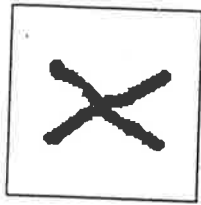
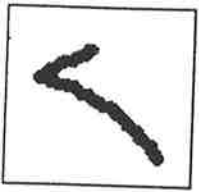
Record time and mistakes.

Important things to remember:

- Do not pause at the end of the line, it is important while you are demonstrating to the child.
- If a child makes one or two mistakes during practice correct him immediately, also remind the child if he/she does the opposite to what he/she is supposed to do. For example if during the test he/she is supposed to say the opposite and he/she says it forwards, remind him by saying *"Remember to say the opposite"*. Remind him/her once only, in order not to stop the child carrying on with the test.

The stopwatch should be started when the child starts to say the first word and stopped immediately after he finishes saying the last word. All mistakes made by the child should be counted and recorded even if the child corrects himself, together with all marks missed out during testing.

- If a child starts the test before stopwatch is started tell him to stop and start again.
- Make sure that different pages are used for both demonstration and testing.



Appendix 16: Oral Arithmetic test

Tarehe |__|__| |__|__| |__|__|

Darasa |__|

Jina _____

Shule _____

IDNO _____

Vidole 3

$9 > 6$

Penseli 9 - penseli 3

$4 < 8$

$41 > 28$

$30 < 40$

Vidole 8

Machungwa 3 + Machungwa 3

Maandazi 3 - andazi 1



3

5

6

71

Appendix 17: Written form of arithmetic test

HISABATI

Tarehe _____ Jina _____

Darasa _____ Shule _____

IDNO _____

Kujumlisha (+)

$5 + 1 =$

$6 + 1 + 2 =$

$5 + 7 =$

$$\begin{array}{r} 32 \\ 24 \\ + 40 \\ \hline \end{array}$$

$$\begin{array}{r} 75 \\ + \underline{8} \\ \hline \end{array}$$

$$\begin{array}{r} 452 \\ 137 \\ + \underline{245} \\ \hline \end{array}$$

$$\begin{array}{r} 48 \\ 2853 \\ 724 \\ 82 \\ + \underline{6215} \\ \hline \end{array}$$

Kutoa (-)

$8 - 0 =$

$5 - 4 =$

$14 - 8 =$

$$\begin{array}{r} 84 \\ - \underline{36} \\ \hline \end{array}$$

$$\begin{array}{r} 745 \\ - \underline{368} \\ \hline \end{array}$$

$$\begin{array}{r} 6204 \\ - \underline{530} \\ \hline \end{array}$$

$$\begin{array}{r} 700904 \\ - \underline{9018} \\ \hline \end{array}$$

Kuzidisha (x)

$$\begin{array}{r} 4 \\ \times 2 \\ \hline \end{array}$$

$$\begin{array}{r} 23 \\ \times 3 \\ \hline \end{array}$$

$$\begin{array}{r} 420 \\ \times 4 \\ \hline \end{array}$$

$$\begin{array}{r} 834 \\ \times 7 \\ \hline \end{array}$$

$$\begin{array}{r} 636 \\ \times 208 \\ \hline \end{array}$$

$$\begin{array}{r} 420.3 \\ \times 29 \\ \hline \end{array}$$

$$\begin{array}{r} 7.952 \\ \times 3.7 \\ \hline \end{array}$$

Kugawanya (÷)

$$4) \overline{8}$$

$$9) \overline{72}$$

$$6) \overline{968}$$

$$3) \overline{9.105}$$

$$31) \overline{6263}$$

$$5.2) \overline{572}$$

$$536) \overline{4762}$$

Kujumlisha sehemu (+)

$$\frac{1}{3} + \frac{1}{3} =$$

$$\frac{1}{2} + \frac{1}{4} + \frac{1}{8} =$$

$$2\frac{4}{5} + 7\frac{2}{5} =$$

$$3\frac{2}{5} + 2\frac{1}{4} + 1\frac{5}{6} =$$

$$6\frac{1}{5} + 4 + \frac{2}{7} + 9\frac{4}{5} =$$

Kutoa sehemu (-)

$$\frac{7}{8} - \frac{3}{8} =$$

$$4\frac{2}{3} - 2\frac{2}{3} =$$

$$8 - 1\frac{3}{5} =$$

$$7\frac{1}{6} - \frac{3}{4} =$$

$$19\frac{1}{5} - 14\frac{2}{3} =$$

Appendix 18: The weight of each collected maize samples from Tanzania and Zimbabwe

Maize samples as described below arrived in October 1999 and were stored under quarantine conditions within the locked fridge. Maize samples were hammer milled on site on the 10/11/99, weighed and returned to storage for analysis

Sample ID	Sample description	Weight(g)
Maize from Zimbabwe		
1	Catete stock # DCJ	113.4
2	High Fe+Zn stock#295 1-21	166.2
3	Low Fe+Zn F1 stock#295 22-42	180.0
4	Stock #295-7	100.2
5	Stock #295-2	96.6
6	Stock #295-15	99.0
7	Stock #295-1	99.2
Maize from Tanzania		
1	Vbeya region A- mountainous	37.5
2	Mbeya region B- low area	40.7
3	Kilimanjaro region A mountainous	46.4
4	Kilimanjaro region B- low area	52.0
5	Morogoro region A- mountainous	57.3
6	Morogoro region B- low area	56.2

Appendix 19: Procedure for determination of iron concentrations from maize samples

In order to compare the iron concentrations from samples of maize from Tanzania and those from Zimbabwe, iron concentration was analysed. For each sample of ground maize mill, 0.5g of maize powder in a test tube was weighed using Sartorius 2003 MP weighing balance. The balance was tarred to zero before each weight measurement was taken.

After the weight was measured each sample was digested as follows: 5mls of Nitric acid (HNO_3) batch No. K₂6055167(BDH) Anala R[®] was added. Three to four acid washed glass beads were dropped into the tubes and 10 drops of 70% Perchloride acid (HClO_4) batch No. B324173 908 Anala R[®] was further added. The samples were then placed on a warm plate and left on the fume chamber overnight. To speed the digestion reaction, the tubes were heated on the following morning by a burner (High performance gas 85% butane, and 15% propane) until all the nitric acid fumes were vanished. The samples were then washed with water and left on a hot plate for half an hour to ensure that complete evaporation of the nitric acid. Samples were poured into plastic test tubes and diluted with milliQ water to fill up to 10mls and was ready to be analysed for iron concentrations using standard operating procedures of Varian Spectra AA 400 spectrophotometer. The Varian Spectra AA 400 Spectrophotometer prints out samples' iron concentrations instantly.

Appendix 20: Iron concentrations from different maize samples from Tanzania and Zimbabwe

Sample ID	Tube No.	Wt digested	Final Vol	Fe ug/ml	Fe ug/ml-blk	Fe ug/g	Av Fe ug/g	SD
Entry 3a	1	0.5029	10	0.9	0.875	17.4		
Entry 3b	2	0.5	10	0.88	0.855	17.1		
Entry 3c	3	0.5064	10	0.92	0.895	17.7	17.4	0.29
Entry 4a	4	0.5012	10	1.35	1.325	26.4		
Entry 4b	5	0.5031	10	2.36	2.335			
Entry 4c	6	0.5007	10	1.47	1.445	28.9	27.6	1.71
Entry 6a	7	0.5009	10	1.39	1.365	27.3		
Entry 6b	8	0.5007	10	1.29	1.265	25.3		
Entry 6c	9	0.5009	10	1.22	1.195	23.9		
Entry 6d	10	0.5029	10	1.58	1.555	30.9	26.8	3.07
Entry 1a	11	0.502	10	1.52	1.495	29.8		
Entry 1b	12	0.503	10	1.68	1.655	32.9		
Entry 1c	13	0.5031	10	1.63	1.605	31.9	31.5	1.59
Entry 7a	14	0.5	10	1.22	1.195	23.9		
Entry 7b	15	0.503	10	1.39	1.365	27.1		
Entry 7c	16	0.5	10	1.41	1.385	27.7	26.2	2.05
MoroB: a	17	0.506	10	0.76	0.735	14.5		
b	18	0.5026	10	0.77	0.745	14.8		
c	19	0.5019	10	0.81	0.785	15.6	15.0	0.58
KilimaA: a	20	0.5052	10	0.98	0.955	18.9		
b	21	0.5054	10	0.98	0.955	18.9		
c	22	0.505	10	1.08	1.055	20.9	19.6	1.15
MoroA: a	23	0.5054	10	0.95	0.925	18.3		
b	24	0.5	10	0.91	0.885	17.7		
c	25	0.505	10	1	0.975	19.3	18.4	0.81
KilimaB: a	26	0.5003	10	0.93	0.905	18.1		
b	27	0.5027	10	1.01	0.985	19.6		
c	28	0.5029	10	1.04	1.015	20.2	19.3	1.08
MbeyaB: a	29	0.504	10	1.03	1.005	19.9		
b	30	0.5026	10	1.27	1.245	24.8		
c	31	0.5034	10	1.19	1.165	23.1	22.6	2.46
Mbeya A:	32	0.5029	10	1.15	1.125	22.4		
b	33	0.5049	10	1.4	1.375	27.2		
c	34	0.5031	10	1.15	1.125	22.4	24.0	2.81
Entry 5: a	35	0.5004	10	1.11	1.085	21.7		
b	36	0.5038	10	1.08	1.055	20.9		
c	37	0.5008	10	1.11	1.085	21.7	21.4	0.42
Entry 2: a	38	0.5018	10	1.11	1.085	21.6		
b	39	0.506	10	1.17	1.145	22.6		
c	40	0.501	10	1.16	1.135	22.7	22.3	0.59
Control	41	0.5014	10	1.88	1.855	37.0		
Control	42	0.5033	10	1.84	1.815	36.1	36.5	

Appendix 21: Procedure used for determination phytates in the maize samples

In order to establish the inhibition effects of phytate in each sample, it deemed important to determine the concentration of phytate in the samples. As such, the two samples which were chosen for in vitro analysis were analysed to determine the concentrations of phytate. Phytate determination was conducted in duplicate for each sample. Samples were run through anion exchange columns and collected in digestion tubes. After addition of nitric and perchloric acid (HNO_3 & HClO_4), evaporation and digestion were performed to finally produce white HClO_4 fumes. Samples were finally made to a total volume of 25ml and then phosphorus determined by colourmetric method on addition of vanadate and molybdate reagents. Phytate concentrations from the samples were then printed and read from the colourmeter.

Appendix 22: Caco-2 cell model Bioavailability procedures

The caco-2 cell bioavailability method entailed the following procedures:

Media preparation

1. An amount of 1800ml milliQ H₂O was placed in a 2 L beaker
2. Then, 2x1L powdered media was added to H₂O (Dulbecco's modified Eagles medium-Sigma D-7777)
3. Powdered media was dissolved and the following were added: 200mg streptomycin, 120mg penicillin and 9.532g HEPES buffer were added to 1.5g NaHCO₃ after all other solid had been dissolved.
4. The pH was checked and adjusted to 7.0 with 1N NaOH
5. In a pre-sterile media bottles, the following were added to the bottle: 100µl fungizone/100ml final media volume. (Amphostat B @ 250µg/ml) 10ml of FBS heat inactivated /100final media volume.
6. Sterile filtered media was added to appropriate volume for each bottle.
7. A small volume of media was transferred to a 50mL yellow-capped container and was kept in incubator for 5 days to check for bacteria.

After 5 days the cells were read and were plated at 250,000cells/ml and 2ml/well in 6 well plates.

All tests were done in triplicate.

The media was changed every 2 days while the cells became confluent and differentiated before use. (This was 12 days after plating).

Preparations of the Transwell inserts.

The dialysis tubing (SpectraPor 2.1 MWCO: 15,000) were cut into appropriate lengths. The tubing were made wet in milliQH₂O and cut along one seam. It was then opened out and placed over the bottom of the Transwell insert. It was then secured with an O ring of a tight fit, while making sure that the dialysis membrane is tight and wrinkle free.

While working in a Biohazard hood, each insert was dipped into EtOH (AR grade) for about 10 seconds, then removed, shaken off excess EtOH and washed in a bath of sterile water. This was repeated four times in different sources of water and finally left in a yellow capped sterile 50ml container with sterile water to cover the inserts.

Preparation of the samples.

The treatments described below were used in triplicate.

Control AA* only	Fe [†] + AA. Fe at 15μM	Maize (Catete)+ AA	Maize(Morogoro)+AA
AA+ Vitamin A at 10μM	Fe AA+ Vitamin A at 10μM	Maize (Catete) + Vitamin A at 10μM	Maize (Morogoro) + AA+ Vitamin A at 10μM
AA+Vitamin A at 50μM	Fe +AA +Vitamin A at 50μM	Maize(Entry1-Catete) +AA+Vitamin A at 50μM	Maize(Morogoro) +AA+Vitamin A at 50μM

• AA = Ascorbic Acid

1. †= iron

Preparation of Ascorbic acid solution.

150mM of ascorbic acid (AA) was prepared in milliQ H₂O =264.2mg/10ml

100μl was added to each 10ml sample to give a final concentration of 1.5mM prior to digestion.

Preparation of Fe solution

15mM FeSO₄ in milliQ H₂O was prepared =417mg FeSO₄/100ml

500μl of 15mM solution was diluted with 500μl H₂O to make 7.5mM

200μl 15mM was diluted with 800μl H₂O to make 3.0mM

100μl 15mM was diluted with 900μl H₂O to make 1.5mM

Finally, the added iron concentration at each sample was at 15μM

Preparation of pepsin

0.2g pepsin was dissolved in 5ml of 0.1M HCl. 2.5g of Chelex-100 was added and the mixture shaken for 30 minutes. Centrifugation was done and the supernatant was removed to a clean tube. 5ml of 0.1M HCl was added and again shaken for 5 minutes, then centrifuged. The supernatant which formed about 8ml was then combined.

Preparation of pancreatin/Bile solution

0.05g pancreatin and 0.3g bile extract were dissolved in 25ml 0.1M NaHCO₃. 12.5g of chelex-100 was added and the mixture was shaken for 30 minutes. Centrifugation was done and the supernatant transferred to a clean tube. A further 10ml of 0.1M NaHCO₃ was added and shaken for another 5minutes then centrifuged. The supernatant was combined and formed about 27ml.

Preparation of vitamin A

Vitamin A was prepared to form 10 μ M and 50 μ M of the required two concentrations. 14.33mg of retinol was dissolved in 10mL THF (and kept in frozen state until when was ready to use)

In vitro digestion of maize:

The following samples were prepared

1. 200 μ l Ascorbic acid (AA) +19.8 ml milliQ H₂O
2. 200 μ l AA+200 μ l Fe at 1.5mM +19.6ml milliQ H₂O
3. 1.0g maize (Catete) +19.8ml milliQ H₂O+200 μ lAA
4. 1.0g maize (Morogoro) + 19.8ml milliQ H₂O +200 μ lAA

Samples were made and pH adjusted to 2.0 using 5M HCl

Different concentration of vitamin A were added at 10 μ mol for level₁ and 50 μ mol for level₂. 0.5 ml of pepsin was added to the samples and were incubated at 37^o for 60 minutes. After incubation, the pH was adjusted to approximately 5 using 1M NaHCO₃ using about 60 μ l of NaHCO₃. 2.5ml of pancreatin/bile solution were added and pH adjusted to 7.0. The volumes were adjusted to 30 ml with 5mM KCl.

Plates were randomly allocated to different treatment each before incubation each round.

Treatment of cells

When the simulated digest was completed the media was removed from cells in 6 well plates. 1 ml of fresh media was added to each well. Sterile Transwell inserts were placed into wells while making sure that as much water as possible was removed before hand. 1.5ml of intestinal digest solution was then added to the inserts while determining that the media was in touch with the membrane to allow Fe exchange. The lid was placed on and the plates were incubated at 37^oC. Gentle motion of the wells were made every 15 minutes to avoid settling of sediments. After 2 hours the inserts were removed and as much media as possible was removed from the wells. An extra 1ml of fresh media/well was added. The plates were then returned to the incubator and left for further 24 hours.

Harvesting the wells

After 24hours, the media was removed from the wells by a pipette(very carefully so as not to disturb the cells). The media was kept for determination of iron (Fe). 2ml milliQ H₂O was added

and the pipette was used to wash all cells from the plate surface. The cell solution was transferred to a labelled yellow capped tubes and frozen at -20°C until ready for ferritin assay.

Ferritin Assay

Ferritin assays were done by using standard Bioclone method (IRMA kit 20170125). Each cell solution in duplicate along with certified serum samples containing known levels of ferritin the analysis was done as follows. The tubes were numbered and $50\mu\text{L}$ of standard or samples added. $500\mu\text{L}$ of ^{125}I antiferritin (yellow solution) was added, vortexed and incubated for 10minutes at room temperature. Another $500\mu\text{L}$ of antiferritin magnetic solid phase(blue green) was also added to each tube, vortexed and incubated for 1 hour at 37°C . Centrifugation was done at 3000rpm for 5minutes and the supernatant was transferred and the tubes drained. A $500\mu\text{L}$ of wash solution was then added, vortexed, centrifuged and decanted as before. Pellets were counted to obtain count per minute. Analysis of ferritin content of the experimental solutions and digests were conducted using Gamma Counter.

Amount of iron digested

To determine the amount of iron digested, the concentrations of iron available in the media were assessed by AA photometric method, and the amount of ferritin for each sample was printed out.